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May 20, 2021

VIA FEDERAL EXPRESS
Robert Singer, MD, FACS
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VIA EMAIL AND FEDERAL EXPRESS
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Garden Grove, CA 92841
TheAestheticSociety@surgery.org

Re: Your False and Deceptive YouTube Video Publication and "Clarification"

Dear Dr. Singer:

We represent Dr. Charles E. Runels Jr. and have been asked to investigate the online YouTube video at https://www.youtube.com/watch?v=t-_HQWmcM4M, which is a video of a presentation you gave to The Aesthetic Meeting held in Miami Beach, Florida from April 29 – May 3, 2021, apparently on behalf of The Aesthetic Society ("TAS"). TAS issued an alleged "Clarification" of your video on May 18, 2021. In this video and the subsequent "Clarification," you and TAS attack the right of certain medical doctors to perform cosmetic PRP procedures. The premise upon which you and TAS base your attack, however, is demonstrably false by the very FDA guidance you purport to cite. For the reasons set forth more fully below, we request that you and TAS immediately (1) issue a retraction of your false statements and (2) ensure that the offending video is removed from YouTube and all other places where it may have been published.

As we understand you are aware, Dr. Runels performs, teaches, and licenses others to perform and teach (via a contract) his own brand of cosmetic PRP procedures under various trademarks, some of which trademarks you reference in your video presentation. Many other doctors perform their own brands of cosmetic PRP therapy. You state in your presentation that the "FDA indicated [that they] view PRP as a 'biological,' [which] would require an IND." If this were true (which it is not), this admonition would obviously directly harm the medical doctors who perform these procedures and who, not so coincidentally, compete with surgeons such as you.

You further explain in your presentation that the FDA has made a recent distinction between "stem cells," which it does consider to be a "biologic drug," and therefore subject to FDA regulation, and "autologous fat grafting," which the FDA does not consider to be a "biologic drug" subject to regulation. You cite no support, however, for your statement that the "FDA indicated" it views PRP as a "biological," but you imply that PRP is closer to stem cell therapy and that the FDA is treating it equivalently. You then explicitly warn doctors to stop performing cosmetic PRP procedures and to beware of alleged

enforcement by the FDA, including potential fines and criminal prosecution. You even go further to imply that state medical board discipline and licensing issues could arise. The TAS "Clarification" doubles down on the attack. In that regard, it makes the following statement about the FDA guidance at issue: "as it applies to physicians that use PRP, the update to the FDA guidance will prohibit . . . [a]dvertising or promoting off-label use/claims of benefits of PRP without an IND or IRB."

Without ever quoting from them, you refer to a set of November 2017 guidelines issued by the FDA throughout your presentation. Not only does the November 2017 guidance not pronounce PRP to be a biological drug, but the November 2017 guidance specifically states that PRP is not subject to FDA regulation:

> As noted in the Background section of this document, this guidance only applies to products and establishments that are subject to FDA's regulations in 21 CFR Part 1271. Establishments that meet the same surgical procedure exception in 21 CFR 1271.15(b) are not subject to FDA's regulations in 21 CFR Part 1271. This guidance also does not apply to products that fall outside the definition of HCT/P in 21 CFR 1271.3(d). For example, platelet rich plasma (PRP, blood taken from an individual and given back to the same individual as platelet rich plasma) is not an HCT/P under 21 CFR Part 1271 because it is a blood product. Accordingly, FDA does not apply the criteria in 21 CFR 1271.10(a) to PRP, and PRP is outside the scope of this guidance.

See Section V.A., p. 21, of the 2017 Guidance, which is attached as Exhibit A. The July 2020 Guidance, which is the current guidance, (1) supersedes the November 2017 Guidance, (2) was available almost a year before and in effect at the time of your presentation, and (3) states the identical exclusion from the guidance for PRP. See Section V.A., p.22, of the 2020 Guidance (which is identical to the above quoted paragraph), attached as Exhibit B.

You also state in your presentation that "we need verifiable scientific data," and you imply that there is no such data or research available for cosmetic PRP therapy. There is, however, already a great deal of scientific data and research available on the subject. It is clear that you and TAS chose simply not to look for it. In that regard, please see the twelve research papers attached collectively as Exhibit C, which represent only a fraction of the available research on the topic. While there are other false and

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misleading statements in your presentation and the TAS "Clarification," the above highlights the obvious attempt to deter doctors from performing cosmetic PRP therapy.

Taken as a whole, the statements you and TAS make are not only deceptive and misleading, but demonstrably false. As such, you and TAS are subject to a claim of deceptive trade practices (which, by the way, can also be brought by the FTC), along with claims for fraud, unfair competition, unjust enrichment, disparagement/trade libel, and intentional interference with contractual relations and/or prospective economic advantage. *See, e.g., Capaci v. Sports Research Corp.*, 445 F. Supp.3d 607 (C.D. Cal. 2020) (false and misleading statements regarding ingredients on label of dietary supplement provided basis for plausible fraud claim); *In re Seagate Tech. L.L.C. Litigation*, 233 F. Supp.3d 776, 791 (S.D. Cal. 2017) (finding plaintiffs adequately pled fraud claim based on Defendant's misrepresentations regarding the effectiveness of its products in certain (i.e., RAID) applications). Moreover, your actions are eerily reminiscent of the anti-competitive practices of the American Medical Association that were long ago ruled to be violations of the antitrust law. *See Wilk v. American Med. Assoc.*, 895 F.2d 352 (7th Cir. 1990) (holding medical association had engaged in illegal restraint of trade by making statements aimed at medical physicians expressing opinions that chiropractic care was unsafe).

In view of the above, we again demand that you immediately issue a retraction and perform a complete take down of your video and withdrawal of the TAS "Clarification" by the close of business tomorrow. If you and TAS do not comply with this request, please understand that Dr. Runels (and perhaps a class of similarly damaged doctors) will not hesitate to take legal action against you, TAS, and all those acting in concert, or who may be conspiring, with you in this matter.

Very truly yours,

Patrick J. Arnold Jr.

Patrick J. Arnold Jr.

cc: James M. Chao, MD, CEO **(VIA FEDERAL EXPRESS)**
Prime Plastic Surgery Associates Corp., d/b/a Prime Plastic Surgery & Med Spa
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EXHIBIT A

Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use

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Guidance for Industry and Food and Drug Administration Staff

For questions on the content of this guidance, contact Center for Biologics Evaluation and Research (CBER), Office of Communication, Outreach, and Development (OCOD) at 240-402-8010 or 800-835-4709. For questions about this document concerning products regulated by Center for Devices and Radiological Health (CDRH), contact the Office of the Center Director at 301-796-5900. If you need additional assistance with regulation of combination products, contact the Office of Combination Products (OCP) at 301-796-8930.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
Center for Devices and Radiological Health
Office of Combination Products
November 2017
Corrected December 2017

Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use

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Guidance for Industry and Food and Drug Administration Staff

Additional copies are available from:
Office of Communication, Outreach and Development
WO71, Room 3103
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10903 New Hampshire Ave.
Silver Spring, MD 20993
Phone: 800-835-4709 or 240-402-8010
ocod@fda.hhs.gov
https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

or
Office of the Center Director
Guidance and Policy Development
Center for Devices and Radiological Health
Food and Drug Administration
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Silver Spring, MD 20993
Phone: 301-796-5900
https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/default.htm

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Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use

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Guidance for Industry and Food and Drug Administration Staff

The final guidance document was issued in November 2017. This document was updated December 2017 to correct language on page 21 to remove references to autologous and non-autologous (allogeneic) products in Section V.B. (Compliance and Enforcement Policy Regarding Certain Regulatory Requirements).

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
Center for Devices and Radiological Health
Office of Combination Products
November 2017
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Contains Nonbinding Recommendations

Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use

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Guidance for Industry and Food and Drug Administration Staff

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

We, FDA, are providing you, human cells, tissues, and cellular and tissue-based product (HCT/P) manufacturers, healthcare providers, and FDA staff, with our current thinking on the criteria under Title 21 of the Code of Federal Regulations (CFR) Part 1271, specifically the 21 CFR 1271.10(a)(1) criterion of minimal manipulation and the 21 CFR 1271.10(a)(2) criterion of homologous use. The interpretation of the minimal manipulation and homologous use criteria and definitions of related key terms have been of considerable interest to industry stakeholders since the criteria and definitions were first proposed.[1] This guidance is intended to improve stakeholders' understanding of the definitions of minimal manipulation in 21 CFR 1271.3(f) and homologous use in 21 CFR 1271.3(c). It will also facilitate stakeholders' understanding of how the regulatory criteria in 21 CFR 1271.10(a)(1) and (2) apply to their HCT/Ps.[2] In addition, we are informing manufacturers, healthcare providers, and other interested persons that over the next 36 months, we intend to exercise enforcement discretion under limited conditions with respect to the investigational new drug (IND) application and premarket approval (biologics license application (BLA)) requirements, for certain HCT/Ps.

This guidance finalizes the document entitled "Minimal Manipulation of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps); Draft Guidance for Industry and Food Administration Staff" dated December 2014, and "Homologous Use of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps); Draft Guidance for Industry and FDA Staff" dated October 2015. This guidance also finalizes certain material related to adipose tissue that was included in draft guidance entitled "Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) from Adipose Tissue: Regulatory Considerations; Draft Guidance for

[1] "Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products" 63 FR 26744 at 26748-26749 (May 14, 1998). (Tissue Registration and Listing; Proposed Rule)

[2] This guidance does not address the classification and/or assignment of HCT/Ps that do not meet the criteria for regulation solely under section 361 of the Public Health Service (PHS) Act and 21 CFR Part 1271.

Industry" dated December 2014 (Adipose Draft Guidance). This material, together with the material related to adipose tissue included in the final guidance entitled "Same Surgical Procedure Exception under 21 CFR 1271.15(b); Questions and Answers Regarding the Scope of the Exception" dated November 2017, supersedes the Adipose Draft Guidance. Accordingly, we do not intend to finalize the Adipose Draft Guidance, which is now withdrawn. Finally, this guidance supersedes the document entitled "Minimal Manipulation of Structural Tissue (Jurisdictional Update); Guidance for Industry and FDA Staff" dated September 2006 (2006 Guidance).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word "*should*" in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

HCT/Ps are defined in 21 CFR 1271.3(d) as articles containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human recipient.[3] Because of the unique nature of HCT/Ps, FDA proposed and in 2005 implemented a tiered, risk-based approach to the regulation of HCT/Ps. Although FDA is authorized to apply the requirements in the Federal Food, Drug, and Cosmetic Act (FD&C Act) and the Public Health Service Act (PHS Act) to those products that meet the definition of drug, biologic, or device, under this tiered, risk-based approach, those HCT/Ps that meet specific criteria or fall within detailed exceptions do not require premarket review and approval. In developing the tiered, risk-based approach the agency focused on public health and regulatory concerns, including how transmission of communicable disease can be prevented; what processing controls are necessary to prevent contamination that could result in an unsafe or ineffective product, and to preserve integrity and function so that the products will work as they are intended; and how clinical safety and effectiveness can be assured. The tiered, risk-based approach is contained in a set of regulations commonly referred to as the "tissue rules," issued by FDA through notice and comment rulemaking, under the communicable disease authority of section 361 of the PHS Act

[3] Examples of HCT/Ps include, but are not limited to, bone, ligament, skin, dura mater, heart valve, cornea, hematopoietic stem/progenitor cells derived from peripheral and cord blood, manipulated autologous chondrocytes, epithelial cells on a synthetic matrix, and semen or other reproductive tissue. The following articles are not considered HCT/Ps: (1) Vascularized human organs for transplantation; (2) Whole Blood or blood components or blood derivative products subject to listing under 21 CFR Parts 607 and 207, respectively; (3) Secreted or extracted human products, such as milk, collagen, and cell factors, except that semen is considered an HCT/P; (4) Minimally manipulated bone marrow for homologous use and not combined with another article (except for water, crystalloids, or a sterilizing, preserving, or storage agent, if the addition of the agent does not raise new clinical safety concerns with respect to the bone marrow); (5) Ancillary products used in the manufacture of HCT/P; (6) Cells, tissues, and organs derived from animals other than humans; (7) In vitro diagnostic products as defined in 21 CFR 809.3(a); and (8) Blood vessels recovered with an organ, as defined in 42 CFR 121.2 that are intended for use in organ transplantation and labeled "For use in organ transplantation only." (21 CFR 1271.3(d))

Please note, the regulatory status of products identified as not being HCT/Ps is beyond the scope of this guidance.

(42 U.S.C. 264). These regulations explain the types of HCT/Ps that do not require premarket approval; and the registration, manufacturing, and reporting steps that must be taken to prevent the introduction, transmission, and spread of communicable disease by these HCT/Ps. These regulations can be found in 21 CFR Part 1271[4].

In 21 CFR 1271.10, the regulations identify the criteria for regulation solely under section 361 of the PHS Act and 21 CFR Part 1271. An HCT/P is regulated solely under section 361 of the PHS Act and 21 CFR Part 1271 if it meets all of the following criteria (21 CFR 1271.10(a)):

1) The HCT/P is minimally manipulated;
2) The HCT/P is intended for homologous use only, as reflected by the labeling, advertising, or other indications of the manufacturer's objective intent;
3) The manufacture of the HCT/P does not involve the combination of the cells or tissues with another article, except for water, crystalloids, or a sterilizing, preserving, or storage agent, provided that the addition of water, crystalloids, or the sterilizing, preserving, or storage agent does not raise new clinical safety concerns with respect to the HCT/P; and
4) Either:
    i) The HCT/P does not have a systemic effect and is not dependent upon the metabolic activity of living cells for its primary function; or
    ii) The HCT/P has a systemic effect or is dependent upon the metabolic activity of living cells for its primary function, and:
        a) Is for autologous use;
        b) Is for allogeneic use in a first-degree or second-degree blood relative; or
        c) Is for reproductive use.

If an HCT/P does not meet the criteria set out in 21 CFR1271.10(a), and the establishment that manufactures the HCT/P does not qualify for any of the exceptions in 21 CFR 1271.15[5], the HCT/P will be regulated as a drug, device, and/or biological product under the FD&C Act, and/or section 351 of the PHS Act (42 U.S.C. 262), and applicable regulations, including 21 CFR Part 1271, and premarket review will be required.

Minimal Manipulation

Section 1271.10(a)(1) (21 CFR 1271.10(a)(1)) provides that one of the criteria for an HCT/P to be regulated solely under section 361 of the PHS Act and the regulations in Part 1271 is that the HCT/P is minimally manipulated. As defined in 21 CFR 1271.3(f), minimal manipulation means:

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[4] *See* "Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing;" Final Rule, 66 FR 5447 (January 19, 2001); "Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products;" Final Rule, 69 FR 29786 (May 25, 2004); "Current Good Tissue Practice for Human Cell, Tissue, and Cellular and Tissue-Based Product Establishments; Inspection and Enforcement;" Final Rule, 69 FR 68612 (November 24, 2004).

[5] See the "Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception; Guidance for Industry" dated November 2017 available at: https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/UCM419926.pdf

1) For structural tissue, processing that does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement;
2) For cells or nonstructural tissues, processing that does not alter the relevant biological characteristics of cells or tissues.

FDA discussed these terms in the preamble to the HCT/P Establishment Registration and Listing final rule[6] and the 2006 Guidance. However, we have received requests from stakeholders to provide additional guidance that explains our current thinking related to meeting the criterion in 21 CFR 1271.10(a)(1). This guidance supersedes the 2006 Guidance.

Please note that if information does not exist to show that the processing meets the definition of minimal manipulation, FDA considers the processing of an HCT/P to be "more than minimal manipulation" that cannot qualify for regulation solely under section 361 of the PHS Act and 21 CFR Part 1271.[7]

Homologous Use

Section 1271.10(a)(2) (21 CFR 1271.10(a)(2)) provides that one of the criteria for an HCT/P to be regulated solely under section 361 of the PHS Act and the regulations in Part 1271 is that the "HCT/P is intended for homologous use only, as reflected by the labeling, advertising, or other indications of the manufacturer's objective intent." As defined in 21 CFR 1271.3(c), homologous use means the repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues with an HCT/P that performs the same basic function or functions in the recipient as in the donor. This criterion reflects the Agency's conclusion that there would be increased safety and effectiveness concerns for HCT/Ps that are intended for a non-homologous use, because there is less basis on which to predict the product's behavior, whereas HCT/Ps for homologous use can reasonably be expected to function appropriately (assuming all of the other criteria are also met)[8].

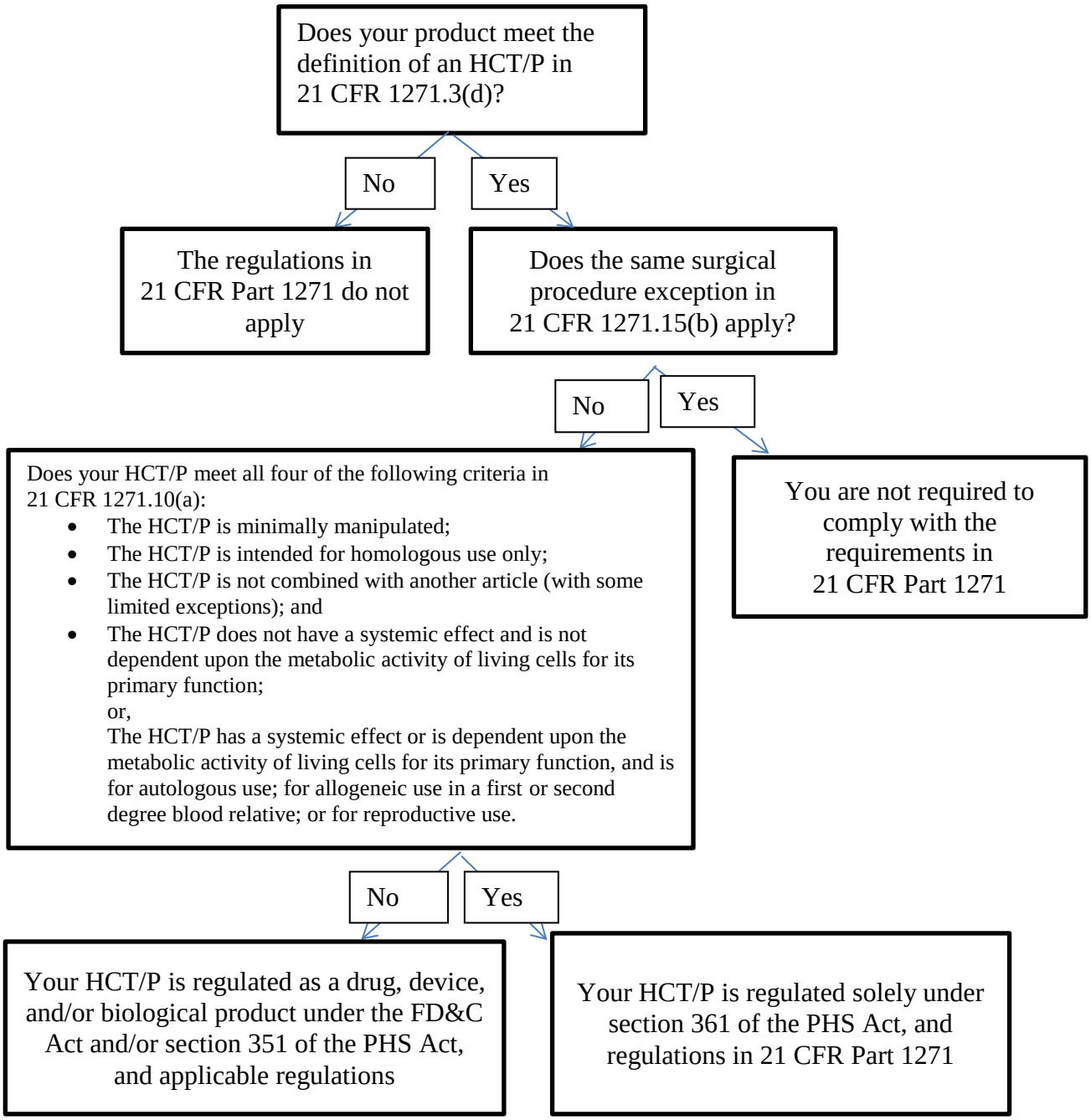
In applying the homologous use criterion, FDA will determine what the intended use of the HCT/P is, as reflected by the labeling, advertising, and other indications of a manufacturer's objective intent, and will then apply the homologous use definition.

FDA has received many inquiries from manufacturers about whether their HCT/Ps meet the minimal manipulation and/or homologous use criteria. Additionally, transplant and healthcare providers often need to know this information about the HCT/Ps that they are considering for use in their patients. This guidance provides examples of different types of HCT/Ps and how the regulations in 21 CFR 1271.10(a)(1) and (2) apply to them, and provides general principles that can be applied to HCT/Ps that may be developed in the future. In some of the examples, the

[6] "Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing" 66 FR 5447 at 5457 (January 19, 2001). (Tissue Registration and Listing; Final Rule).
[7] See the proposed rule, "Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products" 63 FR 26744 at 26748-26749 (May 14, 1998). (Tissue Registration and Listing; Proposed Rule)
[8] "Proposed Approach to Regulation of Cellular and Tissue-Based Products," dated February 28, 1997 62 FR 9721 (March 4, 1997) page 19. (1997 Proposed Approach).

HCT/Ps may fail to meet more than one of the four criteria in 21 CFR 1271.10(a). The following flowchart illustrates how manufacturers and healthcare providers should apply the criteria outlined in 21 CFR 1271.15(b)[9] and 1271.10(a) for HCT/Ps:

Flowchart to illustrate how to apply the criteria in 21 CFR 1271.15(b) and 1271.10(a)



[9] For additional information about applying the exception in 21 CFR 1271.15(b), see the "Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception; Guidance for Industry" dated November 2017.

III. QUESTIONS AND ANSWERS REGARDING MINIMAL MANIPULATION

A. General Concepts

1. How do the regulations define minimal manipulation?

Section 1271.3(f) provides two definitions of minimal manipulation, one that applies to structural tissue and one that applies to cells or nonstructural tissues. For structural tissue, minimal manipulation means that the processing of the HCT/P does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement (21 CFR 1271.3(f)(1)). For cells or nonstructural tissues, minimal manipulation means that the processing of the HCT/P does not alter the relevant biological characteristics of cells or tissues (21 CFR 1271.3(f)(2)).

Original relevant characteristics of structural tissues generally include the properties of that tissue in the donor that contribute to the tissue's function or functions. Similarly, relevant biological characteristics of cells or nonstructural tissues generally include the properties of the cells or nonstructural tissues in the donor that contribute to the cells or tissue's function(s). Processing that alters the original characteristics of the HCT/P, raises increased safety and effectiveness concerns for the HCT/P because there would be less basis on which to predict the product's function after transplantation.[10] Thus, the determination of whether an HCT/P is minimally manipulated is based on the effect of manufacturing on the original relevant characteristics of the HCT/P as the HCT/P exists in the donor, and not based on the intended use of the HCT/P in the recipient.

2. Why is there a different definition of minimal manipulation for structural tissue and for cells or nonstructural tissue?

Under the regulations, HCT/Ps are considered either structural tissues or cells/nonstructural tissue, based on the characteristics of the tissue in the donor. This distinction, which was first described in the 1997 Proposed Approach for the regulation of cell and tissue-based products, is reflected in the definitions of minimal manipulation for structural tissue and cells/nonstructural tissue. Structural HCT/Ps generally raise different safety and efficacy concerns than do cells or nonstructural tissues.[11]

[10] See the "Proposed Approach to Regulation of Cell and Tissue-Based Products" page 19. (1997 Proposed Approach)

[11] See the "Proposed Approach to Regulation of Cell and Tissue-Based Products" page 20 (many structural HCT/Ps are conventional tissues with a long established history of safe use). (1997 Proposed Approach)

3. How do I determine whether an HCT/P is structural tissue or cellular/nonstructural tissue for purposes of applying the minimal manipulation criterion?

To apply the minimal manipulation criterion, you first determine whether the HCT/P is structural or cellular/nonstructural. This determination is made based on the characteristics of the HCT/P in the donor, before recovery and before any processing that takes place. Then, you apply the appropriate definition to determine whether the HCT/P has been minimally manipulated.

HCT/Ps may perform multiple functions and FDA acknowledges that structural tissues contain cells. FDA also acknowledges that some manufacturers assert that an HCT/P has both a structural and cellular/nonstructural function. However, under the regulations, HCT/Ps are considered either structural tissues or cells/nonstructural tissues. HCT/Ps that physically support or serve as a barrier or conduit, or connect, cover, or cushion are generally considered structural tissues for the purpose of applying the HCT/P regulatory framework. Examples of structural tissue are provided in question 6 (see section III.B. of this document). HCT/Ps that serve metabolic or other biochemical roles in the body such as hematopoietic, immune, and endocrine functions, are generally considered cells/nonstructural tissues for the purpose of applying the HCT/P regulatory framework. Examples of cells or nonstructural tissues are provided in question 15 (see section III.C. of this document).

4. What is processing of an HCT/P?

Processing is defined as any activity performed on an HCT/P, other than recovery, donor screening, donor testing, storage, labeling, packaging, or distribution, such as testing for microorganisms, preparation, sterilization, steps to inactivate or remove adventitious agents, preservation for storage, and removal from storage (21 CFR 1271.3(ff)). Processing also includes cutting, grinding, shaping, culturing, enzymatic digestion, and decellularization.

5. Which processing steps should be considered in determining whether an HCT/P is minimally manipulated?

You should consider all of the processing steps.

B. Structural Tissue

6. What types of tissues are considered structural tissues?

Tissues that physically support or serve as a barrier or conduit, or connect, cover, or cushion in the donor are generally considered structural tissues for the purposes of determining the applicable regulatory definition.

Examples of structural tissues include:

- Bone;
- Skin;
- Amniotic membrane and umbilical cord;
- Blood vessel;
- Adipose tissue;
- Articular cartilage;
- Non-articular cartilage; and
- Tendon or ligament

7. Why is adipose tissue considered a structural tissue for the purpose of applying the HCT/P regulatory framework?

Adipose tissue is typically defined as a connective tissue composed of clusters of cells (adipocytes) surrounded by a reticular fiber network and interspersed small blood vessels, divided into lobes and lobules by connective tissue septa.[12] Additionally, adipose tissue contains other cells, including preadipocytes, fibroblasts, vascular endothelial cells, and macrophages.[13] Adipose tissue provides cushioning and support for other tissues, including the skin and internal organs, stores energy in the form of lipids, and insulates the body, among other functions. While adipose tissue has multiple functions, because it is predominantly composed of adipocytes and surrounding connective tissues that provide cushioning and support to the body, FDA considers adipose tissue to be a structural tissue for the purpose of applying the HCT/P regulatory framework.

To evaluate whether processing of adipose tissue would meet the regulatory definition of minimal manipulation, you should consider whether the processing alters the original relevant characteristics of the adipose tissue relating to its utility to provide cushioning and support.

8. If my HCT/P is a structural tissue, how do I determine whether it is minimally manipulated?

To evaluate whether processing of a structural tissue would meet the regulatory definition of minimal manipulation, you should consider whether the processing alters an original relevant characteristic of the tissue, relating to the tissue's utility for reconstruction, repair, or replacement as structural tissue.

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[12] Chapter 6. Adipose Tissue. In: Mescher AL. eds. *Junqueira's Basic Histology: Text & Atlas, 13e.* New York: McGraw-Hill; 2013. http://accessmedicine.mhmedical.com/content.aspx?bookid=574&Sectionid=42524592.

[13] Brown SA, Levi, B, Lequeux, C, et al. Basic Science Review on Adipose Tissue for Clinicians. *Plast. Reconstr. Surg.* 126:1936, 2010.

9. What are original relevant characteristics of structural tissues?

Original relevant characteristics of structural tissues generally include the properties of that tissue in the donor that contribute to the tissue's function or functions. For purposes of determining whether a structural HCT/P is minimally manipulated, a tissue characteristic is "original" if it is present in the tissue in the donor. A structural tissue characteristic is "relevant" if it could have a meaningful bearing on the tissue's utility for reconstruction, repair, or replacement. The structural tissue's utility for reconstruction, repair, or replacement relates to how that tissue functions in the donor. Examples of relevant characteristics of structural tissues include strength, flexibility, cushioning, covering, compressibility, and response to friction and shear.

10. How does changing the size or shape of the structural tissue affect whether an HCT/P is minimally manipulated?

Structural tissues may be processed by various machining and other mechanical methods to change the size or shape of the HCT/P. Such processing can be either minimal manipulation or more than minimal manipulation depending on whether the processing alters the original relevant characteristics of the structural tissue relating to its utility for reconstruction, repair, or replacement.

Example 10-1: Original relevant characteristics of bone relating to its utility to support the body and protect internal structures include strength, and resistance to compression. Milling, grinding, and other methods for shaping and sizing bone may generally be considered minimal manipulation when they do not alter bone's original relevant characteristics relating to its utility to support the body and protect internal structures.

a. A manufacturer performs threading and other mechanical machining procedures to shape bone into dowels, screws, and pins. The HCT/Ps are generally considered minimally manipulated because the processing does not alter the bone's original relevant characteristics relating to its utility to support the body and protect internal structures.

b. A manufacturer grinds bone to form bone chips and particles. The HCT/Ps would generally be considered minimally manipulated because the processing does not alter the bone's original relevant characteristics relating to its utility to support bodily structures.[14]

[14] Refer to the "Jurisdictional Update: Human Demineralized Bone Matrix" dated January 19, 2001 (DBM Guidance) https://www.fda.gov/CombinationProducts/JurisdictionalInformation/JurisdictionalUpdates/ucm106586.htm for information relating to the regulatory classification of demineralized bone matrix (DBM). The guidance remains applicable to HCT/Ps that are DBM products. However, the DBM Guidance is based on factors that are specific to DBM products. The DBM Guidance document does not inform analyses to determine whether HCT/Ps other than DBM have been minimally manipulated and we do not consider it to be applicable to HCT/Ps other than DBM.

c. A manufacturer exposes bone to acid at elevated temperature to demineralize bone and dissolve collagen in order to form a gel. The HCT/P is generally considered more than minimally manipulated because the processing alters the bone's original relevant characteristics relating to its utility to support the body and protect internal structures.

Example 10-2: Original relevant characteristics of amniotic membrane relating to its utility to serve as a barrier generally include the tissue's physical integrity, tensile strength, and elasticity.

a. A manufacturer processes amniotic membrane to preserve it and package it in sheets. The HCT/P generally is considered minimally manipulated because the processing does not alter the original relevant characteristics of the HCT/P relating to its utility to serve as a barrier.

b. A manufacturer grinds and lyophilizes amniotic membrane and packages it as particles. The HCT/P generally is considered more than minimally manipulated because the processing alters the original relevant characteristics of the HCT/P relating to its utility to serve as a barrier.

Example 10-3: Original relevant characteristics of fascia lata, relating to its utility to cover muscle and aid in movement, generally include its strength, flexibility, and its fibrous, sheet-like configuration. A manufacturer grinds sheets of fascia lata into particles. The HCT/P generally is considered more than minimally manipulated because the processing alters the original relevant characteristics of the HCT/P relating to its utility to cover muscle and aid in movement.

Example 10-4: The original relevant characteristics of skin relating to its utility to serve as a protective covering generally include its large surface area, keratinized, water-resistant epithelial layer (epidermis), and dense, strong, and flexible connective tissue layer (dermis).

a. A manufacturer processes skin by mechanical meshing and cryopreservation and packages it in sheets as meshed skin. The HCT/P generally is considered minimally manipulated because the processing does not alter the original relevant characteristics of the skin relating to its utility as a protective covering.

b. A manufacturer processes skin by removing the epidermis and then grinding the dermis into particles. The HCT/P generally is considered more than minimally manipulated because the processing alters the original relevant characteristics of skin related to its utility as a protective covering.

11. How does removal of cells from structural tissue affect whether an HCT/P is minimally manipulated?

Structural tissues may contain both extracellular matrix and cellular components, and any alteration of these components that relates to the structural tissue's utility for reconstruction, repair, or replacement generally would be considered more than minimal manipulation. However, separation of structural tissue into components in which the original relevant characteristics relating to the tissue's utility for reconstruction, repair, or replacement are not altered generally would be considered minimal manipulation. For example, extraction or separation of cells from structural tissue in which the remaining structural tissue's original relevant characteristics relating to its utility for reconstruction, repair, or replacement remain unchanged generally would be considered minimal manipulation.[15]

While some structural tissues may undergo processing that alters the cellular or extracellular matrix components without altering the original relevant characteristics of the tissue, the same processing may alter the original relevant characteristics of a different structural tissue. Therefore, to assess whether a processing step alters the original relevant characteristics of a structural tissue relating to its utility for reconstruction, repair, or replacement, you should consider the effects of the processing on the properties that contribute to the specific tissue's function in the donor, for each type of tissue you manufacture.

Example 11-1: Original relevant characteristics of adipose tissue relating to its utility to provide cushioning and support generally include its bulk and lipid storage capacity. A manufacturer processes adipose tissue by removing the cells, which leaves the decellularized extracellular matrix portion of the HCT/P. The HCT/P generally is considered more than minimally manipulated because the processing alters the original relevant characteristics of the HCT/P relating to its utility to provide cushioning and support.

Example 11-2: Original relevant characteristics of the amniotic membrane related to its utility to serve as a barrier generally include its physical integrity, tensile strength, and elasticity. A manufacturer processes amniotic tissue to remove the chorion and other cells. The HCT/P generally is considered minimally manipulated because the processing does not alter the original relevant characteristics of the HCT/P relating to its utility to serve as a barrier.

Example 11-3: The original relevant characteristics of skin relating to its utility to serve as a protective covering generally include its large surface area, keratinized, water-resistant epithelial layer (epidermis), and dense, strong, and flexible connective tissue layer (dermis). A manufacturer processes skin to remove epidermis and freeze-dries and packages the remaining connective tissue, as

[15] See the Proposed Rule "Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products" 63 FR 26744 at 26748. (May 14, 1998). (Tissue Registration and Listing; Proposed Rule)

decellularized dermis. The HCT/P generally is considered minimally manipulated because the processing does not alter the original relevant characteristics of the HCT/P relating to its utility to serve as a protective covering**.**

12. How does changing the physical state of the structural HCT/P affect whether it is minimally manipulated?

In addition to mechanical methods, there are other types of processing that may alter the physical state of a structural tissue, such as chemical modification. If the mechanical, chemical, or other method of modification alters the HCT/P's physical state relating to its utility for reconstruction, repair, or replacement, then the HCT/P is generally considered more than minimally manipulated.

Example 12-1: The original relevant characteristics of cartilage relating to its utility to perform its load-bearing and other physical functions generally include firmness, smoothness, flexibility, and resistance to deformation. A manufacturer processes cartilage allograft by homogenizing it into a slurry. The HCT/P generally is considered more than minimally manipulated because the processing alters the original relevant characteristics of the HCT/P relating to its utility to absorb shock and reduce friction between joints.

Example 12-2: The original relevant characteristics of ligament relating to its utility to attach bone to bone and aid in movement and stability generally include its tensile strength which is imparted by the bundled fibrous collagen. A manufacturer processes ligament to disaggregate the collagen fibers. The HCT/P generally is considered more than minimally manipulated because the processing alters the original relevant characteristics of the HCT/P relating to its utility to aid in movement and stability.

13. How does storage affect whether a structural tissue is minimally manipulated?

Storage that does not alter the original relevant characteristics of a structural tissue relating to its utility for reconstruction, repair, or replacement would generally be considered minimal manipulation. For example, an HCT/P that is placed in a tissue medium and refrigerated, such as stored in a buffer solution; or an HCT/P that is cryopreserved and stored in liquid nitrogen vapor, would generally meet the minimal manipulation criterion.[16]

[16]According to 21 CFR 1271.10(a)(3), to meet the criteria for regulation solely under section 361 of the PHS Act and 21 CFR Part 1271, the manufacture of the HCT/P does not involve the combination of the cells or tissues with another article, except for water, crystalloids, or a sterilizing, preserving, or storage agent, provided that the addition of water, crystalloids, or the sterilizing, preserving, or storage agent does not raise new clinical safety concerns with respect to the HCT/P.

14. I isolate cells from structural tissue to produce a cellular therapy product. What definition of minimal manipulation would apply?

If you isolate cells from structural tissue, the definition of minimal manipulation for structural tissue applies, regardless of the method used to isolate the cells. This is because the assessment of whether the HCT/P is a structural tissue or cellular/nonstructural tissue is based on the characteristics of the HCT/P as it exists in the donor, prior to recovery and any processing that takes place.

Example 14-1: Original relevant characteristics of adipose tissue relating to its utility to provide cushioning and support generally include its bulk and lipid storage capacity. A manufacturer recovers adipose tissue by tumescent liposuction and processes (e.g., enzymatically digests, mechanically disrupts, etc.) the adipose tissue to isolate cellular components (with or without subsequent cell culture or expansion), commonly referred to as stromal vascular fraction, which is considered a potential source of adipose-derived stromal/stem cells. The definition of minimal manipulation for structural tissue applies.

In this example, the HCT/P generally is considered more than minimally manipulated because the processing breaks down and eliminates the adipocytes and the surrounding structural components that provide cushioning and support, thereby altering the original relevant characteristics of the HCT/P relating to its utility for reconstruction, repair, or replacement.

C. Cells or Nonstructural Tissues

Under the regulatory framework for HCT/Ps, minimal manipulation of cells or nonstructural tissues is defined as processing that does not alter the relevant biological characteristics of cells or tissues (21 CFR 1271.3(f)(2)).

15. What types of tissue are considered cells or nonstructural tissues?

Cells or nonstructural tissues are generally those that serve predominantly metabolic or other biochemical roles in the body such as hematopoietic, immune, and endocrine functions.

Examples of cells or nonstructural tissues include:

- Reproductive cells or tissues (e.g., oocytes);
- Hematopoietic stem/progenitor cells (e.g., cord blood)[17];
- Lymph nodes and thymus;

[17] Bone marrow is a source of hematopoietic stem/progenitor cells.
Minimally manipulated bone marrow for homologous use and not combined with another article (with certain exceptions), is not considered an HCT/P (21 CFR 1271.3(d)(4)). However, bone marrow that is more than minimally manipulated, intended by the manufacturer for a non-homologous use, or combined with another article with limited exceptions, meets the definition of an HCT/P and is subject to the regulations in 21 CFR Part 1271.

- Parathyroid glands;
- Peripheral nerve; and
- Pancreatic tissue.

Secreted body fluids (e.g., amniotic fluid) are generally not considered HCT/Ps.[18] Cells from secreted body fluids are generally considered HCT/Ps, and the definition of minimal manipulation for cells or nonstructural tissues would apply.

16. What are relevant biological characteristics of cells or nonstructural tissues?

Relevant biological characteristics of cells or nonstructural tissues generally include the properties of the cells or nonstructural tissues in the donor that contribute to the cells or tissue's function or functions.

Examples of relevant biological characteristics of cells or nonstructural tissues include differentiation and activation state, proliferation potential, and metabolic activity. Processing that alters any relevant biological characteristics of cells or nonstructural tissues generally would be considered more than minimal manipulation.

Example 16-1: Relevant biological characteristics of hematopoietic stem/progenitor cells generally include the ability to repopulate the bone marrow by self-renewal and by differentiating along myeloid and lymphoid cell lines.

a. Hematopoietic stem/progenitor cells are circulating in increased numbers in the peripheral blood of a donor after administration of mobilizing agent. A manufacturer performs cell selection on the mobilized peripheral blood apheresis product to obtain a higher concentration of hematopoietic stem/progenitor cells for transplantation. The HCT/P would generally be considered minimally manipulated because the concentrated peripheral blood stem/progenitor cells are not altered with regard to their relevant biological characteristics to repopulate the bone marrow.

b. A manufacturer uses hematopoietic stem/progenitor cells to produce terminally differentiated cells by culturing the cells under specific conditions. This HCT/P derived from hematopoietic stem/progenitor cells would generally be considered more than minimally manipulated because the processing alters the cells' relevant biological characteristics of multipotency and capacity for self-renewal.

[18] 21 CFR 1271.3(d) states, "…The following articles are not considered HCT/Ps:…(3) secreted or extracted human products such as milk, collagen, and cell factors, except that semen is considered an HCT/P".

c. A manufacturer of a placental/umbilical cord blood product performs cell selection and incubates the selected cells in a laboratory vessel containing culture media and growth factors to achieve large numbers of cells capable of long-term repopulation of the bone marrow. This HCT/P derived from cord blood would generally be considered more than minimally manipulated because the processing affects the production of intracellular or cell-surface proteins and other markers of cell lineage, activation state, and proliferation, thereby altering the cells' relevant biological characteristics of multipotency and capacity for self-renewal.

IV. QUESTIONS AND ANSWERS REGARDING HOMOLOGOUS USE

17. What is the definition of homologous use?

Homologous use means the repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues with an HCT/P that performs the same basic function or functions in the recipient as in the donor (21 CFR 1271.3(c)), including when such cells or tissues are for autologous use. We generally consider an HCT/P to be for homologous use when it is used to repair, reconstruct, replace, or supplement:

- Recipient cells or tissues that are identical (e.g., skin for skin) to the donor cells or tissues, and perform one or more of the same basic functions in the recipient as the cells or tissues performed in the donor; or,

- Recipient cells or tissues that may not be identical to the donor's cells or tissues, but that perform one or more of the same basic functions in the recipient as the cells or tissues performed in the donor.[19]

Example 17-1: A heart valve is transplanted to replace a dysfunctional heart valve. This is homologous use because the donor heart valve performs the same basic function in the donor as in the recipient of ensuring unidirectional blood flow within the heart.

Example 17-2: Pericardium is intended to be used as a wound covering for dura mater defects. This is homologous use because the pericardium is intended to serve as a covering in the recipient, which is one of the basic functions it performs in the donor.

If an HCT/P is intended for use as an unproven treatment for a myriad of diseases or conditions, the HCT/P is likely not intended for homologous use only.[20]

[19] "Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products" 63 FR 26744 at 26748-49 (May 14, 1998). (Tissue Registration and Listing; Proposed Rule)
[20] "Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing" 66 FR 5447 at 5457 (January 19, 2001). (Tissue Registration and Listing; Final Rule)

18. What does FDA mean by repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues?

Repair generally means the physical or mechanical restoration of tissues, including by covering or protecting. For example, FDA generally would consider skin removed from a donor and then transplanted to a recipient in order to cover a burn wound to be a homologous use. Reconstruction generally means surgical reassembling or re-forming. For example, reconstruction generally would include the reestablishment of the physical integrity of a damaged aorta.[21] Replacement generally means substitution of a missing tissue or cell, for example, the replacement of a damaged or diseased cornea with a healthy cornea or the replacement of donor hematopoietic stem/progenitor cells in a recipient with a disorder affecting the hematopoietic system that is inherited, acquired, or the result of myeloablative treatment. Supplementation generally means to add to, or complete. For example, FDA generally would consider the implantation of dermal matrix into the facial wrinkles to supplement a recipient's tissues and the use of bone chips to supplement bony defects to be homologous uses. Repair, reconstruction, replacement, and supplementation are not mutually exclusive functions and an HCT/P could perform more than one of these functions for a given intended use.

19. What does FDA mean by "the same basic function or functions" in the definition of homologous use?

For the purpose of applying the HCT/P regulatory framework, the same basic function or functions of HCT/Ps are considered to be those basic functions the HCT/P performs in the body of the donor, which, when transplanted, implanted, infused, or transferred, the HCT/P would be expected to perform in the recipient. It is not necessary for the HCT/P in the recipient to perform all of the basic functions it performed in the donor in order to meet the definition of homologous use. However, to meet the definition of homologous use, any of the basic functions that the HCT/P is expected to perform in the recipient must be a basic function that the HCT/P performed in the donor.

The basic function of an HCT/P is what it does from a biological/physiological point of view, or is capable of doing when in its native state.[22] By "basic" we mean the function or functions that are commonly attributed to the HCT/P as it exists in the donor. Basic functions are well understood; it should not be necessary to perform laboratory, pre-clinical, or clinical studies to demonstrate a basic function or functions for the purpose of applying the HCT/P regulatory framework. Also, clinical effects of the HCT/P in the

[21] "Current Good Tissue Practice for Human Cell, Tissue, and Cellular and Tissue-Based Product Establishments; Inspection and Enforcement" 69 FR 68612 at 68643 (November 24, 2004) states, "HCT/Ps with claims for "reconstruction or repair" can be appropriately regulated solely under section 361 of the PHS Act, if such HCT/P meets all the criteria in § 1271.10, including minimal manipulation and homologous use."

[22] "Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products," 63 FR 26744 at 26749 (May 14, 1998). (Tissue Registration and Listing; Proposed Rule)

recipient that are not basic function or functions of the HCT/P in the donor would generally not be considered basic function or functions of the HCT/P for the purpose of applying the definition of homologous use.

Basic functions of a structural tissue would generally be to perform a structural function for example, to physically support or serve as a barrier or conduit, or connect, cover, or cushion.

Basic functions of a cellular or nonstructural tissue would generally be a metabolic or biochemical function, such as, hematopoietic, immune, and endocrine functions.

Example 19-1: Sources of hematopoietic stem/progenitor cells (HPCs) include cord blood, peripheral blood, and bone marrow.[23] The basic functions of HPCs include forming and replenishing the lymphohematopoietic system.

a. HPCs from mobilized peripheral blood are intended for transplantation into an individual with a disorder affecting the hematopoietic system that is inherited, acquired, or the result of myeloablative treatment. This is homologous use because the peripheral blood product performs the same basic function of reconstituting the hematopoietic system in the recipient.

b. HPCs from bone marrow are intended for infusion into an artery with a balloon catheter for the purpose of limiting ventricular remodeling following acute myocardial infarction. This is not homologous use because limiting ventricular remodeling is not a basic function of bone marrow.

c. HPCs from cord blood are intended for intravenous infusion to treat cerebral palsy purportedly through the repair of damaged tissue in the brain through paracrine signaling or differentiation into neuronal cells. This is not homologous use because there is insufficient evidence to support that repair of neurologic tissue through paracrine signaling or differentiation into neuronal cells is a basic function of these cells in the donor.

Example 19-2: The basic functions of the cornea include protecting the eye and serving as its outermost lens. A corneal graft is transplanted to a patient with corneal blindness. This is homologous use because a corneal graft performs the same basic functions in the donor as in the recipient.

Example 19-3: The basic functions of a vein or artery include serving as a conduit for blood flow throughout the body. A cryopreserved vein or artery is used for arteriovenous access during hemodialysis. This is homologous use because the vein or artery is supplementing the vessel as a conduit for blood flow.

[23] See "Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue Based Products," 63 FR 26744 at 26749 (May 14, 1998). (Tissue Registration and Listing; Proposed Rule)

Example 19-4: The basic functions of amniotic membrane include serving as a selective barrier for the movement of nutrients between the external and in utero environment, protecting the fetus from the surrounding maternal environment, and serving as a covering to enclose the fetus and retain fluid in utero.

a. Amniotic membrane is used for bone tissue replacement to support bone regeneration following surgery to repair or replace bone defects. This is not a homologous use because bone regeneration is not a basic function of amniotic membrane.

b. An amniotic membrane product is used for wound healing and/or to reduce scarring and inflammation. This is not homologous use because wound healing and reduction of scarring and inflammation[24] are not basic functions of amniotic membrane.

c. An amniotic membrane product is applied to the surface of the eye to cover or offer protection from the surrounding environment in ocular repair and reconstruction procedures. This is homologous use because serving as a covering and offering protection from the surrounding environment are basic functions of amniotic membrane.[25]

Example 19-5: The basic functions of pericardium include covering, protecting against infection, fixing the heart to the mediastinum, and providing lubrication to allow normal heart movement within chest. Autologous pericardium is used to replace a dysfunctional heart valve in the same patient. This is not homologous use because facilitating unidirectional blood flow is not a basic function of pericardium.

The use of an HCT/P from adipose tissue for the repair, reconstruction, replacement, or supplementation of adipose tissue would be considered a homologous use. In these situations, FDA would consider the HCT/P from adipose tissue to be performing the same basic function in the recipient as in the donor. In contrast, the use of an HCT/P from adipose tissue for the treatment of a degenerative, inflammatory, or demyelinating disorder would generally be considered a non-homologous use.

Example 19-6: The basic functions of adipose tissue include providing cushioning and support for other tissues, including the skin and internal organs, storing energy in the form of lipids, and insulating the body.

[24] Reducing scarring, angiogenesis, and inflammation are potential clinical effects in the recipient but are not basic functions of amniotic membrane in the donor; therefore, they are not considered homologous uses of amniotic membrane.

[25] Bio-Tissue 2001 RFD available at: https://www.fda.gov/downloads/CombinationProducts/JurisdictionalInformation/RFDJurisdictionalDecisions/RedactedDecisionLetters/UCM113701.pdf.

a. Adipose tissue is used to fill voids in the face or hands (e.g., for cosmetic reasons). This is homologous use because providing cushioning and support, is a basic function of adipose tissue.[26]

b. An HCT/P from adipose tissue is used to treat musculoskeletal conditions such as arthritis or tendonitis by regenerating or promoting the regeneration of articular cartilage or tendon. This is generally not considered a homologous use because regenerating or promoting the regeneration of cartilage or tendon is not a basic function of adipose tissue.

c. An HCT/P from adipose tissue is used to treat neurological disorders such as multiple sclerosis by limiting the autoimmune reaction and promoting remyelinization. This is generally not considered a homologous use because limiting the autoimmune reaction and promoting remyelinization are not basic functions of adipose tissue.

d. Adipose tissue is used for transplantation into the subcutaneous areas of breast for reconstruction or augmentation procedures. This is homologous use because providing cushioning and support is a basic function of adipose tissue.[27]

20. Does my HCT/P have to be used in the same anatomic location to perform the same basic function or functions?

An HCT/P may perform the same basic function or functions even when it is not used in the same anatomic location where it existed in the donor.[28] A transplanted HCT/P could replace missing tissue, or repair, reconstruct, or supplement tissue that is missing or damaged, either when placed in the same or different anatomic location, as long as it performs the same basic function(s) in the recipient as in the donor.

Example 20-1: The basic functions of skin include covering, protecting the body from external force, and serving as a water-resistant barrier to pathogens or other damaging agents in the external environment. The dermis is the elastic connective tissue layer of the skin that covers, provides support and protects the body from mechanical stress.

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[26] Some cosmetic procedures involving reimplantation of autologous adipose tissue that is only rinsed or cleansed may meet the exception in 21 CFR 1271.15(b). For additional information about applying the exception in 21 CFR 1271.15(b), see the "Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception; Guidance for Industry" dated November 2017.

[27] Some breast reconstruction or augmentation procedures involving re-implantation of autologous adipose tissue that is only rinsed or cleansed may meet the exception in 21 CFR 1271.15(b). For additional information about applying the exception in 21 CFR 1271.15(b), see the "Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception; Guidance for Industry" dated November 2017.

[28] "Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing" 66 FR 5447 at 5457 (January 19, 2001). (Tissue Registration and Listing; Final Rule)

a. An acellular dermal product is used for supplemental support, protection, reinforcement, or covering for a tendon. This is homologous use because in both anatomic locations, the dermis provides support and protects the soft tissue structure from mechanical stress.

b. An acellular dermal product is used for tendon replacement or repair. This is not homologous use because serving as a connection between muscle and bone is not a basic function of dermis.

Example 20-2: The basic functions of bone are supporting the body and protecting internal structures such as the brain. Allogeneic mineralized or demineralized cortical human bone is used to supplement the recipient's bone for repair, replacement, and reconstruction of bony voids or gaps involving the extremities, cranium, and spinal column; or for augmentation for posterior lateral fusions in the spinal column. These are homologous uses because in all locations, the HCT/P is supplementing the recipient's bone, for the purpose of supporting the body or protecting internal structures.

Example 20-3: The basic functions of pancreatic islets include regulating glucose homeostasis within the body. Pancreatic islets are transplanted into the liver through the portal vein for preservation of endocrine function after pancreatectomy. This is homologous use because the regulation of glucose homeostasis is a basic function of pancreatic islets.

21. What does FDA mean by "intended for homologous use" in 21 CFR 1271.10(a)(2)?

The regulatory criterion in 21 CFR 1271.10(a)(2) states that the HCT/P is intended for homologous use only, as reflected by the labeling, advertising, or other indications of the manufacturer's objective intent.

Labeling includes the HCT/P label and any written, printed, or graphic materials that supplement, explain, or are textually related to the product, and which are disseminated by or on behalf of its manufacturer.[29] Advertising includes information, other than labeling, that originates from the same source as the product and that is intended to supplement, explain, or be textually related to the product (e.g., print advertising, broadcast advertising, electronic advertising (including the Internet), statements of company representatives).[30]

An HCT/P is intended for homologous use when its labeling, advertising, or other indications of the manufacturer's objective intent refer to only homologous uses for the HCT/P. When an HCT/P's labeling, advertising, or other indications of the manufacturer's objective intent refer to non-homologous uses, the HCT/P would not meet the homologous use criterion in 21 CFR 1271.10(a)(2).

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[29] "Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing" 66 FR 5447 at 5457 (January 19, 2001). (Tissue Registration and Listing; Final Rule)

[30] *Id.*

22. What does FDA mean by "manufacturer's objective intent" in 21 CFR 1271.10(a)(2)?

A manufacturer's objective intent is determined by the expressions of the manufacturer or its representatives, or may be shown by the circumstances surrounding the distribution of the article. A manufacturer's objective intent may, for example, be shown by labeling claims, advertising matter, or oral or written statements by the manufacturer or its representatives. It may be shown by surrounding circumstances under which a HCT/P is offered for a purpose for which it is neither labeled nor advertised.

V. REGULATORY SCOPE AND COMPLIANCE POLICY

A. Scope of FDA's Regulation of HCT/Ps

As noted in the Background section of this document, this guidance only applies to products and establishments that are subject to FDA's regulations in 21 CFR Part 1271. Establishments that meet the same surgical procedure exception in 21 CFR 1271.15(b) are not subject to FDA's regulations in 21 CFR Part 1271. This guidance also does not apply to products that fall outside the definition of HCT/P in 21 CFR 1271.3(d). For example, platelet rich plasma (PRP, blood taken from an individual and given back to the same individual as platelet rich plasma) is not an HCT/P under Part 1271 because it is a blood product. Accordingly, FDA does not apply the criteria in 21 CFR 1271.10(a) to PRP, and PRP is outside the scope of this guidance.

B. Compliance and Enforcement Policy Regarding Certain Regulatory Requirements

To give manufacturers time to determine if they need to submit an IND or marketing application in light of this guidance and, if such an application is needed, to prepare the IND or marketing application, for the first 36 months following issuance of this guidance FDA generally intends to exercise enforcement discretion with respect to the IND and the premarket approval requirements for HCT/Ps that do not meet one or more of the 21 CFR 1271.10(a) criteria, provided that use of the HCT/P does not raise reported safety concerns or potential significant safety concerns.

FDA intends to focus enforcement actions on products with higher risk, including based on the route and site of administration. For example, actions related to products with routes of administration associated with a higher risk (e.g., those administered by intravenous injection or infusion, aerosol inhalation, intraocular injection, or injection or infusion into the central nervous system) will be prioritized over those associated with a lower risk (e.g., those administered by intradermal, subcutaneous, or intra-articular injection). HCT/Ps that are intended for non-homologous use, particularly those intended to be used for the prevention or treatment of serious and/or life-threatening diseases and conditions, are also more likely to raise significant safety concerns than HCT/Ps intended for homologous use because there is less basis on which to predict the product's behavior

in the recipient, and use of these unapproved products may cause users to delay or discontinue medical treatments that have been found safe and effective through the New Drug Application or BLA approval processes.

Regenerative medicine is a complex and rapidly evolving field. Accordingly, FDA will continue to reassess its application of the HCT/P regulatory framework, including the minimal manipulation and homologous use criteria in 21 CFR 1271.10(a), as additional scientific evidence emerges in this field.

VI. ADDITIONAL INFORMATION

23. What regulations apply if my HCT/P is regulated as a biological product?[31]

HCT/Ps that are regulated as biological products are subject to section 351 of the PHS Act and the FD&C Act and require premarket approval. Such HCT/Ps are subject to the applicable drug regulations, including the requirements in 21 CFR Parts 210 and 211, and the applicable requirements in 21 CFR Parts 600 through 680. Such products are also regulated under section 361 of the PHS Act and are subject to requirements in Part 1271 designed to prevent the introduction, transmission, and spread of communicable diseases. Pursuant to these regulations, you are required to register as an establishment, and list your HCT/Ps (21 CFR 1271.1(b)(2)) (see section VI. question 25 of this document).

In order to lawfully market a biological product, a biologics license must be in effect (PHS Act) (42 U.S.C. 262(a)). Such licenses are issued only after a determination by FDA that the establishment(s) and the biological products meet the applicable requirements to ensure the continued safety, purity, and potency of such products (21 CFR 601.2(d)). For clinical studies of investigational drug products, the sponsor must have an IND application in effect in accordance with the FD&C Act (21 U.S.C. 355(i)) and FDA regulations (21 CFR Part 312 and 21 CFR 601.21). See section VI. question 27 of this document about obtaining more information regarding the IND process.

24. What must I do if my HCT/P meets the criteria for regulation solely under section 361 of the PHS Act and Part 1271?

If you are a domestic or foreign establishment that manufactures an HCT/P that is regulated solely under section 361 of the PHS Act and 21 CFR Part 1271, you must, in accordance with 21 CFR 1271.1(b)(1):

1) Register with FDA (See section VI. question 25 of this document);
2) Submit to FDA a list of each HCT/P manufactured; and
3) Comply with all applicable requirements contained in 21 CFR Part 1271.

[31] Some HCT/Ps may be regulated as devices. For more information about device regulation, see CDRH's webpage Device Advice – Overview of Medical Device Regulation at https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/default.htm.

Establishment means a place of business under one management, at one general physical location that engages in the manufacture of HCT/Ps, including:

> 1) Any individual, partnership, corporation, association, or other legal entity engaged in the manufacture of HCT/Ps; and
> 2) Facilities that engage in contract manufacturing services for a manufacturer of HCT/Ps (21 CFR 1271.3(b)).

Manufacture means, but is not limited to, any or all steps in the recovery, processing, storage, labeling, packaging, or distribution of any human cell or tissue, and the screening and testing of the cell or tissue donor (21 CFR 1271.3(e)).

25. Must I register as an HCT/P manufacturer?

FDA regulations require establishments that perform one or more steps in the manufacture of HCT/Ps to register and submit a list of products with the Agency. If you are a manufacturer that is required to register, you must do so within five days after beginning operations (21 CFR 1271.21(a)). Registrations must be updated annually in December (21 CFR 1271.21(b)), except if the ownership or location of the establishment changes, or if there is a change in the United States agent's name, address, telephone number, or email address, in which case, you must submit an amendment to the registration within 30 calendar days of the change (21 CFR 1271.26).

26. How can I get more information about the appropriate regulatory considerations for my HCT/P?

The Agency provides two mechanisms through which a manufacturer may obtain a recommendation or decision regarding the classification of an HCT/P:

> 1) The Tissue Reference Group, a group that includes representatives from CBER and the Center for Devices and Radiological Health (CDRH), provides product sponsors with an informal process through which they may obtain an Agency recommendation regarding the application of the criteria in 21 CFR 1271.10(a) to their HCT/Ps for a given indication. Information about this process as well as what you may want to include to facilitate review of your request can be found at: https://www.fda.gov/BiologicsBloodVaccines/TissueTissueProducts/RegulationofTissues/ucm152857.htm.
>
> 2) A Request for Designation (RFD) may be submitted to the Office of Combination Products (OCP) to obtain a formal Agency decision regarding the regulatory identity or classification of an HCT/P (21 CFR Part 3). A description of that process and information on how to submit an RFD can be found at: https://www.fda.gov/CombinationProducts/RFDProcess/default.htm. Additional information may be found at https://www.fda.gov/Regulatoryinformation/Guidances/ucm126053.htm. You may also submit a Pre-RFD to OCP to obtain preliminary feedback on the

classification for your HCT/P as well as assistance on how to prepare an RFD. Additional information may be found at https://www.fda.gov/Regulatoryinformation/Guidances/ucm126053.htm.

27. How can I obtain more information about the IND process for my HCT/P that requires premarket approval?

Further information about IND requirements for biological products may be obtained through the Division of Regulatory Project Management, Office of Tissues and Advanced Therapies, at 240-402-8190 or mailto:OTATRPMS@fda.hhs.gov.

EXHIBIT B

Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use

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Guidance for Industry and Food and Drug Administration Staff

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
Center for Devices and Radiological Health
Office of Combination Products
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Contains Nonbinding Recommendations

Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use

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Guidance for Industry and Food and Drug Administration Staff

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Guidance for Industry and Food and Drug Administration Staff

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

We, FDA, are providing you, human cells, tissues, and cellular and tissue-based product (HCT/P) manufacturers, healthcare providers, and FDA staff, with our current thinking on the criteria under Title 21 of the Code of Federal Regulations (CFR) Part 1271, specifically the 21 CFR 1271.10(a)(1) criterion of minimal manipulation and the 21 CFR 1271.10(a)(2) criterion of homologous use.

This guidance supersedes the guidance of the same title dated November 2017 and corrected December 2017.[1] This guidance revises section V. to extend the period of enforcement discretion through May 31, 2021.

The interpretation of the minimal manipulation and homologous use criteria and definitions of related key terms have been of considerable interest to industry stakeholders since the criteria and definitions were first proposed.[2] This guidance is intended to improve stakeholders' understanding of the definitions of minimal manipulation in 21 CFR 1271.3(f) and homologous use in 21 CFR 1271.3(c). It will also facilitate stakeholders' understanding of how the regulatory criteria in 21 CFR 1271.10(a)(1) and (2) apply to their HCT/Ps.[3] In addition, the November 2017 version of the guidance informed manufacturers, healthcare providers, and other interested persons that over the next 36 months (through November 2020), we intended to

[1] This document was updated December 2017 to correct language to remove references to autologous and non-autologous (allogeneic) products in section V.B. (Compliance and Enforcement Policy Regarding Certain Regulatory Requirements).

[2] "Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products" 63 FR 26744 at 26748-26749 (May 14, 1998). (Tissue Registration and Listing; Proposed Rule)

[3] This guidance does not address the classification and/or assignment of HCT/Ps that do not meet the criteria for regulation solely under section 361 of the Public Health Service (PHS) Act and 21 CFR Part 1271.

exercise enforcement discretion under limited conditions with respect to the investigational new drug (IND) application and premarket approval (biologics license application (BLA)) requirements, for certain HCT/Ps. The current version of this guidance explains that FDA intends to exercise such enforcement discretion for a longer period of time: through May 2021.

The November 2017 version of the guidance finalized the document entitled "Minimal Manipulation of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps); Draft Guidance for Industry and Food Administration Staff" dated December 2014, and "Homologous Use of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps); Draft Guidance for Industry and FDA Staff" dated October 2015. It also finalized certain material related to adipose tissue that was included in draft guidance entitled "Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) from Adipose Tissue: Regulatory Considerations; Draft Guidance for Industry" dated December 2014 (Adipose Draft Guidance). This material, together with the material related to adipose tissue included in the final guidance entitled "Same Surgical Procedure Exception under 21 CFR 1271.15(b); Questions and Answers Regarding the Scope of the Exception" dated November 2017, superseded the Adipose Draft Guidance. Accordingly, we did finalize the Adipose Draft Guidance, and that draft guidance was withdrawn in November 2017. Finally, the November 2017 version of the guidance superseded the document entitled "Minimal Manipulation of Structural Tissue (Jurisdictional Update); Guidance for Industry and FDA Staff" dated September 2006 (2006 Guidance).

In general, FDA's guidance documents,including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

HCT/Ps are defined in 21 CFR 1271.3(d) as articles containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human recipient.[4] Because of the unique nature of HCT/Ps, FDA proposed and in 2005 implemented a tiered, risk-based approach to the regulation of HCT/Ps. Although FDA is authorized to apply

[4] Examples of HCT/Ps include, but are not limited to, bone, ligament, skin, dura mater, heart valve, cornea, hematopoietic stem/progenitor cells derived from peripheral and cord blood, manipulated autologous chondrocytes, epithelial cells on a synthetic matrix, and semen or other reproductive tissue. The following articles are not considered HCT/Ps: (1) Vascularized human organs for transplantation; (2) Whole Blood or blood components or blood derivative products subject to listing under 21 CFR Parts 607 and 207, respectively; (3) Secreted or extracted human products, such as milk, collagen, and cell factors, except that semen is considered an HCT/P; (4) Minimally manipulated bone marrow for homologous use and not combined with another article (except for water, crystalloids, or a sterilizing, preserving, or storage agent, if the addition of the agent does not raise new clinical safety concerns with respect to the bone marrow); (5) Ancillary products used in the manufacture of HCT/P; (6) Cells, tissues, and organs derived from animals other than humans; (7) In vitro diagnostic products as defined in 21 CFR 809.3(a); and (8) Blood vessels recovered with an organ, as defined in 42 CFR 121.2 that are intended for use in organ transplantation and labeled "For use in organ transplantation only" (21 CFR 1271.3(d)).

Please note, the regulatory status of products identified as not being HCT/Ps is beyond the scope of this guidance.

the requirements in the Federal Food, Drug, and Cosmetic Act (FD&C Act) and the Public Health Service Act (PHS Act) to those products that meet the definition of drug, biologic, or device, under this tiered, risk-based approach, those HCT/Ps that meet specific criteria or fall within detailed exceptions do not require premarket review and approval. In developing the tiered, risk-based approach the agency focused on public health and regulatory concerns, including how transmission of communicable disease can be prevented; what processing controls are necessary to prevent contamination that could result in an unsafe or ineffective product, and to preserve integrity and function so that the products will work as they are intended; and how clinical safety and effectiveness can be assured. The tiered, risk-based approach is contained in a set of regulations commonly referred to as the "tissue rules," issued by FDA through notice and comment rulemaking, under the communicable disease authority of section 361 of the PHS Act (42 U.S.C. 264). These regulations explain the types of HCT/Ps that do not require premarket approval; and the registration, manufacturing, and reporting steps that must be taken to prevent the introduction, transmission, and spread of communicable disease by these HCT/Ps. These regulations can be found in 21 CFR Part 1271[5].

In 21 CFR 1271.10, the regulations identify the criteria for regulation solely under section 361 of the PHS Act and 21 CFR Part 1271. An HCT/P is regulated solely under section 361 of the PHS Act and 21 CFR Part 1271 if it meets all of the following criteria (21 CFR 1271.10(a)):

1) The HCT/P is minimally manipulated;
2) The HCT/P is intended for homologous use only, as reflected by the labeling, advertising, or other indications of the manufacturer's objective intent;
3) The manufacture of the HCT/P does not involve the combination of the cells or tissues with another article, except for water, crystalloids, or a sterilizing, preserving, or storage agent, provided that the addition of water, crystalloids, or the sterilizing, preserving, or storage agent does not raise new clinical safety concerns with respect to the HCT/P; and
4) Either:
    i) The HCT/P does not have a systemic effect and is not dependent upon the metabolic activity of living cells for its primary function; or
    ii) The HCT/P has a systemic effect or is dependent upon the metabolic activity of living cells for its primary function, and:
        a) Is for autologous use;
        b) Is for allogeneic use in a first-degree or second-degree blood relative; or
        c) Is for reproductive use.

If an HCT/P does not meet the criteria set out in 21 CFR1271.10(a), and the establishment that manufactures the HCT/P does not qualify for any of the exceptions in 21 CFR 1271.15[6], the

[5] *See* "Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing;" Final Rule, 66 FR 5447 (January 19, 2001); "Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products;" Final Rule, 69 FR 29786 (May 25, 2004); "Current Good Tissue Practice for Human Cell, Tissue, and Cellular and Tissue-Based Product Establishments; Inspection and Enforcement;" Final Rule, 69 FR 68612 (November 24, 2004).

[6] See the "Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception; Guidance for Industry" dated November 2017 available at: https://www.fda.gov/media/89920/download.

HCT/P will be regulated as a drug, device, and/or biological product under the FD&C Act, and/or section 351 of the PHS Act (42 U.S.C. 262), and applicable regulations, including 21 CFR Part 1271, and premarket review will be required.

Minimal Manipulation

Section 1271.10(a)(1) (21 CFR 1271.10(a)(1)) provides that one of the criteria for an HCT/P to be regulated solely under section 361 of the PHS Act and the regulations in Part 1271 is that the HCT/P is minimally manipulated. As defined in 21 CFR 1271.3(f), minimal manipulation means:

1) For structural tissue, processing that does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement;
2) For cells or nonstructural tissues, processing that does not alter the relevant biological characteristics of cells or tissues.

FDA discussed these terms in the preamble to the HCT/P Establishment Registration and Listing final rule[7] and the 2006 Guidance. However, we have received requests from stakeholders to provide additional guidance that explains our current thinking related to meeting the criterion in 21 CFR 1271.10(a)(1). This guidance supersedes the 2006 Guidance.

Please note that if information does not exist to show that the processing meets the definition of minimal manipulation, FDA considers the processing of an HCT/P to be "more than minimal manipulation" that cannot qualify for regulation solely under section 361 of the PHS Act and 21 CFR Part 1271[8].

Homologous Use

Section 1271.10(a)(2) (21 CFR 1271.10(a)(2)) provides that one of the criteria for an HCT/P to be regulated solely under section 361 of the PHS Act and the regulations in Part 1271 is that the "HCT/P is intended for homologous use only, as reflected by the labeling, advertising, or other indications of the manufacturer's objective intent." As defined in 21 CFR 1271.3(c), homologous use means the repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues with an HCT/P that performs the same basic function or functions in the recipient as in the donor. This criterion reflects the Agency's conclusion that there would be increased safety and effectiveness concerns for HCT/Ps that are intended for a non-homologous use, because there is less basis on which to predict the product's behavior, whereas HCT/Ps for homologous use can reasonably be expected to function appropriately (assuming all of the other criteria are also met)[9].

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[7] "Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing" 66 FR 5447 at 5457 (January 19, 2001). (Tissue Registration and Listing; Final Rule).

[8] See the proposed rule, "Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products" 63 FR 26744 at 26748-26749 (May 14, 1998). (Tissue Registration and Listing; Proposed Rule)

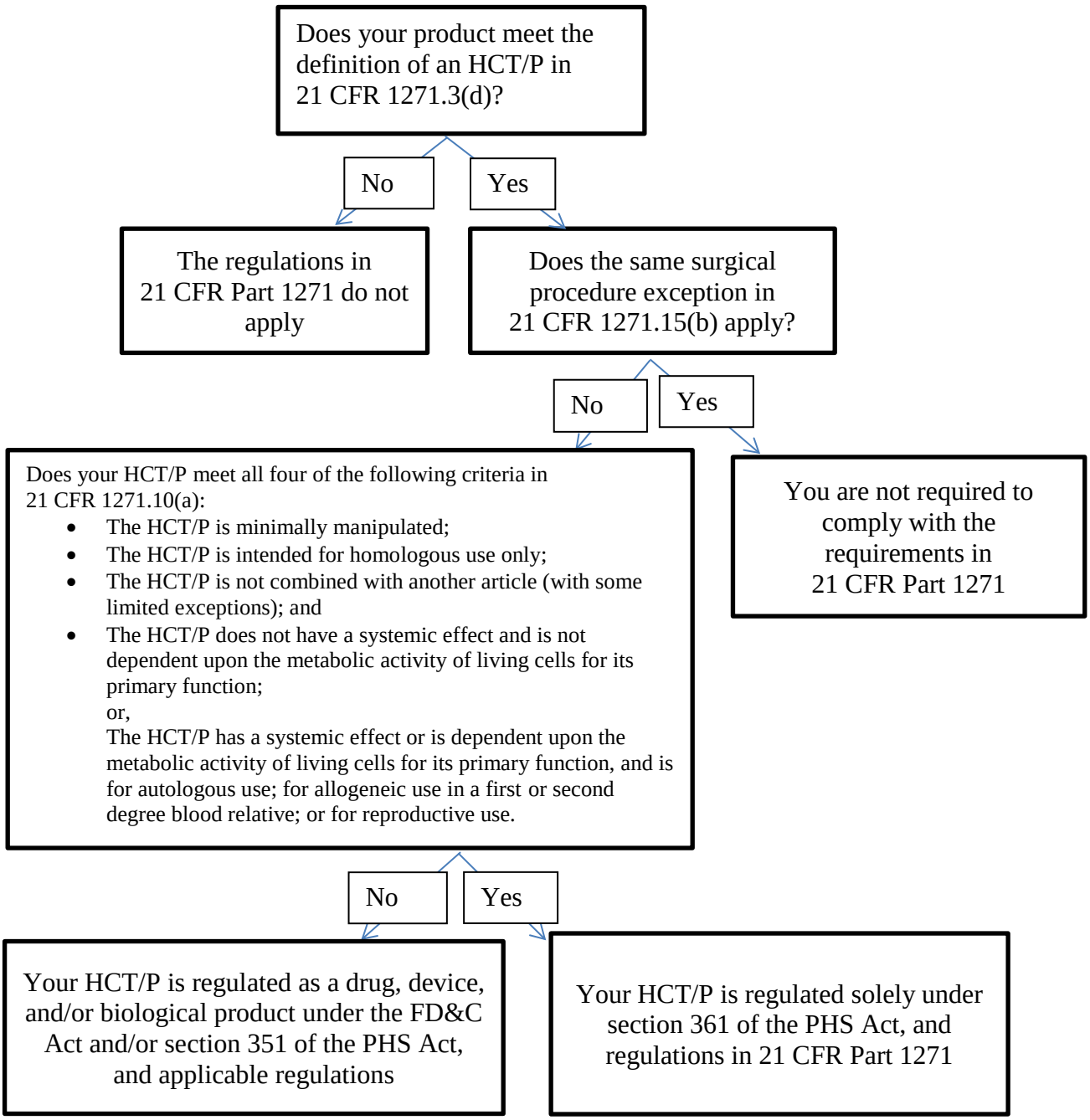
[9] "Proposed Approach to Regulation of Cellular and Tissue-Based Products," dated February 28, 1997 62 FR 9721 (March 4, 1997) page 19. (1997 Proposed Approach).

In applying the homologous use criterion, FDA will determine what the intended use of the HCT/P is, as reflected by the labeling, advertising, and other indications of a manufacturer's objective intent, and will then apply the homologous use definition.

FDA has received many inquiries from manufacturers about whether their HCT/Ps meet the minimal manipulation and/or homologous use criteria. Additionally, transplant and healthcare providers often need to know this information about the HCT/Ps that they are considering for use in their patients. This guidance provides examples of different types of HCT/Ps and how the regulations in 21 CFR 1271.10(a)(1) and (2) apply to them, and provides general principles that can be applied to HCT/Ps that may be developed in the future. In some of the examples, the HCT/Ps may fail to meet more than one of the four criteria in 21 CFR 1271.10(a). The following flowchart illustrates how manufacturers and healthcare providers should apply the criteria outlined in 21 CFR 1271.15(b)[10] and 1271.10(a) for HCT/Ps:

[10] For additional information about applying the exception in 21 CFR 1271.15(b), see the "Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception; Guidance for Industry" dated November 2017.

Flowchart to illustrate how to apply the criteria in 21 CFR 1271.15(b) and 1271.10(a)



III. QUESTIONS AND ANSWERS REGARDING MINIMAL MANIPULATION

A. General Concepts

1. How do the regulations define minimal manipulation?

Section 1271.3(f) provides two definitions of minimal manipulation, one that applies to structural tissue and one that applies to cells or nonstructural tissues. For structural tissue, minimal manipulation means that the processing of the HCT/P does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement (21 CFR 1271.3(f)(1)). For cells or nonstructural tissues, minimal manipulation means that the processing of the HCT/P does not alter the relevant biological characteristics of cells or tissues (21 CFR 1271.3(f)(2)).

Original relevant characteristics of structural tissues generally include the properties of that tissue in the donor that contribute to the tissue's function or functions. Similarly, relevant biological characteristics of cells or nonstructural tissues generally include the properties of the cells or nonstructural tissues in the donor that contribute to the cells or tissue's function(s). Processing that alters the original characteristics of the HCT/P, raises increased safety and effectiveness concerns for the HCT/P because there would be less basis on which to predict the product's function after transplantation.[11] Thus, the determination of whether an HCT/P is minimally manipulated is based on the effect of manufacturing on the original relevant characteristics of the HCT/P as the HCT/P exists in the donor, and not based on the intended use of the HCT/P in the recipient.

2. Why is there a different definition of minimal manipulation for structural tissue and for cells or nonstructural tissue?

Under the regulations, HCT/Ps are considered either structural tissues or cells/nonstructural tissue, based on the characteristics of the tissue in the donor. This distinction, which was first described in the 1997 Proposed Approach for the regulation of cell and tissue-based products, is reflected in the definitions of minimal manipulation for structural tissue and cells/nonstructural tissue. Structural HCT/Ps generally raise different safety and efficacy concerns than do cells or nonstructural tissues.[12]

[11] See the "Proposed Approach to Regulation of Cell and Tissue-Based Products" page 19. (1997 Proposed Approach)

[12] See the "Proposed Approach to Regulation of Cell and Tissue-Based Products" page 20 (many structural HCT/Ps are conventional tissues with a long established history of safe use). (1997 Proposed Approach)

3. How do I determine whether an HCT/P is structural tissue or cellular/nonstructural tissue for purposes of applying the minimal manipulation criterion?

To apply the minimal manipulation criterion, you first determine whether the HCT/P is structural or cellular/nonstructural. This determination is made based on the characteristics of the HCT/P in the donor, before recovery and before any processing that takes place. Then, you apply the appropriate definition to determine whether the HCT/P has been minimally manipulated.

HCT/Ps may perform multiple functions and FDA acknowledges that structural tissues contain cells. FDA also acknowledges that some manufacturers assert that an HCT/P has both a structural and cellular/nonstructural function. However, under the regulations, HCT/Ps are considered either structural tissues or cells/nonstructural tissues. HCT/Ps that physically support or serve as a barrier or conduit, or connect, cover, or cushion are generally considered structural tissues for the purpose of applying the HCT/P regulatory framework. Examples of structural tissue are provided in question 6 (see section III.B. of this guidance). HCT/Ps that serve metabolic or other biochemical roles in the body such as hematopoietic, immune, and endocrine functions, are generally considered cells/nonstructural tissues for the purpose of applying the HCT/P regulatory framework. Examples of cells or nonstructural tissues are provided in question 15 (see section III.C. of this guidance).

4. What is processing of an HCT/P?

Processing is defined as any activity performed on an HCT/P, other than recovery, donor screening, donor testing, storage, labeling, packaging, or distribution, such as testing for microorganisms, preparation, sterilization, steps to inactivate or remove adventitious agents, preservation for storage, and removal from storage (21 CFR 1271.3(ff)). Processing also includes cutting, grinding, shaping, culturing, enzymatic digestion, and decellularization.

5. Which processing steps should be considered in determining whether an HCT/P is minimally manipulated?

You should consider all of the processing steps.

B. Structural Tissue

1. What types of tissues are considered structural tissues?

Tissues that physically support or serve as a barrier or conduit, or connect, cover, or cushion in the donor are generally considered structural tissues for the purposes of determining the applicable regulatory definition.

Examples of structural tissues include:

- Bone;
- Skin;
- Amniotic membrane and umbilical cord;
- Blood vessel;
- Adipose tissue;
- Articular cartilage;
- Non-articular cartilage; and
- Tendon or ligament

2. Why is adipose tissue considered a structural tissue for the purpose of applying the HCT/P regulatory framework?

Adipose tissue is typically defined as a connective tissue composed of clusters of cells (adipocytes) surrounded by a reticular fiber network and interspersed small blood vessels, divided into lobes and lobules by connective tissue septa.[13] Additionally, adipose tissue contains other cells, including preadipocytes, fibroblasts, vascular endothelial cells, and macrophages.[14] Adipose tissue provides cushioning and support for other tissues, including the skin and internal organs, stores energy in the form of lipids, and insulates the body, among other functions. While adipose tissue has multiple functions, because it is predominantly composed of adipocytes and surrounding connective tissues that provide cushioning and support to the body, FDA considers adipose tissue to be a structural tissue for the purpose of applying the HCT/P regulatory framework.

To evaluate whether processing of adipose tissue would meet the regulatory definition of minimal manipulation, you should consider whether the processing alters the original relevant characteristics of the adipose tissue relating to its utility to provide cushioning and support.

3. If my HCT/P is a structural tissue, how do I determine whether it is minimally manipulated?

To evaluate whether processing of a structural tissue would meet the regulatory definition of minimal manipulation, you should consider whether the processing alters an original relevant characteristic of the tissue, relating to the tissue's utility for reconstruction, repair, or replacement as structural tissue.

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[13] Chapter 6. Adipose Tissue. In: Mescher AL. eds. *Junqueira's Basic Histology: Text & Atlas, 13e.* New York: McGraw-Hill; 2013. http://accessmedicine.mhmedical.com/content.aspx?bookid=574&Sectionid=42524592.

[14] Brown SA, Levi, B, Lequeux, C, et al. Basic Science Review on Adipose Tissue for Clinicians. *Plast. Reconstr. Surg.* 126:1936, 2010.

4. What are original relevant characteristics of structural tissues?

Original relevant characteristics of structural tissues generally include the properties of that tissue in the donor that contribute to the tissue's function or functions. For purposes of determining whether a structural HCT/P is minimally manipulated, a tissue characteristic is "original" if it is present in the tissue in the donor. A structural tissue characteristic is "relevant" if it could have a meaningful bearing on the tissue's utility for reconstruction, repair, or replacement. The structural tissue's utility for reconstruction, repair, or replacement relates to how that tissue functions in the donor. Examples of relevant characteristics of structural tissues include strength, flexibility, cushioning, covering, compressibility, and response to friction and shear.

5. How does changing the size or shape of the structural tissue affect whether an HCT/P is minimally manipulated?

Structural tissues may be processed by various machining and other mechanical methods to change the size or shape of the HCT/P. Such processing can be either minimal manipulation or more than minimal manipulation depending on whether the processing alters the original relevant characteristics of the structural tissue relating to its utility for reconstruction, repair, or replacement.

Example 10-1: Original relevant characteristics of bone relating to its utility to support the body and protect internal structures include strength, and resistance to compression. Milling, grinding, and other methods for shaping and sizing bone may generally be considered minimal manipulation when they do not alter bone's original relevant characteristics relating to its utility to support the body and protect internal structures.

a. A manufacturer performs threading and other mechanical machining procedures to shape bone into dowels, screws, and pins. The HCT/Ps are generally considered minimally manipulated because the processing does not alter the bone's original relevant characteristics relating to its utility to support the body and protect internal structures.

b. A manufacturer grinds bone to form bone chips and particles. The HCT/Ps would generally be considered minimally manipulated because the processing does not alter the bone's original relevant characteristics relating to its utility to support bodily structures.[15]

[15] Refer to the "Jurisdictional Update: Human Demineralized Bone Matrix" dated January 19, 2001 (DBM Guidance) https://www.fda.gov/combination-products/jurisdictional-updates/jurisdictional-update-human-demineralized-bone-matrix for information relating to the regulatory classification of demineralized bone matrix (DBM). The guidance remains applicable to HCT/Ps that are DBM products. However, the DBM Guidance is based on factors that are specific to DBM products. The DBM Guidance document does not inform analyses to determine whether HCT/Ps other than DBM have been minimally manipulated and we do not consider it to be applicable to HCT/Ps other than DBM.

c. A manufacturer exposes bone to acid at elevated temperature to demineralize bone and dissolve collagen in order to form a gel. The HCT/P is generally considered more than minimally manipulated because the processing alters the bone's original relevant characteristics relating to its utility to support the body and protect internal structures.

Example 10-2: Original relevant characteristics of amniotic membrane relating to its utility to serve as a barrier generally include the tissue's physical integrity, tensile strength, and elasticity.

a. A manufacturer processes amniotic membrane to preserve it and package it in sheets. The HCT/P generally is considered minimally manipulated because the processing does not alter the original relevant characteristics of the HCT/P relating to its utility to serve as a barrier.

b. A manufacturer grinds and lyophilizes amniotic membrane and packages it as particles. The HCT/P generally is considered more than minimally manipulated because the processing alters the original relevant characteristics of the HCT/P relating to its utility to serve as a barrier.

Example 10-3: Original relevant characteristics of fascia lata, relating to its utility to cover muscle and aid in movement, generally include its strength, flexibility, and its fibrous, sheet-like configuration. A manufacturer grinds sheets of fascia lata into particles. The HCT/P generally is considered more than minimally manipulated because the processing alters the original relevant characteristics of the HCT/P relating to its utility to cover muscle and aid in movement.

Example 10-4: The original relevant characteristics of skin relating to its utility to serve as a protective covering generally include its large surface area, keratinized, water-resistant epithelial layer (epidermis), and dense, strong, and flexible connective tissue layer (dermis).

a. A manufacturer processes skin by mechanical meshing and cryopreservation and packages it in sheets as meshed skin. The HCT/P generally is considered minimally manipulated because the processing does not alter the original relevant characteristics of the skin relating to its utility as a protective covering.

b. A manufacturer processes skin by removing the epidermis and then grinding the dermis into particles. The HCT/P generally is considered more than minimally manipulated because the processing alters the original relevant characteristics of skin related to its utility as a protective covering.

6. How does removal of cells from structural tissue affect whether an HCT/P is minimally manipulated?

Structural tissues may contain both extracellular matrix and cellular components, and any alteration of these components that relates to the structural tissue's utility for reconstruction, repair, or replacement generally would be considered more than minimal manipulation. However, separation of structural tissue into components in which the original relevant characteristics relating to the tissue's utility for reconstruction, repair, or replacement are not altered generally would be considered minimal manipulation. For example, extraction or separation of cells from structural tissue in which the remaining structural tissue's original relevant characteristics relating to its utility for reconstruction, repair, or replacement remain unchanged generally would be considered minimal manipulation.[16]

While some structural tissues may undergo processing that alters the cellular or extracellular matrix components without altering the original relevant characteristics of the tissue, the same processing may alter the original relevant characteristics of a different structural tissue. Therefore, to assess whether a processing step alters the original relevant characteristics of a structural tissue relating to its utility for reconstruction, repair, or replacement, you should consider the effects of the processing on the properties that contribute to the specific tissue's function in the donor, for each type of tissue you manufacture.

Example 11-1: Original relevant characteristics of adipose tissue relating to its utility to provide cushioning and support generally include its bulk and lipid storage capacity. A manufacturer processes adipose tissue by removing the cells, which leaves the decellularized extracellular matrix portion of the HCT/P. The HCT/P generally is considered more than minimally manipulated because the processing alters the original relevant characteristics of the HCT/P relating to its utility to provide cushioning and support.

Example 11-2: Original relevant characteristics of the amniotic membrane related to its utility to serve as a barrier generally include its physical integrity, tensile strength, and elasticity. A manufacturer processes amniotic tissue to remove the chorion and other cells. The HCT/P generally is considered minimally manipulated because the processing does not alter the original relevant characteristics of the HCT/P relating to its utility to serve as a barrier.

Example 11-3: The original relevant characteristics of skin relating to its utility to serve as a protective covering generally include its large surface area, keratinized, water-resistant epithelial layer (epidermis), and dense, strong, and flexible connective tissue layer (dermis). A manufacturer processes skin to remove epidermis and freeze-dries and packages the remaining connective tissue, as

[16] See the Proposed Rule "Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products" 63 FR 26744 at 26748. (May 14, 1998). (Tissue Registration and Listing; Proposed Rule)

decellularized dermis. The HCT/P generally is considered minimally manipulated because the processing does not alter the original relevant characteristics of the HCT/P relating to its utility to serve as a protective covering**.**

7. How does changing the physical state of the structural HCT/P affect whether it is minimally manipulated?

In addition to mechanical methods, there are other types of processing that may alter the physical state of a structural tissue, such as chemical modification. If the mechanical, chemical, or other method of modification alters the HCT/P's physical state relating to its utility for reconstruction, repair, or replacement, then the HCT/P is generally considered more than minimally manipulated.

Example 12-1: The original relevant characteristics of cartilage relating to its utility to perform its load-bearing and other physical functions generally include firmness, smoothness, flexibility, and resistance to deformation. A manufacturer processes cartilage allograft by homogenizing it into a slurry. The HCT/P generally is considered more than minimally manipulated because the processing alters the original relevant characteristics of the HCT/P relating to its utility to absorb shock and reduce friction between joints.

Example 12-2: The original relevant characteristics of ligament relating to its utility to attach bone to bone and aid in movement and stability generally include its tensile strength which is imparted by the bundled fibrous collagen. A manufacturer processes ligament to disaggregate the collagen fibers. The HCT/P generally is considered more than minimally manipulated because the processing alters the original relevant characteristics of the HCT/P relating to its utility to aid in movement and stability.

8. How does storage affect whether a structural tissue is minimally manipulated?

Storage that does not alter the original relevant characteristics of a structural tissue relating to its utility for reconstruction, repair, or replacement would generally be considered minimal manipulation. For example, an HCT/P that is placed in a tissue medium and refrigerated, such as stored in a buffer solution; or an HCT/P that is cryopreserved and stored in liquid nitrogen vapor, would generally meet the minimal manipulation criterion.[17]

[17] According to 21 CFR 1271.10(a)(3), to meet the criteria for regulation solely under section 361 of the PHS Act and 21 CFR Part 1271, the manufacture of the HCT/P does not involve the combination of the cells or tissues with another article, except for water, crystalloids, or a sterilizing, preserving, or storage agent, provided that the addition of water, crystalloids, or the sterilizing, preserving, or storage agent does not raise new clinical safety concerns with respect to the HCT/P.

9. I isolate cells from structural tissue to produce a cellular therapy product. What definition of minimal manipulation would apply?

If you isolate cells from structural tissue, the definition of minimal manipulation for structural tissue applies, regardless of the method used to isolate the cells. This is because the assessment of whether the HCT/P is a structural tissue or cellular/nonstructural tissue is based on the characteristics of the HCT/P as it exists in the donor, prior to recovery and any processing that takes place.

Example 14-1: Original relevant characteristics of adipose tissue relating to its utility to provide cushioning and support generally include its bulk and lipid storage capacity. A manufacturer recovers adipose tissue by tumescent liposuction and processes (e.g., enzymatically digests, mechanically disrupts, etc.) the adipose tissue to isolate cellular components (with or without subsequent cell culture or expansion), commonly referred to as stromal vascular fraction, which is considered a potential source of adipose-derived stromal/stem cells. The definition of minimal manipulation for structural tissue applies.

In this example, the HCT/P generally is considered more than minimally manipulated because the processing breaks down and eliminates the adipocytes and the surrounding structural components that provide cushioning and support, thereby altering the original relevant characteristics of the HCT/P relating to its utility for reconstruction, repair, or replacement.

C. Cells or Nonstructural Tissues

Under the regulatory framework for HCT/Ps, minimal manipulation of cells or nonstructural tissues is defined as processing that does not alter the relevant biological characteristics of cells or tissues (21 CFR 1271.3(f)(2)).

1. What types of tissue are considered cells or nonstructural tissues?

Cells or nonstructural tissues are generally those that serve predominantly metabolic or other biochemical roles in the body such as hematopoietic, immune, and endocrine functions.

Examples of cells or nonstructural tissues include:

- Reproductive cells or tissues (e.g., oocytes);
- Hematopoietic stem/progenitor cells (e.g., cord blood)[18];
- Lymph nodes and thymus;

[18] Bone marrow is a source of hematopoietic stem/progenitor cells. Minimally manipulated bone marrow for homologous use and not combined with another article (with certain exceptions), is not considered an HCT/P (21 CFR 1271.3(d)(4)). However, bone marrow that is more than minimally manipulated, intended by the manufacturer for a non-homologous use, or combined with another article with limited exceptions, meets the definition of an HCT/P and is subject to the regulations in 21 CFR Part 1271.

- Parathyroid glands;
- Peripheral nerve; and
- Pancreatic tissue.

Secreted body fluids (e.g., amniotic fluid) are generally not considered HCT/Ps.[19] Cells from secreted body fluids are generally considered HCT/Ps, and the definition of minimal manipulation for cells or nonstructural tissues would apply.

2. What are relevant biological characteristics of cells or nonstructural tissues?

Relevant biological characteristics of cells or nonstructural tissues generally include the properties of the cells or nonstructural tissues in the donor that contribute to the cells or tissue's function or functions.

Examples of relevant biological characteristics of cells or nonstructural tissues include differentiation and activation state, proliferation potential, and metabolic activity. Processing that alters any relevant biological characteristics of cells or nonstructural tissues generally would be considered more than minimal manipulation.

Example 16-1: Relevant biological characteristics of hematopoietic stem/progenitor cells generally include the ability to repopulate the bone marrow by self-renewal and by differentiating along myeloid and lymphoid cell lines.

a. Hematopoietic stem/progenitor cells are circulating in increased numbers in the peripheral blood of a donor after administration of mobilizing agent. A manufacturer performs cell selection on the mobilized peripheral blood apheresis product to obtain a higher concentration of hematopoietic stem/progenitor cells for transplantation. The HCT/P would generally be considered minimally manipulated because the concentrated peripheral blood stem/progenitor cells are not altered with regard to their relevant biological characteristics to repopulate the bone marrow.

b. A manufacturer uses hematopoietic stem/progenitor cells to produce terminally differentiated cells by culturing the cells under specific conditions. This HCT/P derived from hematopoietic stem/progenitor cells would generally be considered more than minimally manipulated because the processing alters the cells' relevant biological characteristics of multipotency and capacity for self-renewal.

[19] 21 CFR 1271.3(d) states, "…The following articles are not considered HCT/Ps:…(3) secreted or extracted human products such as milk, collagen, and cell factors, except that semen is considered an HCT/P".

c. A manufacturer of a placental/umbilical cord blood product performs cell selection and incubates the selected cells in a laboratory vessel containing culture media and growth factors to achieve large numbers of cells capable of long-term repopulation of the bone marrow. This HCT/P derived from cord blood would generally be considered more than minimally manipulated because the processing affects the production of intracellular or cell-surface proteins and other markers of cell lineage, activation state, and proliferation, thereby altering the cells' relevant biological characteristics of multipotency and capacity for self-renewal.

IV. QUESTIONS AND ANSWERS REGARDING HOMOLOGOUS USE

1. What is the definition of homologous use?

Homologous use means the repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues with an HCT/P that performs the same basic function or functions in the recipient as in the donor (21 CFR 1271.3(c)), including when such cells or tissues are for autologous use. We generally consider an HCT/P to be for homologous use when it is used to repair, reconstruct, replace, or supplement:

- Recipient cells or tissues that are identical (e.g., skin for skin) to the donor cells or tissues, and perform one or more of the same basic functions in the recipient as the cells or tissues performed in the donor; or,

- Recipient cells or tissues that may not be identical to the donor's cells or tissues, but that perform one or more of the same basic functions in the recipient as the cells or tissues performed in the donor.[20]

Example 17-1: A heart valve is transplanted to replace a dysfunctional heart valve. This is homologous use because the donor heart valve performs the same basic function in the donor as in the recipient of ensuring unidirectional blood flow within the heart.

Example 17-2: Pericardium is intended to be used as a wound covering for dura mater defects. This is homologous use because the pericardium is intended to serve as a covering in the recipient, which is one of the basic functions it performs in the donor.

If an HCT/P is intended for use as an unproven treatment for a myriad of diseases or conditions, the HCT/P is likely not intended for homologous use only.[21]

[20] "Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products" 63 FR 26744 at 26748-26749 (May 14, 1998). (Tissue Registration and Listing; Proposed Rule)
[21] "Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing" 66 FR 5447 at 5457 (January 19, 2001). (Tissue Registration and Listing; Final Rule)

2. What does FDA mean by repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues?

Repair generally means the physical or mechanical restoration of tissues, including by covering or protecting. For example, FDA generally would consider skin removed from a donor and then transplanted to a recipient in order to cover a burn wound to be a homologous use. Reconstruction generally means surgical reassembling or re-forming. For example, reconstruction generally would include the reestablishment of the physical integrity of a damaged aorta.[22] Replacement generally means substitution of a missing tissue or cell, for example, the replacement of a damaged or diseased cornea with a healthy cornea or the replacement of donor hematopoietic stem/progenitor cells in a recipient with a disorder affecting the hematopoietic system that is inherited, acquired, or the result of myeloablative treatment. Supplementation generally means to add to, or complete. For example, FDA generally would consider the implantation of dermal matrix into the facial wrinkles to supplement a recipient's tissues and the use of bone chips to supplement bony defects to be homologous uses. Repair, reconstruction, replacement, and supplementation are not mutually exclusive functions and an HCT/P could perform more than one of these functions for a given intended use.

3. What does FDA mean by "the same basic function or functions" in the definition of homologous use?

For the purpose of applying the HCT/P regulatory framework, the same basic function or functions of HCT/Ps are considered to be those basic functions the HCT/P performs in the body of the donor, which, when transplanted, implanted, infused, or transferred, the HCT/P would be expected to perform in the recipient. It is not necessary for the HCT/P in the recipient to perform all of the basic functions it performed in the donor in order to meet the definition of homologous use. However, to meet the definition of homologous use, any of the basic functions that the HCT/P is expected to perform in the recipient must be a basic function that the HCT/P performed in the donor.

The basic function of an HCT/P is what it does from a biological/physiological point of view, or is capable of doing when in its native state.[23] By "basic" we mean the function or functions that are commonly attributed to the HCT/P as it exists in the donor. Basic functions are well understood; it should not be necessary to perform laboratory, pre-clinical, or clinical studies to demonstrate a basic function or functions for the purpose of applying the HCT/P regulatory framework. Also, clinical effects of the HCT/P in the

[22] "Current Good Tissue Practice for Human Cell, Tissue, and Cellular and Tissue-Based Product Establishments; Inspection and Enforcement" 69 FR 68612 at 68643 (November 24, 2004) states, "HCT/Ps with claims for "reconstruction or repair" can be appropriately regulated solely under section 361 of the PHS Act, if such HCT/P meets all the criteria in § 1271.10, including minimal manipulation and homologous use."

[23] "Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products," 63 FR 26744 at 26749 (May 14, 1998). (Tissue Registration and Listing; Proposed Rule)

recipient that are not basic function or functions of the HCT/P in the donor would generally not be considered basic function or functions of the HCT/P for the purpose of applying the definition of homologous use.

Basic functions of a structural tissue would generally be to perform a structural function for example, to physically support or serve as a barrier or conduit, or connect, cover, or cushion.

Basic functions of a cellular or nonstructural tissue would generally be a metabolic or biochemical function, such as, hematopoietic, immune, and endocrine functions.

Example 19-1: Sources of hematopoietic stem/progenitor cells (HPCs) include cord blood, peripheral blood, and bone marrow.[24] The basic functions of HPCs include forming and replenishing the lymphohematopoietic system.

a. HPCs from mobilized peripheral blood are intended for transplantation into an individual with a disorder affecting the hematopoietic system that is inherited, acquired, or the result of myeloablative treatment. This is homologous use because the peripheral blood product performs the same basic function of reconstituting the hematopoietic system in the recipient.

b. HPCs from bone marrow are intended for infusion into an artery with a balloon catheter for the purpose of limiting ventricular remodeling following acute myocardial infarction. This is not homologous use because limiting ventricular remodeling is not a basic function of bone marrow.

c. HPCs from cord blood are intended for intravenous infusion to treat cerebral palsy purportedly through the repair of damaged tissue in the brain through paracrine signaling or differentiation into neuronal cells. This is not homologous use because there is insufficient evidence to support that repair of neurologic tissue through paracrine signaling or differentiation into neuronal cells is a basic function of these cells in the donor.

Example 19-2: The basic functions of the cornea include protecting the eye and serving as its outermost lens. A corneal graft is transplanted to a patient with corneal blindness. This is homologous use because a corneal graft performs the same basic functions in the donor as in the recipient.

Example 19-3: The basic functions of a vein or artery include serving as a conduit for blood flow throughout the body. A cryopreserved vein or artery is used for arteriovenous access during hemodialysis. This is homologous use because the vein or artery is supplementing the vessel as a conduit for blood flow.

[24] See "Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue Based Products," 63 FR 26744 at 26749 (May 14, 1998). (Tissue Registration and Listing; Proposed Rule)

Example 19-4: The basic functions of amniotic membrane include serving as a selective barrier for the movement of nutrients between the external and in utero environment, protecting the fetus from the surrounding maternal environment, and serving as a covering to enclose the fetus and retain fluid in utero.

a. Amniotic membrane is used for bone tissue replacement to support bone regeneration following surgery to repair or replace bone defects. This is not a homologous use because bone regeneration is not a basic function of amniotic membrane.

b. An amniotic membrane product is used for wound healing and/or to reduce scarring and inflammation. This is not homologous use because wound healing and reduction of scarring and inflammation[25] are not basic functions of amniotic membrane.

c. An amniotic membrane product is applied to the surface of the eye to cover or offer protection from the surrounding environment in ocular repair and reconstruction procedures. This is homologous use because serving as a covering and offering protection from the surrounding environment are basic functions of amniotic membrane.[26]

Example 19-5: The basic functions of pericardium include covering, protecting against infection, fixing the heart to the mediastinum, and providing lubrication to allow normal heart movement within chest. Autologous pericardium is used to replace a dysfunctional heart valve in the same patient. This is not homologous use because facilitating unidirectional blood flow is not a basic function of pericardium.

The use of an HCT/P from adipose tissue for the repair, reconstruction, replacement, or supplementation of adipose tissue would be considered a homologous use. In these situations, FDA would consider the HCT/P from adipose tissue to be performing the same basic function in the recipient as in the donor. In contrast, the use of an HCT/P from adipose tissue for the treatment of a degenerative, inflammatory, or demyelinating disorder would generally be considered a non-homologous use.

Example 19-6: The basic functions of adipose tissue include providing cushioning and support for other tissues, including the skin and internal organs, storing energy in the form of lipids, and insulating the body.

[25] Reducing scarring, angiogenesis, and inflammation are potential clinical effects in the recipient but are not basic functions of amniotic membrane in the donor; therefore, they are not considered homologous uses of amniotic membrane.

[26] Bio-Tissue 2001 RFD available at: https://www.fda.gov/media/74873/download .

a. Adipose tissue is used to fill voids in the face or hands (e.g., for cosmetic reasons). This is homologous use because providing cushioning and support, is a basic function of adipose tissue.[27]

b. An HCT/P from adipose tissue is used to treat musculoskeletal conditions such as arthritis or tendonitis by regenerating or promoting the regeneration of articular cartilage or tendon. This is generally not considered a homologous use because regenerating or promoting the regeneration of cartilage or tendon is not a basic function of adipose tissue.

c. An HCT/P from adipose tissue is used to treat neurological disorders such as multiple sclerosis by limiting the autoimmune reaction and promoting remyelinization. This is generally not considered a homologous use because limiting the autoimmune reaction and promoting remyelinization are not basic functions of adipose tissue.

d. Adipose tissue is used for transplantation into the subcutaneous areas of breast for reconstruction or augmentation procedures. This is homologous use because providing cushioning and support is a basic function of adipose tissue.[28]

20. Does my HCT/P have to be used in the same anatomic location to perform the same basic function or functions?

An HCT/P may perform the same basic function or functions even when it is not used in the same anatomic location where it existed in the donor.[29] A transplanted HCT/P could replace missing tissue, or repair, reconstruct, or supplement tissue that is missing or damaged, either when placed in the same or different anatomic location, as long as it performs the same basic function(s) in the recipient as in the donor.

Example 20-1: The basic functions of skin include covering, protecting the body from external force, and serving as a water-resistant barrier to pathogens or other damaging agents in the external environment. The dermis is the elastic connective tissue layer of the skin that covers, provides support and protects the body from mechanical stress.

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[27] Some cosmetic procedures involving reimplantation of autologous adipose tissue that is only rinsed or cleansed may meet the exception in 21 CFR 1271.15(b). For additional information about applying the exception in 21 CFR 1271.15(b), see the "Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception; Guidance for Industry" dated November 2017.

[28] Some breast reconstruction or augmentation procedures involving re-implantation of autologous adipose tissue that is only rinsed or cleansed may meet the exception in 21 CFR 1271.15(b). For additional information about applying the exception in 21 CFR 1271.15(b), see the "Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception; Guidance for Industry" dated November 2017.

[29] "Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing" 66 FR 5447 at 5457 (January 19, 2001). (Tissue Registration and Listing; Final Rule)

a. An acellular dermal product is used for supplemental support, protection, reinforcement, or covering for a tendon. This is homologous use because in both anatomic locations, the dermis provides support and protects the soft tissue structure from mechanical stress.

b. An acellular dermal product is used for tendon replacement or repair. This is not homologous use because serving as a connection between muscle and bone is not a basic function of dermis.

Example 20-2: The basic functions of bone are supporting the body and protecting internal structures such as the brain. Allogeneic mineralized or demineralized cortical human bone is used to supplement the recipient's bone for repair, replacement, and reconstruction of bony voids or gaps involving the extremities, cranium, and spinal column; or for augmentation for posterior lateral fusions in the spinal column. These are homologous uses because in all locations, the HCT/P is supplementing the recipient's bone, for the purpose of supporting the body or protecting internal structures.

Example 20-3: The basic functions of pancreatic islets include regulating glucose homeostasis within the body. Pancreatic islets are transplanted into the liver through the portal vein for preservation of endocrine function after pancreatectomy. This is homologous use because the regulation of glucose homeostasis is a basic function of pancreatic islets.

21. What does FDA mean by "intended for homologous use" in 21 CFR 1271.10(a)(2)?

The regulatory criterion in 21 CFR 1271.10(a)(2) states that the HCT/P is intended for homologous use only, as reflected by the labeling, advertising, or other indications of the manufacturer's objective intent.

Labeling includes the HCT/P label and any written, printed, or graphic materials that supplement, explain, or are textually related to the product, and which are disseminated by or on behalf of its manufacturer.[30] Advertising includes information, other than labeling, that originates from the same source as the product and that is intended to supplement, explain, or be textually related to the product (e.g., print advertising, broadcast advertising, electronic advertising (including the Internet), statements of company representatives).[31]

An HCT/P is intended for homologous use when its labeling, advertising, or other indications of the manufacturer's objective intent refer to only homologous uses for the HCT/P. When an HCT/P's labeling, advertising, or other indications of the

[30] "Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing" 66 FR 5447 at 5457 (January 19, 2001). (Tissue Registration and Listing; Final Rule)
[31] *Id.*

manufacturer's objective intent refer to non-homologous uses, the HCT/P would not meet the homologous use criterion in 21 CFR 1271.10(a)(2).

22. What does FDA mean by "manufacturer's objective intent" in 21 CFR 1271.10(a)(2)?

A manufacturer's objective intent is determined by the expressions of the manufacturer or its representatives, or may be shown by the circumstances surrounding the distribution of the article. A manufacturer's objective intent may, for example, be shown by labeling claims, advertising matter, or oral or written statements by the manufacturer or its representatives. It may be shown by surrounding circumstances under which a HCT/P is offered for a purpose for which it is neither labeled nor advertised.

V. REGULATORY SCOPE AND COMPLIANCE POLICY

A. Scope of FDA's Regulation of HCT/Ps

As noted in the Background section of this guidance, this guidance only applies to products and establishments that are subject to FDA's regulations in 21 CFR Part 1271. Establishments that meet the same surgical procedure exception in 21 CFR 1271.15(b) are not subject to FDA's regulations in 21 CFR Part 1271. This guidance also does not apply to products that fall outside the definition of HCT/P in 21 CFR 1271.3(d). For example, platelet rich plasma (PRP, blood taken from an individual and given back to the same individual as platelet rich plasma) is not an HCT/P under 21 CFR Part 1271 because it is a blood product. Accordingly, FDA does not apply the criteria in 21 CFR 1271.10(a) to PRP, and PRP is outside the scope of this guidance.

B. Compliance and Enforcement Policy Regarding Certain Regulatory Requirements

To give manufacturers time to determine if they need to submit an IND or marketing application in light of this guidance and, if such an application is needed, to prepare the IND or marketing application, FDA generally intends to exercise enforcement discretion through May 31, 2021,[32] with respect to the IND and the premarket approval requirements for HCT/Ps that do not meet one or more of the 21 CFR 1271.10(a) criteria, provided that use of the HCT/P does not raise reported safety concerns or potential significant safety concerns.

FDA intends to focus enforcement actions on products with higher risk, including based on the route and site of administration. For example, actions related to products with routes of administration associated with a higher risk (e.g., those administered by

[32] The November 2017 guidance, corrected in December 2017 provided a period of 36 months, through November 2020, in which FDA intended to exercise enforcement discretion. This enforcement discretion period now extends through May 2021.

intravenous injection or infusion, aerosol inhalation, intraocular injection, or injection or infusion into the central nervous system) will be prioritized over those associated with a lower risk (e.g., those administered by intradermal, subcutaneous, or intra-articular injection). HCT/Ps that are intended for non-homologous use, particularly those intended to be used for the prevention or treatment of serious and/or life-threatening diseases and conditions, are also more likely to raise significant safety concerns than HCT/Ps intended for homologous use because there is less basis on which to predict the product's behavior in the recipient, and use of these unapproved products may cause users to delay or discontinue medical treatments that have been found safe and effective through the New Drug Application or BLA approval processes.

Regenerative medicine is a complex and rapidly evolving field. Accordingly, FDA will continue to reassess its application of the HCT/P regulatory framework, including the minimal manipulation and homologous use criteria in 21 CFR 1271.10(a), as additional scientific evidence emerges in this field.

VI. ADDITIONAL INFORMATION

23. What regulations apply if my HCT/P is regulated as a biological product?[33]

HCT/Ps that are regulated as biological products are subject to section 351 of the PHS Act and the FD&C Act and require premarket approval. Such HCT/Ps are subject to the applicable drug regulations, including the requirements in 21 CFR Parts 210 and 211, and the applicable requirements in 21 CFR Parts 600 through 680. Such products are also regulated under section 361 of the PHS Act and are subject to requirements in 21 CFR Part 1271 designed to prevent the introduction, transmission, and spread of communicable diseases. Pursuant to these regulations, you are required to register as an establishment, and list your HCT/Ps (21 CFR 1271.1(b)(2)) (see section VI. question 25 of this document).

In order to lawfully market a biological product, a biologics license must be in effect (PHS Act 42 U.S.C. 262(a)). Such licenses are issued only after a determination by FDA that the establishment(s) and the biological products meet the applicable requirements to ensure the continued safety, purity, and potency of such products (21 CFR 601.2(d)). For clinical studies of investigational drug products, the sponsor must have an IND application in effect in accordance with the FD&C Act (21 U.S.C. 355(i)) and FDA regulations (21 CFR Part 312 and 21 CFR 601.21). See section VI. question 27 of this document about obtaining more information regarding the IND process.

[33] Some HCT/Ps may be regulated as devices. For more information about device regulation, see CDRH's webpage Device Advice – Overview of Medical Device Regulation at https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/default.htm.

24. What must I do if my HCT/P meets the criteria for regulation solely under section 361 of the PHS Act and 21 CFR Part 1271?

If you are a domestic or foreign establishment that manufactures an HCT/P that is regulated solely under section 361 of the PHS Act and 21 CFR Part 1271, you must, in accordance with 21 CFR 1271.1(b)(1):

1) Register with FDA (see section VI. question 25 of this guidance);
2) Submit to FDA a list of each HCT/P manufactured; and
3) Comply with all applicable requirements contained in 21 CFR Part 1271.

Establishment means a place of business under one management, at one general physical location that engages in the manufacture of HCT/Ps, including:

1) Any individual, partnership, corporation, association, or other legal entity engaged in the manufacture of HCT/Ps; and
2) Facilities that engage in contract manufacturing services for a manufacturer of HCT/Ps (21 CFR 1271.3(b)).

Manufacture means, but is not limited to, any or all steps in the recovery, processing, storage, labeling, packaging, or distribution of any human cell or tissue, and the screening and testing of the cell or tissue donor (21 CFR 1271.3(e)).

25. Must I register as an HCT/P manufacturer?

FDA regulations require establishments that perform one or more steps in the manufacture of HCT/Ps to register and submit a list of products with the Agency. If you are a manufacturer that is required to register, you must do so within five days after beginning operations (21 CFR 1271.21(a)). Registrations must be updated annually in December (21 CFR 1271.21(b)), except if the ownership or location of the establishment changes, or if there is a change in the United States agent's name, address, telephone number, or email address, in which case, you must submit an amendment to the registration within 30 calendar days of the change (21 CFR 1271.26).

26. How can I get more information about the appropriate regulatory considerations for my HCT/P?

The Agency provides two mechanisms through which a manufacturer may obtain a recommendation or decision regarding the classification of an HCT/P:

1) The Tissue Reference Group (TRG), a group that includes representatives from CBER and CDRH, provides product sponsors with an informal process through which they may obtain an Agency recommendation regarding the application of the criteria in 21 CFR 1271.10(a) to their HCT/Ps for a given indication. Information about this process as well as what you may want to include to

facilitate review of your request can be found at: https://www.fda.gov/vaccines-blood-biologics/tissue-tissue-products/tissue-reference-group .

2) A Request for Designation (RFD) may be submitted to the Office of Combination Products (OCP) to obtain a formal Agency decision regarding the regulatory identity or classification of an HCT/P (21 CFR Part 3). A description of that process and information on how to submit an RFD can be found at: https://www.fda.gov/combination-products/rfd-process. Additional information may be found at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/how-write-request-designation-rfd. You may also submit a Pre-RFD to OCP to obtain preliminary feedback on the classification for your HCT/P as well as assistance on how to prepare an RFD. Additional information may be found at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/how-write-request-designation-rfd.

27. How can I obtain more information about the IND process for my HCT/P that requires premarket approval?

Further information about IND requirements for biological products may be obtained through the Division of Regulatory Project Management, Office of Tissues and Advanced Therapies, at 240-402-8190 or mail to:OTATRPMS@fda.hhs.gov.

EXHIBIT C

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Research Article Open Access

A Pilot Study of the Effect of Localized Injections of Autologous Platelet Rich Plasma (PRP) for the Treatment of Female Sexual Dysfunction

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Abstract

Currently, accepted treatments for Female Sexual Dysfunction (FSD) are limited to psychological, behavioral, hormonal and psychopharmacologic interventions. Because of the complex and multifactorial nature of FSD, current therapeutic options may leave a subset of women suffering with sexual dysfunction without clinical improvement. As a simple, safe, and natural alternative therapeutic option for treating female sexual dysfunction, a pilot study was undertaken to test the effect, if any, of vaginal and clitoral injections of autologous Platelet Rich Plasma (PRP) on women desiring treatment for painful intercourse or anorgasmia. Two standardized sexuality tests, the Female Sexual Function Index and the Female Sexual Distress Scale, were administered before and after treatment and were used to measure the response to this therapeutic intervention. Our data indicated some degree of improvement in FSD, including positive changes in isolated sexual difficulties and in the reduction of levels of sexual distress. However, the limited number of participants in this pilot study restricts conclusions. Our initial observations do suggest that further investigation of PRP therapy for the treatment of female sexual dysfunction is indicated.

Keywords: Dyspareunia; Platelet rich plasma; Female sexual dysfunction; Female sexual arousal disorder; Female orgasmic disorder; Hypoactive sexual arousal disorder; Anorgasmia; Injection

Introduction

Because of the many possible etiologic factors involved in female sexual dysfunction and the variability in the response to existing treatment modalities, this area requires research to develop new safe and effective therapeutic alternatives [1]. A recent review of treatment options, when surgically correctable pathology has been ruled out, listed psychological therapeutics and short-term testosterone as the only Level A therapies [1]. A woman with normal hormonal levels or a contraindication to hormonal therapy and no surgical pathology has only psychological therapies as Level A choices for all four classes of sexual dysfunction (i.e. for hyposexual desire disorder, arousal disorder, orgasmic disorder, and dyspareunia) [1]. Although psychological therapies do help many women, there are no other Class A therapeutic alternatives. This indicates that there is a need for further research in this area.

As one possible strategy, a variety of materials have been injected in the periurethral area to treat both sexual dysfunction and urinary incontinence [2]. For example, Calcium Hydroxyapatite Crystals (CHAC) are FDA approved (Coaptite®) for periurethral injection in the treatment of urinary incontinence. However, such therapy may create a discrete constriction that can be associated with urinary obstruction, erosion, infection, and granuloma formation requiring surgical removal [2-6]. With CHAC, no reports show improvement in sexual dysfunction.

Similarly, the injection of hyaluronic acid fillers (the "G-Shot") has been used as a treatment to enhance orgasmic intensity by the amplification of a controversial anatomical area in the anterior vaginal wall (the Graffian Spot). Due to the potential incidence of granuloma formation by hyaluronic acid fillers at the injection site, this therapy has been condemned by the American College of Obstetrics and Gynecology [2-4].

Since investigators have studied the injection of various substances into the vaginal or periurethral areas for treatment of both urinary incontinence and sexual issues, the mechanics and technique of injection into these anatomic sites appears to be safe and well tolerated. The limiting factor seems to be finding a material that, when injected, produces the desired therapeutic effect without causing untoward side effects.

In contrast to the above mentioned synthetic materials, Platelet Rich Plasma (PRP) has been demonstrated to be effective and without serious side effects in multiple studies in the areas of wound care, orthopedics, dental surgery and in a variety of cosmetic procedures [7-9]. PRP activates pluripotent stem cells in the area of injection, resulting in rejuvenation and even enhancement of damaged or undamaged tissue [10-12]. Moreover, the medical literature contains many articles demonstrating the safety of PRP, with no reports of granuloma formation, infection, or any other serious side effects when FDA approved preparation kits are used [13,14]. Should the PRP be prepared using improperly sterilized tubes, there is the potential for a serious local inflammation or life threatening sepsis. Since PRP is completely autologous, there are no known contraindications to its administration. Technically, PRP injection also offers the advantage of flowing into tissue as a non-viscous liquid and not as a gel (as with hyaluronic acid fillers) or as a particulate slurry (as with calcium hydroxyapatite). The aqueous nature of PRP allows injection through a small bore needle and an even distribution throughout the tissue surrounding the injection site.

Considering the precedent of PRP use in clinical practice, as well as its proven safety, women who presented with complaints of dyspareunia or other symptoms related to sexual dysfunction were offered PRP injections into the periurethral area of the Skenes glands and the clitoris and were observed for their responses to this treatment.

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This pilot study measures the responses of women with varying degrees of sexual dysfunction who received this intervention.

Methods

Eleven females, ages 24-64, presenting with complaints associated with female orgasmic disorder, hypoactive sexual arousal disorder, anorgasmia, or dysparunia, participated in the study. The patients were seen in clinical private practices and were not paid either to receive the procedure or to complete the survey. All patients were fully informed of the innovative therapeutic and experimental nature of the localized PRP injection and consented to the procedure.

The materials and equipment included the following: (1) 5cc syringes, (2) 27 gauge needles, (3) two separate centrifuges with proprietary collection systems, (4) calcium chloride 10% (for activation of PRP), (5) and a topical anesthetic cream compounded with a base that prevents irritation and promotes absorption through the vaginal mucosa. Active ingredients were as follows: bupivicaine, lidocaine, and tetracaine with percent concentrations of 20/8/8 respectively.

First, a topical anesthetic cream was applied to the anterior vaginal wall. The clitoral hood was retracted and cream applied to the clitoris. Delaying the PRP injection for 20 minutes after anesthetic application achieved complete or near complete analgesia for the procedure. Peripheral blood was drawn from the arm and centrifuged to yield 5 cc of PRP. One of either of two FDA-approved, proprietary collection systems were used according to the standard recommendations for each system: (1) Regen® or (2) TruPRP® [15,16]. Both systems use centrifugation to separate and concentrate PRP. The TruPRP® system concentrates 5 ml of PRP from 60 ml of whole blood using a laser device that visualizes the buffy coat to separate the PRP from RBC's. The Regen® system concentrates 5ml of PRP from 10 ml of whole blood using a gel separator.

After isolation of the PRP, calcium chloride (0.5ml) was added to the 5 ml of PRP isolate to activate the thrombin cascade, thereby causing degranulation of platelets, releasing growth factors and cytokines, and starting the transformation of the PRP to platelet rich fibrin matrix (PRFM) [17]. Before the PRFM became too gelatinous for passing through a needle (less than 10 minutes), two injections were given through a 27-gauge needle, one injection into each of two specific sites: (1) the anterior vaginal wall into a space between vagina and urethra most distal from bladder), and (2) into the clitoris. All authors were trained by Dr. Runels and agreed to perform the procedure in a uniform manner.

Exclusion criteria

Patients presenting with pregnancy, infection, prior genital tract surgery, malignancy or inappropriate affect were not considered eligible for the procedure.

Ethics

This study falls in the category of Medical Practice and Innovative Therapy, which describes an activity that is designed solely to benefit individual patient(s) and does not require IRB review (University of Virginia Institutional Review Board Health Science for Health Science Research).

Data collection

Two standardized tests to monitor the effects of the procedure on sexual function were employed: (1) the standardized Female Sexual Function Index (FSFI) questionnaire and (2) the Female Sexual Distress Scale Revised (FSDS-R) [18,19]. The FSFI questionnaire measures arousal, desire, pain, orgasm, satisfaction, and lubrication [18]. The FSDS-R questionnaire measures sexually related distress in Females With Sexual Dysfunction (FSD) [19,20]. The FSFI and the FSDS-R were administered before and after the procedure by the patient. Data was obtained at the time of the injection and at 12-16 weeks after receiving treatment.

Previous PRP studies of other tissue types suggests that collection of follow up data at approximately twelve weeks after the procedure allows adequate time to observe therapeutic effects attributed to stem activation and transformation [7-11]. The outcomes measured were the patients' responses to the FSDS-R and FSFI surveys prior to and after receiving the intervention.

Results

Eleven females presenting with dysparunia (not related to vulvodynia or vaginismus) or with one of the previously mentioned categories of sexual dysfunction, ages 24-64, were included the pilot study group. Of the 11 patients treated, seven (64%) demonstrated some degree of improvement (Table 1). Five of the 7 women who started with elevated levels of sexual distress in the FSDS-R, in which the threshold of distress is defined as a score of 11 or more, dropped their scores to less than 11. Therefore, according to the test criteria, 71% of the women improved from being "distressed" to being "not distressed" after the procedure.

Two patients (18%) showed no change in their levels of distress, but both of these women started with low distress levels. Interestingly, two women (18%), according to their FSDS-R, actually became more distressed after treatment. One of the two who reported more distress subsequent to her PRP injection attributed the worsening of her distress to the loss of her sexual partner. The other woman who reported increased distress explained that after the procedure, her libido increased to a point that it exceeded the ability of her partner to satisfy her.

The mean FSDS-R score dropped 10 points, from 17 to 7 (p=0.04) (Table 2). Nine (82%) of 11 women showed improvement in total FSFI scores. Two (18%) did not experience improvement. The range of improvement in total FSFI scores was from 1.6 to 14.3. The difference between the mean pre-treatment and post-treatment totals was 5.5 (p=0.01) (Table 2). The mean scores for arousal improved by 1.2 (p=0.009), for lubrication improved by 1.28 (p=0.002), for desire 0.82 (p=0.06) and orgasm 1.08 (p=0.05). Since the pre-injection and post-injection scores are observed from the same individual (a repeated-measures design), a paired sample t-test was used, assuming that the

Patient #	PRE-Shot	POST-shot
1	31	2
2	2	2
3	21	35
4	1	2
5	7	4
6	30	4
7	3	3
8	34	14
9	28	0
10	21	7
11	12	7

Table 1: Results for Female Sexual Distress Scale: A score of ≥ 11 effectively discriminates between women with FSD and no FSD.

	Pre PRP Injection		Post PRP Injection*		Difference	
	n	Mean	n	Mean	P value**	95% CI
		Score		Score		
FSDS-R	11	17.27	11	7.27	0.04	-19.57 to -0.43
FSFI (Total)	11	24.13	11	29.63	0.01	1.48 to 9.52
Desire	11	3.60	11	4.42	0.06	0.03 to 1.67
Arousal	11	3.95	11	5.18	0.009	0.39 to 2.07
Lubrication	11	4.17	11	5.45	0.002	0.57 to 1.99
Orgasm	11	4.11	11	5.19	0.05	0.02 to 2.14
Satisfaction	11	4.04	11	4.40	0.28	-0.35 to 1.08
Pain	11	4.25	11	4.98	0.25	-0.59 to 2.04

*10-16 weeks post treatment **unadjusted

Table 2: Mean scores before and after injection with Plate-Rich Plasma (PRP) of the Female Sexual Distress Scale Revised (FSDS-R) and the Female Sexual Function Index (FSFI). The FSFI individually measures Desire, Arousal, Lubrication, Orgasm, Satisfaction, and Pain. The Wilcoxon signed rank sum test was used to assess the difference between the Pre PRP Injection and Post PRP Injection scores.

differences between paired observations are normally distributed. When breaking down the FSFI into the individual domains: desire, arousal, lubrication, and orgasm were significantly increased after injection with PRP. There was no statistically significant effect on satisfaction and pain, although there was a trend toward improvement in those domains.

Side effects

Extreme sexual arousal occurred in 2 patients and included the following: sexual arousal with urination, continuous sexual arousal, ejaculatory orgasm, and spontaneous orgasm. Except for ejaculation, these responses only lasted 1 to 2 weeks and occurred in younger patients who received the procedure with an initial score indicating minimal dysfunction. Other than ejaculatory orgasm, these side effects resolved without further treatment. No other unfavorable side effects were reported.

Discussion

Our results suggest that some cases of female sexual dysfunction, manifested by decreases in sexual desire, arousal, lubrication and orgasmic responsiveness, may be treated with specifically directed injections of autologous Platelet Rich Plasma (PRP) in the area of the Skene's glands and the clitoris. The issue of female sexual dysfunction is quite common. Data shows that sexual difficulties may be experienced by more than 40% of the sexually active adult female population at some time in their lives [21,22]. This percentage represents a sub-set of women who are psychologically distressed by their dysfunction, but do not necessarily consult a physician. Consequently, this statistic may actually be under reported and the condition under-diagnosed because data indicates that only 14% of women may have a conversation with their physician about their sexuality [1]. For sexually dysfunctional women who have not responded to hormonal or psychologic therapies, previous attempts to develop an effective injectable therapy to treat dyspareunia, female orgasmic difficulties, and urinary incontinence have been limited by complications related to the material injected. Autologous PRP injections, on the other hand, have been shown to be safe in other therapeutic areas, since PRP is nonantigenic and contains no synthetic agents that could cause an untoward local or systemic reactions. From our literature search, we could find no reports of granuloma formation, infection, or local tissue necrosis with the use of any of the kits approved by the FDA for preparation of PRP. Since PRP is derived from the patient's own blood, with no foreign or synthetic substances employed, the body will not react to it immunologically [12]. Hence, there are no reports of allergic reactions to PRP injection.

Studies have demonstrated that PRP induces regrowth of new tissue by of the activation of pluripotent stem cells that are indigenous to most parts of the body. These cells are capable of differentiating into several tissue types, when stimulated by growth factors produced by activated platelets [4-7]. We therefore postulate that when PRP is activated and injected into the anatomic areas involved in sexual responsiveness, growth factors and cytokines may cause differentiation of pluripotent stem cells resulting in neoangiogenesis, fibroblast growth, glandular proliferation (Skene's glands), and new neuronal growth—resulting in improved physiologic responsiveness. Improved vascularity and neuronal regrowth in the vagina and in the clitoral area could restore or possibly enhance sexual responsiveness and sensitivity by increasing blood flow to the area, especially in cases where hormonally independent vaginal atrophy contributes to FSD. In addition to increased blood flow, collagen and sensory nerve regrowth might relieve coital discomfort as well as enhance vaginal sensitivity. Also, increased blood flow in the clitoris, if induced by PRP injections, could also lead to improved arousal and orgasm.

The extreme sexual arousal observed in two of our patients may have resulted from a volumetric effect of the PRP injection, causing continuous pressure on the urethra and the Skene's glands. This effect can result from Platelet Rich Fibrin Matrix (PRFM), in which the PRP interacts with thrombin to form a matrix. If Platelet Rich Fibrin Matrix in the periurethral tissue behaves as it does in the dermis of the arm, then this matrix would resolve within 2 weeks to become replaced over the following 8 weeks with new tissue growth [12].

There are certain obvious limitations to this study. Because of the small number of patients in this pilot study, the statistical power of this study is limited and, as such, only suggests a possible effect of our intervention. Furthermore, due to the complexity of the female sexual response and the importance of emotional factors in sexual responsiveness, a placebo effect must be considered when evaluating our findings. Another possible limitation of our pilot study is its observational and subjective nature, despite the use of standardized diagnostic questionnaires. Despite the potential methodologic problems inherent in a pilot study involving female sexuality, because of the patient's positive response to our intervention, without the incidence of complications, future prospective, placebo controlled studies are planned.

Conclusions

The preliminary results of this pilot study suggests that specifically placed intravaginal and intraclitoral PRP injections could be an effective method to treat certain types of female sexual dysfunction, especially in the areas of desire, arousal, lubrication and orgasm. Improvement in satisfaction and pain were noted, but were not statistically significant.

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Safety and feasibility of platelet rich fibrin matrix injections for treatment of common urologic conditions

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Purpose: Autologous platelet rich plasma (PRP) is used increasingly in a variety of settings. PRP injections have been used for decades to improve angiogenesis and wound healing. They have also been offered commercially in urology with little to no data on safety or efficacy. PRP could theoretically improve multiple urologic conditions, such as erectile dysfunction (ED), Peyronie's disease (PD), and stress urinary incontinence (SUI). A concern with PRP, however, is early washout, a situation potentially avoided by conversion to platelet rich fibrin matrix (PRFM). Before clinical trials can be performed, safety analysis is desirable. We reviewed an initial series of patients receiving PRFM for urologic pathology to assess safety and feasibility.

Materials and Methods: Data were reviewed for patients treated with PRFM at our center from November 2012 to July 2017. Patients were observed immediately post-injection and at follow-up for complications and tolerability. Where applicable, International Index of Erectile Function (IIEF-5) scores were reviewed before and after injections for ED and/or PD. Pad use data was collected pre/post injection for SUI.

Results: Seventeen patients were identified, with a mean receipt of 2.1 injections per patient. Post-procedural minor adverse events were seen in 3 men, consisting of mild pain at injection site and mild penile bruising. No patients experienced complications at follow-up. No decline was observed in men completing pre/post IIEF-5 evaluations.

Conclusions: PRFM appears to be a safe and feasible treatment modality in patients with urologic disease. Further placebo-controlled trials are warranted.

Keywords: Erectile dysfunction; Penile induration; Platelet-rich fibrin; Platelet-rich plasma; Urinary incontinence, stress

INTRODUCTION

Platelet-derived therapies are a growing trend across multiple medical and surgical specialties [1-5]. Evidence suggests that platelets play an important role in tissue repair, vascular remodeling and inflammatory and immune responses through secretion of growth factors, cytokines and chemokines [6,7]. These biologically active proteins include transforming growth factor-β, platelet-derived growth factor, platelet-derived epithelial growth factor, insulin-like growth factor, vascular endothelial growth factor, basic fibroblast growth factor, as well as many others [8]. These

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growth factors are implicated in many aspects of natural wound healing, including chemotaxis, cell proliferation, cell differentiation and angiogenesis. They also control and conduct synthesis, modification and degeneration of extracellular matrix proteins. Coordination of these cellular and molecular processes is integral to proper wound healing and tissue regeneration [9]. The key role of platelets in these processes makes them an attractive candidate for therapies aimed at accelerating natural healing.

One of the most well described platelet-based therapies is autologous platelet-rich plasma (PRP) [10]. PRP is derived from the centrifugation of whole blood with a separator gel to remove the red and white blood cells. The resulting supernatant has a greater than four-fold increase in platelets and other plasma proteins [11]. This concentrate is then administered via injection. Newer strategies to prolong the anti-inflammatory and wound healing properties of platelets have focused on creating a fibrin matrix (platelet rich fibrin matrix, PRFM) to bind the platelets and prevent extravasation from the site of injection, thereby addressing the concern of early washout with PRP [12]. In addition, PRFM offers a potential scaffold for tissue ingrowth and may allow continued release of platelet-related factors for a longer duration.

Autologous blood-based biomaterials are promising therapeutic options for varied pathology. Rapid generation of therapeutic material following collection allows for point-of-care therapy [13]. Furthermore, an autologous therapy avoids the need for immunosuppression and eliminates concern of rejection. Within urology, as with many other specialties, there are numerous conditions where tissue regeneration is desirable. In a prior rodent model, Wu et al. [14,15] performed intracavernosal injection of PRP after cavernous nerve crush injury and noted increased myelinated axons and improved recovery of erectile function. Currently, there are no reports of PRP or PRFM for the treatment of urologic conditions in humans, and thus, no assessment of safety. The aim of this study was to evaluate the safety and feasibility of PRFM injections in a subset of patients treated for erectile dysfunction (ED), Peyronie's disease (PD), or stress urinary incontinence (SUI).

MATERIALS AND METHODS

The study was approved by the Institutional Review Board of Wake Forest School of Medicine (approval number: IRB00042919). Data was prospectively collected and retrospectively reviewed for patients treated with PRFM for ED, PD, or SUI by a single surgeon from November 2012 to July 2017 as part of our novel therapeutics program. Informed consent was obtained and patients were aware of off-label use. Demographic data, clinical pathology, procedural details, outcomes data, and pre- and post-procedural International Index of Erectile Function (IIEF-5) questionnaires (for male patients) were collected. Each participant was injected with autologous PRFM using a proprietary system (Selphyl, Aesthetic Factors Inc., Wayne, NJ, USA).

1. Preparation and injection process

Venipuncture was performed in the clinic. Two separate collection tubes were filled with 9 mL of whole blood. The samples were centrifuged at 6,000 RPMS for six minutes, and the supernatant was separated from the remaining blood sample using a proprietary system. Ten percent calcium chloride solution was then added to the PRP in a 1:10 ratio, converting fibrinogen to fibrin. This process would generally yield approximately 5.5 mL of injectable PRFM per tube with patients receiving either 1 or 2 tubes. PRFM, referred to some as 'activated PRP' was chosen so as to allow better local retention of product and thus avoid early washout. Administration was performed within ten minutes of final preparation.

Injections were performed based on the targeted genitourinary pathology. Between 4 and 9 mL of PRFM was injected per treatment session. Intracavernosal injection was performed for ED. For patients with PD, an artificial erection was induced with 20 μg of alprostadil to assess curvature, and injections were placed directly into tunical plaques under ultrasound guidance. After a thorough discussion of potential risks and benefits, three patients elected needle fracture of plaque(s) with concomitant 10 mL saline injection prior to PRFM injections. For SUI, a pediatric cystoscope and transurethral injection needle were used to inject PRFM into the urethral submucosa, distal to the bladder neck.

Patients were observed in the clinic for 20–30 minutes post-procedurally for potential complications or side effects. Clinical information, safety related questions, survey data, and IIEF-5 questionnaires were collected at the time of clinical follow-up and telephone calls were used to evaluate for possible adverse events for which no medical attention was sought.

RESULTS

Seventeen patients underwent injections for the treatment of organic ED (4), PD (11), coexisting ED with

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Table 1. Demographic breakdown (n=17)

Demographic	Value
Male	16
Female	1
Mean age (y)	46 (27–61)
Mean body mass index (kg/m^2)	25.5
Urologic diseases treated	
ED	4
PD	11
ED+PD	1
Stress urinary incontinence	1
Mean of injections	2.1 (1–8)

Values are presented as number only or mean (range).
ED, erectile dysfunction; PD, Peyronie's disease.

Table 2. Demonstrates the minor adverse effect rate (n=17)

Adverse event	No. (%)
Minor	
Overall	4 (23.5)
Mild pain	4 (23.5)
Bruising	1 (5.9)
Major	
Overall	0
Bleeding	0
Infection	0
Compartment syndrome	0

PD (1), and female SUI (1) (Table 1). Cited reasons for ED included vasculogenic, penile fracture, medication-related and electrical injury to the genitalia. Mean patient age at time of first injection was 46 years (range, 27–61 years). Patients received an average of 2.1 (range, 1–8) injection procedures during the study period. Additional injections were provided upon patient request. Injections were well tolerated in all cases. Three patients reported mild pain at the injection site, one of whom also noted mild penile bruising after the injection (Table 2). All patients who noted bruising were PD patients who were given intracavernosal alprostadil were also given planned injection of 250 μg of phenylephrine at the conclusion of the procedure to detumescence. No systemic complications were noted initially or during follow-up. Mean follow-up was 15.5 months.

Among ED and/or PD patients queried with IIEF-5 (7), no patient reported a worsening of overall score or of any individual domain score. IIEF-5 scores improved by an average of 4.14 points after PRFM therapy. In patients with PD with subsequent follow-up, 80% (4/5) initially reported subjective improvement in their degree of curvature. One female patient underwent transurethral injection for SUI with 50% reduction in pad usage. When asked whether they would be likely to undergo further PRFM injections, 80% of patients answered affirmatively.

DISCUSSION

Platelet based therapies are being increasingly utilized in multiple medical settings, including dermatology, ophthalmology, cardiology, colorectal surgery, and plastic surgery [1,11]. PRP has been frequently used for orthopedic conditions such as bone and soft tissue trauma, inflammatory conditions, and chronic pain syndromes [1,7,10]. Across multiple disciplines, PRP has been used both as a primary treatment modality and as a supplement to other therapies in hopes of supplementing wound healing, tissue regeneration, and angiogenesis. Although most of the studies focusing on PRP injections have been relatively small and heterogenous, they largely support safety and efficacy. Additionally, the concept of autologous therapy may be particularly attractive to some patients [16].

ED affects as many as 1 in 4 men, and evidence indicates the incidence is rising [17,18]. The pathophysiology is multifactorial, but a significant proportion results from endothelial dysfunction secondary to inflammation [19]. The most common treatments for ED aim to improve endothelial function through augmentation of the nitric oxide pathway [20]. To date, there are no treatments that address the underlying cause of endothelial dysfunction. Platelet-derived therapies targeting inflammation and promoting tissue regeneration may represent a potential treatment option.

PD, while less common than ED, affects roughly 1%–8% of men [21]. The pathophysiology appears to involve increased inflammation from tissue disruption, followed by aberrant wound healing resulting in fibrotic plaques [22]. Current treatment regimens include plaque injection, plication, grafting, or insertion of penile prosthesis to restore appropriate form and function. Currently there are no therapies targeting either the inflammatory processes or the aberrant wound healing that causes PD. Furthermore, therapies focusing on disrupting the fibrotic plaques through mechanical manipulation, or more recently, collagenase injection, do not address appropriate wound healing or regeneration of the damaged tissue [23]. Theoretically, injection of PRFM could combine mechanical disruption of the plaque, via needle fracture, while simultaneously neutralizing destructive inflammatory processes in an effort to promote a better wound-healing response and stabilize the disrupted plaque.

Biologic materials have been used for decades in the

treatment of SUI. Multiple products have been used as bulking agents to supplement urethral coaptation. While generally less efficacious than surgical repairs, injectable agents remain attractive given their relative ease of administration and lack of need for implantable mesh-based materials. When it was previously available, glutaraldehyde cross-linked bovine collagen was the most commonly injected biomaterial used to treat female SUI and was associated with a cure rate of 53% [24]. Theoretically, injection of autologous PRFM could provide both urethral bulking and potential regenerative effects to a damaged female urethra.

Investigations of PRFM for the urologic conditions noted in this report have not been previously reported. Wu et al. [15] investigated the effects of several different preparations of PRP injections in rat models with bilateral cavernous nerve crush injuries. Their data suggest that an "optimized" PRP formulation with a high level of growth factors was more stable than other preparations of PRP. Rats receiving this formulation showed significantly greater increases in intracavernosal pressure, higher mean arterial pressure, higher levels of nitric oxide synthase, and greater recovery of erectile function than those receiving saline injections or other formulations of PRP. Tang et al. [25] also showed that PRP injections at the site of cavernous nerve crush injuries helped facilitate nerve regeneration and erectile function in a rat model. More recently, Shirvan et al. [26] described injection of PRP and interposition platelet rich fibrin glue into the fistulous tracts of 12 patients with vesicovaginal fistulas (most <5 mm). All patients showed significant improvement with 11 patients cured at six-month follow-up, both subjectively and by examination.

We recognize that a variety of preparations, delivery modalities, and dosing schedules are available for PRP/PRFM therapies. A mean of 2.1 injection procedures per patient were performed during the study period. In our study, the PRP was added to a calcium chloride preparation to create PRFM. This was done to theoretically prevent rapid washout of the PRP from the corpora. One potential safety concern about using a colloid/hydrogel type of material in the corpora was the possibility of interrupting corporal blood flow, creating the possibility of a 'penile compartment syndrome,' akin to priapism. This did not occur in any of the ED or PD patients in our study, as each of these injections was well tolerated.

Data from this report regarding functional assessments must be interpreted with caution. This was not a prospective study, and we believe a significant placebo effect exists for research involving male sexual health. Objective improvements in the IIEF-5 score (4.14 points, 9.1%) were seen in patients receiving PRFM therapy for ED and PD. This level of improvement was similar to the average IIEF score increase (4.45 points, [3.42, 5.29]) seen in patients using PDE5Is after nerve sparing prostatectomy in a recent meta-analysis [20]. At follow-up interviews, patients expressed specific improvements in the rigidity of erections and improvements in satisfaction due to increased confidence. Of PD patients available for follow-up, 80% noticed an initial subjective improvement in their degree of curvature. Additionally, the one patient who received PRFM injections for SUI noted a 50% decrement in pad usage. Patients injected with silicone polymers (Macroplastique, Cogentix, Minnetonka, MN, USA) reported a 77% subjective cure rate but only a 9% objective cure rate on urodynamic testing [27]. No conclusions can be drawn from a single patient, but a 50% objective improvement from a transurethral injection procedure using an autologous product seems promising. With regards to feasibility of the procedure, there were no concerns related to the preparation of the PRFM or the injection process itself into the corpora cavernosa, tunical plaques, or urethral submucosa for patients with ED, PD, or SUI, respectively.

While this study attests to safety in this selected population, it has multiple limitations. This was a retrospective review of a small cohort of patients with a spectrum of pathology that may not be representative of the general population. As an autologous product, we expect that reabsorption rates are high, such that repetitive therapy will be required. This raises the possibility of treatment-related fibrosis from injection site trauma. As mentioned, although there was no detriment in IIEF score, the lack of a placebo arm prevents a detailed context. Future work will involve placebo control, with structured assessments for efficacy.

CONCLUSIONS

Our initial experience suggests that PRFM injections for ED, PD, and female SUI are feasible and safe. Although the limited data is suggestive of efficacy, a placebo control will be required in subsequent efforts for confirmation. Future studies evaluating efficacy of PRFM injections for genitourinary pathology appear warranted.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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A pilot study: effectiveness of local injection of autologous platelet-rich plasma in treating women with stress urinary incontinence

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The study aims to evaluate the effectiveness of local injection of autologous platelet rich plasma (A-PRP) as a treatment for women suffering from stress urinary incontinence (SUI). In a prospective intervention study, twenty consecutive women suffering from SUI were treated with A-PRP injection at anterior vaginal wall where mid-urethra locates. Self-reported questionnaires were used to measure pre-treatment, 1 month and 6 months post-treatment symptom severity. Secondary outcomes of sexual function and treatment effect sorted by age were analyzed with valid statistical methods. A-PRP is effective in relieving SUI symptoms at both 1 month and 6 months post-treatment without significant adverse reactions reported. It seems to have a trend that treatment success rate with cured and improved symptoms was slightly higher in the younger group, although it did not reach statistical significance ($P = 0.07$). No significant changes in sexual function before and after the treatment were reported by the patients. This pilot study is the first to report A-PRP treatment effect for SUI in women. The result suggested that A-PRP is a considerable treatment option for mild to moderate SUI cases. It also opens up further research opportunities for A-PRP's clinical applications.

Platelet is rich in various kinds of growth factors and cytokines that promote soft tissue healing. Insulin-like growth factor (IGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), transforming growth factors-beta (TGF-β), vascular endothelial growth factors (VEGF), connective tissue growth factor (CTGF) hepatocyte growth factor (HGF) and interleukin 8 (IL-8) are only a few to name[1]. Each has its role in enhancing cell migration, cell recruitment, cell replication, extracellular matrix scaffolding, tissue regeneration and neo-angiogenesis. These processes are activated upon stimulation by exposure to thrombin, calcium or collagen in vivo[2]. Collectively, they repair damaged tissues and rejuvenate aged cells. Autologous platelet-rich plasma (A-PRP) is synthesized from patients' own blood after concentration via centrifugation. The autologous nature meant satisfying safety profile for reduced immune reactions, as long as the preparation is handled with care and good sterile techniques.

A-PRP has long-standing history and good outcomes in sports medicine, particularly in treating tendonitis, arthritis, ligament sprains and tears. It is effective in reducing injury pain, fast healing and quick return to regular activities. Other fields with evidence-based applications include dentistry, dermatology[2], sports medicine[3], cardiac surgery, pediatric surgery, urology[4], plastic surgery and ophthalmology[5]. Utilization of A-PRP in the

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Mean age (years)	44.5 ± 9.1
Mean parity	1.6 ± 0.5
Mean BMI (kg/m^2)	22.7 ± 6.3
Pad test	5.8 ± 3.6
Menopause	5 (25.0)
SUI grade (ICIQ-SF)	
Mild	1 (5.0)
Modetate	12 (60.0)
Severe and very severe	7 (35.0)
Follow up (months)	6

Table 1. Demographic data (n = 20) are given as mean ± standard deviation or n(%). BMI, body mass index, Values are expressed as mean ± standard deviation or numbers; SUI grade according to ICIQ-SF: slight (1–5), moderate (6–12), severe (13–18) and very severe (19–21).

				P value*	
N = 20	**Baseline**	**1 months post-Tx**	**6 months post-Tx**	**1 month**	**6 months**
ICIQ-SF	11 (6–18)	6 (0–17)	4 (0–16)	0.012*	0.002*
UDI-6	33.3 (17–72)	22.2 (0–78)	17 (6–72)	0.005*	0.004*
IIQ-7	23.8 (5–90)	4.8 (0–86)	4.8 (0–86)	0.001*	0.016*
OABSS	6 (0–12)	3 (0–12)	4 (0–12)	0.034*	0.229
POPDI-6	4 (0–13)	2 (0–15)	2 (0–14)	0.254	0.232

Table 2. Questionnaire results at baseline and 1, 6 months post-treatment. Tx treatment, ICIQ-SF International Consultation on Incontinence Questionnaire-Short Form, UDI-6 Urogenital Distress Inventory, IIQ-7 Incontinence Impact Questionnaire, OABSS Overactive Bladder Symptom Scores, POPDI-6 Pelvic Organ Prolapse Distress Inventory 6. Values are expressed as median (range). *Statistical significance; Wilcoxon signed rank test.

field of obstetrics and gynecology dated back as early as 2007 by the Fanning J et al., who investigated direct application to operated wounds in total abdominal hysterectomy, laparoscopy-assisted vaginal hysterectomy and urogynecology surgeries. They found significantly reduced procedure related pain as early as post-operation day 1[6]. However, no available literatures published so far have demonstrated potential therapeutic effect of A-PRP in treating women with stress urinary incontinence (SUI).

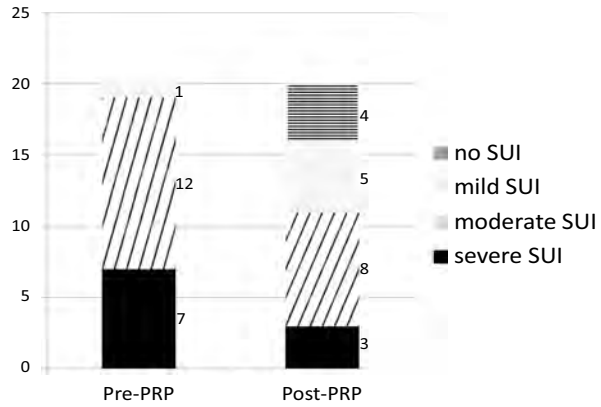
SUI is a bothersome gynecology problem all around the world, with an estimated prevalence of 40% in adult women[7]. Birth trauma, aging, obesity and estrogen deprivation are well-known risk factors. Kunkle and colleague reported approximately 13.12 billion US dollars were spent on SUI, including disposable diapers, laundry, dry cleaning, and sanitary pads[8]. A variety of treatment modalities is currently available: lifestyle modification and pelvic floor muscle exercise might be effective for mild degree of SUI symptom; electrostimulation, biofeedback, and extracorporeal magnetic innervation are non-invasive symptom-control methods.

Anti-incontinence surgeries, such as mid-urethral tapes and colposuspension are effective and durable[9]. Each method has its strengths and limitations that should be adopted according to individual condition, characteristics, disease severity, and economic considerations to reach a shared decision making between the healthcare provider and patients. This study aims to assess A-PRP as an alternative treatment option for the treatment of SUI. Reviewing the literature, this is the first report that demonstrates clinical outcomes of A-PRP application in stress-incontinent women.

Results

Among the 20 patients enrolled, the average age was 44.5 years old with averaged parity of 1.6 times, body mass index 22.7 mg/m^2. Five or 25% of them are menopause. Prior to the PRP treatment, the average pad test was 5.8 g; one (5%) reported mild symptom while 12 (60%) and 7 (35%) reported moderate and severe to very severe diseases, respectively by International Consultation on Incontinence Questionnaire-Short Form (ICIQ-SF) score. All patients followed for at least 6 months (Table 1). Treatment efficacy assessed by ICIQ-SF, Urogenital Distress Inventory (UDI-6), Incontinence Impact Questionnaire (IIQ-7) and Overactive Bladder Symptom Scores (OABSS) showed significant incontinence improvement at both 1 month and 6 months post treatment, but not by Pelvic Organ Prolapse Distress Inventory 6 (POPDI-6) (Table 2).

Changes in grades of SUI following treatment of PRP injection were shown in Fig. 1. These women were then sorted by age, with the cut-off value at 40 years old to investigate age as a factor for treatment efficacy. The result showed no significance between the characteristics of the two groups in terms of mean body mass index, underlying diabetes mellitus, hypertension, history of hysterectomy or history of mid-urethral sling. Only parity number was significantly higher for the older group. However, it seems to have a trend that treatment success



SUI, stress urinary incontinence; PRP, platelet-rich plasma.

Figure 1. Changes in grades of stress urinary incontinence following treatment of PRP injection.

	Post-op						
Pre-op	Cure	Mild	Moderate	Severe	Improved	Unchanged/worse	Efficacy
Mild (n = 1)	1	0	0	0	1	0	1/1 (100%)
Moderate (n = 12)	3	3	5	1	6	6	6/12 (50%)
Severe (n = 7)	0	2	3	2	5	2	5/7 (71.4%)
All (n = 20)	4	5	8	3	12	8	12/20 (60%)
95% CI					95% CI [3.89, 9.01]	95% CI [− 2.59, 0.15]	

Table 3. Treatment efficacy of PRP injection. PRP, platelet-rich plasma; Pre-op, preoperative; Post-op, postoperative; CI, confidence interval.

Parameters	Pre-op (n = 8)	Post-op (n = 8)	*P* value #
Qmax (ml/s)	29.8 ± 5.4	33.7 ± 4.1	0.08
RU (ml)	9.7 ± 5.1	46.7 ± 13.4	0.028 §
FS (ml)	135.8 ± 42.5	163.8 ± 17.0	0.001 §
MCC(ml)	428.0 + 21.5	408.8 ± 41.1	0.17
Pdet (cmH_2O)	24.5 ± 5.4	23.8 ± 5.8	0.52
FUL (mm)	24.8 ± 6.2	25.4 ± 6.8	0.22
MUCP (cmH_2O)	65.5 ± 9.8	54.1 ± 21.5	0.14

Table 4. Urodynamic changes before and 6 months after PRP treatment. Data are given as n(%) or mean ± standard deviation. PRP, platelet-rich plasma; DO, detrusor overactivity; Qmax, maximum flow rate; RU, residual urine; FS, first sensation to void; MCC, maximum cystometric capacity; Pdet, detrusor pressure at peak flow; FUL, functional urethral length; MUCP, maximum urethral closure pressure. #Paired t-test; § significant significance.

rate with cured and improved symptoms was slightly higher in the younger group (75%) compared to that of the older group (50%), although it did not reach statistical significance ($P = 0.07$). The treatment efficacy is also demonstrated by disease severity distribution pre-PRP and 6 months post-PRP (Table 3), with a shift of the majority reporting moderate and severe diseases to moderate and milder diseases. No adverse reactions were reported.

Only 8 women completed the urodynmaic studies before and 6 months after intervention. Residual urine and bladder volume at first sensation to void increased significantly after injection of PRP. All other urodynamic parameters showed no significant differences following treatment (Table 4). The influence of PRP injection on sexual function was investigated by Female Sexual Function Index (FSFI) questionnaire. No significant changes before and after the treatment were reported by the patients, neither for the total score or for each component (i.e. desire, arousal, lubrication, orgasm, satisfaction and dyspareunia) (Table 5). Further analysis by the factor of age revealed there were no in-between group differences (Table 6).

	Pre-treatment	6 months post-treatment	*P* value
FSFI total scores	17.9 ± 10.2	20.3 ± 11.0	0.25
Desire (1, 2)	2.7 ± 1.3	2.7 ± 1.2	0.79
Arousal (3–6)	2.6 ± 1.8	2.9 ± 0.9	0.46
Lubrication (7–10)	3.2 ± 2.1	3.5 ± 2.1	0.34
Orgasm (11–13)	3.0 ± 1.9	3.4 ± 2.1	0.35
Satisfaction (14–16)	3.2 ± 2.0	3.8 ± 2.3	0.13
Pain (17–19)	3.2 ± 2.0	3.9 ± 2.4	0.11

Table 5. Changes Female Sexual Function Index (FSFI) scores at baseline and 6 months post-treatment with PRP injection. Data are given as median (range) or mean ± standard deviation. Paired's t-test.

	Age < 40 (n = 8)			Age > 40 (n = 12)			
Domains	Pre-op	Post-op	*P* value*	Pre-op	Post-op	*P* value*	Intergroup *P* value^
Sexual desire	2.6 ± 1.4	2.9 ± 1.2^	0.03**	2.8 ± 1.3	2.3 ± 1.1^	0.014**	P^a
Sexual arousal	2.5 ± 1.7	3.2 ± 1.2^	0.19	3.7 ± 1.0	3.0 ± 1.3^	0.029**	P^b
Lubrication	3.5 ± 1.8	4.3 ± 1.1^	0.30	4.0 ± 1.5	3.6 ± 2.0^	0.23	P^c
Orgasm	2.8 ± 1.5	3.6 ± 1.6^	0.27	4.2 ± 1.3	4.1 ± 1.7^	0.71	P^d
Satisfaction	3.1 ± 1.7	4.2 ± 1.7^	0.22	4.4 ± 1.3	4.5 ± 1.9^	0.77	P^e
Dyspareunia	3.8 ± 1.7	4.7 ± 1.2^	0.24	3.4 ± 1.6	3.7 ± 2.3^	0.39	P^f
Total scores	18.3 ± 8.5	22.8 ± 6.7^	0.21	24.3 ± 6.1	24.1 ± 6.7^	0.89	P^g

Table 6. Changes in scores of Female Sexual Function Index in both groups before and 6 months after treatment. Data are given as mean ± standard deviation. Pre-op, preoperatively; Post-op, postoperatively. *Paired *t*-test; ^Student's *t*-test; **Statistical significance. $P^a = 0.72$, $P^b = 0.59$, $P^c = 0.48$, $P^d = 0.13$, $P^e = 0.20$, $P^f = 0.22$, $P^g = 0.59$.

Discussion

The current study demonstrates A-PRP is effective in treating women with SUI for as long as 6 months post treatment. The outcome is evidenced by multiple self-reported questionnaires before, 1 month and 6 months after the treatment. ICIQ-SF, UDI-6, IIQ-7 and OABSS questions all revealed significant and lasting effectiveness, while POPDI-6 showed a trend of improved symptom scores although not reaching statistical significance (Table 2). Figure 1 further illustrated a shift of disease severity distribution to milder diseases after PRP treatment. According to the integral theory, the most important factor in cases of female SUI is a pubourethral ligament defect[10]. Nikolopoulos and colleagues have advocated the plausibility of A-PRP in restoring pubourethral ligament integrity to treat SUI in as early as 2016[11]. He promoted his hypothesis by laying out various animal models that proved the rejuvenating abilities of A-PRP compositions, such as VEGF, IGF-1, PGDF, HGF, TGF-β and FGF. Injecting bulking agents to provide mechanical support of urethral, thereby storing normal pelvic anatomy and reducing urethral hypermobility, is not new in treating SUI. A-PRP serves a superior agent than previously employed paraffin, bovine collagen, polydimethylsiloxane, polyacrylamide gel, and hyaluronic acids[1], for its autologous nature and minimal, if any, allergic reactions. A-PRP is not only biocompatible, durable and non-migratory; its reparative ability can repair damaged ligaments and potentially prolong treatment effectiveness.

A secondary analysis of this study observed a superior treatment outcome in the patient group younger than 40 years old. It is postulated that being younger might pose better rejuvenating abilities, and thus better treatment effect of A-PRP. Also, aged patients are likely to be impacted with additional variables such as more parity number, menopausal state, more severe SUI symptoms and more underlying systemic conditions such as diabetes mellitus and hypertension, history of hysterectomy, abdominal surgeries and previous anti-continence treatments. The reparative ability in aged people's plasma might be reduced is another hypothesis to be tested. These potential confounding factors are demonstrated by a trend in our small study groups, although not reaching statistical significance. Further study with greater patient number and variable-adjusted analysis is required to validate the assertion. If proven, these factors would be very helpful for patient selection that will benefit the most from PRP treatment.

Sexual function is intimately related to urinary incontinence, thus warranting a secondary analysis in the treatment of SUI with A-PRP. The current study utilizes FSFI questionnaires pre- and post-PRP treatment but failed to reveal significantly improved composite sexual function score, regardless of length of follow up (Table 2). However, when examining each domain of FSFI, an improved sexual desire was noted in both age groups (Table 3). This signifies how stress urinary incontinence impacts the patient's self-image, confidence and fear of embarrassment when it comes to sexual functions. Therefore, an improvement in SUI symptoms readily enhances sexual desire. Interestingly, our finding is in contrary to Runels and colleagues' 2014 report, which demonstrated significantly improved FSFI performance in total scores, desire, arousal, lubrication and orgasm domains in 11

women receiving PRP for sexual disorders[12]. The authors also reported prolonged prolong arousal, ejaculatory orgasm, spontaneous orgasm in younger women as side effects that resolve in 2 weeks without treatment. The observation is not revealed by our study, which is mainly attributed to disparity in injection sites. Runels' group, who specifically aimed at treating sexual dysfunctions, injected A-PRP at both clitoris and a spot of anterior vaginal wall most distal from the urinary bladder. Our study that aimed at treating urinary incontinence had a different injection site. Cultural difference where sexual topics are not as openly discussed in Asian populations might also contribute to the differential results.

PRP it has only gained more attention in the field of obstetrics and gynecology in recent years. Targeted conditions encompass symptomatic ectopic cervix[13], lichen sclerosis at vulvovaginal[14], vesicovaginal fistula[15], pelvic organ prolapse[16,17], ovarian function rejuvenation[18], endometrial receptivity[19], female sexual dysfunction[12], membrane sealant in preterm pre-labor ruptured membrane[20] and cesarean section wound aesthetics[21]. We ever reported that the treatment efficacy for the vaginal Er:YAG laser for SUI at 6-month follow-up was 75.5%[22]. Promising impact of PRP and carbon dioxide laser for SUI was also noted recently[23].

However, the effect of PRP alone on SUI was rarely reported.

The current study is the first report of A-PRP application on women with SUI, with treatment outcomes demonstrated by before and after treatment questionnaires scores. The results are analyzed objectively with valid statistical methods. An intermediate follow-up time of 6 months was investigated. Secondary analysis of impacts of age and sexual functions were also reported. Limitations of the work included small sample size and lack of a controlled group. Future work is encouraged to incorporate a randomized controlled trials with longer follow-up period. Comparative study with head-to-head comparisons to other bulking agents is plausible. Other potential areas to investigate may include A-PRP as a preventative role at the time of pelvic floor structure insult, as an adjuvant modality in combination with corrective surgery or other conservative treatments, determination of its lowest effective dose, repeat treatment intervals if necessary and long-term efficacy.

Conclusion

Local injection of autologous platelet rich plasma seems safe with somewhat satisfactory response in treating female SUI both at 1 month and 6 months post treatment. It appears to have a trend that younger women have better treatment outcome, and larger sample sizes might shed more light upon this effect. Yet how long the treatment effect could sustain remains unknown. This innovated intervention could be an alternative treatment for SUI but awaits further explorations.

Material and methods

From June 2018 to November 2018, females with SUI (involuntary loss of urine on effort or physical exertion or on sneezing or coughing), age over 20 years old, the platelet counts within normal limit (150 k–450 k/uL) and Prothrombin Time (PT) was normal, were offered the innovative treatment of autologous platelet-rich plasma (A-PRP) injections. The study employed a prospective interventional design. A total number of 20 consecutive patients consented to enter the trial with full awareness of the experimental nature and treatment process without compensations in any form. The treatment utilizes autologous material that poses minimal adverse reactions to the patients.

The Institutional Review Board (IRB) Committee of Kaohsiung Medical niversity Hospital approved this research (IRB Number: KMUHIRB-F (I)-20170048) and confirmed that all methods were performed in accordance with the relevant guidelines and regulations. This clinical trial was registered in a publically accessible primary register that participates in the WHO International Clinical Trial Registry Platform with Clinical trial registration number ID: NCT04279210) and date of registration (21/02/2020). All procedures involving human participants were in accordance with the ethical standards of Institutional Review Board of Kaohsiung Medical University Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Patients with the following conditions were excluded from the study: known platelet dysfunction, critical thrombocytopenia, hypofibrinogenemia, hemodynamic instability, sepsis, acute or chronic infections, chronic liver disease, anti-coagulant users and known malignancy (Fig. 2). They did not receive concurrent treatment for SUI during the study period.

The study employed commercialized PRP kit from RegenKit (Regenlab, Le Mont-sur-Lausanne, Switzerland). PRP was prepared according to the standardized procedures instructed by the kit. Two tubes preloaded with anticoagulant additive were used to collect individual patient's whole blood, 10 mL each. The content was mixed by inverting the tubes gently 4–5 times. The tubes were centrifuged at 3400 rpm for 15 min. After centrifugation, three-layered content was noted with platelet pellet, separating gel and red blood cells from top to bottom in order. Platelet pellet was remixed with the supernatant by inverting the tubes gently for 5–10 times. The supernatant then was collected in a 5-mL Luer-Lock syringe. The yielded volume was approximately 5 mL from each tube. The Regen system was specifically designed to produce APRP with a platelet concentration of 1.6X. With a 27-gauge needle, PRP was injected into the anterior vaginal mucosa around the patient's mid-urethra, which was approximately 1 cm below the urethra meatus with a depth about 1.5 cm. Two mL underneath mid-urethra and 1.5 mL for each side of urethra (Fig. 3). No anesthesia was used in this procedure. Monthly treatment was given for 3 consecutive months.

Before enrollment, the patients signed informed consent for participation. The primary outcome was the degree of SUI relief, while the secondary outcome assesses sexual function. Urodynmaic studies were scheduled for them before and 6 months after treatment. Questionnaires that assess the severity of their urinary incontinence and sexual dysfunction were distributed. Specifically, they were asked to fill out OABSS, UDI-6, IIQ-7, ICIQ-SF, POPDI-6 and FSFI questionnaires. ICIQ-SF was the primary tool for assessing SUI severity, which was categorized into slight, moderate, severe and very severe according to the total points obtained after answering the

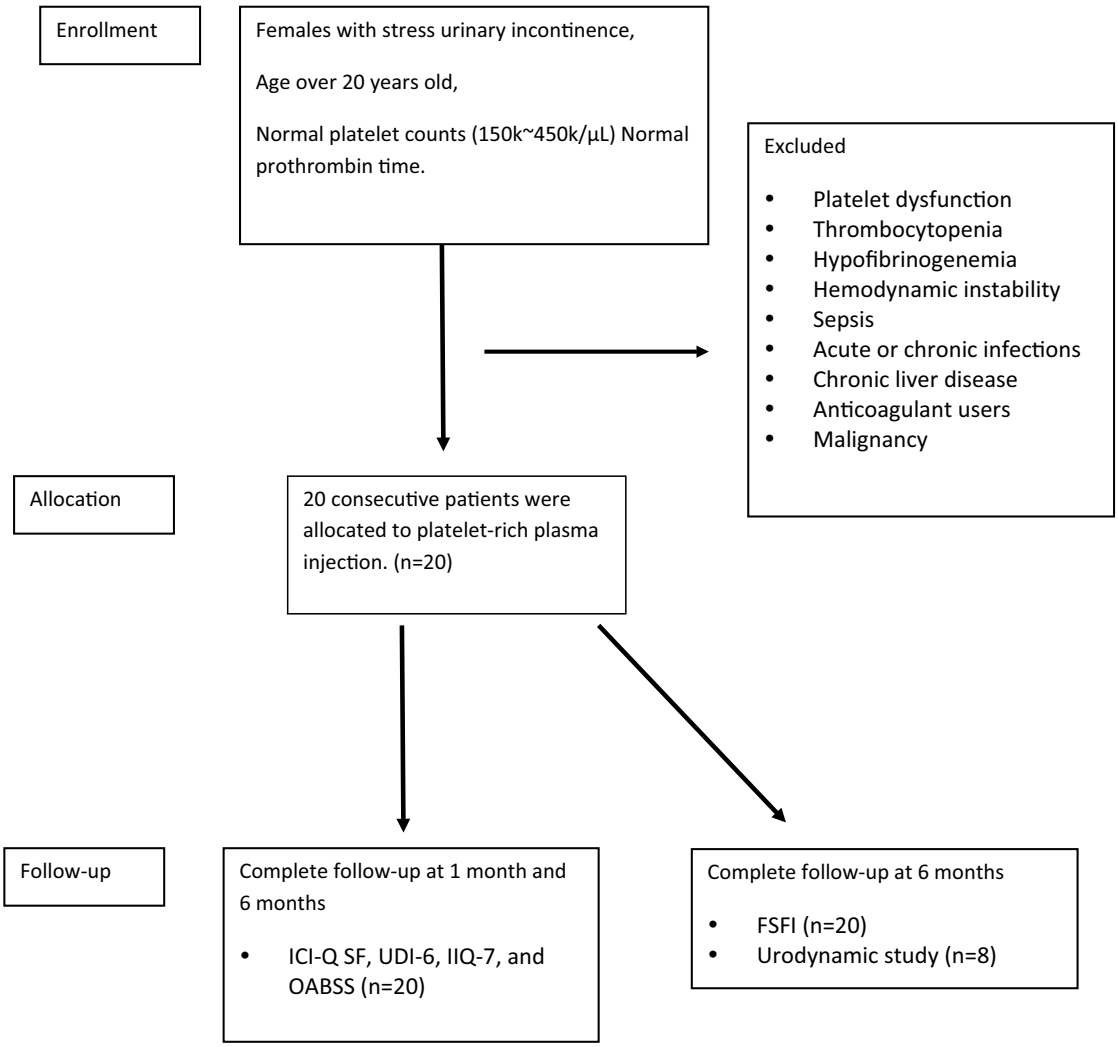


Figure 2. The clinical trial flowchart for platelet-rich plasma injection.

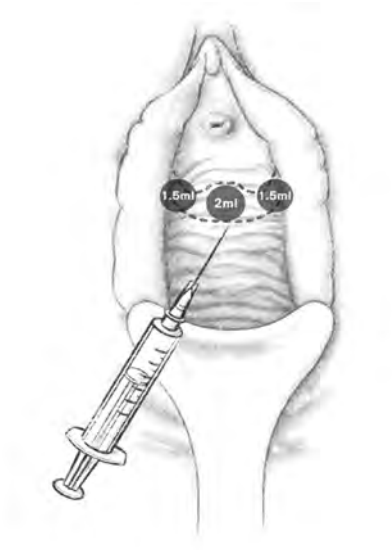


Figure 3. Diagram of the injection site and depth over anterior vaginal mucosa.

questionnaire. The cut-off values were 1–5, 6–12, 13–18 and 19–21 points for slight, moderate, severe and very severe, respectively[24]. Women were deemed for cure if they felt no complaint of SUI after procedure. Symptom improvement was recognized with downgrading of the ICIQ-SF results. Data were collected before, 1 month and 6 months post-treatment. Statistical analysis was performed using Student's t test and paired t-test. A difference was considered statistically significant when $P < 0.05$.

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Author contributions

C.-Y.L. and K.-L.L. have contributed equally for patient recruitment, manuscript writing, project development; C.-R.K., Y.-Y.L. assisted in data acquisition, C.-R.S. and H.-H.H. is responsible for manuscript editing, Z.-X.L. assisted in statistical analysis and while Y.-C.L. conducted the statistical analysis and project development.

Competing interests
The authors declare no competing interests.

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The Future of Vulvar Disease: Ongoing and New Challenges

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The International Society for the Study of Vulvovaginal Disease works diligently to promote teamwork and to collaborate among nationalities and medical disciplines.

Our challenges:
Teamwork: Today´s society rewards individual work for personal benefits. To work as a team, we often must ignore our individual desires. We believe, "If you want to go fast, go alone; if you want to go far, go together". Goals are more easily achieved if we work together.
Become mentors: We believe by sharing our information and our experience by being generous and trying to pass on to others everything we have learned we truly impact the future. If we keep knowledge to ourselves it loses its essence. It becomes pointless. By being a mentor, you can leave a permanent mark on another person´s life.
Spread the knowledge all around the world, especially to underdeveloped countries. It can be challenging, but we must reach out to those that cannot afford to pay for expensive meetings and annual fees to become members of Societies.
We must improve clinical trials on most vulvar diseases. Throughout healthcare fields, clinical trial and observational studies, investigators have recognized the need for development of standard (core) outcomes and instruments to measure them. Core outcome sets/measures are key to the improvement of observational research and clinical trial methods. We will work together on this project.
New approaches to vulvovaginal diseases: Genomics and molecular biology will help us in describing pathogenesis and finding new treatments.
Recognize the importance of the whole woman: Women with vulvovaginal diseases carry a heavy burden that affects their family, social and sexual life. We are not treating a "vulva" we are treating a whole woman. We work for them NOT FOR US.

We all have a challenge, a scientific and a human challenge. To succeed we do not have to lose sight of whom we are working for.

Our mission is to take care of our patients!

Highlights on Clinical Research Regulations: Focus on ICH-GCP and Ethics

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Research means a systematic investigation, designed to develop or contribute to generalizable knowledge and is increasingly playing a role in various medical disciplines.

It is essential that a clinical research practice be based on ethics principles. An investigator in a clinical trial needs to know about the paramount importance to minimize risks of the people who consent to take part of it, as well as being aware of the relevant national and international laws and regulations.

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) is a unique arrangement bringing together the regulatory authorities and pharmaceutical industry to discuss scientific and technical aspects of drug registration.

Directives for Good Clinical Practice (GCP) are recorded in the ICH-GCP Guideline E6. The ICH-GCP are defined as *"international ethics and quality standards for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials that provides assurance that the data and reported results are creditable and accurate, and that the rights, integrity, and confidentiality of trial subjects are protected."* Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki, and the clinical trial data are credible (*www.ich.org*).

In November 2016, the second revision to ICH - GCP E6 (R2), the largest revision to GCP in 20 years, was published.

For the last 50 years, the protection from harm caused by the conduct of research studies and protection of the persons who consent to participate has become crucial for researchers, ethicists and the totality of stakeholders involved in clinical research worldwide. Current laws and regulations aim at such protection by ensuring Good Clinical Practice.

Update on Chronic Recurrent Vulvovaginitis

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Recurrent vulvovaginitis (RVV) is one of the most frequent reasons for a woman to visit a gynecologist. The burden of such infections is high for both patients and health care workers. Despite its frequency, little effect has been achieved over the years to prevent RVV. Dietary measures and behavioral adaptation have failed to decrease recurrent bacterial vaginosis (RBV), recurrent candida vaginitis (RCV), or recurrent aerobic vaginitis (RAV). Reinfection does not seem to be the major cause of the recurrences, rather endogenous, immune-genetic tolerance seems too crucial to maintain the abnormal microbiome in these conditions. Furthermore, in recurrent Trichomonas vaginitis (RTV), where reinfection does play an important role in most cases, resistance to currently used antibiotics has emerged. Vaccination trials have not been sufficiently successful to justify promising patients a clinical useful vaccine, anytime soon, for any of these infections. Concerning treatment, we are aware that the current standard treatment of BV with metronidazole or clindamycin is far from ideal and does not cure more than 2/3 of the relapses. Recently, new disinfectants and antibiotics were shown to have at least equal efficacy compared to standard drugs, opening the possibility to vary and combine treatments for women with RBV. For RCV, most newly introduced triazoles do not have a profile sufficiently different from the existent drugs to promise large improvements in recurrence prevention. Instead a continuous, individualized regressive regimen with fluconazole (ReCiDiF) is the most widely used treatment program in Europe and abroad, keeping women relapse free in 90% and 78% after 6 months and a year, respectively. Non-responders have a higher likelihood to have an atopic constitution, or have switched to a different genotype of Candida albicans or even to a non-albicans infection. For these women, switching to non-azole treatments is advised (boric acid, amphotericin B or flucytosine). These patients are often managed in specialized centers. Glucose or yeast lowering diets, as well as several other behavioral changes, are seldom helpful and should only be maintained if they were proven to reduce recurrences. For RAV several antibiotics such as local kanamycin or moxifloxacin can help for a short while, but tailored treatment according to the microscopic findings of the level of atrophy, number and type of micro-organisms and inflammation give the best long-term results and satisfaction for patients. Theoretically, based on antimicrobial properties in lab circumstances, different probiotics are pushed forward as ideal, natural and efficient ways to treat and prevent recurrences of RVV. A word of caution: most products, both for oral and vaginal use, are widely promoted despite a lack of clinical evidence of efficacy. We advocate using only those products that can submit proper clinical effects on delineated indications.

Approaching Vulvovaginal Candidiasis: Developing Drugs for Treatment – FDA Draft Guidance for Industry

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The Food and Drug Administration (FDA) announced the availability of a draft guidance for industry entitled "*Vulvovaginal Candidiasis: Developing Drugs for Treatment."* The comments on this draft guidance were submitted to the Agency until September 29, 2016.

This guideline, prepared by the FDA Center for Drug Evaluation and Research (CDER) - Division for Anti-Infective Drug Products-, supersedes 1998 FDA draft guidance: "Vulvovaginal Candidiasis: Developing Antimicrobial Drugs for Treatment."

In a Federal Register notice dated August 7, 2013, FDA announced that such 1998 draft guidance was being withdrawn as new information, scientific developments, and emerging technologies require a revision.

The purpose of the guidance we are approaching is to assist sponsors in the overall clinical development program and clinical trial designs to support drugs being developed for the treatment of VVC. In addition, it reflects recent developments in scientific information that pertain to such drugs.

In general, this guidance focuses only on developing antifungal drugs (discovery and clinical research) for the treatment of uncomplicated vulvovaginal candidiasis (VVC), generally defined as a single episode of vulvogaginal inflammation caused by *Candida* yeast in an otherwise healthy female. For complicated VVC the dose, duration, or formulation of antifungal treatment may be

different in comparison to uncomplicated VVC, thus they are not discussed in this document.

This document helps define enrollment criteria for VVC trials, and recommends that such trials be superiority trials against placebo or active control. The recommended efficacy endpoint is resolution of clinical signs and symptoms.

Remarkably, this guidance does not discuss trial designs for development programs for nonprescription treatments of VVC. Neither does this contain a discussion on general issues in drug development or general issues of statistical analysis or clinical trial design.

Update-2 Management of Ulcers - Consensus

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Vulvar ulcers are painful, debilitating, and severely compromise the quality of life (QoL) of the individual. Etiology is varied ranging from infections (herpes simplex, herpes zoster, impetigo, candidiasis), inflammatory (erosive lichen planus, lichen sclerosus, irritant contact dermatitis,) blistering disorders (pemphigus, bullous pemphigoid, linear IgA, cicatricial pemphigoid, Hailey-Hailey disease) and premalignancies (high grade vulvar intraepithelial lesion (HSIL) of the vulva), and malignancies (squamous carcinomas, basal cell carcinomas, extra-mammary Paget's disease, and Langerhans cell histiocytosis). Clinical features are often overlapping, and diagnosis depends on exhaustive history taking, and histopathology. Except for the management of infections, where clinical, diagnostic, and therapy guidelines have been laid out; other diseases suffer from a paucity of randomized studies, and small sample sizes, with no definite consensus criteria. An attempt is made to lay down recommendations for diagnosing and managing vulvar ulcers, by collating data from existing publications, from the Medline (PubMed) database.

Vulvodynia Update

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The objectives of this update are to: (1) selectively review the published literature on vulvodynia; (2) highlight specific advances in knowledge; and (3) speculate on how this knowledge may translate into advances in clinical management and or research. A search was made in Medline/PubMed (time period 2015-2017) using the words vulvodynia/vestibulodynia and the following terms etiology, epidemiology, diagnosis, and treatment/management and research. Select articles were chosen for presentation and discussion. This update will review the currently accepted terminology and classification of persistent vulvar pain and vulvodynia. Current practice patterns will be analyzed by reviewing the recommendations of experts regarding the management of vulvodynia; and then comparing these to evidence regarding practice patterns for the management of vulvodynia in the United States. Topical medications are a popular intervention for vulvodynia and recent research has identified specific inflammatory responses in the vestibular mucosa that may contribute to the onset or amplification of vulvar pain. Treatment studies report a positive effect of medical (topical steroid), psychological (Cognitive Behavioral Therapy), and physical therapeutic interventions on reducing pain. Analysis of the research methodology in the field demonstrates that researchers are reporting on a broader range of treatment outcomes that: measure the bio psychosocial health of women with vulvodynia; and reflect the six core outcome domains for chronic pain clinical trials recommended by the Initiative on Methods, Measurement and Pain Assessment in Clinic Trials (IMMPACT). The Vulvar Pain Assessment Questionnaire Inventory (VPAQ) is an example of a multidimensional assessment tool specifically developed for vulvodynia. A comprehensive definition of treatment success may provide guidance to health care providers and patients regarding vulvodynia therapies.

Quality of Life After Surgery vs. Conservative Treatment for Local Provoked Vulvodynia

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Objectives: Surgical removal of painful tissue (posterior vestibulectomy) is considered the last treatment option for local, provoked vulvodynia (LPV) if conservative treatments fail to reduce the pain sufficiently. The aim of our study was to compare quality of life (QoL) of LPV patients (surgery vs. conservative treatment) with validated questionnaire. QoL data obtained from LPV patients was also compared to population-based QoL data obtained from healthy age-matched women.
Methods: Our study consisted of a retrospective patient cohort of surgically (n = 13) treated LPV patients and conservatively treated controls (n = 23). Data were collected by review of patient records and postal questionnaires. A Finnish validated version of RAND-36 questionnaire was used for QoL measurement.
Results: QoL between surgeries vs. conservative treatment groups did not differ significantly. However, conservatively treated LPV patients had a statistically significant lower quality of life than healthy women in general health (62.1 SD 23.9 vs. 74.9 SD 17.8 p = 0.018), emotional role functioning (56.5 SD 43.9 vs. 76.7 SD 34.0 p = 0.049), and pain dimension (64.7 SD 24.5 vs. 80.5 SD 21.2 p = 0.005).
Conclusions: Patients treated conservatively for LPV suffer from poorer quality of life than healthy women at same age in general health, emotional role functioning and pain dimension. This study suggests that surgery may be superior to conservative treatment in LPV with respect to quality of life.

Mindfulness-Based Group Cognitive Behavior Therapy for Women with Provoked Localized Vulvodynia: A Randomized Controlled Trial

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Objectives: The purpose of this study is to evaluate the effectiveness of mindfulness-based group cognitive behavior therapy (M-gCBT) compared to education-support group therapy alone for the treatment of the pain and distress associated with provoked localized vulvodynia (PLV).
Methods: A randomized controlled trial was conducted using two cohorts of participants for a total of thirty-one participants randomized to M-gCBT or education-support group therapy. Pre-study questionnaires were administered and the cohorts subsequently underwent an 8-week long program. M-gCBT participants attended weekly sessions. Participants in the education-support group had 8 weeks of online-education with 3 in-person support visits. Vaginal insertion pain (Tampon Test) was the primary outcome. Sexual function and quality of life were also evaluated. Questionnaires were administered at the completion of the study period, at 3 months, and at 6 months.
Results: A total of 32 participations were enrolled and 31 were randomized. There were 14 participants who were randomized and completed M-gCBT and 17 that were randomized and completed education-support group therapy. Baseline characteristics did not differ significantly with respect to baseline tampon test, BMI, relationship status, age of menarche, use of hormonal contraception, or previous pregnancy. Age was significantly different between the two groups. On average, vaginal insertion pain measured by the Tampon Test decreased at the end of study though was not significantly different between the two groups (p = 0.764). The Female Sexual Function Index (FSFI) and Female Sexual Distress Score (FSDS) showed improvement in both groups at the end of the study, though it was significantly more improved in the M-gCBT group (p = 0.013 and p = 0.005, respectively). All sexual function and quality of life measures trended towards improvement in both groups.
Conclusions: Based on the results of this RCT, both m-gCBT and education-support can be effective in reducing pain and sexual distress associated with PLV. However, an 8-week long m-gCBT program may be more effective at reducing sexual distress in particular. Women expressed a high amount of satisfaction, which was reflected in the statistically significant improvement in quality of life and sexual function outcome scores. Longer term data are currently being collected.

Acupuncture Augmentation of Lidocaine Treatment for Provoked Localized Vulvodynia – A Pilot Study

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Objectives: To assess acupuncture's augmentation of lidocaine therapy in the treatment of provoked localized vulvodynia (PLV).

Methods: In a randomized, single blind controlled trial over 12 weeks, 19 women with moderate to severe PLV were randomized to either 18 sessions each of classical acupuncture (CA) or non-classical acupuncture (NCA). Women in the CA group received alternating sessions of manual or electro-acupuncture. The NCA group received minimal needling with no stimulation. All participants applied lidocaine 5% cream four times daily to the vestibule. The primary outcome was change in Tampon Test scores using a 100-point visual analogue scale (VAS) from baseline and week 12.

Results: Participants in both groups reported significant pain reduction over 12 weeks. Follow up scores were obtained in 14 women at week 24; 5 women withdrew from the study. Women in the CA group (n = 7) experienced a within group mean difference (MD) of 42.419.4 from baseline and 12 weeks (p = 0.001), and was maintained at week 24 (MD 35.717.8, p = 0.002). In the NCA group (n = 7), women experienced a within group MD 28.728.5 at 12 weeks (p = 0.04), and further improved at week 24 (MD 36.717.7, p = 0.002). There was no difference between groups.

Conclusions: In this early-phase research, acupuncture augmentation of lidocaine was acceptable and may reduce vestibular pain in women with PLV more than lidocaine alone. Both acupuncture techniques showed a favorable effect.

A Comparison of the Lidocaine Test to the Vulvalgesiometer for the Diagnosis of Localized Provoked Vulvodynia (LPV)

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Objectives: The purpose of this study was to compare the lidocaine test (LT) to the vulvalgesiometer (VG) for diagnosing patients with localized provoked vulvodynia (LPV).

Methods: Reproductive-age women with LPV were evaluated by cotton swab test (CST) at 6 sites and VG at 2 sites with the order of initial test randomized. Participants reported pain using a numeric rating scale 0-10 (NRS). For the VG, up to 6 pressures were tested as tolerated (10, 25, 50, 100, 200 and 300 grams). Lidocaine 4% topical solution was then applied for three minutes and the CST and VG repeated in order of randomization. Demographics were analyzed using t-tests and Fisher's exact tests. Changes in NRS with lidocaine application were analyzed using paired t-tests. Pain threshold pressure (NRS > 4) and max tolerated pressure differences (pre-and post lidocaine) were analyzed using Wilcoxon signed rank tests. The relationship between the CST and VG was analyzed by computing Spearman's correlation coefficients.

Results: 16 patients completed the study, 8 in each arm (CST or VG first). Lidocaine significantly reduced pain with the CST and VG at all pressure points (P < 0.05). Max tolerated pressure with application of lidocaine increased significantly (p < 0.005). After lidocaine application, most participants reported pain (NRS > 4) at pressures of 100-200grams.

Conclusions: Lidocaine application extinguishes mucosal vestibular tenderness provoked by both diagnostic tools. Pressures above 100-200 grams may evoke pain other than mucosal allodynia of LPV since lidocaine was less able to extinguish pain at these pressure levels.

Lower Urinary Tract Symptoms in a Chronic Pain Population

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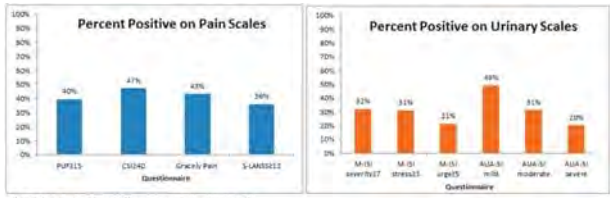
Objectives: To characterize the prevalence of lower urinary tract symptoms (LUTS) in a chronic pain population.

Methods: Patients referred to a female pelvic pain clinic at our institution completed several validated questionnaires regarding pain and bladder symptoms including the pelvic pain and urgency/frequency patient symptom scale (PUF), central sensitization inventory (CSI), the American Urological Association symptom index (AUA-SI) and the Michigan incontinence symptom index (M-ISI). Patients were excluded for interstitial cystitis/bladder pain syndrome. Survey responses were analyzed via Pearson's correlation coefficients and logistic regression.

Results: A total of 144 patients were included in the final analysis. Baseline scores are shown in figures 1a and 1b. Between 39 and 47% of patients screened positive on the PUF and CSI questionnaires. AUA-SI data showed 48.8% of patients had mild, 31.2% had moderate and 20% had severe symptoms. M-ISI data showed 31.9% had a severity score ≥7, 31% had a stress incontinence score ≥ 3 and 21% had an urgency incontinence score ≥5. The CSI and PUF were moderately correlated with AUA-SI total scores (PUF r = .54, p < .0001, CSI r = .65, p < .0001). Logistic regression models demonstrated that the CSI and PUF are both significantly associated with having moderate to severe symptoms on AUA-SI, and that a diagnosis of vestibulodynia had a protective effect (Table 1).

Conclusions: There is a high prevalence of LUTS among patients with chronic pelvic pain. Vestibulodynia was associated with a lower likelihood of bladder symptoms and high PUF and CSI scores were significantly associated with moderate to severe bladder symptoms.



PUF: Pelvic Pain and Urgency/Frequency patient symptom scale
CSI: the Central Sensitization Inventory
Gracely Pain: Gracely Pain Intensity Scale – pain outside of interpourse
S-LANSS :Self-Administered Leeds Assessment of Neuropathic Symptoms and Signs
AUA-SI: American Urological Association Symptom Index
M-ISI: Michigan Incontinence Symptom Index (M-ISI)

Predictors of Moderate-Severe symptoms on AUA-SI

	Unadjusted Odds Ratio	Adjusted Odds Ratio	95% Confidence Interval	p
Age	1.02	1.02	(0.99-1.05)	0.31
BMI	1.01	1.01	(0.95-1.08)	0.78
Vestibular Pain	0.35	0.28	(0.11-0.74)	0.01
PUF	7.58	4.4	(1.58-12.2)	0.005
CSI	5.99	4.39	(1.67-11.52)	0.003

BMI: Body Mass Index
PUF: Pelvic Pain and Urgency/Frequency patient symptom scale
CSI: Central Sensitization Inventory

These included the Pelvic Pain and Urgency/Frequency patient symptom scale (PUF), the Central Sensitization Inventory (CSI), the Gracely Pain Intensity Scale, the Self-Administered Leeds Assessment of Neuropathic Symptoms and Signs (S-LANSS), the PROMIS Anxiety 7a and Depression 8a forms, the McGill Pain Questionnaire, the Gracely pain questionnaire, a visual analog scale (VAS), the American Urological Association Symptom Index (AUA-SI) and the Michigan Incontinence Symptom Index (M-ISI).

Physiotherapeutic Treatment in Vulvodynia

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Objectives: Highlight an interdisciplinary physiotherapeutic approach for the treatment of patients with vulvodynia.

Methods: From 2010 to 2016, 259 patients were assisted with generalized and localized vulvodynia. The techniques for rehabilitation are: The initial evaluation is outlined in table 1.

Results: 259 patients completed 9 sessions; 66% received physiotherapy alone, and 34% received pharmacotherapy and physiotherapy. The mean age was 41 years (range:19-82). 83% reported substantial clinical improvement. 45% were asymptomatic after treatment. 38% reported moderate improvement.

TABLE 1. Illustrates the clinical record, physical examination techniques and treatment for this form of therapy for vulvodynia.

Clinical record, physical examination	Technique
External evaluation in order to observe the state of the skin and the presence of scars.	Visual
Internal evaluation to determine the vaginal opening, trigger points, muscle retractions, contraction capacity, and pelvic floor muscle tone	Manual techniques (aiming to desensitize the area and decrease pain, improve the circulation and normalize the muscle tone). Biofeedback to evaluate the capacity of muscle relaxation (muscle stretching technique). Therapeutic exercises (individualized to each patient to maintain and improve patient outcome; includes global exercises to mobilize the pelvis, abdomen and lower extremities). Auto massage and stretching of the pelvic floor muscles, especially the perineum.
Treatments	
Electrotherapy	Neuromodulation of the posterior tibial nerve.
Behavioral changes	Implemented at the beginning of the treatment (modify risk factors).

Conclusions: The interdisciplinary treatment demonstrates that physiotherapy represents a good alternative for the management of patients with vulvodynia.

Risk Factors for Vulvodynia: A Systematic Review

H Graetz[1], A White[2], and HC Frawley, PhD[3,4]. [1]The Royal Hospital for Women, Sydney, Australia. [2] Darlinghurst Physiotherapy, Sydney, Australia. [3]Monash University, Frankston, Victoria, Australia, [4] Cabrini Health, Malvern, Victoria, Australia.

Objectives: The objective was to systematically review the literature on the risk factors for vulvodynia.
Methods: A systematic review was conducted as per preferred reporting items for Systematic reviews and meta-analyses (PRISMA) guidelines. The search was undertaken for risk factors for vulvodynia; across PubMed, Ovid, ProQuest and Science Direct databases. The methodological quality was assessed by two independent reviewers using standardized criteria before analysis of main results.
Results: 18 observational studies covering 11 different cohorts, including 3 cohorts, 4 cross-sectional and 11 case control studies fulfilled inclusion criteria. Fifty-seven factors were assessed across three different domains: gynecological (19), medical (24), and psychosocial (14). Reported incidence of vulvodynia was between 3/100 and 11/100. Factors consistently identified as contributing to the risk of vulvodynia included oral contraceptive use (ever used, duration of use greater than 2-6 years and younger than 17 years of age at commencement of oral contraceptive pill); and combination or single reports of urogenital infection and comorbid pain conditions. Anxiety and a childhood experience of severe abuse were psychosocial factors highly linked with vulvodynia. The predictive ability and degree of risk was difficult to categorically determine because of confounders, heterogeneity of populations, and methodologies, and a lack of cohort studies.
Conclusions: Many gynecological, medical and psychosocial risk factors for vulvodynia have been demonstrated to exist. Recent investigations have widened the scope of etiological considerations for vulvodynia and with recently updated vulvar pain terminology. This review may assist in the identification of risk and guide efforts in the prevention of vulvodynia.

Vulvar and Clitoral Pain after Female Genital Mutilation/Cutting: Diagnosis and Treatments

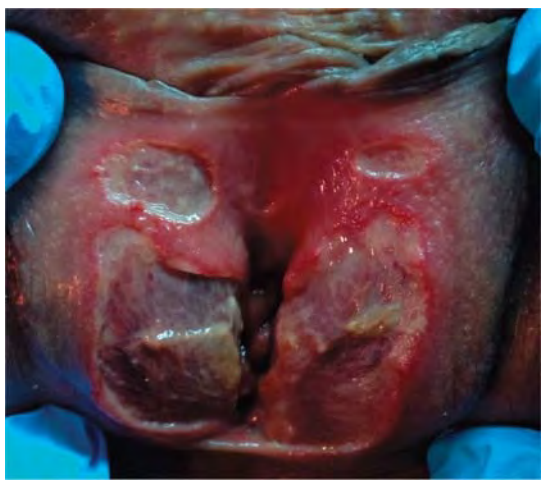
J Abdulcadir[1], and P Petignat[1]. [1]Geneva University Hospitals, University of Geneva, Geneva, Switzerland.

Objectives: Female genital mutilation/cutting (FGM/C) involves the partial or total removal of the female genitalia for non-therapeutic reasons. It can involve the cutting of the clitoris and cause psychological, sexual, and physical complications. Pain from FGM/C originates from scarring complications. A recent literature review regarding management for clitoral/vulvar pain after FGM/C identified no studies. Our aim is to present the management of 6 cases of clitoral/vulvar pain after FGM/C.
Methods: This is a case study of 6 patients who presented to the specialized clinic for women with FGM/C of the Geneva University Hospitals. Multidisciplinary care is offered by a gynecologist; a sexual counselor; midwives trained in pelvic floor muscle training; a psychologist and a psychiatrist (both sex therapists).
Results: We present a series complications after female genital mutilation/cutting a) 3 cases of painful clitoral cysts managed with surgical excision. Histopathology revealed a post-traumatic clitoral neuroma and granuloma, a Mullerian cyst, and a clitoral epidermoid cyst. Clitoral pain was resolved 1-year post-surgical revision. b) 2 cases of superficial dyspareunia after FGM/C type III managed with anterior, and anterior and posterior defibulation (opening of the vulva with reconstruction of the labia minora). c) 1 case of chronic and provoked clitoral pain without a mass, successfully managed through clitoral reconstructive surgery. Following excision of pericliteral fibrosis a clitoral neuroma was identified and excised. In this patient, post-traumatic stress disorder (due to recall of the FGM/C experience) manifested following surgery. Psychotherapy and medical treatment were successful with resolution of clitoral pain symptoms.
Conclusions: Despite the lack of evidence, there are successful treatments for pain after FGM/C.

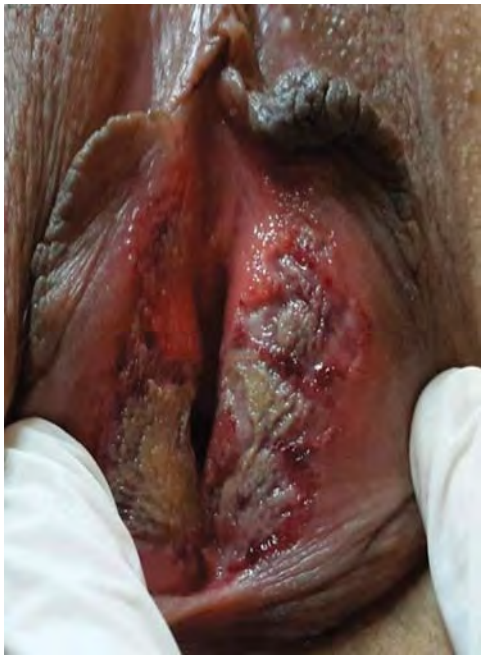
Acute Idiopathic Ulcers: A Case Series and Review of the Literature

GD Saike[1], CE Marchitelli[2], MC Sluga[1], MS Peremateu[1], MM Domenech[1], MJ Martinez[3] and S Gogorza[4]. [1]Center of Vulvar Disease, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina. [2]Chief of Center for Vulvar Disease, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina. [3]Fellow of Center for Vulvar Disease, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina. [4]Chief of Gynecology, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina.

Objectives: To report our cases at the Italian Hospital of Buenos Aires over a 6-year time period.
Methods: Ten patients presented with acute vulvar ulcers that appeared in the context of a febrile syndrome. The ages ranged from 11 to 49 years. Adolescents under the age of 18 had not initiated sexual activity. A complete history, physical examination, and laboratory testing were carried out to rule out both venereal diseases and other usual causes of vulvar ulcers including: blood testing with serology for human immunodeficiency virus type 1 and 2, venereal disease research laboratory test (VDRL), toxoplasmosis, cytomegalovirus (CMV), herpes simplex types 1 and 2, and Epstein- Barr virus were performed. Mycoplasma culture of the lesions and polymerase chain reaction (PCR) for detection of viral DNA (herpes and EBV) were done. A biopsy was performed in only two cases.
Results: The lesions were unilateral in 1 case and bilateral in 9 cases. All patients presented with leukocytosis with a predominance of polymorphonuclear cells. In 4 patients, an increase in AC IG G anti-HSV type 1 and anti-VCA IgG (viral capsule antigen) EB was observed. In two patients,



serology was positive for CMV, and one for Mycoplasma. Serologies were negative in five patients. Cultures were negative in all patients. The ulcers healed spontaneously.
Conclusions: This rare entity usually occurs in women without prior sexual intercourse, in the context of an influenza-like illness. The diagnosis is made by exclusion. In recent studies, it has been associated with primary infection by EBV. Laboratory tests and histopathology are often nonspecific. We were able to demonstrate the relationship with the EBV in 4 cases. The clinical course is self-limited and resolves spontaneously with no sequelae.



Hidradenoma Papilliferum of the Vulva: Report of 19 Cases

ML Absi, MD[1], C Marchitelli, MD[2], MC Sluga, MD[3], G Secco, MD[3], M Martinez, MD[4], A Wernicke, MD[5], and S Gogorza, MD[6]. [1]Resident of Obstetrics and Gynecology, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina. [2]Chief of the Center for Vulvar Disease, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina. [3]Center for Vulvar Disease, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina. [4]Fellow of the Center for Vulvar Disease, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina. [5]Pathology service, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina. [6]Chief of gynecology section, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina.

Objectives: To describe the main characteristics and treatment course of 19 cases of hidradenoma papilliferum of the vulva treated at the department of gynecology, Hospital Italiano of Buenos Aires, Argentina.
Methods: We reviewed 19 cases of hidradenoma papilliferum in women who were diagnosed between January 2008 and January 2016. The mean age was 47 years (27-78 years). The most frequent location was labia majora (9/19), followed by the interlabial sulci (6/19). The size of the tumors ranged between a few millimeters to 3-4 cm. Though the disease is generally asymptomatic (14/19), it may also present with pruritus (4/19), or pain (1/19).
Results: The diagnosis of hidradenoma papilliferum is confirmed by histology. The standard treatment is wide local excision secondary to the association with malignancy, although this occurs infrequently. All our patients were surgically treated, and simple excision was curative. There have not been any recurrences during follow-up.
Conclusions: Hidradenoma papilliferum is the most frequent glandular tumor of the vulva. They arise from the apocrine sweat glands but, since they often occur along the known distribution of mammary-like glands, some authors consider them to be the cutaneous counterpart of mammary intraductal papillomas. They can be a challenge for health care providers because of their variable clinical presentation. Differential diagnoses are pyogenic granuloma, angioma, Bartholin cyst, or squamous cell carcinoma when ulceration is present. Rare cases of malignant changes have been reported; therefore, the simple excision is the recommended treatment.

A Retrospective Audit of Patients attending a New Community Multidisciplinary Vulva Clinic: The Invisible Vulva

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Objectives: Vulvovaginal complaints are common but poorly managed issues of sexual and reproductive health. We initiated a multidisciplinary vulva clinic based on a perceived need for improved community care. We performed an audit of the patients attending our clinic to establish an ongoing requirement for our services and to appropriately target education within our referral base to enhance the care of women in our community.
Methods: A retrospective review of the records of all the patients attending The Jean Hailes Vulva Clinic between July 1, 2015 and December 30, 2016 was made. Information was entered into a database and analyzed.
Results: 101 patients attended as new patients to the Jean Hailes Multidisciplinary Vulva Clinic over a period of eighteen months. The most common diagnosis was lichen sclerosus 50/101 (49.5%), either proven on biopsy or visual diagnosis; vulvar pain and/or dyspareunia 33/101, (32.7%) and candida 26/101 (25.7%). Within our population, we identified one patient with extra mammary Paget's disease and lichen sclerosus, one patient with high grade squamous intraepithelial lesions of the vulva (HSIL) and lichen sclerosus, and 2 patients with HSIL of the vulva. Common associations were lichen sclerosus and vulvar pain or dyspareunia (N = 7, 6.9%) and pain and candida. If a patient presented with pain, either vulvar pain or dyspareunia (N = 35, 34.6% patients) then 40% had candida albicans or candida glabrata as a cofactor. Vulvar pain, with no other associations was found in 7 patients (6.9%). Our patients were drawn from a 130 km radius to our clinic with a small percentage 3/101 (2.8%) from interstate and the majority travelling 50 kms or less. Patients had seen multiple practitioners prior to attending: mean 2.3 (range 0 to >5). Symptom duration varied from two months to ≥ 5 years (classified as long term). 45% of our new patients (N =46) had had symptoms for 5 or more years at presentation (range 2 months to 30 years).
Conclusions: Vulvovaginal complaints are a frequent reason for women to visit their primary care practitioner. We found that within a multidisciplinary vulva clinic placed within easy access to the community there were varied presentations and a high demand for services. Our patient profile confirmed previous findings that women's care was compromised by delayed and lengthy time to diagnosis. We found many varied diagnoses including 3 premalignant conditions and one malignancy. We demonstrated that there were several key diagnoses we could target within our referral base, with simple algorithms, to improve the outcomes for women in our catchment area.

Vulvar Endometriosis: A Series of 4 Cases

MF Marcos[1], C Marchitelli, MD[2], C Sluga[1], G Secco[1], S Gogorza[1], and M Martinez[3]. [1] Department of Gynecology, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina, [2]Chief of Center for Vulvar Disease, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina.[3]Department of Surgical Pathology, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina.

Objectives: The objective of this study is to present four patients with the diagnosis of vulvar endometriosis, evaluated in the Service of Gynecology of the Italian Hospital of Buenos Aires.
Methods: Endometriosis is a common gynecological pathology characterized by growth of glands and endometrial stroma outside the endometrium. Implants are commonly found in the pelvis, but may appear in different sites. Vulvar endometriosis is a rare condition with an estimated incidence of less than 0.5%. It often develops after abdominal-pelvic surgery.
Results: Four women of reproductive age were diagnosed with vulvar endometriosis. The lesions were painful in all patients and surgery was performed in all cases. The mean follow-up was 5 years. No recurrences were observed.
Conclusions: Vulvar endometriosis is a relatively uncommon phenomenon, rarely localized to the vulva. Clinically, a painful bluish nodule can be observed in women of reproductive age. The diagnosis should be based

on review of previous medical records and clinical appearance; it should be confirmed by histological examination. Treatment is surgical resection with wide margins or laser therapy. A thorough gynecological evaluation should be performed, especially if the patient has symptomatic pelvic endometriosis.

Assessment of Efficacy of Er: YAG Laser Treatment for Female SUI Using 1 Hour Pad Test - Pilot Study with 12 Months Follow-Up

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Objectives: The scope of this study was to demonstrate the efficacy and safety of a non-surgical, minimally invasive Erbium laser (Er:YAG laser) treatment for stress urinary incontinence (SUI) and to compare the results to a control group.
Methods: Two groups were recruited: LASER GROUP with 43 patients. Aged 33-64 Average BMI: 27.4. They underwent thermal Er: YAG laser treatments. Patients received three treatment sessions with intervals of 1 month in between the sessions. CONTROL GROUP: with 29 patients aged 31-60. Average BMI: 26.7 received 2 weekly sessions of pelvic floor muscles exercises with perineometry, during three months. Only patients with valsalva leak point pressure (VLPP) > 60 mmHg were recruited. A standardized 1-hour pad-test was used to evaluate the efficacy of the treatment.
Results: Follow-ups were performed at 3, 6, and 12 months. The pad weight decreased in both groups at the 3-month follow-up. This was more pronounced in the laser group (66% - 72% - 70%) in contrast to the control group (58% - 47% - 29%). This effect remained constant up to the 12-month, while the results in the control group showed a diminishing trend.
Conclusions: Erbium laser for stress urinary incontinence is a safe and effective method for the management of SUI. Mid-term follow-up results show that the results can last for at least a year after treatment. Since the treatment is non-invasive, it could also be repeated once the results start diminishing.

Long-Term Experience with the Bidimensional Labia Minora Reduction

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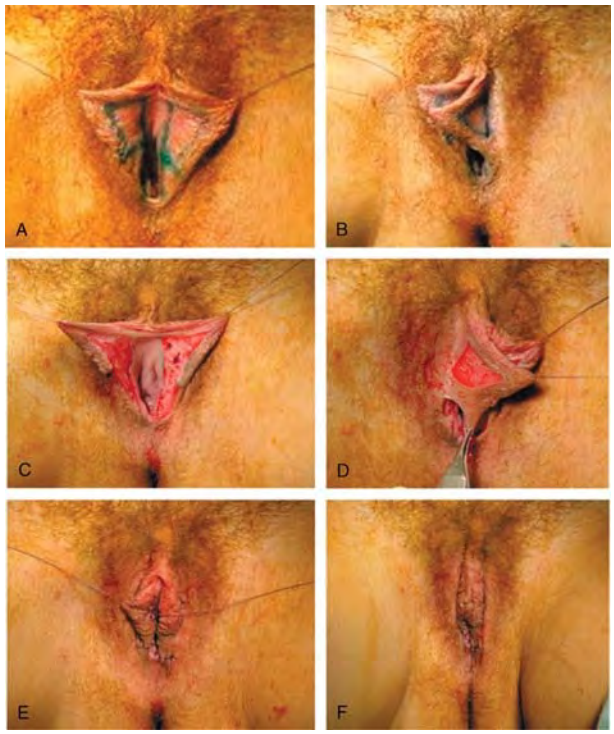
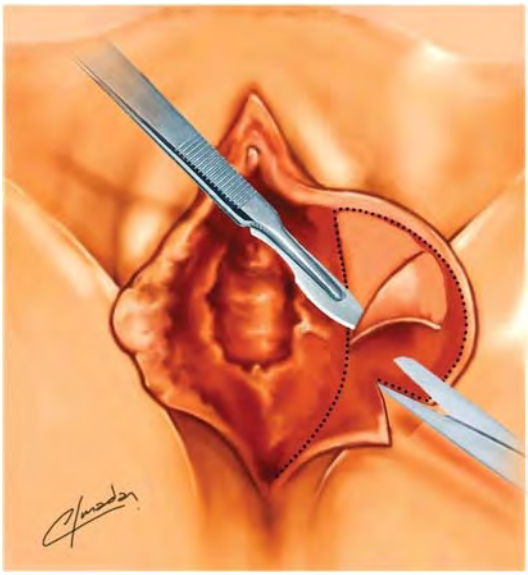
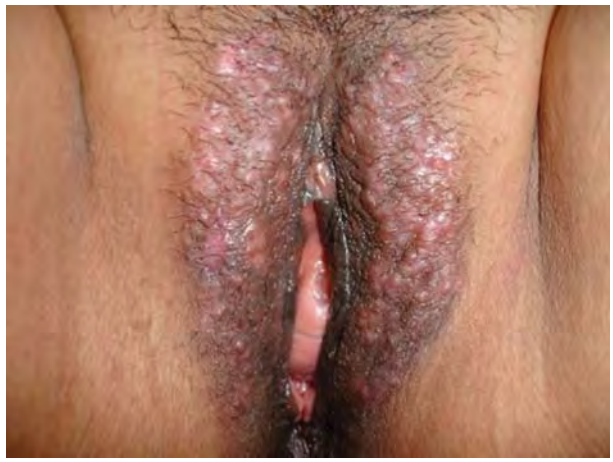
Objectives: To present clinical experience with the bidimensional technique.
Methods: A retrospective review of all patients' clinical records that underwent the bidimensional technique was undertaken. A long-term follow-up was carried out by telephone. Patients' overall satisfaction with the procedure and final result was rated on a scale of 1 to 5, where 1 was poor, 2 was fair, 3 was good, 4 was very good, and 5 was excellent.
Results: From October 2005 to December 2016, 36 women with an average age of 27 years (range 18–47) underwent this technique. In all patients, the wound healed very well. There were no reports of tip flap necrosis. Two patients had immediate postoperative bleeding and another one developed a small hematoma that drained spontaneously. One patient developed an infection that responded well to antibiotics. By a telephone survey, 22 patients rated the procedure and results as excellent, 10 patients as very good, and 2 as good. Two patients were not reached.
Conclusions: The bidimensional technique provides a tension-free closure and adequate vascularization to the healing edges of the superior labial flap, which reduces the chance of wound dehiscence. The associated resection of a full thickness posterior wedge avoids a festooned appearance and the resulting scar is posteriorly placed, providing excellent cosmetic results and long term overall satisfaction.



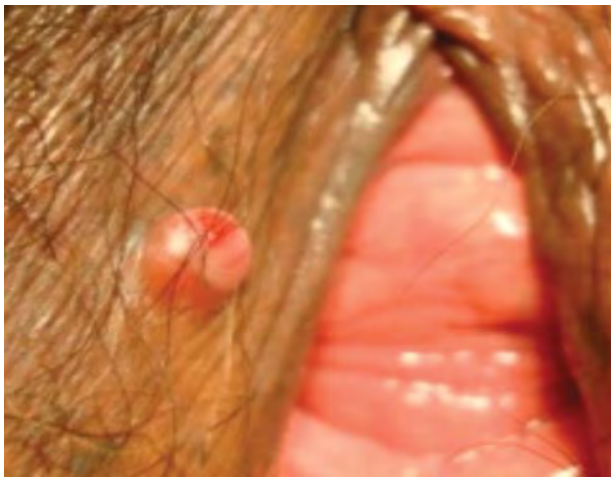


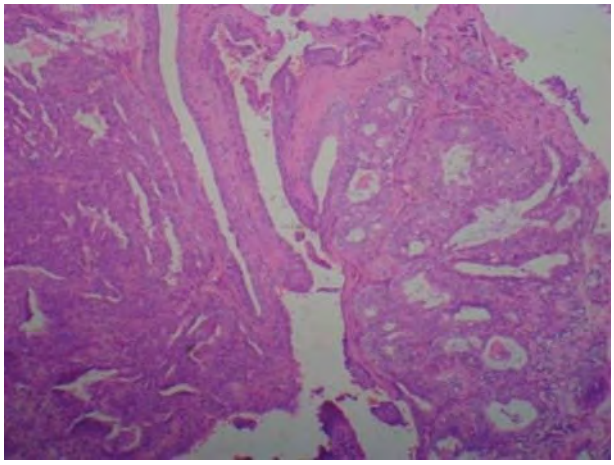


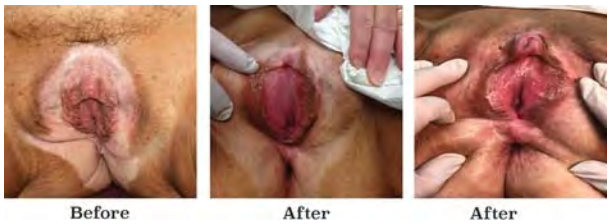
Vulvar Sudoriferous Gland Epithelial Tumors

L Tinoco[1], S Carrillo[2], A Nicolalde[3], D Tinoco[4], V Tinoco[5], R Tinoco[6], D Ayala[2], R Masaquiza[1], and Z Paz[1]. [1]Hospital Oncologico Solca-Quito, Quito, Ecuador. [2]Ginecomast, Quito, Ecuador. [3]Solca-Quito, Quito, Ecuador. [4]Hospital San Francisco de Quito, Quito, Ecuador. [5]Hospital Rodriguez Zambrano, Manta, Ecuador. [6]Hospital Luz Elena Arismendi "Nueva Aurora", Quito, Ecuador.

Objectives: To identify the most frequent vulvar sudoriferous (sweat) gland epithelial tumors.
Methods: A descriptive epidemiological study was performed including women with vulvar sweat gland tumors surgically treated between 2004 and 2016 at Ginecomast and Oncologico Hospital "Solca" Quito.
Results: Ten apocrine gland tumors (hidradenoma papilliferum) were identified within the labia minora among women with an average age of 45 years (range 33 to 59). All were surgically excised. Eccrine gland tumors (syringomas and nodular hidradenoma) were identified among women with an age range of 28 to 68 years. Six syringomas were identified within the labia majora. The main symptomatology was pruritus. The treatment was surgical and in three patients an excisional biopsy and laser were performed. A scissile biopsy was performed on a nodular hidradenoma in a 28-year-old patient with a 2-cm. lesion. Histologically it was established as a clear cell variant.
Conclusions: Tumors of apocrine sweat glands were characterized by having associated ulceration, located mainly on labia minora. Eccrine glandular tumors were present on the labia majora and were characterized by intense pruritus. The treatment in extensive glandular lesions was surgical, with combined biopsy and laser performed in smaller lesions.





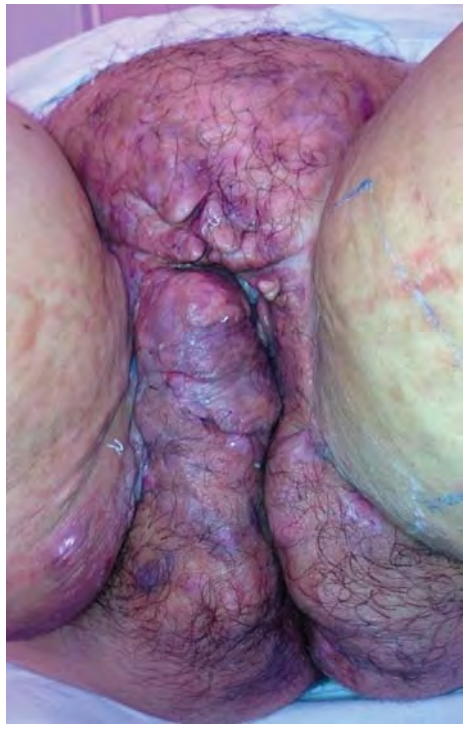


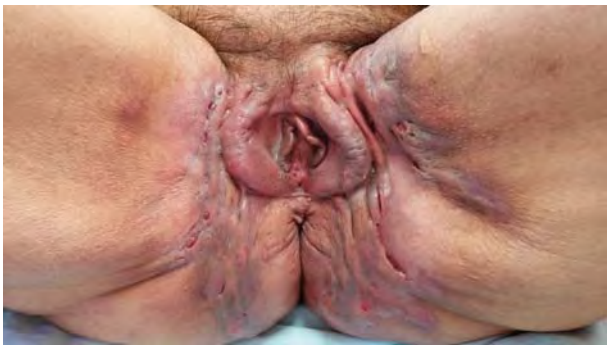
Before After After

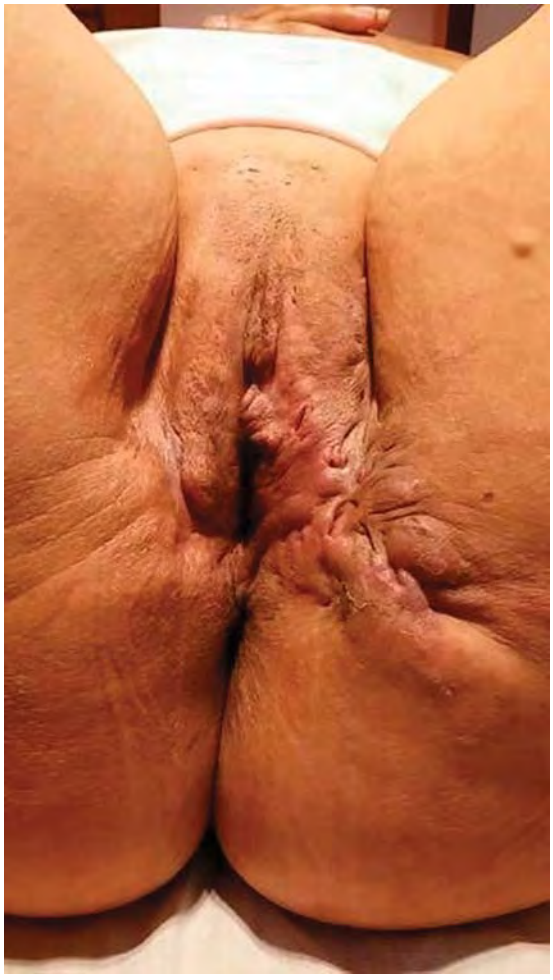
Hidradenitis Suppurativa

V Fonseca, MD[1]. [1]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

Objectives: Hidradenitis suppurativa is a chronic and recurrent inflammatory disease caused by occlusion and rupture of follicular units, in which the inflammatory response may secondarily involve the apocrine glands, leading to the formation of papules and nodules, which can progress to pustules, abscesses and fistulas. It is classified by Hurley´s criteria stage I, II, III, according to the severity and extent of the disease. We present 3 women who required surgical treatment for severe hidradenitis suppurativa.
Methods: A retrospective review was performed, including three patients with severe hidradenitis suppurativa (Hurley's stage III) from the Vulvar Pathology Clinic of the Gynecology Service of HUCFF-UFRJ.
Results: Three patients 39, 41, and 60 years of age with Hurley's stage III hidradenitis suppurativa were included. Each had previously used antibiotics, zinc gluconate, isotretinoin and dapsone without satisfactory therapeutic response. Examinations revealed multiple interconnected sinus tracts, mons venus and perianal scarring, and abscesses involving the entire vulva and the bilateral inguinal area in each patient. All 3 were underwent surgical treatment consisting of wide excision or wide unroofing of affected areas - with healing by secondary intention or the use of skin grafts to close the defect.
Conclusions: Hidradenitis suppurativa is difficult to diagnose and manage. There is no ideal treatment that provides good functional and aesthetic results for all patients. In the cases presented, the surgical approach provided good results. Faced with the significant impact on the quality of life generated by this disease, it is up to the physician to use the best therapeutic strategies available.







Chronic Genital Malodor in the Absence of Infection: A Possible Manifestation of Olfactory Reference Syndrome

A Machefsky, MD[12], C Miller, MSW[2], KT Smith, PhD, CRNP,[1] P Nyirjesy, MD[1]. [1]Drexel Vaginitis Center, Department of Obstetrics and Gynecology, Drexel University, Philadelphia, PA, USA. [2]Department of Obstetrics, Gynecology and Women's Health, St. Louis University, St Louis, MO, USA.

Objectives: While bacterial vaginosis (BV) is a common cause of vaginal malodor (VM), many affected women have no objective findings. These women present a challenge since there is no satisfactory diagnosis or treatment. Olfactory reference syndrome (ORS) is a psychiatric disorder characterized by a preoccupation with the belief that there is a foul offensive body odor, often from the genital region. We studied women with chronic VM and no infection, as they may have ORS.
Methods: We performed a case-control study of 50 women with VM and 51 women with recurrent BV. All patients underwent a standardized evaluation. Historical and clinical variables were collected. Differences in demographic characteristics, medical/psychological comorbidities, and symptoms were assessed.
Results: The median age was 36.5 years in cases vs. 33.0 in controls. 40.0% vs. 29.4% were Caucasian. 16% vs. 10% were menopausal. The median duration of symptoms was 2 years vs. 1 year. All of the VM complained of malodor (described most commonly as fishy) compared to only 74.5% of the BV patients. 92% of the VM and 78.4% of the BV group had been previously treated for BV ($p = 0.06$). Both groups had similar rates of diagnosed psychiatric conditions (8% vs. 7.8%). After the initial evaluation showed no infection, 62% of the VM patients attempted some sort of intervention, with 79% reporting no change.
Conclusions: Women with VM are demographically similar to women with recurrent BV. Standard interventions to address abnormal odor are frequently unhelpful. These women may have a manifestation of ORS.

Recurrent Vulvovaginitis and its Relationship with Low-Grade Vaginal Intraepithelial Neoplasia in Ecuadorian Women

PA Yánez, MD, PhD (c)[12], K Simbana, IR[1], L Gómez, IR[1], JA Duran, MD, PhD (c)[12], S Quintana, MD, PhD[3], and E Terán, MD, PhD[4]. [1]Department of Clinical Pharmacology, Central University of Ecuador, Quito, Ecuador. [2]Unit of Colposcopy and Pathology of the Lower Genital Tract, Provida Basic Hospital, Latacunga, Ecuador. [3]Department of Gynecology and Obstetrics, Faculty of Medicine of Ribeirão Preto, University of Sao Paulo, Sao Paulo, Brazil. [4]Colegio de Ciencias de la Salud, Universidad San Francisco de Quito, Pichincha, Ecuador.

Objectives: To investigate the association between recurrent vulvovaginitis with the presence of vaginal low-grade squamous intraepithelial lesions (LSILs) in Ecuadorian women.
Methods: This retrospective and descriptive study was performed in a group of 96 women attending the outpatient clinics at the Unit of Inferior Genital Tract Pathology and Colposcopy of Provida Basic Hospital in Latacunga, Ecuador between 2014 and 2016 for chronic itching of the vulva. Data from medical records were analyzed using the computer software Statistical Package for the Social Sciences (SPSS) 11.0.
Results: Out of 77 cases of histopathological samples, 92.2% (n = 71) presented with intraepithelial lesions. Vaginal LSIL was as follows: VAIN 1 64.8% (n = 46), squamous papillomas 28.2% (n = 20), condyloma 5.6% (n = 4). 7.8% of the patients had a negative result. In addition, on histology, the possible presence of human papillomavirus (HPV) was reported in 58.4% (n = 45).
Conclusions: This study shows that a large percentage of patients with recurrent vulvovaginitis also have benign histopathological alterations associated with HPV infection. A prospective study is necessary to explain the relationship between vaginal microbiota and HPV infection.

Diazepam Serum Levels are Minimal Following Vaginal Administration

L Edwards, MD[1]. [1]Southeast Vulvar Clinic, Charlotte, NC, USA.

Objectives: To evaluate the systemic absorption of diazepam following vaginal use of diazepam for vulvodynia.
Methods: Fourteen consecutive women with vulvodynia treated with compounded 10 mg vaginal diazepam suppositories were assessed. One hour after insertion, serum diazepam levels were measured.
Results: Thirteen of the fourteen patients had undetectable levels of diazepam. One patient had nordiazepam level of .1 mg/mL, the lowest detectable level. No patient had side effects such as sedation that would be expected with systemic absorption of the diazepam.
Conclusions: Compounded 10 mg vaginal diazepam suppositories appear to produce minimal systemic absorption, so that driving following insertion is most likely safe, and addiction and withdrawal symptoms unlikely. Efficacy was not measured.

Histopathological Findings in Patients with Chronic Vulvar Itching: Experience in an Ecuadorian Population

JA Duran-Chávez, MD, PhD (c)[1,2,3], L Gómez, IR[1], K Simbana, IR[1], S Quintana, MD, PhD[3], PA Yánez, MD, PhD (c)[1,2], and E Terán, MD, PhD[4]. [1]Department of Clinical Pharmacology, Central University of Ecuador, Quito, Ecuador, [2]Unit of Colposcopy and Pathology of the Lower Genital Tract, Provida Basic Hospital, Latacunga, Ecuador, [3]Department of Gynecology and Obstetrics, Faculty of Medicine of Ribeirão Preto, University of Sao Paulo, Sao Paulo, Brazil, [4]Colegio de Ciencias de la Salud, Universidad San Francisco de Quito, Ecuador.

Objectives: To investigate the association between chronic vulvar itching with the presence of vulvar low-grade squamous intraepithelial lesions (LSIL).
Methods: This retrospective and descriptive study was performed in a group of 33 women, attending to the outpatient clinics at the Unit of Inferior Genital Tract Pathology and Colposcopy of Provida Basic Hospital in Latacunga, Ecuador between 2014 and 2016 with chronic vulvar itching. A biopsy was performed in all 33 patients with pigmented lesions, and condyloma/verrucous lesions, to confirm the diagnosis. Data from medical records were analyzed using the computer software statistical package for the social sciences (SPSS) 11.0.
Results: The mean age was 28 ± 5 years (range 19 to 53). Out of 33 cases, 93.9% (n = 31) were confirmed to have LSIL on histology, with lichen simplex chronicus identified in the remaining 6.1 % (n = 2). Additionally, the presence of human papillomavirus (HPV) was reported in 60.6% (n = 20).
Conclusions: This study suggests that the majority of women with vulvar LSIL complained of vulvar itching.

Pelvic Floor Dysfunction: Women's Sexual Activity at Risk

AE Sampietro[1] and ME Alcoba[1]. [1]Austral University Hospital, Pilar, Buenos Aires, Argentina.

Objectives: Female sexual function is complex, involving physical, emotional, and psychological factors. Female patients with pelvic floor diseases may suffer from several sexual disorders and sexual life impairments. The aim of this study was to evaluate sexual dysfunction in female patients presenting with urinary incontinence (UI), pelvic organ prolapse (POP) or both.
Methods: A retrospective analysis was performed of a prospectively collected database of women referred to the pelvic floor section, who completed the Spanish validated pelvic organ prolapse/incontinence sexual questionnaire-12 (PISQ-12) at the first visit. Statistical analysis was performed to evaluate and compare sexual dysfunction between patients with UI, POP and both, as well as published data on the general population.
Results: 176 patients were identified, 98 (55%) who reported sexual activity were analyzed: 52 had UI (53%), 24 POP (25%) and 22 both (22%). Major sexual impairment (PISQ-12 < 30) was found in 42 patients (42.8%). The mean PISQ-12 (32.59 ± 7.2) score was 6 points lower than those reported in the general population from PISQ-validating studies ($p < 0.05$). When stratified by pelvic floor dysfunction, the UI group had higher PISQ scores, reflecting less sexual dysfunction than the POP and the UI + POP groups.
Conclusions: Sexual dysfunction is prevalent among patients suffering from UI and POP, and questionnaires are useful in recognizing these patients. The physical effect of prolapse and incontinence is one of the contributing factors for sexual dysfunction.

The Prevalence of High Tone Pelvic Floor Dysfunction with Symptomatic Vulvovaginal Atrophy

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Objectives: Vulvovaginal atrophy (VVA) is a leading cause of dyspareunia in postmenopausal women. Clinical experience suggests that some women with VVA will also have high tone pelvic floor dysfunction (HTPFD). How often VVA and HTPFD coexist has not been studied. Treating VVA without treatment of HTPFD leaves a cohort of women still struggling with sexual pain. Our aim was to assess the frequency with which HTPFD coexists with VVA in women seen in a sexual medicine clinic as well as to determine if differences exist in women with VVA alone when compared to women with VVA/HTPFD.
Methods: A retrospective chart review was performed. Women seen for sexual disorders in a sexual medicine clinic were included. Previously collected demographic data, pertinent past medical history, and Female Sexual Function Index (FSFI) scores were extracted and analyzed.
Results: Of 80 women with VVA, 31 (38.8%) were diagnosed with HTPFD. Co-existing conditions were similar between women with and without HTPFD. A greater proportion of women with HTPFD had a chief complaint of dyspareunia (93.5% vs. 69.4%; $p = 0.02$). A higher proportion of women with HTPFD had a history of endometriosis (16.7% vs. 2.1%; $p = 0.05$). No statistically significant differences emerged when comparing FSFI scores between women with and without HTPFD.
Conclusions: More than a third of women with VVA had HTPFD. Many clinicians don't routinely check for HTPFD. Clinicians may be under-treating HTPFD in women with VVA and limiting restoration of normal sexual function and painless intercourse.

Long-Term Outcome of Total Vestibulectomy

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Objectives: To evaluate long term follow-up of patients who underwent total (anterior and posterior) vestibulectomy over 10 years ago, by one surgeon.
Methods: The follow-up was conducted through face-to-face interviews with 32 patients who were operated before 2003 and chosen randomly. All of them were operated on by one surgeon (JB). The interviews tested the frequency of sexual intercourse, the degree of pain in carrying out various activities, satisfaction from the surgery's results, and willingness to recommend the surgery to other women.
Results: 100% of the patients experienced painful sexual intercourse at some point after surgery, and the median time was 4 months until first painless intercourse. Prior to surgery, introital penetration was the most painful activity to all patients, averaging 9.12 on a pain scale (0-10). It dropped to 0.47 currently ($P < 0.001$). Using a tampon, post-coital urination, and other activities were also improved significantly after surgery. None of the patients reported reappearance of pain over time. 94% of the operated patients were highly satisfied of the operation they underwent, 97% would undergo the surgery again knowing the outcome, and 100% would recommend it to a friend suffering from the same condition.
Conclusions: Our work demonstrated high surgical success rates of total vestibulectomy. We did not find any evidence of deterioration or resurgence of pain over more than a decade after the surgery, and even witnessed an improvement. Overall patient satisfaction was high, and the general attitude toward the surgery was positive.

Improvements in FSFI Scores using Vaginal CO2 Microablative Laser

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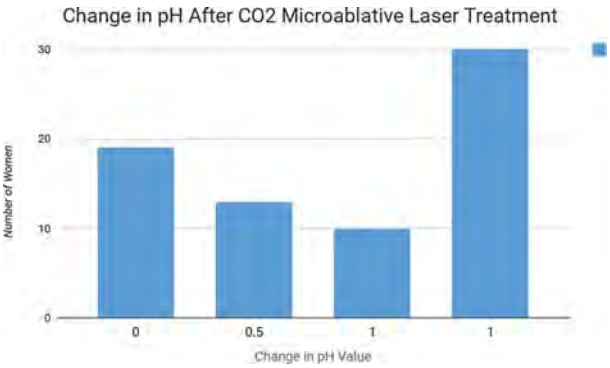
Objectives: To examine the clinical efficacy of a carbon dioxide (CO2) laser treatment in improving female sexual function, using a validated Female Sexual Function Index (FSFI) questionnaire.
Methods: 62 postmenopausal women aged 46-75 underwent three SmartXide (DEKA, Florence, Italy) vaginal treatments six weeks apart. Patients completed FSFI questionnaires before and after the treatment cycle. Half the women received laser treatments alone (Group 1) and half received concurrent therapy with either vaginal estrogen or ospemifene (Group 2).

Table 1. FSFI prior and post MLT treatment study results and individual domain results

	FSFI Scores*	Desire Scores*	Arousal Scores*	Lubrication Scores*	Orgasm Scores*	Satisfaction Scores*	Pain Scores*
Raw Percentages of improvement	(51/62) 82.3% showed an increased FSFI score.	(34/62) 54.8% showed increased sexual desire	(39/62) 62.9% showed increased sexual arousal.	(46/62) 74.1% showed increased lubrication.	(32/62) 51.6% showed enhanced orgasms.	(38/62) 61.3% showed increased sexual satisfaction.	(43/62) 69.4% showed less pain with vaginal penetration.
Mean of Difference between Pre and Post Scores with 95% Confidence	6.49 ± 2.79	0.72 ± 0.36	0.73 ± 0.57	1.58 ± 0.64	2.8 ± 0.58	0.74 ± 0.52	1.78 ± 0.63
Number of Participants (n)	62	62	62	62	62	62	62
t-statistic and degrees of freedom (df)	6.19 with 61 df	5.27 with 61 df	8.37 with 61 df	6.57 with 61 df	12.87 with 61 df	3.76 with 61 df	6.95 with 61 df
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Statistical Significance	Yes, at α = 0.01	Yes, at α = 0.01	Yes, at α = 0.01	Yes, at α = 0.01	Yes, at α = 0.01	Yes, at α = 0.01	Yes, at α = 0.01

FSFI SCORES PRE- AND POST-CO2 LASER TREATMENT.
*Each section compared pre- and post-treatment scores using a Matched Pairs T-test.



Results: As a primary analysis, we compared pre- and post-laser FSFI scores for each of the six subdomains and the overall score for statistical significance using a matched pairs t-test. Each of the seven p-values demonstrated statistical significance with $p < 0.01$. As a secondary analysis, we tested the difference in the FSFI scores between Groups 1 and 2. The mean difference was calculated using a 95% confidence interval and was found to be 2.09 ± 4.23. A two-sample t-test resulted in a t-statistic of 1.009 with degrees of freedom of 30. A p-value greater than 0.20 but less than 0.15 was obtained.
Conclusions: 82.3% of patients showed statistically significant improvement in overall FSFI. All subdomains showed statistically significant change as well. In our secondary analysis, we had presumed an additive or synergistic effect from dual therapy but statistical analysis did not support our hypothesis. Vaginal CO2 laser therapy is a promising clinical tool; higher powered sham and head-to-head comparison studies are needed.

Connecting the Dots: Relationships Between Review of Systems Responses and Chronic Urogynecologic Pain

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Objectives: Patients with functional disorders have characteristic responses to the review of systems (ROS) that can be used to facilitate diagnostic assessment and treatment allocation. We aimed to identify patterns of ROS symptoms affirmed/denied by genitourinary pain patients and assess potential associations with chronic pain severity and narcotic use.
Methods: New patients referred to a urogenital/pelvic pain clinic (N = 158) completed standardized questionnaires, including ROS. ROS symptoms reported by ≥10 patients were subjected to multiple correspondence analysis (MCA). Patient reports of symptoms in two contrastive clusters identified in the MCA were summed, creating two scores which were then individually correlated with patient visual analogue pain scale (VAS, 1–10) responses and compared across self-identified narcotic users/non-users.
Results: Two MCA dimensions of variance collectively accounted for 21.4% of the ROS response variation. The first dimension contrasted symptom affirmations and denials, while the second identified two symptom affirmation clusters: 1) uro-psych (N = 20 symptoms, e.g., dysuria, frequency, urgency, anxiety, premenstrual syndrome) and 2) thoraco-abdominal (N = 32 symptoms, e.g., nausea, diarrhea, chest pain, and cough). There was a significant positive correlation between VAS and thoraco-abdominal scores ($R = 0.20$, $p = 0.02$), but not uro-psych scores ($R = 0.15$, $p = 0.10$). Total number of symptom affirmations also correlated positively with VAS responses ($R = 0.21$, $p = 0.02$). Narcotic users had more uro-psych, thoraco-abdominal, and overall symptom affirmations (x-users = 14.6, x-non = 9.15; $p < 0.01$) compared to non-users.
Conclusions: Greater numbers of reported symptoms on ROS—particularly those related to thoraco-abdominal concerns—are associated with more severe chronic pain in urogenital/pelvic pain patients. Narcotic users had significantly more cross-category ROS symptom affirmations than non-users.

Refining a Pain Mapping Tool for Vulvodynia

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Objectives: The study sought to establish the validity of pain mapping points that would reliably differentiate between women with vulvodynia, bladder pain syndrome (BPS), general gynecology, and asymptomatic controls.
Methods: A total of 320 pain maps were included in the study. Of these 238 were of women diagnosed with vulvodynia. Of these 238 vulvodynia cases, 119 were diagnosed with vulvodynia only and 119 with vulvodynia and BPS. These groups were compared with 29 women diagnosed with BPS only, 21 general gynecology cases, and 32 asymptomatic controls. A total of 52 points were assessed in three anatomical regions (Map A, B & C). The study was approved by the University of South Australia's Human Research Ethics Committee.
Results: All groups were comparable in terms of age and parity. The summary of pain scores for all groups are shown in Figure A. Pain mapping score comparisons of Vulvodynia, BPS, Vulvodynia and BPS, General Gynecology & Controls. On Map A women with vulvodynia only reported higher scores than those with vulvodynia and BPS or BPS only. On Map B and C vulvodynia and BPS reported higher scores. Thirteen points on Map A and all points on Map B and Map C differentiated between pain groups and controls. For all pain groups the highest pain ratings were reported in the paraurethral area.

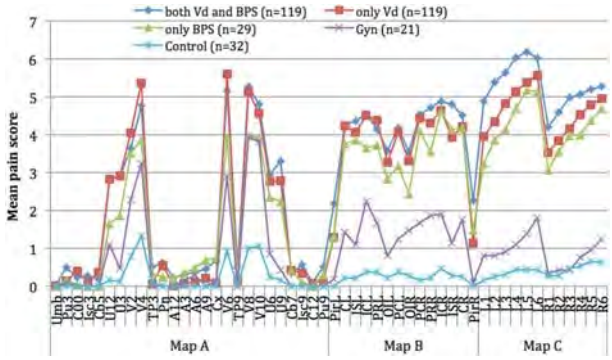

FIGURE. Comparison of mean pain scores for Vd, BPS and controls

Conclusions: For diagnostic and classification purposes the focus to date has been on the external urogenital area. Insufficient attention has been directed to other sources of symptoms and pain, such as the pelvic muscles and the paraurethral area that reliably and consistently differentiate between asymptomatic and symptomatic women.

Keratin Pearls as a Cause of Clitorodynia: A Series of Five Cases

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Objectives: Clitorodynia is a form of localized vulvodynia. Limited research has been published regarding the etiologies clitorodynia, however, several conditions have been associated with clitorodynia including lichen sclerosus, vestibulodynia, multiple sclerosis, and pudendal neuralgia. A keratin pearl is a focus of central keratinization within concentric layers of squamous cells. It is thought to be a result of accumulation of smegma, which is the combination of secretion from sebaceous glands and desquamated epithelial cells beneath the prepuce in females or the foreskin in males. We describe the cases of five women who presented with clitorodynia and associated keratin pearls.
Methods: Retrospective review including five women who presented to a vulvar disorders clinic with clitorodynia with associated keratin pearls.
Results: Our patients presented with three months to fourteen years of clitoral pain. All complained of both non-provoked and provoked clitoral pain exacerbated by touch and certain movements. One patient also had sensations consistent with persistent genital arousal disorder (PGAD). All patients had adhesions between the prepuce and glans clitoris and keratin pearls. (Figure 1). Mild erythema and inflammation were present (balanitis) and there was clitoral allodynia. Following application of a topical anesthetic, a lacrimal probe was used to lyse the adhesions and excise the keratin pearls. All five patients reported improvement in their clitorodynia; three having complete remission.

Conclusions: Clitorodynia is a poorly understood and under-reported form of vulvar pain. Our five cases demonstrate that adhesion and keratin pearls may be a cause of clitorodynia. In addition, adhesiolysis and removal of keratin pearls can provide improvement in clitoral pain.

Lessons from Postmenopausal Patients about Estrogen and Excisable Vulvar Pain – A Case Series

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Objectives: To describe cases of localized vulvodynia that demonstrate estrogen sensitivity, and the utility of aqueous lidocaine for diagnosis, and the benefits of excision for pain relief.
Methods: Five patients, seen between 2013 and 2016 with severe dyspareunia or severe chronic unprovoked vulvar pain, are presented. Criteria: several minutes of 4% lidocaine topical solution extinguished severe vestibule allodynia and pain, and surgical excision corrected this pain 80-100%.
Results: Three patients had provoked pain after 1- 2 years without estrogen; two had severe unprovoked pain after 30 & 17 years without estrogen. Patient #1 had had postpartum vestibular dyspareunia at age 32, cured with a localized superficial vestibulectomy, but after menopause vestibulodynia recurred during two periods without estrogen, and resolved with systemic estrogen therapy. Patients #2 and 3 were breast cancer survivors who had localized vestibulodynia amenable to office excisions of peri-Bartholin duct mucosa, correcting their severe dyspareunia. Patients #4 and 5 had late-age debilitating vulvar pain that responded partially to estrogen, and excision corrected the pain in one and reduced it 80% in the other.
Conclusions: The vulvar vestibule is sensitive to dramatic drops or prolonged absence of estrogen. Postpartum anovulation can initiate localized provoked vulvodynia, which, once corrected, can return with estrogen depletion of menopause. Prolonged estrogen lack after menopause can first cause severe dyspareunia but then progress to severe unprovoked pain. Late postmenopausal estrogen supplementation can ameliorate pain, but surgical excision may be an option in severe cases or in breast cancer survivors for whom estrogen is contraindicated.

Sexual Satisfaction in Women Under 45 Years with Vulvar Dermatoses that Obtain Colposcopy Service at the Ambulatory Attention Center and Day Hospital "El Batán" During the Period January-June 2016

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Objectives: To determine the sexual satisfaction and alterations of the sexual function in women under 45 years old who came for vulvar dermatoses, using the Female Sexual Function Index (FSFI).
Methods: A cross-sectional descriptive observational study was performed in 50 women who attended the Service of Pathology of the Lower Genital Tract and Colposcopy of the Center of Ambulatory Attention and Day Hospital "El Batán", between January and June of 2016 who agreed to participate in the investigation.
Results: The mean age of patients was 42 years. The majority lived in an urban area (83.75%). 96.58% were married. 49.57% were multiparous. 57.26% had a secondary education. A sexual satisfaction disorder was present in 46% (n = 23), within which a severe lubrication disorder was observed in 47.83% (n = 11), a moderate disorder in the sexual desire 69.56% (n = 16), excitation in 52.17% (n = 12), and orgasm in 43.48% (n = 10). There was a statistically significant relationship between the level of sexual satisfaction and the presence of vulvar condylomata (p = 0.019).
Conclusions: The sexual satisfaction of patients presenting with vulvar dermatoses was altered in 46% of cases, mainly due to an alteration in sexual arousal.

The Burden and Impact of Moderate-to-Severe Genital Psoriasis on Female Patients

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Objectives: Genital psoriasis (GP) is common, impacting patients' sexual and physical activity, as well as health-related quality of life (HRQoL). We conducted a survey to better understand symptoms and burden of GP in female patients.
Methods: We conducted semi-structured phone interviews with 20 US patients, aged ≥18 years, with a physician diagnosis of plaque psoriasis (duration ≥6 months) and self-reported, dermatologist confirmed current/recent (≤3 months) moderate-to-severe GP (per Patient Global Assessment ≥4). We designed interviews to identify the impact of GP symptoms on sexual health and HRQoL. We coded and analyzed transcripts to identify key themes and responses. Here we report results for the 11 female patients, who were on average 49.5 years of age with 5.1 years of GP.
Results: Common GP symptoms were itch (100%), discomfort (100%), redness (91%), stinging/burning (91%), pain (73%), and scaling (73%). Itch (55%), pain (36%), and stinging/burning (36%) were most bothersome. Common nonsexual impacts of GP on HRQoL were mood/emotion (91%), physical activities (64%), daily activities (64%), and general impacts not covered by other categories (64%). Patients most frequently reported the following sexual impacts of GP: worsening GP symptoms after sexual activity (73%), physical effects on sexual experience (73%), decreased sexual frequency (73%), avoidance of sexual relationships (64%), and reduced sexual desire (45%).
Conclusions: In these qualitative interviews, the most burdensome symptoms of GP in female patients were itch, pain, and stinging/burning. In the majority of women we interviewed, GP negatively impacted HRQoL, including sexual and nonsexual impacts, and requires assessment and follow-up.

Assessing Patient Satisfaction and Compliance with Compounded Clobetasol Propionate 0.05% and Estradiol 0.01% for the Management of Lichen Sclerosus with Coexisting Genitourinary Syndrome of Menopause

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Objectives: The aim of this study was to assess both patient satisfaction and compliance with compounded clobetasol propionate 0.05% and estradiol 0.01% in an emollient base for treatment of lichen sclerosus (LS) with coexisting genitourinary syndrome of menopause (GSM) compared to prior treatment regimens of separate commercially available preparations of clobetasol ointment and estrogen cream.
Methods: Seventeen women from the Center for Vulvar Disorders at the University of Arkansas for Medical Sciences were identified for study participation. Patients were given a prescription for compounded clobetasol propionate 0.05% and estradiol 0.01% in an emollient base. After a treatment trial with the compounded medication, 14 of the 17 subjects completed a short survey about their level of satisfaction and compliance. Descriptive statistics were obtained, and the Wilcoxon signed rank test was used to compare the compliance level for the compounded medical regimen and the previous medical regimen.
Results: Most patients were very satisfied overall with the compounded medication regimen (85.7%) and were more satisfied with the compounded medication compared to their previous medication regimen (92.9%). The compliance levels of the two medication regimens were statistically significantly different (p = 0.0010) with compliance for the compounded medication regimen being higher.
Conclusions: As satisfaction and medication compliance levels appear to be higher for the compounded clobetasol 0.05% and estradiol 0.01% than for separate preparations of commercially available clobetasol ointment and estrogen cream, the compounding of these medications for treatment of LS with coexisting GSM may warrant further investigation.

The Link Between Management, Compliance, and Clinical Outcomes in Women with Vulvar Lichen Sclerosus

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Objectives: To investigate the relationship between strength of topical steroid regimen, treatment compliance, and clinical outcomes in women with vulvar lichen sclerosus (VLS).

Methods: Retrospective case series of women with VLS at a vulvovaginal specialty clinic from 2012-2017. IRB approval was obtained. Data were extracted by chart review. Analysis included descriptive statistics and panel data logistic regression.
Results: 151 women attended 788 visits (median 5 per patient, range 1-17). At presentation, the mean age was 61 ± 11 years (range 31-90), and 22% were premenopausal. 77% were Caucasian, 4% Hispanic, 2% African-American, 1% Asian, and 16% unidentified. 52% had atopic disease, 34% had hypothyroidism, and 24% were nulliparous. There was no significant difference in likelihood of clinical improvement between super-high and high potency steroid ($p = 0.67$). Clinical improvement was more likely with use of super-high or high-potency steroid compared to medium- or low-potency steroid (OR 3.8, $p = 0.03$). Patients reported perfect compliance with topical steroid at 65% of visits. 27% reported imperfect use, and 8% reported no use at all. Compared to no use, perfect use strongly predicted clinical improvement (OR 9.8, $p < 0.01$); less-than-perfect use also predicted clinical improvement but to a lesser extent (OR 4.9, $p = 0.04$). At presentation, 48% were not sexually active due to pain. Of these, 53% became sexually active during follow-up.
Conclusions: Women with VLS are ten times more likely to improve clinically with perfect use of topical steroid, but only four times more likely to improve with imperfect use. Super-high and high-potency steroids demonstrate equivalent outcomes.

Plasma Cell Vulvitis (Zoon´s Vulvitis) in 3 Patients

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Objectives: To present 3 cases of plasma cell vulvitis (PCV) evaluated at the Gynecology Service at the Italian Hospital in Buenos Aires.
Methods: Three patients were seen complaining of dyspareunia (vaginal stinging pain). During their physical examination, the vaginas appeared erythematous, shiny, with well-defined vestibular plaques. A biopsy was consistent with plasma cell vulvitis (Zoon's vulvitis). The patients were managed with clobetasol and estrogen vaginal cream.
Results: With this treatment, the patients (ages 32, 56, and 57 years) all had partial remission of their symptomatology. They experienced exacerbations during their follow up. Nevertheless, all the patients experienced some degree of improvement.
Conclusions: Plasma cell vulvitis (PCV) or Zoon´s vulvitis is a benign disease without a known cause. Multiple factors have been found to predispose patients to this condition, such as chronic infections, trauma, heat, profuse sweating, and lack of hygiene (accompanying an inflammatory response). This disease affects women between 50 and 80 years of age. The clinical signs are bright erythematous well defined, unique, or multiple plaques in the vestibular mucosa, labia minora, and periurethral areas. The accompanying symptoms are pruritus, pain, stinging, and dyspareunia. Nevertheless, sometimes it can be asymptomatic. The diagnosis of this disease is performed by observing the distinctive histopathology of a dense lichenoid infiltrate, which is mainly constituted by plasma cells, a vascular proliferation, dilation of capillary vessels, extravasation of erythrocytes, and hemosiderin deposits. While a variety of treatments have been tried for plasma cell vulvitis, many patients do not respond to them.

Review of Women Diagnosed with Plasma Cell Vulvitis Treated by the Harvard Vanguard Vulvovaginal Service

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Objectives: To review the medical records of all women with plasma cell (Zoon's) vulvitis treated by the Harvard Vanguard Medical Associates Vulvovaginal Service from 2002 through 2016 with respect to presenting symptoms, treatment modalities, comorbidities, and long term follow-up.
Methods: Record review of all women with a diagnosis code for plasma cell vulvitis resulted in 32 who met inclusion criteria, which included: biopsy proven disease, referral to our specialty service, and age over 18. Age at diagnosis, presenting symptoms, number of visits and therapeutic treatments were analyzed. Women were followed longitudinally with evaluation of disease response to various treatments.
Results: The mean age at diagnosis was 56.5 (range 28-85). Women presented with multiple symptoms. Most common were: dyspareunia (22), burning (16), pruritus (13), contact dysuria (10), vaginal discharge (9), pain (9), irritation (5) and bleeding (3). The average number of visits was 11.5 (range 2-51). Treatment modalities included: topical, intralesional, vaginal, intramuscular and oral steroids; compounded clobetasol/oxytetracycline/nystatin cream (Zoon's paste); topical calcineurin inhibitors; topical and systemic estrogen; pelvic floor physical therapy; acupuncture; fluconazole and clotrimazole for suppression of candida; gabapentin and amitriptyline for neuropathic pain. The most commonly used and effective treatment was clobetasol ointment. The average number of months in treatment was 43 with 18 women in ongoing care.
Conclusions: Plasma cell vulvitis is difficult to treat and is commonly associated with sexual dysfunction as well as vulvar and vaginal symptoms. Multiple treatment approaches are necessary. Although women usually improve, most require long-term treatment and monitoring.

Evaluating the Benefit of Quadrivalent HPV Vaccine on Genital Warts in Opportunistic Vaccination Setup

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Objectives: Genital warts are the most common sexually transmitted disease and have a detrimental impact on quality of life. Genital warts could be prevented by prophylactic human papilloma virus (HPV) vaccination. To study the real-life benefit of opportunistic HPV vaccination setup on age and gender specific incidence of genital warts.
Methods: A register-based population cohort study was performed from the second largest publicly funded health-care provider in Israel, during two time frame intervals: 2006-2008 (pre-vaccination effect period) and 2013-2016 (post-vaccination effect period), with an average annual number of members of 1,765,481 and 2,042,678 in the years 2006-2008 and 2013-2016, respectively. Main outcomes and measures: genital warts incidence.
Results: Among females, the annual incidence of genital warts per 100,000 women decreased from 210.43 to 146.8 (OR 0.69, 95%CI 0.66-0.72, $p < 0.001$) between pre- and post-vaccination effect period with absolute risk reduction of 63.62 cases per 100,000 women per year. Among males, the annual incidence of genital warts per 100,000 men decreased from 262.85 to 234.01 (OR 0.88, 95%CI 0.86-0.91, $p < 0.001$) between pre- and post-vaccination effect period with absolute risk reduction of 28.84 cases per 100,000 men per year.
Conclusions: We have shown that there is a potential benefit in reducing the incidence of genital warts even in an opportunistic setup. This information might be relevant for health-care providers in countries where national immunization programs do not include HPV vaccines.

Radiofrequency Treatment in Patients Under 21 Years Old with Florid Vulvar Condyloma Resistant to Conservative Treatments

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Objectives: Human papillomavirus (HPV) infection is the most prevalent sexually transmitted disease worldwide. Clinical treatment of anogenital warts is conservative, however, in extreme cases conservative therapy is insufficient and surgical excision is required. The objective of this study is to present four patients under 21 years of age with florid vulvar condyloma, who were treated with radiofrequency excision after topical treatments with imiquimod 5% cream or topical tricloroacetic (TCA) acid failed.
Methods: Retrospective review including 4 patients with florid vulvar condyloma.
Results: All patients attended the public hospital, were under 21 years old, non-immunized with HPV vaccine, non-smokers, without co-morbid diseases, with sexual initiation at age 15 and 2 sexual partners. The outstanding feature in each case included exacerbation with increasing expression of the condyloma despite treatment. Four patients of 16, 17, 18 and 20 years respectively received local destructive treatment with 80% TCA acid for 2 to 3 months. Subsequently, all of them received imiquimod 5% 3 times per week, for 6 months. In view of the lack of response to conservative treatments, all patients were referred to our public

hospital in Buenos Aires and a shaving procedure was performed with Radiofrequency. This is the only device available in our public hospital for the treatment of condyloma. Patients are free of disease after 12 months of follow-up.
Conclusions: Radiofrequency represents a good alternative treatment for vulvar condyloma in young patients in cases of non-response to more conservative treatments.

Vulvar Telangiectasia: An Important Warning Sign of Excessive Topical Steroid Use

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Objectives: To analyze three cases of vulvar telangiectasia in association with topical steroid use.
Methods: This study reviews three cases of vulvar telangiectasia related to superpotent topical steroid use. The women in this study presented to a community hospital vulvar disease clinic for routine management of vulvar dermatoses. All three women were using superpotent topical steroids at the time of their initial visit. Following an initial diagnosis of vulvar telangiectasia, each woman was tapered to the use of a lower potency steroid and was followed no less often than every three months with visual inspection and review of symptoms.
Results: All three women showed improvement or resolution of telangiectasia within three months of switching to a lower potency topical steroid, while maintaining symptomatic and architectural control of their underlying vulvar dermatoses.
Conclusions: Classically, the first line therapy for vulvar lichenoid dermatoses involves the use of superpotent topical steroids. Topical steroids can have deleterious effects on the vulvar skin when used too frequently or in formulations with excessive potency over extended periods of time. Vulvar telangiectasia is one particular effect. This study emphasizes the need for regular follow up for patients undergoing treatment with superpotent topical steroids, as well as the importance of provider recognition of steroid effects and adjustment of therapy to avoid continued insult to the vulvar skin.

The Prevalence of Clitoral Adhesions in Women Presenting to a Sexual Medicine Practice

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Objectives: Physical examination of the clitoris during gynecological examination involves concomitant cephalad preputial retraction to evaluate the presence of the clitoral glans corona. Failure to see the corona implies that there are adhesions of adjacent prepuce skin to the glans covering the corona. The presence of clitoral adhesions is especially relevant in women with clitorodynia, sexual pain concerns and persistent genital arousal disorder. Beneath the clitoral adhesions, an underlying balanitis may exist that is perpetuated by a closed-compartment environment, often with several millimeter-sized keratin pearls observed lying underneath the adhesions. Since physical examination of the glans clitoris is not routinely performed by health care providers during the clinical examination, there are limited data on the prevalence of clitoral adhesions.
Methods: Vulvoscopic photographs taken in our sexual medicine practice from 2007–2015 were reviewed and evaluated for the presence of clitoral adhesions by two independent reviewers. Criteria for clitoral adhesions included: clitoral glans fully visualized, photograph in good focus, and cephalad preputial retraction noted.
Results: 1261 vulvoscopy photographs were reviewed and 767 photographs were considered adequate. Clitoral adhesions were suspected in 175 women (23%).
Conclusions: More than one in five women attending a sexual medicine practice has clitoral adhesions. We strongly encourage health care providers to carefully examine the clitoris with magnified view and ensure visualization of the corona by retracting the clitoral prepuce as a routine part of the gynecological examination. Understanding normal clitoral anatomy is a key part to being able to identify and diagnose clitoral disorders when they arise.

Diagnosis of Genital Infection with PCR Technique and the Relationship to Premature Birth in Patients Admitted to Santiago Oriente Hospital Between January 2015 and January 2016

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Objectives: To describe the epidemiological profile of pregnant women with genital tract infections detected by polymerase chain reaction (PCR) technique, results of the examination, treatments received and the association between the presence of infectious agents and preterm birth.
Methods: The identification of the total PCR Pack Pregnant performed between January 2015 and January 2016 were obtained from the records of the Laboratory of the Santiago Oriente Hospital. The patients' clinical records were then requested and their epidemiological profile, treatments received, and obstetric results were reviewed. The data were tabulated and analyzed using the Excel 2010 program.
Results: 197 PCR results were analyzed, with 143 positive results. Patients with preterm birth had more PCR + testing, especially with Ureaplasma urealyticum, as well as a higher amount of chorioamnionitis associated with PCR + for Ureaplasma parvum. The number of patients treated with antibiotics according to the isolated agent was low in both groups.
Conclusions: The findings are very similar to those described in the literature, both the isolated germs and the low antibiotic coverage given to patients with positive tests, which makes us consider the need to generate strategies to follow up our patients to ensure they receive the appropriate managements for their clinical conditions.

Human Papillomavirus-Induced Squamous Intraepithelial Lesions in Vulvar Lichen Planus

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Objectives: Approximately 50% of vulvar cancers arise after transforming infections with human papilloma virus (HPV) in precursor squamous intraepithelial lesions (SILs). Lichen planus (LP)-associated vulvar cancers are typically HPV negative and arise in differentiated vulvar intraepithelial neoplasia (d-VIN).
Methods: An index case of vulvar high-grade squamous intraepithelial lesions (HSIL) in a patient with LP prompted this 12-year retrospective analysis to study the frequency of HPV-induced SILs. 785 biopsies of 584 patients with vulvar LP were analyzed. All squamous intraepithelial lesions were analyzed for p53 and p16 overexpression and for the presence of 32 HPV DNA subtypes.
Results: Nine (1.6%) of 584 women had lichen planus (papular (3) and mucosal erosive LP (6)). High-grade squamous intraepithelial lesions (HSILs) were present in 7 patients and low-grade squamous intraepithelial lesions (LSILs) were present in 2 patients. All SIL harbored HPV16-DNA and showed p16-overexpression. Concomitant immune suppression included T-suppressor lymphocyte deficit (1), and cortisone therapy (systemic (1), and topical (2)). HSILs regressed spontaneously in 1/7 or after imiquimod therapy (3/7). The remaining 3/7 women with erosive LP discontinued imiquimod because of side effects and had laser destruction (1), skinning vulvectomy (1), and surgery (1) for definitive treatment. Two women had recurrent vulvar SILs, and 1 woman progressed to invasive squamous cell carcinoma (SCC). In the same patient population, 16 of 584 women had d-VIN, with 9 of 16 progressing to SCC.
Conclusions: HSILs in patients with vulvar LP are rare and may occur in the setting of various risk factors. If clinical suspicion arises, biopsy and histological examination assist in correct etiologic classification of a precancerous lesion and subsequent therapy decisions. The minimal risk for HSIL development in vulvar LP patients should not preclude therapy of LP.

hELP! A Multi-Center Four Armed Pilot Randomized Controlled Trial for Vulvar Erosive Lichen Planus: Results and Lessons Learned

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Objectives: Erosive lichen planus affecting the vulva (ELPV) is distressing and impacts upon quality of life. First-line therapy is often inadequate and evidence for second-line treatments is poor. 'hELP' (systemic therapy for vulvar erosive lichen planus) was a feasibility study to assess if a definitive randomized controlled trial (RCT) of second-line systemic therapy for ELPV was possible.
Methods: 'hELP' was a multicenter, four-arm, open-label, pragmatic, pilot RCT. Randomized participants received a six-month course of hydroxychloroquine, methotrexate or mycophenolate mofetil, or a four-week reducing regimen of prednisolone (comparator group). The primary feasibility outcome was the proportion of eligible participants randomized. Secondary outcomes were the proportion of patients adhering to treatment, quality and suitability of clinical images, suitability of trial design and chosen clinical outcomes. The primary clinical outcome, measured at 6 months, combined patient global assessment and blinded assessment of images to determine 'success'.
Results: Over 14 months, 180 patients with ELPV were identified from 12 sites. Only 44/180 (24%) were eligible. The main reason for ineligibility was disease too mild (n=50). Of those eligible, 22/44 (50%) were randomized. Four participants did not start study medications, four stopped trial treatment early and two were lost to follow-up. Only 14/22 participants had complete before and after images, of which 10 were suitable for clinical assessment. Treatment success only occurred in the hydroxychloroquine (2/6, 33%) and mycophenolate mofetil (2/5, 40%) groups.
Conclusions: 'hELP' demonstrated reluctance to take systemic therapy and highlighted some specific limitations of a multi-arm study design. Better evidence is required on which to base treatment in this setting but, on the basis of these findings, a full-scale trial using this design did not proceed. It also emphasized the lack of suitable outcome measures available for vulvar disorders. To improve quality of vulvar skin trials in the future, core outcomes need to be agreed upon by the international community.

Stem Cell Enriched Lipotransfer as a Regenerative Treatment for Vulvar Lichen Sclerosus: Results of A Prospective Open Cohort Study

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Objectives: Lichen sclerosus is a chronic inflammatory condition that affects genital skin in the male and female. Recently, minimally invasive regenerative therapies including autologous fat transfer, adipose-derived stem cells (ASCs), or platelet-rich plasma (PRP) have been proposed as an additional option to treat the patients who are non-respondent to steroid treatment. The aim of the study was to evaluate the effect of lipotransfer in a cohort of women presenting with lichen sclerosus of the vulva.
Methods: A series of 20 prospective patients were treated with 1 to 2 autologous lipotransfers in the affected areas following enrichment through centrifugation. Standardized pre and post-operative assessments were used. These included clinical observation, photography, and a vulvar grading scale. Symptoms were measured with: 1) a validated visual analogic scale (VAS); 2) sexual function (female sexual function index); 3) sexual distress (female sexual distress scale); 4) psychological assessment (hospital anxiety and depression scale) and 5) intimacy (relationship assessment scale).
Results: The clinical score showed a significant improvement in all treated areas ($p<0.05$). A significant improvement was reported in the VAS for itching ($p<0.05$) and soreness ($p<0.05$). Sexual function was significantly improved after treatment ($p<0.05$), as was the distress associated with sexuality ($p<0.05$). The patients also reported a significant improvement in the level of anxiety ($p<0.05$) and depression ($p<0.05$).
Conclusions: Stem cell enriched lipotransfer is an effective treatment for vulvar lichen sclerosus. It reverses skin fibrosis, ameliorates the disease manifestations and patients' quality of life. Despite the encouraging results, further invitro studies and prospective clinical trials are required to better understand the mechanism of action and to confirm the efficacy and safety of this potentially transformative regenerative treatment.

In Office Surgery and Use of Platelet Rich Plasma for Treatment of Vulvar Lichen Sclerosus to Alleviate Painful Sexual Intercourse

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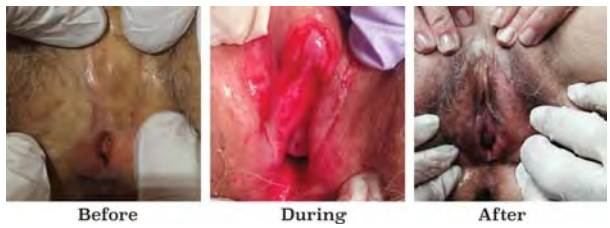
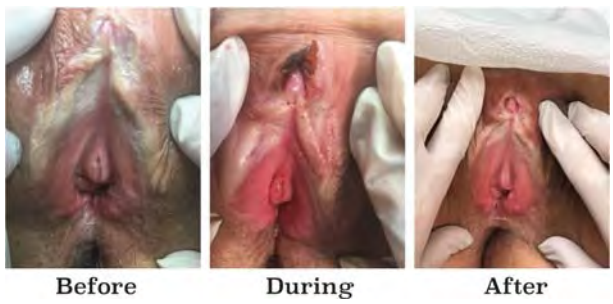
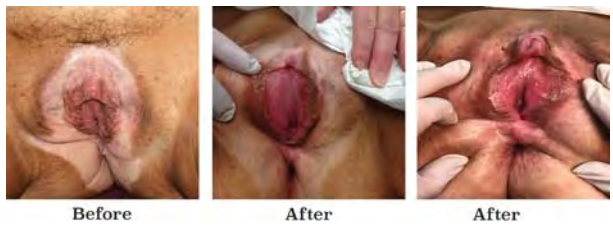
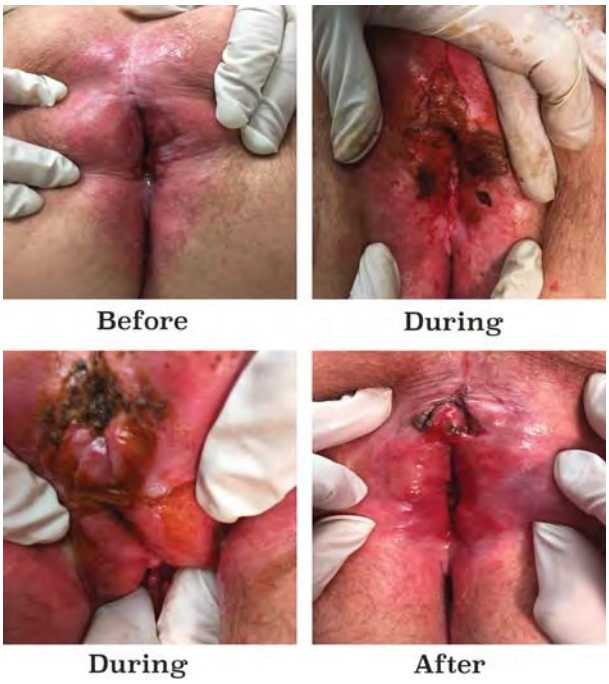
Objectives: To evaluate patients' symptoms and signs of vulvar lichen sclerosus and the ability to have pain free sexual intercourse after in office surgery to remove adhesions, followed by platelet rich plasma (PRP).
Methods: Fourteen patients with biopsy proven vulvar lichen sclerosus were seen in a private clinic between August 2014 and December 2016. All had some adhesions involving the clitoris, labia minora, and/or introitus. All experienced itching and pain. Of those who were sexually active, all had painful intercourse. The fourteen patients responded to a six-question survey, eight to ten weeks after initial treatment. The treatment consisted of in office surgery followed by PRP to the related sites. One provider performed all in office surgeries, as well as injections with PRP.
Results: All patients completed the questionnaire and recommended this treatment. Six patients were sexually active before treatment and nine patients after treatment. Four patients had no partner and one was gay. Of the sexually active patients, after treatment with surgery and PRP, 67% had sexual intercourse with no pain; 33% reported less pain. Of these same patients, 70% reported no bleeding; 30 % reported less bleeding. The itching went away in 50% of all patients treated; improved in 43%; and no change in 7%. Ninety-three percent of the patients were happy with the treatment. Before and after photos of four of these patients are included in this abstract.
Conclusions: Patients reported improvement in their symptoms and signs of lichen sclerosus, including the ability to have pain free sexual intercourse. Surgery with PRP is an option to help improve the scarring associated with lichen sclerosus and decrease pain with sexual intercourse.









Vulvar Surgery in Young Women with Lichen Sclerosus and Dyspareunia or Apareunia

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Objectives: Lichen sclerosus (LS) is a chronic inflammatory condition, which affects the vulva. Many patients who report dyspareunia have introital stenosis on physical examination. Thus, patients have less frequent and less satisfying sexual activity than women without this disease. The objective of this study is to review the indications of surgery for introital stenosis related to vulvar lichen sclerosus, and to show its results.

Methods: The records of 11 patients who underwent surgery due to introital stenosis caused by LS in the Hospital Italiano of Buenos Aires were analyzed retrospectively. The mean follow-up period was 30 months (range, 6–84).

Results: The mean age of patients was 31 (18–46) years. The main complaints were dyspareunia, inability to achieve vaginal penetration, and urinary obstruction in three of the patients. In 10 cases, a perineoplasty was made. One patient refused perineoplasty and only lysis of adhesions was performed. Patients were able to initiate vaginal intercourse one month after surgery, with considerable relief of symptoms. 60% reported some degree of dyspareunia. One wound dehiscence was observed. Topical application of clobetasol propionate ointment and promestriene cream was continued post-operatively, and no relapses were detected.

Conclusions: Surgery is indicated in women with LS who experience sexual pain owing to anatomic changes and who wish to resume vaginal intercourse. In our experience, vulvar anatomy was restored with a low rate of surgical complications. While improved, resolution of dyspareunia may be incomplete. Discussion of sexual activities other than intercourse is essential. Maintenance with corticosteroids is also advised.

Genomic Profiling of Vulvar Lichen Sclerosus Patients Reveals Possible Pathogenetic Mechanisms of Disease

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Objectives: To explore the genetic profiling of patients with vulvar lichen sclerosus (VLS) to potentially uncover pathogenetic mechanisms, which may lead to improvements in targeted therapeutics or future areas of clinical research.

Methods: Whole exome sequencing (WES) was performed on seven patients affected by VLS from two unrelated families along with one unaffected paternal aunt from one pedigree. The results of WES were compared to population-specific allele frequency databases to prioritize variants likely to be contributing to VLS development.

Results: Recurrent germ-line variants in four genes were identified as likely to be deleterious to proper protein function in all of the seven probands and not in the unaffected control. The genes with variants included CD177 (neutrophil activation), ANKRD18a (ankyrin repeat protein), CD200 (inhibitory signal to macrophages), and LATS2 (co-repressor of androgen signaling). Studies are currently underway to determine the prevalence of these or similar variants impacting the same pathways in VLS patients generally. Although clinical significance of these genetic alterations is uncertain at this time, previous research suggests that neutrophil activation and macrophage inhibition may be related to granulomatous/autoimmune diseases, while Ankyrin repeat protein and co-repressor androgen signaling have been linked to tumor suppressor activities.

Conclusions: This is the first report to detail genetic profiling of VLS patients and may provide insight into the pathogenetic mechanism of this disease. Future research should focus on identifying whether these similarities are present in other affected families with VLS in order to better understand the pathophysiology of this condition in an effort to guide treatment.

Development of the Adult Vulvar Lichen Sclerosus Severity Scale –a Delphi Consensus Exercise for Item Generation

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Objectives: To generate a list of items through international consensus for inclusion in a scale to measure the severity of adult vulvar lichen sclerosus.

Methods: This study was carried out as a three-stage Delphi consensus exercise. Following an extensive literature review, any items used to determine disease severity in previous clinical trials were compiled into a survey. The Delphi participants were recruited from the International Society for the Study of Vulvovaginal Disease (ISSVD). Participants were asked to rate the importance of items and in determining vulvar lichen sclerosus severity and were asked to indicate how these items should be measured. Consensus was defined as 75% agreement.

Results: Of approximately 400 members of the ISSVD, 66 participated in the study. Of the 14 symptoms presented, 7 reached consensus for inclusion (itch, pain unrelated to intercourse, pain with intercourse, bleeding and pain with intercourse, skin tearing with intercourse, quality of life, changes/decrease in sexual function). Of the 23 signs presented, 11 reached consensus for inclusion (fissures, whitening, crinkly/fine wrinkling of skin/parchment-like skin, extent of disease, erosions, ulcerations, hyperkeratosis, excoriations, lichenification, elasticity, sclerosis) and 1 reached consensus for exclusion (telangiectasia). Of the 6 architectural changes presented, all 6 reached consensus for inclusion (clitoral hood fusion, labial fusion/resorption, narrowing of the introitus, anterior changes, perianal involvement, and formation of posterior commissure bands/fourchette webs). No consensus was reached regarding method of measurement for any of the symptoms/signs.

Conclusions: International consensus was reached for a variety of signs and symptoms that should be included when assessing the severity of vulvar lichen sclerosus.

Co-existing Conditions in Patients with Lichen Sclerosus

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Objectives: To determine the frequency of co-existing conditions in patients with lichen sclerosus (LS).
Methods: Patient data between 7/1/07 and 7/1/12 with clinical signs of LS presenting to a Mayo Clinic practice specializing in vulvovaginal disorders were reviewed. The degree of LS was graded by the progression of clinical signs technique. Quantitative wet preparations were performed on vaginal secretions to determine the presence of coexisting conditions that could be implicated in vulvar symptoms.
Results: A total of 498 cases were reviewed. Lichen sclerosus was ranked severe in 95 (19%), moderate in 88 (17.7%), and mild in 315 (63.3%). Symptoms in decreasing order of frequency were itching (n= 296 (59.4%)), burning (n=196 (39.4%)), a dry/chafed sensation (n=152 (30%)), cracks/paper cuts (n=152 (30%)), vaginal discharge (n=103 (20.7%)) and vaginal odor (n= 43 (9%)). Clinical signs of precancerous conditions were present in 17 (3.4%) with 7 (41%) of biopsies positive (1.4% of total patients). One-hundred ninety-one patients had burning without fissures plus vaginal discharge or odor. These symptoms are not attributable to LS. Of the 191, 158 (82.7%) had altered vaginal micro-flora patterns (32% of total).
Conclusions: Coexisting precancer is rare with in patients with lichen sclerosus. Patients with lichen sclerosus present with co-existing altered vaginal micro-flora.

Is Non-Response to Fluconazole Maintenance Therapy for Recurrent Candida Vaginitis Related to Host Immunity Disorders?

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Objectives: Patients suffering from atopic disease and other autoimmune phenomena can develop recurrent vulvovaginal candidiasis (RVVC). We analyzed the relationship between failure to respond to maintenance fluconazole treatment for RCVV and the presence of immune-mediated disorders.
Methods: A prospective multi-center open label follow-up study, named ReCiDiF, was performed on women suffering from RCVV in ambulatory gynecology centers in Belgium. We analyzed the medical history, physical status, family history, and vaginal immune response of optimal and poor responders (N=71) to fluconazole maintenance treatment. We analyzed the results using Chi-square, Fisher exact, and Spearman rho tests, and compared means using the Mann-Whitney U test. We developed a multivariate logistic regression model and receiver operating characteristic (ROC) curve.
Results: Sociodemographic characteristics of 33 non-responding women were similar to those of 38 optimal responders. Responders with a score ≥ 3 on vulvar excoriation had a higher risk of non-response to therapy (OR = 3.99, CI 95% 1.41-11.23). Univariate regression analysis showed that family history of atopy (OR 3.61, CI 95 % 1.17-11.18), and eczema (OR 5.76, CI 95 % 1.13 – 29.44) were also related to non-response. Vulvar excoriation at entry was the only significant predictive factor for non-response in multivariate analysis with specificity 78.9 % and sensitivity 54.0%.
Conclusions: Women with RCVV, who have family history of atopy and eczema, especially if presenting with severe vaginal excoriation, are at increased risk not to respond to maintenance fluconazole treatment. This finding is important for clinicians for predicting possible treatment outcomes.

The Mutable Profile of Infectious Candida Species and Resistance to Antifungal Agents: A Clinical and Laboratorial Study

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Objectives: Identify candida species in patients with vulvovaginitis and determine their sensitivity to antifungal agents.
Methods: Eighty-four vaginal secretion samples of patients seen at the Brasilia University Hospital Gynecology outpatient clinic were analyzed. Nineteen patients were asymptomatic and 65 patients disclosed at least one of the following symptoms: vaginal discharge, vulvar hyperemia or edema, and localized itching or burning sensation. Candida phenotype was identified by culture, and confirmed by matrix assisted laser desorption ionization time-of-flight (MALDI TOF). The sensitivity profile of Candida species for flucytosine, fluconazole, voriconazole, amphotericin B, capsofungin and mycofungin was determined by the minimal inhibitory concentration (MIC).
Results: Sample analysis of the 65 symptomatic patients showed 73% (48) positivity, with 75% (36 of 48) of the phenotypes identified as Candida albicans, 22.9% (11 of the 48) as non-albicans species (respectively, 8.3% C. glabrata, 6.2% C. parapsilosis, 4.2% C. tropicalis, 2.1% C. krusei, 2.1% C. zeylanoides) and 2.1% (1 of 48) Rhodotorula mucilaginosa. C. albicans species were sensitive to all antifungal agents with the exception of one of the species that showed an intermediate sensitivity to amphotericin B (2.1%). Resistance was found among non-albicans species to fluconazole in 2.1% (C. glabrata), and to voriconazole in 2.1% (C. krusei).
Conclusions: In view of significantly increased infectivity of non-albicans species, with some phenotypes demonstrating resistance to usual antifungal agents, our results emphasize the need to precisely identify the Candida species to abrogate possible treatment failure and repetitive episodes of vulvovaginitis.

Reduced Antifungal Susceptibility of Vulvovaginal Candida Species at Normal Vaginal pH Levels: Clinical Implications

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Objectives: Antifungal susceptibility testing is routinely performed at pH 7 but the pH of the normal and yeast infected vagina is closer to 4. Our objective was to assess for differences in susceptibility at pH 7 and pH 4.
Methods: 310 yeast isolates were collected from 217 patients. The first isolates collected from each patient were included in the analysis. In patients who had more than one species cultured, the first isolate of each species was included. 173 C. albicans, 15 C. glabrata, and 29 from 8 other species first isolates underwent susceptibility testing at pH 7 and pH 4 against fluconazole, itraconazole, miconazole, clotrimazole, terconazole and nystatin.
Results: Geometric mean (GM) MICs for all antifungals were significantly higher when tested at pH 4 ($p<0.001$). For C. albicans, the largest GM MIC differences were observed for terconazole (0.17 pH 7 vs. 6.17 pH 4) and clotrimazole (0.04 vs. 0.24). For fluconazole, 5.2% of susceptible isolates at pH 7 (MIC<2) had MIC <4 at pH 4. For terconazole, 97.7% of the isolates had MIC<1 at pH 7 but 83.2% had MIC>4 at pH 4. For C. glabrata, terconazole (0.26 pH 7 vs. >64 pH 4), clotrimazole (0.13 vs. 6.96), miconazole (0.06 vs. 0.76) and fluconazole (3.17 vs. 26.60) were most affected. Nine of 15 C. glabrata isolates had MIC<2 for fluconazole at pH 7, but 14 of 15 had MIC>16 at pH 4. All C. glabrata isolates had MIC values of <1 for terconazole at pH 7, but one had MIC=8 and the other 14 were highly resistant (MIC>64) at pH 4.
Conclusions: Antifungals have reduced potency when tested at lower pH. C. glabrata is more affected than C. albicans. Although the clinical implication is unknown, the impact may be greatest for terconazole and C. glabrata.

Accuracy of DNA Probe Based Testing for Candida Vaginitis Compared with Office Microscopy

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Objectives: To compare DNA probe based testing using AFFIRM with office microscopy to diagnose Candida vaginitis. Comparisons were made regarding agreement of the two tests in diagnosing candida vaginitis, cost of the tool, and time delay until patients received results.
Methods: There were 89 patients who met the inclusion criteria. At the initial visit a 10% KOH and saline slide with vaginal secretions were examined under a phase contrast microscope. Positive testing was visualization of hyphae, spores, or mycelia. At the initial visit all patients had a sample sent for PCR testing using AFFIRM. PCR is the gold standard for diagnosis. Patients were contacted two weeks after the initial visit to assess resolution of symptoms. The Kappa test

score was calculated to determine agreement between microscopy and PCR testing. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using the PCR test as the gold standard.
Results: Kappa agreement between the PCR and microscopy was good (K=0.79, (0.66, 0.92). There were 31 patients had both a positive microscopy and PCR. 47 patients had a negative microscopy and PCR for Candida. Eight patients had a positive microscopy and negative PCR, and 1 patient had a negative microscopy and positive PCR for Candida.
Conclusions: Our study supports use of microscopy as an initial tool to diagnosis Candida vaginitis in the office. Microscopy has lower cost, provides faster ability to treat symptomatic patients, and high concurrence to PCR testing.

Vulvar Melanosis: Clinical, Dermoscopic and Histologic Correlation and Approach To Management

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Objectives: Vulvar melanosis and melanoma are particularly challenging clinical diagnoses, may result in patient and physician anxiety, and can lead to unnecessary, potentially disfiguring surgical procedures. We define the clinical, dermatoscopic and histopathologic characteristics of vulvar melanosis and propose an approach to its diagnosis and management.
Methods: A retrospective analysis of 5 patients with vulvar pigmented lesions with clinical diagnosis of suspected vulvar melanosis and/or melanoma was performed. Patient clinical records, clinical characteristics of pigmented areas, dermatoscopic features and histologic findings were evaluated for all pigmented lesions. All patients were referred to our Dermatology Department of the Italian Hospital of Buenos Aires, Argentina.
Results: The lesions varied in color, tan to dark brown/black, and size. In the detailed dermoscopic analysis we found: a ring-like pattern, structureless and globule-like patterns, a parallel pattern, as well as cobblestone-like, and reticular-like patterns. Histologic findings showed vulvar melanosis in all patients with increased melanin in the basal layer and a normal or slightly increased number of melanocytes arranged as single units at the dermoepidermal junction.
Conclusions: The diagnosis and management of vulvar melanosis should be based on a clinical and dermoscopic correlation to allow differentiation of vulvar melanosis from early melanoma. One or multiple biopsy specimens should be considered if the differential diagnosis between melanosis and melanoma cannot be made. Because vulvar melanosis is more commonly found among perimenopausal women, it is important that clinicians, dermatologists, and gynecologists carry out an adequate evaluation so as to avoid unnecessary treatments.

Vulvar Squamous Cell Carcinomas (VSCC). A 10- Year, Single Institution Review from The Hospital Italiano de Buenos Aires (HIBA)

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Objectives: To examine clinical and pathological features of vulvar squamous cell carcinoma (VSCC) in a period of 10 years at our institution.
Methods: Patients diagnosed with VSCC between 2006 and 2016 were retrieved from the pathology files of the Hospital Italiano de Buenos Aires (HIBA). Clinical and pathologic diagnosis information were analyzed.
Results: A total of 52 VSCC were identified. 43 tumors (82%) were of conventional keratinizing type, 2 (4%) were non-keratinizing, 2 (4%) warty, 1 (2%) basaloid, and 4 (8%) verrucous type. Squamous intraepithelial lesions adjacent to VSCC were found in 36 (69%) cases, 12 (33%) were high-grade vulvar squamous intraepithelial lesions (HSIL) and 24 (67%) were differentiated vulvar intraepithelial neoplasia (dVIN). Within the group of patients with VSCC and HSIL, the mean age was of 62 years. 7 patients (58%) had multicentric HPV related lesions. No other vulvar dermatoses were found in the specimens nor in their previous clinical records. 4 (33%) were immunocompromised patients. 2 patients recurred, but none died of this disease. In the group of patients with VSCC with adjacent dVIN or with no HSIL, the mean age was of 74 years: 21 (53%) cases had no previous history of dermatoses; 7 patients had previous history of dVIN; 7 lichen sclerosus; 1 lichen simplex chronicus; and 1 vulvar acanthosis with altered differentiation. Only 1 patient had previous history of cervical HSIL. 16 (45%) patients recurred, and 9 of these died of this disease.
Conclusions: We report our experience in VSCC. 23% of our cases showed a link to HR-HPV pathway, and this group showed different clinical and pathologic history compared to the group with dVIN or no HSIL associated.

Inmunomodulatory Treatment with Imiquimod 5% Cream in High-Grade Vulvar Squamous Intraepithelial Lesions

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Objectives: To evaluate the effectiveness and safety of imiquimod 5% cream for the treatment of high- grade squamous intraepithelial lesions (HSIL) of the vulva.
Methods: Retrospective cohort of 92 patients with a mean age of 47 years (23–72) with histopathological diagnosis of vulvar HSIL, treated with imiquimod 5% cream including mean follow up of 27 months (6–48) was performed. Patients applied imiquimod 3 times a week until total clearance of lesions or up to a maximum 6 months. Response was categorized as complete when there was no clinical evidence of lesions, partial when the area diminished >50%, and progressive when there was an increase of the lesion area. A biopsy was performed at the end of treatment and follow up was carried out monthly to evaluate adverse effects.
Results: Total clearance of lesions was observed in 54 of the 92 patients (58.69%) after 12–24 weeks. Twenty-eight patients (30.43%) had a partial response and 10 patients (10.86%) were non-responders; all of them received surgical treatment. No invasion was observed in the specimens. Eleven patients with complete response (11.95%) had recurrences during a mean of 27 months (6 – 48) follow-up. Of the 28 who had partial response, 5 were immunocompromised and 3 had poor adherence to treatment.
Conclusions: In this initial series, imiquimod proved to be an effective and less invasive for the treatment of vulvar HSIL compared to surgical options. The treatment was well tolerated; only local reactions were observed. Imiquimod represents a good alternative for the management of this disease.

Epidemiological, Clinical, Histological, and Immunohistochemical Aspects of Vulvar Intraepithelial Neoplasia Based on Five Cases

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Objectives: To study the epidemiological, clinical, histological, and immunohistochemical characteristics of vulvar high-grade intraepithelial lesions (HSIL) and differentiated vulvar intraepithelial neoplasia (dVIN) at the Vulvar Pathology Section.
Methods: Clinical examination of patients age, smoking status, immunodeficiency history, and pathologic diagnosis of condyloma or neoplasia in the lower genital tract was performed. Tissue samples were analyzed to describe the histological patterns. We performed immunohistochemistry for p16 and p53 on the specimens.
Results: Out of the 5 patients with squamous intraepithelial lesions, 4 were HSIL and 1 was differentiated VIN. The 4 cases of HSIL included 1 basaloid and 3 warty variants. The average age of patients with vulvar HSIL was 49 years. 3 patients were former smokers and had a history of cervical HSIL. 1 patient developed invasive cervical carcinoma. 1 patient was immunocompromised from treatment for autoimmune hepatitis. The patient with differentiated VIN was 62 years old. Her histopathological analysis demonstrated lichen sclerosus adjacent to the lesion. At clinical examination, the 4 patients with vulvar HSIL had multifocal lesions, while the patient with dVIN had only 1 lesion. All cases of vulvar HSIL were positive for p16. Co-expression of p16 and p53 was found in 1 case. The case of dVIN was negative for p16 and positive for p53.
Conclusions: Two types of vulvar squamous intraepithelial lesions have been described. One is related to HPV infection and the other is not HPV-related but is associated with inflammatory dermatoses. Each type of squamous intraepithelial lesion has a different etiology, epidemiology, clinical and immunohistochemistry presentation.

Vulvar Basal Cell Carcinoma

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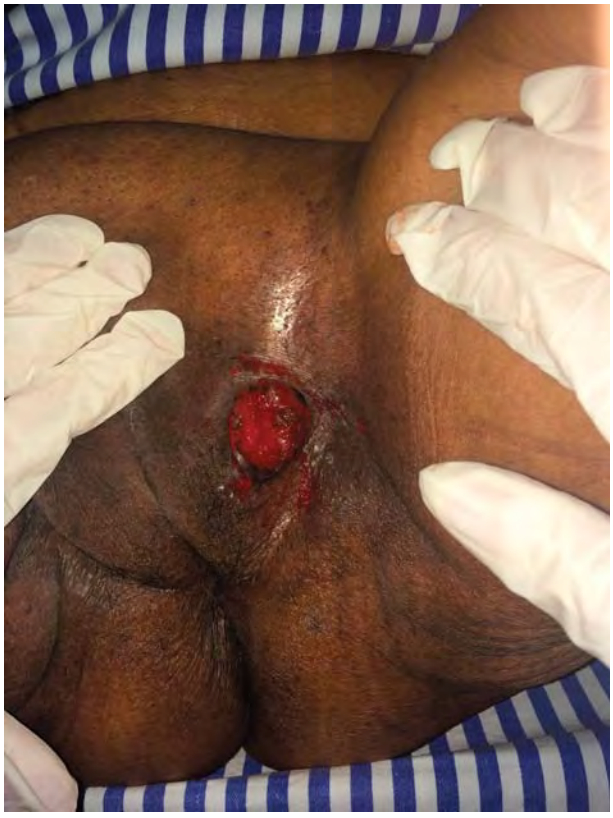
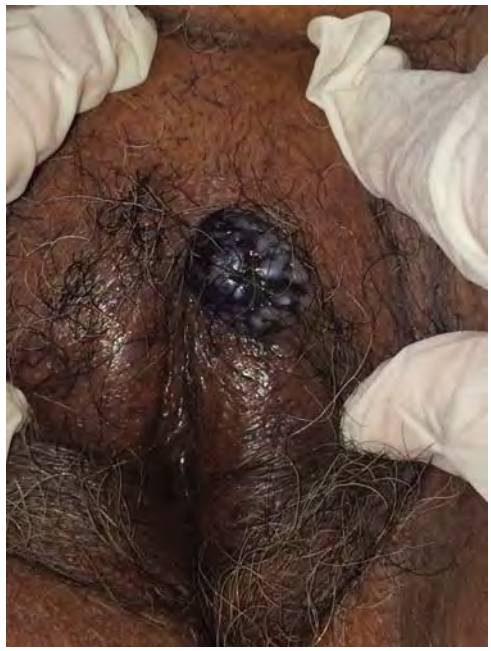
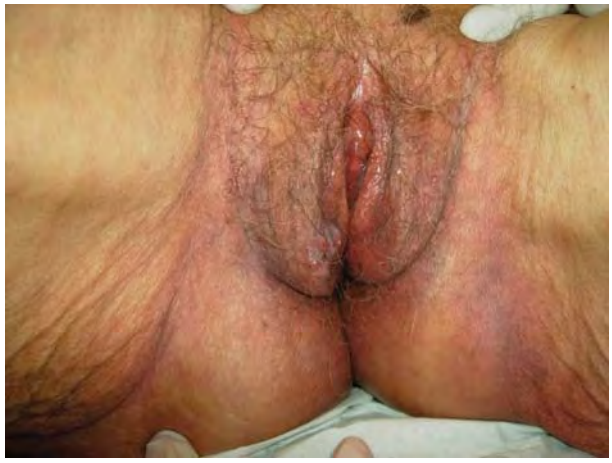
Objectives: To report 3 cases of vulvar basal cell carcinoma.
Methods: A retrospective review of cases of vulvar basal cell carcinoma including three women, aged 75–81 years, referred to the Vulvar Pathology Service of the University Hospital Antônio Pedro (HUAP) of the Federal University of Fluminense (UFF) was performed.
Results: Three women, aged 75–81 years, were referred for solitary vulvar lesions, each around 5 cm, and large labia. In each case the clinical presentation was distinct: dark colored nodule, ulcerated nodule, and polychromic nodule. In each case a biopsy was performed with pathological confirmation of basal cell carcinoma. One included immunohistochemistry with positivity with BCL2 (focal), BER-EP4 and CK17 and negativity with EMA. All were treated with extensive lesion excision. They remain in follow-up at the HUAP Vulvar Pathology Service.
Conclusions: Basal cell carcinoma is the most common cutaneous malignant neoplasm in humans, mainly affecting individuals with light skin. The primary risk factor is exposure to ultraviolet radiation, being more prevalent in exposed regions. Only 2% of basal cell carcinomas occur on the vulva, affecting predominantly menopausal patients as was noted in our series. It corresponds to only 2% of the total cases of vulvar cancer, but should be considered among the differential diagnoses of vulvar lesions, which are often asymptomatic. Excision with clear margins of the lesion is the treatment of choice. Long-term follow-up is necessary because of the risk of local recurrence and onset of other primary cancers.







Vulvar Basal Cell Carcinoma: An Unusual Location

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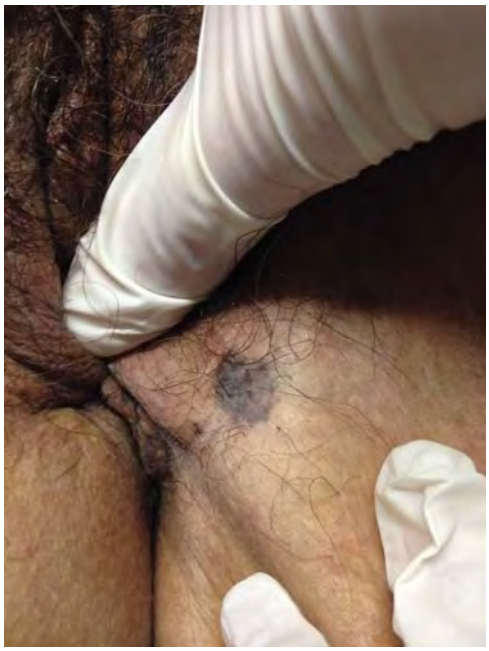
Objectives: To present three cases of vulvar basal cell carcinoma (BCC), their histology, treatment and follow up.
Methods: We present three patients, aged 52 (image 1), 59 (image 2) and 65 (image 3), with a diagnosis of vulvar BCC, from September 2006 to June 2016, in our hospital.
Results: Three patients presented with pigmented lesions localized to the labia majora. All of them were asymptomatic. A biopsy was performed and the pathologic diagnosis was pigmented basal cell carcinoma. The surgical procedure consisted of resection of the lesions, with free margins of at least 5 mm. There were no recurrences after a mean follow-up of 5 years (2–8 years).



Conclusions: BCC is the most common cutaneous neoplasm in the white population. The main risk factor involved in its genesis is chronic sun exposure. The location in non-exposed areas is infrequent, less than 2%, and suggests the existence of etiological factors that are still unknown. The frequency of vulvar BCC is approximately 1%. The labium majus is the most frequent location. The pigmented variant described in the three cases presented is one of the less frequent histopathological varieties of this type of tumor. The growth of these lesions is slow and the frequency of metastasis is low, so an early diagnosis will allow adequate treatment with minimal impact on morbidity and mortality.

Clinicopathological Features of Vulvar Granular Cell Tumor

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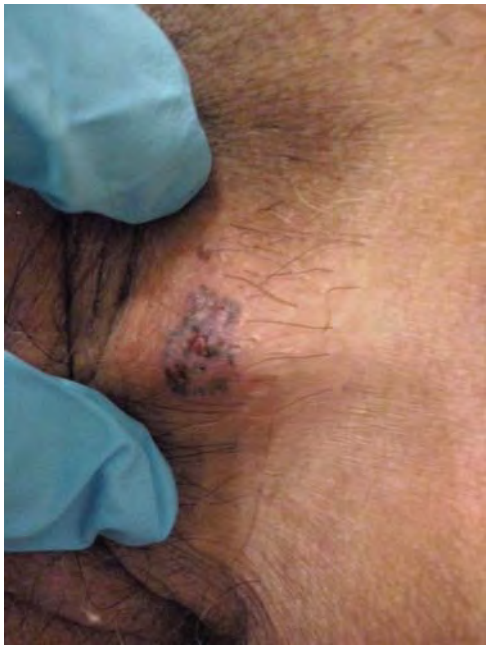

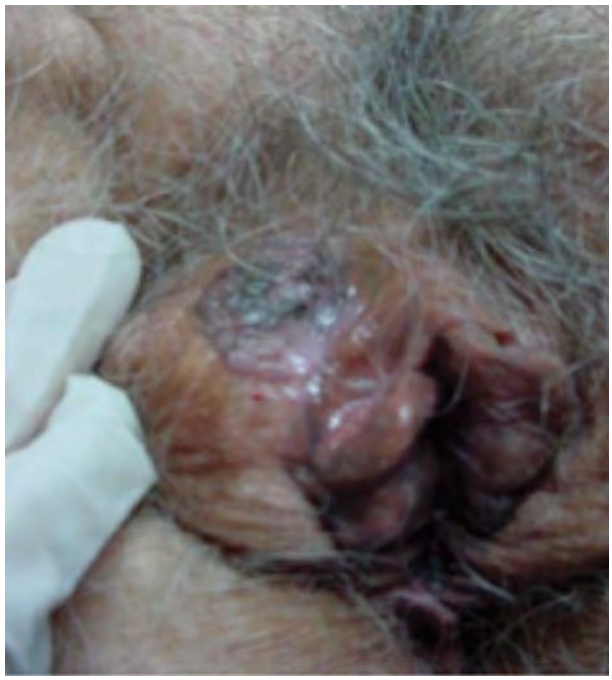

Objectives: Granular cell tumors (GCT) are infrequent soft tissue neoplasms that have been found throughout the body, including the female genital tract. They occur in both children and adults, with a higher incidence in the fourth to sixth decade. Most cases are benign. Malignant variants are described in 1-3% of cases. The vulvar involvement ranges from 5 to 16%. The purpose of this study was to review the clinicopathological features, surgical management and follow-up.
Methods: We conducted a retrospective chart review of all patients that were diagnosed in a 15-year period (01/07/2001–30/06/2016). Three cases could be identified. Clinical data, histologic features, surgical treatment, and follow-up were reviewed.
Results: The median age was 52 years. The patients presented with a unique firm nodule located in the labia majora without pruritus or pain. The median tumor size was 2.3 cm. All cases were managed surgically with wide local excision. The samples were submitted for histopathological study with confirmation of vulvar GCT without malignant histological criteria and negative margins. The patients continue with annual gynecological exam as follow-up. Recurrence and metastasis have not been found.
Conclusions: We present 3 patients with GCTs confined to the labia majora as a firm nodule without other symptoms. The clinical diagnosis should include all vulvar masses. The histopathological diagnosis is mandatory but does not guarantee clinical behavior since there have been reports of malignant tumors retaining a benign histological appearance. The treatment of choice is wide local excision. The patient must be counseled to follow-up regularly with physical exams to detect recurrence, regional lymphadenopathy, or metastasis.

Invasive Vulvar Carcinoma and Lymphovascular Space Invasion: Presentation of Three Cases

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Objectives: To present three clinical cases of radical vulvectomy for vulvar squamous cell carcinoma (VSCC) with free margins and negative lymphadenectomy with inguinofemoral recurrence within 24 months.
Methods: Retrospective review of three patients with VSCC, mean age, 60 years.
Results: The three patients underwent radical vulvectomy with bilateral lymphadenectomy. The three were stage II (FIGO 2009). Histopathology demonstrated squamous cell carcinoma with an average depth invasion 2 mm, free margins (1.5 –3 cm), negative nodes, presence of lymphovascular space

invasion, and normal computed tomographic scan of the abdomen and pelvis. Inguinal recurrence occurred in 12 months average time. Mortality occurred within 24 months after the initial diagnosis for each.
Conclusions: We present 3 cases in which cancer recurrence occurred within the first two years. The presence of lymphovascular space invasion was present in each and may represent a prognostic factor for recurrence; consideration for adjunctive radiotherapy treatment may be indicated.

Verrucous Carcinoma versus Giant Condyloma of the Vulva: A 15 Years Retrospective Study

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Objectives: The aim of the study was to assess the clinicopathological characteristics of patients with verrucous carcinoma (VC) and giant condyloma (GC) of the vulva.
Methods: We reviewed data on the age, disease course, pathologic diagnosis, treatment and follow-up of patients with VC or GC who were treated at our hospital during the past 15 years.
Results: Six cases of VC were identified. The mean age of patients was 65 years. Primary signs and symptoms were exophytic neoplasms with pruritus and/or pain. Surgical treatment included radical local excision and radical hemivulvectomy with inguinofemoral lymphadenectomy when invasion was found. Vulvar verrucous carcinoma occurred simultaneously with differentiated vulvar intraepithelial neoplasia (DVIN) in 3 cases, vulvar acanthosis with altered differentiation (VAAD) in 2 cases, and well-differentiated squamous cell carcinoma in 1 case. 2 patients with VC had a history of previous DVIN, and 1 patient had a history of VAAD. HPV testing was negative in 5 cases. The mean follow-up time period was 44.8 months. Local relapse developed in 2 cases (recurrence rate = 33%). None of the patients died of VC disease. Three cases of GC were identified; the mean age of these patients was 61. The primary signs were exophytic neoplasms. Surgical treatment included wide local excision and simple vulvectomy. GC was associated with a previous history of vulvar HSIL in all cases. pl61NK4a (p16) by immunohistochemical staining was performed in all 3 cases of GC; all results were positive. The mean follow-up was 10 months with no recurrences.
Conclusions: Vulvar VC is a distinct type of slow- growing, nonmetastatic tumor with unclear etiology. These tumors should be distinguished from GC. Histological examination is the standard for diagnosis, although evaluation of HPV status may aid in the diagnosis. Surgery is the most effective treatment.

Verrucous Carcinoma of the Vulva: Report of Three Cases

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Objectives: To present three cases of verrucous carcinoma of the vulva.
Methods: Retrospective review involving three patients with verrucous carcinoma of the vulva.
Results: Three patients (mean age 63 years old) with initial diagnosis of squamous cell hyperplasia and suspicious lesions underwent local resection of the vulva with confirmation of invasive vulvar verrucous carcinoma and coexisting squamous carcinoma in two of the three cases. Subsequently, all patients underwent radical vulvectomy and inguinofemoral lymphadenectomy, with free margins and no involvement of lymph nodes. One patient had differentiated vulvar intraepithelial neoplasia two years later and had local excision. Another patient had recurrence of verrucous carcinoma and underwent radical surgery with chemotherapy. The third patient continues follow-up with no lesions.
Conclusions: Verrucous carcinoma represents a rare entity which most commonly presents in postmenopausal women. Surgery is considered the most appropriate treatment and its prognosis is relatively good. Recurrence occurs more frequently in patients with coexisting verrucous and squamous cells carcinoma.

Extramamary Paget's Disease of the Vulva (EMPD): A Novel Therapeutic Approach

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Objectives: Vulvar Paget's disease is an intraepithelial adenocarcinoma which arises from the apocrine glands of the vulvar skin, representing 1-2% of vulvar cancers. It is typically diagnosed in women around 70 years of age and usually presents as a pruritic erythematous squamous lesion. Skin biopsy allows diagnosis, and immunohistochemistry has a key role in differentiating primary and secondary disease. Treatment consists of surgical excision; local recurrence rate is high. The objective of this study is to describe 24 cases of extramammary Paget's Disease (EMPD) of the vulva and compare clinical pathological characteristics and management.
Methods: Observational retrospective study including patients with vulvar EMPD who were followed at the Hospital Italiano of Buenos Aires between June 2006 and June 2016.
Results: The mean age at presentation was 71.2 years. In 23 cases, primary EMPD was the diagnosis and one patient presented with EMPD associated with urothelial carcinoma. Among the 23 cases of primary EMPD, 20 had intraepithelial disease and 3 had invasive disease. Eleven patients underwent primary surgical treatment. Six of the 11 patients presented local recurrence and were treated with 5% topical imiquimod, of whom 5 had complete clinical response. The remaining 13 patients received primary imiquimod treatment, achieving complete response in 7 cases. Clinical resolution was attained after an average of 5 months of treatment. Post-treatment biopsy was performed in all patients with complete clinical response for confirmation of concomitant complete pathologic response. The mean follow-up was 39 months.
Conclusions: Based on our study, we propose 5% imiquimod cream as a valid alternative for the treatment of primary and recurrent vulvar EMPD. It proved to be a safe and effective option, especially in those patients with extensive lesions or those at high risk for surgical treatment because of comorbidities.

Vulvar High-grade Squamous Intraepithelial Lesions in Elderly patients

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Objectives: Vulvar high-grade squamous intraepithelial lesions (HSIL) are commonly detected in young women, smokers, and immunosuppressed, with a history of several sexual partners. However, in the last few years, it has been diagnosed in women older than 60 years, without the mentioned risk factors. We intended to evaluate whether recent sexual contact is a necessary cause for the development of vulvar HSIL and to confirm the presence of these HPV lesions using histological criteria and immunohistochemistry (IHQ) (p16) as a diagnostic tool, correlating it with the results of polymerase chain reaction (PCR).
Methods: We conducted a retrospective analysis of data collected from electronic medical records of patients referred to the vulvar section of the Hospital Italiano de Buenos Aires, who were diagnosed with vulvar HSIL, between October 2006 and March 2017. Information about risk factors as well as diagnostic methodology and biomarker results was obtained.
Results: 26 patients over 60 years old were included, but only 14 (53.8%) who did not have sex at least 10 years prior to the diagnosis were analyzed and found to have HPV. The mean age of this group was 73.8 years old (61–87). None were smokers or immunosuppressed.
Conclusions: The presence of related HPV vulvar lesions was demonstrated in patients without recent sexual contact. This may be due to reactivation of the virus acquired decades ago and not to a new sexual exposure. Histology remains the key to the diagnosis of this entity, with the IHQ as a confirmatory tool.

Vulvar Squamous Cell Carcinoma: Clinical, Histological, and Immunohistochemical Characteristics

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Perrupato Hospital, Mendoza, Argentina. [5]Departement Pathology, Italian Hospital, Mendoza, Argentina. [6]Molecular Biology Laboratory, Mendoza, Argentina.

Objectives: To analyze the histopathology of vulvar squamous cell carcinoma and their clinical and immunohistochemical characteristics.
Methods: We analyzed data for 21 patients diagnosed with invasive vulvar cancer between 2009 and 2015. 15 cases of squamous cell carcinoma (SCC) were included in the study. Histology samples were analyzed for histologic type, associated lesions, depth of invasion, growth pattern, tumor grade, and vascular/lymphatic and lymph node involvement. All samples were immunolabeled for p16 and p53. Patients' treatment and their epidemiological and clinical history were also assessed.
Results: For these 15 SCC patients, SCC subtypes were classified as warty (5), basaloid (2), and keratinizing (8). All tumors were G2, except for 3 warty cases that presented G1. At the time of diagnosis, 9 patients were stage I/II and 6 were stage III/IV. Depth of invasion was <6mm for all warty subtypes and >7mm for keratinizing subtypes (11.7 mm on average). 6 out of the 7 patients with warty and basaloid SCC presented vulvar HSIL close to the invasive lesion. Out of the 8 patients with keratinizing-SCC only 3 presented areas of differentiated-VIN and 5 were diagnosed with lichen sclerosus. p16 overexpression was observed in all warty and basaloid subtypes (7), which were associated to stages I and II, node-negative disease and expansive growth pattern. p53 expression was observed only in 2 keratinizing-SCCs that also presented an infiltrative growth pattern and deeper invasion. There were 11 radical vulvectomies/hemivulvectomies and 6 lymphadenectomies. The sentinel node was identified in 9 patients. Cutaneous flaps were used in 7 patients for reconstruction purposes. 10 patients received adjuvant radiotherapy.
Conclusions: The histologic subtypes of vulvar SCC present different features and specific behavior. The immunohistochemical profile can be very useful in the diagnosis, assessment, and treatment of SCC patients.

"Premalignant and Malignant Lesions of Cervix in Patients with Vulvar Condylomatosis who Attended the Pathology Service of Lower Genital Tract of the Obstetrics Gynecology Isidro Ayora Hospital from January to February Of 2017"

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Objectives: To determine the prevalence of premalignant and malignant lesions of the cervix in patients with vulvar condyloma who presented to the Pathology Service of Lower Genital Tract of the Obstetrics Gynecology Isidro Ayora Hospital from January to February of 2017.
Methods: Observational Descriptive Study of transversal cut in 20 women who attended the Pathology Service because of vulvar lesions.
Results: Twenty patients were observed: average age was 31.15 years, average age at the beginning of sexual life was 19.15 years, and the average number of sexual partners was 1.6. Fourteen patients were using family planning/contraception: hormonal methods in 6 and non-hormonal methods in 8 cases. Additionally, the majority did not smoke, with tobacco use in only 1 case. The vulvar condylomas were studied by histopathology in all cases. Cervical low-grade intraepithelial lesions were identified in 2, and high-grade intraepithelial lesions in 1 cytology. The colposcopy was grade 1 in 13 patients and grade 2 in 2 patients. The histopathological study demonstrated 10 low-grade lesions (50% of the cases) and 2 high-grade lesions.
Conclusions: There exists a high prevalence of premalignant and malignant cervical lesions in patients with vulvar condyloma.

Perception of Sexuality in Patients with Vulvar Warts

A Garcia, MD[1], F Ceragioli, MD[1], S Sanguino, MD[1], M Avalle, MD[1], and M Ledesma, MD[1,2]. [1]Rawson Hospital, Department of Lower Genital Tract, Cordoba, Argentina. [2]Chief of Gynecology of Rawson Hospital, Cordoba, Argentina.
Objectives: This study was aimed at determining the impact of having a diagnosis of HPV regarding sexuality in women with vulvar warts.
Methods: A case series was done in patients with warts diagnosed at the Inferior Genital Tract Department of Rawson Hospital in the city of Córdoba (Argentina). Patients were enrolled from December 2016 to January 2017. Inclusion criteria were current diagnosis of vaginal, vulvar, or anal warts and ability to complete the changes in sexual functioning questionnaire (CSFQ)-14 (the validated Spanish short form). The statistical analysis was done in statistical package for the social sciences (SPSS), analysis of means for continued variables and univariate for categorical variables.
Results: We included 14 patients aged from 18 to 48 years. The mean age of sexual initiation was 16 years and the mean number of sexual partners was 5 (table 1). 85% of the sample claimed to be in a stable relationship. 41% of the patients had HIV and 30% were smokers. The vulva was the most common location of warts. 57% of patients were not having sexual relations due to the presence of warts. From this group, 37.5% had the feeling of shame or discomfort, 12% had fear of infecting others, 12.5% per partner request, 25% due to the pain of the lesions, and 12% for other causes. Question number 2 (sexual intercourse frequency) and number 11 (orgasm frequency) were analyzed (table2).

CSFQ-F® Questions	MODE
2- How frequently do you engage in sexual activity now?	4
3- How often do you desire to engage in sexual activity?	3
11- How often do you experience an orgasm?	3
	1. Never 2. Rarely 3. Sometimes 4. Often 5. Always

Table 1

N=14	AGE	AGE OF SEXUAL INITIATION	NUMBER OF SEXUAL PARTNERS	PREGNANCY
MEAN	25.93	16.64	4.93	1.14

Conclusions: This case series suggests that the diagnosis of warts modifies the perception of sexuality and the sexual behavior in patients who suffer from this condition.

Cosmetic Surgery Committee-Position

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The number of vulvovaginal cosmetic surgeries and procedures is increasing worldwide, despite the lack of scientific evidence supporting either the need for many of these or their efficacy. These are being offered and advertised as common, simple and complication-free procedures, capable of not only improving aesthetic appearance, but also increasing self-esteem and enhancing sexual pleasure for both women and their partners. Complex social factors are actively creating a perceived problem/disease for which surgical intervention is being offered as a cure.

This committee reached a consensus in the following points:

1. There is a wide variation in terms of normalcy of the look of the genitals; physicians must be able to explain this to women.
2. All women undergoing cosmetic genital surgery should previously be evaluated by a gynecologist; attention must be paid to their psychological and social context. Evaluation by a mental health provider should be considered when the motivation for seeking surgery and/or expectations are not clear or realistic.
3. Women should not be submitted to labiaplasty before adulthood.
4. There are no data supporting the performance of hymenoplasty, vulvar bleaching/whitening, vaginal tightening procedures, G-spot augmentation and other procedures aimed at increasing sexual function.
5. Cosmetic genital surgery is not exempt from complications.
6. A thorough informed consent must always be obtained.
7. Surgeons performing cosmetic genital surgery should refrain from solicitous advertising or promoting procedures without scientific basis, including on websites.
8. Surgeons must resist patient's pressure to perform surgeries that they do not agree with and explain their rationale/position.
9. The surgeon must be adequately trained and experienced in performing the surgery and have knowledge of the anatomy, physiology and pathophysiology of the vulva, vagina and adjacent organs.

Guidelines for physicians and clear, scientifically correct information for patients must be made available, in order to minimize the number of ineffective or even deleterious interventions in this area.

Talking to Patients about Sexuality: What the Vulvologist Needs to Know

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All women will likely experience some type of sexual change, difficulty, or dysfunction during their lifetime. Due to the symptoms that accompany vulvar disease, these patients are particularly vulnerable to sexual concerns. However, both patients and medical professionals find sexuality difficult to discuss. Lack of discussion can lead to patient and partner distress, incomplete reporting of medical symptoms and possible treatment non-compliance. Because sexual functioning is much more than a physiological experience, but is heavily influenced by psychological, relational, and cultural factors, clinicians must use a bio-psycho-social lens, rather than the traditional biomedical lens, to sensitively understand and assess the full extent of their patients' sexual concerns. This multidimensional approach informs the "who, when, how and what" of a sexual health assessment integrated into the medical assessment. A basic understanding of the delineation of sexual dysfunction according to the DSM 5 (Diagnostic and Statistic Manual) and ICD 10; sexual identity and orientation issues; complexities of relationship dynamics; and the sequelae of physical, emotional or sexual trauma are important to skillful sexual health assessment and treatment. In addition, multidisciplinary collaboration and timely referral for psychological, couple, and/or sex counseling is a valuable and necessary adjunct to medical treatment.

Core Outcome Set Development for Vulvovaginal Disease

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Clinical trials in medical research have been criticized for lack of reproducibility, for difficulty in cross-comparison of studies, and for lack of clearly defined, well-validated, reliable, and responsive outcome measures. No group of women's health conditions calls for greater improvement in research methods than chronic vulvovaginal disorders, one of the most common ambulatory care complaints frequently associated with chronic pain and sexual dysfunction. Ironically, clinical research in chronic vulvovaginal disorders has lagged behind other women's health disciplines in terms of funding availability and overall volume of high-quality clinical research. Independently developed workgroups, diverse in focus, and geographic location, have begun to address improved research in vulvovaginal disorders. However, the various workgroups lack a unifying focus. It is our intention, under the auspices of two major professional societies, ASCCP and the ISSVD, to provide overall direction, organize a venue for expert panel consensus meetings on outcomes measures, and facilitate publication of consensus results. The vulvovaginal Core Outcome Set (COS) initiative will remain relatively broad in scope, covering a spectrum of at least three large disease categories: 1) vulvovaginal pain, 2) vulvar inflammation/dermatoses, and 3) vulvovaginal infections/chronic discharge. Future consensus meetings will cover the disease categories and focus on three major goals: 1) Delimiting clinical conditions within each disease category and identifying core domains within each condition, 2) Identifying and defining core outcome measure instruments within each domain, and 3). Develop networks on which to base future collaborative randomized clinical trials (RCT's) in the respective disease states. Publication of internationally accepted core outcome measures for clinical trials would enable the synthesis of evidence-based practice and facilitate cross-comparison and meta-analysis of studies.

Workshop: "Clinical Research in Vulvar Disease: A Multidisciplinary Approach"

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Workshops's main objectives:

i) To approach and discuss the ethical, legal, and operative framework for clinical research in vulvovaginal disease, focusing on the particular features of the disease and the study population.
ii) To encourage and fortify the Research capacities of all professionals and non-professionals involved in the ISSVD's study topics.

Pivotal points to be covered:

i) Theoretical - conceptual framework of Clinical Research in Vulvar Disease:
- Who should participate in vulvar and vaginal clinical research, how to do it, why and what for?
- Research capacities in women's health. Acquiring generalizable knowledge while protecting human research participants.
- Highlights on CIOMS 2016- International Ethical Guidelines for Health-related Research Involving Humans.
- Clinical Research in vulnerable persons and populations: Women as clinical research participants.

ii) Clinical Research Sites:
- Organize a site in a proper way in order to provide high quality clinical research standards, applying regulations for rights' protection, safety and welfare of human subjects.
- Identify human and technical resources needs to participate in clinical research and take full responsibility for the proper conduct of the study.

iii) Site operative approach: Clinical research from the investigator's point of view:
- How to obtain valuable information, while protecting and respecting your patients?
- What does it mean in practical terms?
- Investigator responsibilities before initiation, during conducting and following the completion of trial.
- Interactions between the investigator and other actors involved in the trial.
- Site organization: training, patient selection, visits, procedures, CRF.
- Investigator reports. Essential documents.
- Trial Master file/Investigator Site File, etc.

iv) Informed consent as:
a) Requirement to satisfy the ethical principle of autonomy.
b) Investment instead of bureaucratic procedure.

A fundamental tool to generate and/or strengthen the patient researcher relationship.

Update: Vulvar Squamous Intraepithelial Lesions

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Epidemiological data are consistent with an increased incidence rate of invasive vulvar squamous cell carcinoma (VSCC) in women less than 50 years. Unfortunately, unlike cervical disease, there is no screening for Vulvar Highgrade Squamous Intraepithelial Lesions (HSIL) that can only be suspected and biopsied during the visual assessment of the vulvar region.

High-resolution dermoscopy has been suggested as a complement of clinical examination, providing an adjunctive tool to biopsy suspected vulvar HSIL, taking into account that unrecognized invasive carcinoma is reported in more than 10% of patients undergoing surgical excision after an office biopsy of vulvar HSIL.

The 2015 ISSVD terminology for vulvar HSIL represents a cornerstone to underline the dual etiology of VSCC precursor lesions and to ask to clinician and pathologists the best efforts to diagnose them.

Studies on HPV type specific distribution, integrated with studies assessing peripheral and in situ lymphocyte function during HPV persistence in vulvar HSIL might play an important prognostic role in identifying the risk of progression in HPV-driven vulvar cancer.

Compared with vulvar HSIL, differentiated Vulvar Intraepithelial Neoplasia (dVIN) is a more rapidly progressing precursor, that does not show p16ink4a overexpression but often reveals TP53 mutations.

Findings on activating mutations in PI3K/AKT/mTOR pathway underline that researches are needed to identify and treat precursor lesions with mutations that can drive HPV-unrelated carcinogenesis.

On the other side - exploration of the safety, tolerability and clinical efficacy of therapeutic HPV vaccines that eliminate HPV transformed cells is one of the greatest challenges for gynecological oncology.

Morphological Vaginal Changes in Cervical Cancer Survivors Affecting Sexual Health

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Objectives: Women who have been treated for cervical cancer have persistent changes in their sexual function, which result in considerable distress. Advances in treatment have resulted in improvement in survival rates and a rising number of cervical cancer survivors. Still, the morphology of the vaginal wall after treatment with radiotherapy and its role in sexual health is unknown. The objective was to investigate the vaginal morphology in cervical cancer survivors treated with radiotherapy.
Methods: We included 34 patients treated for cervical cancer with radiotherapy and 37 healthy age-matched controls. The patients were treated with radiotherapy with or without surgery and/or chemotherapy. Vaginal biopsies were obtained and analyzed by epithelial morphometry and analyses for elastin and collagen content. Clinical examination was performed for estimating atrophy, amount of telangiectasia and pelvic fibrosis. Questionnaires were used for psychosexual evaluation.
Results: The cancer survivors had marked morphological vaginal changes compared to controls. The epithelial measurement showed atrophy as reduced epithelial volume. Elastosis was found in the connective tissue with altered distribution of the elastin fibers. The collagen fibers were of high density and clinical examination showed different degrees of atrophy, telangiectasia, and pelvic fibrosis. Report of physical sexual dysfunction with reduced vaginal elasticity at intercourse, reduced lubrication, and dyspareunia were common.
Conclusions: Women who have been treated with radiotherapy for cervical cancer have morphological changes in the vaginal wall with atrophy and fibrosis as the predominant findings. Early use of topical estrogen and dilators may improve the sexual function in the survivors.

Vulvar Lichen Planus is not Associated with Squamous Cell Carcinoma

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Objectives: To determine the incidence of vulvar lichen planus (LP) with non-human papillomavirus (HPV)-related squamous cell carcinoma (SCC).
Methods: We performed a clinicohistopathologic review of consecutive vulvectomies and wide local excisions for non-HPV related vulvar or vaginal SCC from 2007 to 2016. Clinical data collected included tumor location and dermatologic diagnosis and treatment. Histopathological review included site of SCC, adjacent precursor lesions, and dermatoses.
Results: There were 39 first presentations of primary non-HPV related vulvar SCC treated by excision, but no cases of primary non-HPV related vaginal cancer. 22 (56%) women had a clinical diagnosis of lichen sclerosus (LS); none had a diagnosis of LP. Topical steroids were prescribed intermittently in 16 (41%) and as a long-term maintenance regimen in 2 (5%). Tumors arose from the labia minora, labia majora, and clitoral region, but not from vestibule or perianus. On histopathological review, LS was present in 38/39 (97%) and there was 1 non-specific lichenoid reaction on hair bearing skin. No erosive, hypertrophic, or typical LP was seen. Differentiated vulvar intraepithelial neoplasia (dVIN) was present in 34/39 (87%), 1 had acanthosis with altered differentiation, and 4 (10%) had no precursor lesion. dVIN had areas of basaloid morphology superficially resembling erosive LP in 9 (26%), and had a similar appearance to hypertrophic LP in 3 (9%).
Conclusions: LP was not seen in association with non-HPV related vulvar SCC, while LS is under-recognized and inadequately treated in this group. Pathologists need to be aware that dVIN may resemble erosive or hypertrophic LP.

Vulvar High-Grade Squamous Intraepithelial Lesion (HSIL): Risk Factors for Recurrence after Excisional Therapy

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Objectives: To identify risk factors associated with recurrence of vulvar high grade squamous intraepithelial lesions (HSIL) following excisional therapy.
Methods: We performed a retrospective cohort study of women who underwent excisional therapy for a histologic diagnosis of HSIL of the vulva (vulvar intraepithelial neoplasia (VIN) 2/3/carcinoma in situ) within Southern California Permanente Medical Group between 1995-2007. Terms HSIL and differentiated-type VIN (DVIN) were not used during our study's timeframe; women with VIN 1, or low-grade squamous intraepithelial lesion (LSIL), were excluded. Medical records/pathology were reviewed to determine potential demographic, medical and pathological risk factors for recurrence. Statistical analyses included Chi-squared and Student's t-tests, cumulative incidence analysis, univariate and multivariate logistic regression.
Results: Among the 418 patients who underwent excisional treatment alone, median age was 50.1 years; 69% were white, 13% Hispanic, and 10% black. Margins were positive in 204 (48.8%) and negative in 214 (51.2%). Median follow-up was 89 months. 104 women had recurrence (25%). Of those with positive margins, 41.7% recurred compared to 8.9% with negative margins ($p<0.0001$). Median time to recurrence was 14.3 months for patients with positive margins and 41.8 months for those with negative margins. Significant risk factors for recurrence in multivariate analysis included positive margins (OR, 8.165; 95% CI 4.595 to 14.509), lichen sclerosus (LSA) (OR, 6.914; 95% CI 1.526 to 31.320) or HPV changes (OR, 2.152; 95% CI 1.259 to 3.680) on adjacent pathology, and immunosuppression (OR, 1.949; 95% CI 1.127 to 3.370). Cumulative-incidence analysis revealed that women with positive margins remain at increased risk for recurrence compared to those with negative margins not only early following treatment (14% vs. 1.4% in first 6 months) but also long-term (26.1% vs. 7.4% post 24 months of follow-up).
Conclusions: Positive margins and VIN-adjacent to lichen sclerosus are the strongest predictors of recurrence after excision of HSIL. Patients with positive margins are at increased risk for both, early and delayed recurrence and require close long-term surveillance.

Vaginal and Vulvar Intra-Epithelial Neoplasia in Young Women Attributed to 14 Human Papillomavirus Genotypes

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Objectives: To estimate the proportion of vulvovaginal lesions in young women attributable to human papillomavirus (HPV) types preventable by the 9vHPV vaccine.

Methods: Prospectively diagnosed vulvar and vaginal low- and high-grade squamous intraepithelial lesions (LSILs and HSILs, respectively) among 8,798 women 15–26 years old enrolled in the placebo arms of two phase 3 randomized HPV-vaccine trials. They were analyzed for the presence of 14 HPV genotypes (6/11/16/18/31/33/35/39/45/51/52/56/58/59).
Results: Overall, 40 vulvar LSILs, 46 vulvar HSILs, 118 vaginal LSILs, and 33 vaginal HSILs were detected with approximately 4 years of follow-up. At least one of the 14 types were detected in 72.5%, 91.3%, 61.9%, and 72.7% of these lesions, and multiple HPV types were detected in 40.3%, 30.4%, 24.1%, and 45.2% of the HPV-positive lesions, respectively. After accounting for co-infections, 60.0-67.5% of vulvar LSILs, 76.1-91.3% of vulvar HSILs, 27.1-43.2% of vaginal LSILs, and 42.4-60.6% of vaginal HSILs were attributable to 9vHPV vaccine types. Among the HPV-positive lesions, 89.4% of vulvar LSILs, 100% of vulvar HSILs, 56.0% of vaginal LSILs, and 78.3% of vaginal HSILs were attributable to 9vHPV vaccine types, accounting for 1.7% of vulvar LSILs, 16.1% of vulvar HSILs, 30.8% of vaginal LSILs, and 20.9% of vaginal HSILs (Table).
Conclusions: Widespread uptake of the 9vHPV vaccine could potentially prevent a sizeable fraction of benign and precancerous HPV-related vulvar and vaginal lesions.

Table. Proportionally weighted percentages of potentially vaccine-preventable HPV-positive vulvovaginal lesions.

Vaccine		2vHPV	4vHPV	9vHPV	None[2]
Targeted HPV genotypes[1]		16/18	6/11/16/18	6/11/16/18/31/33/45/52/58	35/39/51/56/59
Vaginal	LSIL	18.8%	25.2%	56.0%	44.0%
	HSIL	53.1%	57.4%	78.3%	21.7%
Vulvar	LSIL	10.7%	87.7%	89.4%	10.6%
	HSIL	74.3%	83.9%	Up to 100.0%	0.0%

[1]A total of 14 HPV types were assessed. Some lesions not categorized as HPV-related could have been caused by other HPV types for which genotyping was not performed. Some HPV types not genotyped could have been present in lesions categorized as HPV-related.
[2]Not covered by any of the 3 vaccines.

Epidemiology of Anogenital Warts in a Group of Argentinian Women and the Impact on their Quality of Life

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Objectives: To study the epidemiology of anogenital warts (AGWs) in an Argentinian population of women and the knowledge the women have about these lesions, as well as the emotional impact on their quality of life.
Methods: Prospective observational case-control study including 158 women (mean age 29 years), 98 women with AGWs and 60 without lesions. Sociodemographic data were collected and analyzed. Women with AGWs were surveyed. To measure quality of life in the group of patients with AGWs a self-administered validated questionnaire in Spanish called cuestionario específico para condilomas acuminados (CECA) was used. It is a specific questionnaire for condylomata acuminata (copyright© Dr. Xavier Badia, 2005) that evaluates 10 items and two areas (emotional and sexual areas).
Results: Of 98 patients with AGWs 66% had lesions for the first time; 34% were a recurrent episode. 91% knew the name of the lesion; 77% had detected themselves with the lesion; 18% said it was detected by a doctor and three patients by their partners. 64% knew what warts were; 84% knew they were contagious and 84% knew that there was a treatment for warts. 76% knew about human papilloma virus (HPV). Only 28% used condoms before having warts, while 66% responded they started using condoms after having condylomata acuminata. 47% feared that the lesions would not go away; 77% were worried about having the infection forever; 63% were worried that AGWs would become complicated; 41% felt anxious, depressed, or sad.
Conclusions: Women with AGWs are likely to feel anxious. They fear that the lesions won't go away. Many women know about AGWs however, few used condoms before they developed these lesions.

Recurrent Yeast Infections and Vulvodynia: Can We Believe Associations Based on Self-Reported Data?

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Objectives: We determined whether self-reported new or recurrent yeast infections were a risk factor for, and/or consequence of vulvodynia, and then determined the extent to which various levels of misclassification of self-reported yeast infections influenced these results.
Methods: In this case-control study we retrospectively assessed self-reported new and recurrent yeast infections prior and subsequent to first vulvar pain onset among 216 clinically confirmed cases, and during a similar time period for 224 general population controls.
Results: A history of >10 yeast infections prior to vulvodynia onset was strongly, but imprecisely associated with developing the disorder after adjustment for age, age at first intercourse and history of urinary tract infections (aOR=5.5, 95% CI: 1.7-17.8). Likewise, a history of vulvodynia was associated with a 2-fold risk of subsequent new or recurrent onset of yeast infections after adjustment for age, age at first intercourse, and history of yeast infections prior to vulvodynia onset (comparable time period among controls, 95%CI 1.5-2.9). Bias analyses showed that our observed associations were an underestimate of the true association when non-differential misclassification of self-reported yeast infections and certain differential misclassification scenarios were present. However, if women with vulvodynia equally or more accurately self-reported yeast infections when they truly had them compared to controls, but more frequently misreported having them when they truly did not, our observed associations may have been an overestimate of the truth.
Conclusions: To better understand the bi-directional associations between yeast infections and vulvodynia, future validation studies are needed to determine the extent to which misclassification of self-reported yeast infections differ between women with and without vulvodynia.

Survey of ISSVD Membership to Evaluate Areas of Confusion in Pathologist-Clinician Communication

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Objectives: Pathologist-clinician communication has been an ongoing topic in the literature. Pathology reports are geared to assisting clinicians with patient therapy, however at times there are barriers to communication. This survey aims to explore clinicians' understanding of their pathology reports within the membership of the International Society for Vulvovaginal Disease- (ISSVD).
Methods: An email survey was sent to all members of the ISSVD.
Results: Surveys were emailed to 397 members, with 91 responding (23%). Most (76%) of the respondents were gynecologists, with 13% dermatologists and 6% advanced practice nurses. 40% of respondents did not always understand their pathology reports, 62% did not know the difference between levels and recuts, 71% were unclear as to why levels rather than recuts would be ordered, and 26% were not familiar with the term "spongiosis". Over 94% read the gross description on a pathology report. Over 90% reported speaking with their pathologist, which they considered important. They considered having a pathologist with specialty expertise important.
Conclusions: Clinician members of the ISSVD are particularly attuned to the importance of pathology consultation in the care of women with vulvovaginal conditions. There are still areas for potential improvement in educational efforts, particularly providing information on how pathology laboratory processes may impact the report, as well as in further education in dermatopathology terminology.

Care of Male-to-Female Transgender Individuals After Sex Reassignment Surgery: Are Gynecologists/Vulvologists Competent?

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Objectives: The gynecologic care of male-to-female (MTF) transgender patients after sex reassignment surgery (SRS) in a setting of vulvar and colposcopy specialists is reported.
Methods: The evaluation of 29 MTF transgender after SRS has been performed at the Center of Colposcopy and Vulvology in the Section of Gynaecology of our department. For evaluation of "neovulva" and "neovagina" of MTF transgender females a protocol called LATTEX (Lower Anogenital Tract Trangender Examination) has been created.
Results: For neovulva and neovagina evaluation, the parameters were both aesthetic-anatomical and clinical-functional (sexual). Results of neovulva examination showed that labia minora were well defined only in 10/29 patients, while labia majora were well defined in 22/29, and looked "abnormal" in 7/29

patients. The vaginal introitus was widely permitting in 4/29 and sufficiently permitting in 15/29 patients, while in 10/29 it was even difficult to examine the vagina. In 5/19 patients, the neovagina contained hair and in 7/19 sebaceous secretions. In 7/19 cases the neovagina was eutrophic and elastic. Vaginal sexual activity was referred as absent in 12/29, sporadic in 8/29, and regular with a stabile partner in 9/29 cases. Sexual desire, activity, and satisfaction were also evaluated before and after surgery.

Conclusions: Subjects with "sexual dysphoria" (DSM-V) are constantly followed in a multidisciplinary setting, according to World Professional Association for Transgender Health (WPATH) 2011 guidelines. Gynecologists play an important role in caring for transgender patients. In particular, colposcopic and vulvoscopic assessment of neovulva and neovagina, in MTF after SRS, need more standardized criteria, which should be also specific for this special condition.

A Randomized Controlled Trial of Gabapentin in Provoked Vulvodynia: Racial Differences

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Supported by a grant from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the Office of Women's Health Research (HD065740), the University of Tennessee General Clinical Research Center (GCRC) and Depomed, Inc. who provided gabapentin extended release and matching placebo for the study.

Objectives: We compared the efficacy of sustained-released gabapentin compared to placebo in the first multicenter, randomized, double-blind, placebo-controlled trial in women with provoked vulvodynia.

Methods: Eighty-nine participants, 18 years or older, reporting insertional dyspareunia or pain to vulvar touch, fulfilled provoked vulvodynia criteria including localized, vulvar vestibular provoked pain confirmed by cotton swab testing. The primary outcome variable was defined as tampon test pain intensity (numeric rating scale, 0-10) during the last 7 days of the maintenance phase using a randomized, blinded, crossover design with modified intention-to-treat analysis.

Results: Subjects, during the gabapentin crossover phase experienced significantly less pain on the tampon test compared to the placebo phase (mean ±SE, 3.83 ±0.47 vs. 4.28 ±0.47, P = 0.03), but the difference was not considered clinically significant. When stratified by race, white subjects experienced significantly less tampon test pain during the gabapentin crossover phase (3.41 ±0.51 vs. 4.55 ±0.52, P = <.01), whereas black women experienced no significant difference in tampon test pain intensity (5.52 ±1.23 vs. 5.62 ±1.23, P = 0.67). The 1.14 reduction in pain intensity in the primary outcome variable in vulvodynia cases in white women was considered a mild treatment effect.

Conclusions: These data suggest that pharmacologic interventions for pain may be dependent on demographic variables, as white women in this study tended to have a better response, albeit mild response, to gabapentin for vulvodynia pain relief as compared to black women.

Dissecting Vulvodynia Sensory Phenotypes with Intradermal Capsaicin

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Supported by grants from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the Office of Women's Health Research (HD065740), the University of Tennessee General Clinical Research Center (GCRC) and Depomed, Inc. who provided gabapentin extended release and matching placebo for the study.

Objectives: Sensory phenotype research may provide insight into provoked vulvodynia (PVD) pain chronicity and comorbid associations. We hypothesize the existence of two PVD sensory phenotypes: with and without measurable post-capsaicin secondary hyperalgesia, an experimental pain index of central sensitization.

Methods: Demographic, medical history, physical exam, and other measures that include intradermal capsaicin testing, were analyzed from the GABA group, an RCT studying gabapentin efficacy for PVD. Post-capsaicin evoked spontaneous pain and secondary hyperalgesia (punctate hyperalgesia and dynamic allodynia) were assessed, among other data points.

Results: Post-capsaicin response segregated into two distinct sensory phenotypes: (0.70) of the cohort with and (0.30) without secondary hyperalgesia. Demographic data and PVD history were indistinguishable by sensory phenotype. Lower genital tract provoked pain and palpable pelvic floor tenderness were also indistinguishable. In contrast, symptoms of overactive bladder, somatic tender point tenderness, measures of depression and anxiety, and screening for sexual abuse were markedly increased in the subgroup displaying capsaicin evoked secondary hyperalgesia. Independent effects on pain threshold/level also were found by racial category but interaction effects between race and sensory phenotypes were minimal. PVD cohort declined further capsaicin experimental pain testing after baseline.

Conclusions: Capsaicin-evoked pain defines two quantifiable PVD sensory phenotypes associated with comorbidity measures and history of sexual abuse. A high discontinuance rate for intradermal capsaicin testing argues for refinement of alternative methods for future research.

Environmental Exposures and Risk of Vulvodynia: Unrecognized Risk Factors

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Objectives: Vulvodynia is a chronic pain condition associated with sensitivity of the vulva, but risk factors are poorly understood. Due to the potential impact of environmental exposures on neurotoxicity and immunoreactivity, we assessed the association between past environmental exposures and risk of vulvodynia.

Methods: Recalled exposures to 28 past environmental chemicals at home or work were queried at the 24-month survey in the longitudinal population-based Woman-to-Woman Health Study. Vulvodynia case status was defined on biannual surveys from baseline through 24 months as 1) meeting case criteria, or 2) having intermediate symptoms, and each was compared to those never having had vulvodynia symptoms. Multinomial regression models were used to evaluate the relationship between vulvodynia status and environmental exposures (assessed individually and aggregated into 8 categories), with and without adjustment for age, ethnicity, and socio-economic status.

Results: Data on 1,585 women met criteria for inclusion (325 vulvodynia cases, 301 with intermediate symptoms, and 959 controls). Eleven of the 28 individual exposures were increased in vulvodynia cases ($p<0.05$ after adjustment), with increased risk associated with the following categories: occupational exposure to solvents or paints (OR=2.1; 95% CI=1.3-3.4), reporting exposures to chemicals used in or around the home (OR= 1.9; 95%CI=1.3-2.8), employment as a housekeeper (OR=1.8; 95%CI=1.2-2.6), and use of rodent poison or mothballs at home (OR=1.4; 95%CI=1.1-1.9).

Conclusions: Past environmental exposures were associated with current/recent vulvodynia. Future research is needed to confirm these findings, to identify specific chemical agents, dose and timing of exposure, and to identify factors associated with increased susceptibility to these toxins.

Systematic Review of Treatment Outcomes for Vulvodynia

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Objectives: Systematically evaluate the literature regarding treatment outcomes for vulvodynia. Identify reported outcomes of vulvodynia treatments and categorize these into 3 core domains: (1) pain; (2) physical/sexual functioning and (3) emotional functioning.

Methods: Databases (MEDLINE via OVID, PubMed, and PsycINFO) were searched using MeSH terms related to vulvodynia/treatment. Studies were included if: English full text; original research published in a peer-review journal; objective of study or first reported outcome was to evaluate the outcome of a vulvodynia treatment; study population included only women >18 years of age with vulvodynia; and the study design was a randomized trial or a well-designed prospective observational case series. Three authors independently sorted all studies. Selected studies were then analyzed.
Results: There were 206 articles identified for full-text screening, 33 of which met the criteria. The most commonly reported outcome was "pain with intercourse" with 25/33 of studies reporting explicitly about this outcome. The most common standardized instrument used to elicit this outcome and general sexual function was the Female Sexual Function Index (13/33). There was a broad range of investigator-derived questions regarding sexual intercourse that varied in format, content and scales used. The McGill Pain Questionnaire was used in 14/33 studies. Clinical outcomes included: pain with Q-tip exam (11/33), pain threshold of vestibule (7/33), and pain with gynecologic exam (5/33). There was a lack of standardization of the method to obtain and report these outcomes. The most common tool used to report emotional outcomes was the Beck's Depression Index (6/33).
Conclusions: A core set of standard vulvodynia treatment outcomes would support the meta-analysis of research in the field.

Vaginal Flora Influences the Risk of Vulvodynia

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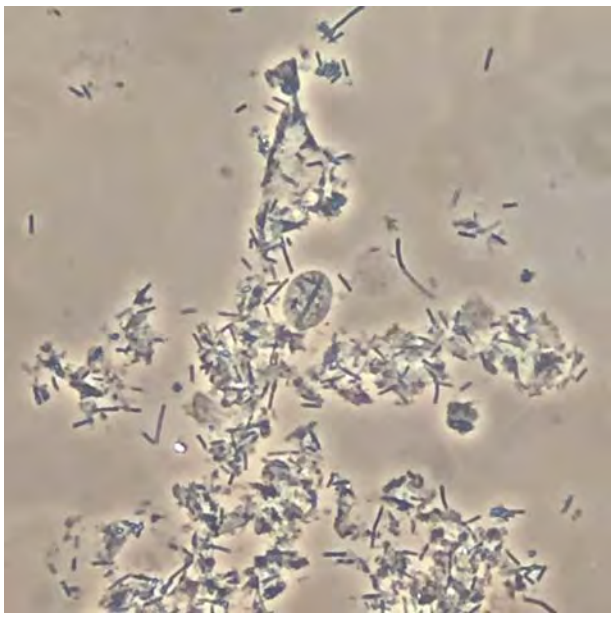
Objectives: The cause(s) of vulvodynia remain largely unresolved. We have postulated that vaginal flora, with inherently different interleukin and pH profiles can play a role in the development or severity of the condition.
Methods: Vaginal slides from vulvodynia patients (97) were compared with an historical control group (women referred for abnormal Pap test or for family planning services). Cases for the control group were randomly age-matched at a ratio 2:1 with the study group. Slides were graded according to Femicare's criteria1. Women in the study group were only considered a case if symptoms (burning/pain) persisted after correction of the first situation.
Results: The presence of bacterial vaginosis and Candida were associated with a lower risk of vulvodynia. On the contrary, cytolytic vaginosis (CyV) (Image 1) was strongly associated with vulvodynia as compared to normal controls (OR 4.6 (CL95 1.89-11.16). See image 2



	Vulvodynia (97)	Control (194)	*OR (CL95)*	*P* value
msAV	10.3%	8.8%	1.197 (0.526-2.723)	0.668
AV (any)	21.6%	20.1%	1.098 (0.604-1.996)	0.759
BV	1.0%	14.9%	0.059 (0.008-0.442)	**0.000**
AVF (LB grade IIb+ III+absent)	33.0%	42.8%	0.658 (0.395-1.096)	0.107
Candida	23.7%	35.6%	0.563 (0.324-0.978)	**0.040**
Cytolytic vaginosis	16.5%	4.1%	4.593 (1.890-11.160)	**0.000**

msAV – moderate or severe aerobic vaginitis; AV –aerobic vaginitis; BV – bacterial vaginosis; AVF – abnormal vaginal flora; LB - lactobacilli

Conclusions: Vaginal flora can affect vulvodynia. This can be due to chronic exposure of the vestibule to very low pH, hydrogen peroxide and acids. The lower risk associated with Candida could be due to a bias as these women are more likely over treated with antifungals. We advise providers to search for CyV in vulvodynia patients and attempt correction if possible, before engaging in more intensive therapies. Also, as moderate/severe Aerobic Vaginitis can be linked to severity of vulvodynia, and CyV to its frequency, wet mount microscopy is fundamental in women with vulvar pain or burning.

Manual Techniques Aimed at Easing Chronic Vulvar Pain Prior to Internal Assessment

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Objectives: Reducing abnormal tension in pelvic floor muscles (PFMs) in women with vestibulodynia (provoked vestibulodynia or PVD) was found by Glazer et al. in 1995 to improve sexual function and decrease complaints of chronic vulvar pain. Multiple studies since have confirmed the presence of abnormal tension in PFMs in women with PVD when compared to matched, asymptomatic controls. Though its pathophysiology remains unclear, PVD may be caused by inciting events in patients' histories. Histories that include recurrent yeast or bacterial infections, urinary or bladder infections, endometriosis, or chronic bowel dysfunction can have a lasting, negative impact, causing PFM dysfunction as well as elevated tension in the affected viscera. Though this may not occur in all, many women with PVD present with both elevated PFM tone and residual abnormal visceral tension. Though PFM dysfunction appears to be a primary driver of chronic vulvar pain, it is possible that the muscular dysfunction is secondary to abnormal tension in the surrounding abdominal and pelvic viscera, fascia, and muscle. Together, these abnormal physical findings make internal assessment, whether digital or with a speculum, painful, difficult at best, or often impossible.
Methods: The faces and verbal responses of 5 women with chronic vulvar pain were recorded by a professional cinematographer prior to, and following instruction in and completion of, 5 patient-performed activities. The activities, meant to decrease tension in abdominal and pelvic viscera, fascia, and muscles, included 1) deep, lateral, diaphragmatic breathing; 2) stretching the urachus on the lower abdominal wall; 3) stretching the deep hip muscles; 4) bridging; and 5) actively contracting and relaxing the PFMs. Changes in subjective vulvar pain were measured using digital palpation at 3, 6, and 9 o'clock on the vulva using the perceived pain index (PPI) of 0-10/10 pain.
Results: All 5 women responded favorably to the simple techniques, reporting immediate increased comfort, decreased palpated vulvar pain, and reduced anxiety. All palpated PPI scores decreased by more than 3 points on the 0-10 scale.
Conclusions: When working with women with PVD using the described techniques, healthcare providers—physicians, nursing professionals, physical therapists—can begin to assure women and to give them hope, while still in the clinic, that their vulvar pain may be manageable from a functional, physical perspective. The techniques introduced are easily transferable to any clinic setting, providing medical practitioners with strategies to immediately decrease tissue tension, reduce anxiety, and lessen palpated vulvar pain prior to performing digital or speculum vaginal exams.

A Much-Needed Model for the Preclinical Testing of New Vulvodynia Therapies

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Objectives: There is no single effective treatment for localized provoked vulvodynia (LPV), the most common cause of dyspareunia. Following our in vitro work that established a link between inflammation and LPV pain, we developed a preclinical model to test new/promising LPV therapeutics and monitor pain/inflammation in vivo.
Methods: We improved an existing mouse model of LPV to assess therapeutic intervention against vulvar pain; we incorporated and validated new techniques (e.g. video monitoring of mouse behavior, the use of an electronic von Frey system for pain testing, weight assessment, and proinflammatory mediator quantification) to facilitate thorough and objective monitoring of pain and inflammation over time.
Results: We established stable allodynia (lasting months) in three mouse strains (one outbred, two inbred); reproducible pain thresholds values were generated using an electronic vonFrey system. During allodynia induction, we found that pain thresholds decreased with weekly subdermal vulvar injections of zymosan (yeast cell wall product), while mice receiving placebo (saline) failed to develop or only developed transient allodynia. In conjunction with reduced pain thresholds, we observed an increase in proinflammatory mediator levels (e.g. prostaglandin E2) within collected vulvovaginal fluids, consistent with previous in vitro findings. After establishing allodynia, we treated with pro-resolving agents that mitigate zymosan-associated inflammation; preliminary results suggest treatment can restore pain thresholds to pre-induction levels.
Conclusions: We have made a significant advance in vulvar pain research through the development of a mouse model to monitor pain and inflammation (in real time), which will facilitate the necessary preclinical testing of new vulvodynia therapies.

Vulvar Cancer: Epidemiology and Treatment in Different Countries

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Vulvar cancer is a rare malignancy with an incidence ranging from 1.6 to 2.4 per 100.000 women. In some countries, an increase in overall incidence rate has been reported: in Germany, the incidence rate has doubled from 1.7 to 3.6 from 1999 to 2011, in Denmark a 1.97% increase in incidence rate per year from 1978 to 2007 has been reported, similarly in USA between 1973 and 2000 a 1.0% per year increase in incidence has been noted. In Australia from 1982 to 2009 a far less increase, from 2.1 to 2.5 per 100.000 women, has been observed.

Surgery still represents the standard treatment for any primary lesion confined to the vulva. However, by the end of the 1980s, the Taussig and Way en block vulvectomy with bilateral groin dissection has been gradually replaced by the 'conservative and individualized approach'. Safe surgical conservative modifications, today unanimously accepted, are: separate skin vulvar-groin incisions, wide local radical excision or partial radical vulvectomy in the case of tumour less than 3-4 cm, omission of groin lymphadenectomy only when the tumor stromal invasion is ≤ 1 mm, unilateral groin lymphadenectomy only in well-lateralized early lesion, and total groin lymphadenectomy with preservation of femoral fascia when full groin resection is needed. Sentinel lymph node biopsy is a reliable method, but should not be routinely employed outside referral centers.

Adjuvant radiation is indicated in presence of groin lymph node metastasis.

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Uncommon Vulvar Lesions

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Objectives: To show uncommon cases of vulvovaginal disease.
Methods: We selected 6 cases of uncommon vulvar diseases seen at the Vulvar Pathology section of the Hospital Italiano of Buenos Aires where the disease diagnosis has been a challenge.
Results: Case 1. Vulvar fixed drug eruption in an 82-year-old woman related to analgesic intramuscular injection. The diagnosis was made by exclusion after ruling out other causes for the vulvar ulcers. The skin lesions resolved immediately when the offending drug was ceased. Case 2. Vulvar pemphigus vegetans (Hallopeau type) in a 56 y.o. patient. The diagnosis was made by clinical presentation, direct immunofluorescence, and histopathology demonstrating hypereosinophilia. Treatment consists of systemic steroids and azathioprine. Case 3. Glomus tumor in a 26 y.o. patient who was evaluated for a painful tumor on the clitoris. The tumor was initially misdiagnosed as an epidermal inclusion cyst. The correct diagnosis was made by histopathology after complete excision of the tumor Case 4. Chondroid syringoma in a 78 y.o. patient. The diagnosis was made by histopathology after removing the entire lesion. Case 5. Ectopic mammary gland tissue in the vulva of a puerperal patient. The diagnosis was made by the clinical presentation, puncture aspiration of a vulvar cyst, and confirmed by histopathology. The treatment consisted of lactation suppression and excision of the remaining cysts. Case 6. Chronic lymphocytic leukemia presenting as vulvar ulcers in a 67 y.o. patient with a history of non B cell Hodgkin lymphoma. Symptomatic treatment was applied locally and systemic management of the leukemia was initiated.
Conclusions: Regardless of their low frequency, we should take these uncommon pathologies into account for differential diagnoses.

Short and Long-Term Efficacy of Focused Ultrasound Therapy for Lichen Simplex Chronicus, Lichen Sclerosus, and Lichen Planus

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Objectives: To investigate the short and long-term efficacy and influential factors of focused ultrasound for the treatment of lichen simplex chronicus, lichen sclerosus, and lichen planus.
Methods: A total of 136 eligible patients with lichen sclerosus and lichen planus were included in this study and treated with focused ultrasound. According to the terminology of the International Society for the Study of Vulvovaginal Disease (ISSVD), 85 of the patients with vulvar disease had lichen simplex chronicus (LSC), 44 patients had lichen sclerosus (VLS), and 7 patients had lichen planus (LP). Patients were followed up regularly after treatment. The efficacy of ultrasound therapy was evaluated based on degrees of itching, physical signs and pathological changes in lesions. The relations between age, course, menopause status, pathological type and improvement with treatment were analyzed. Statistical analysis was performed using the $\chi 2$ (McNemar$\chi 2$) text.
Results: The average follow-up period was 23.8 months (range 3 months to 60 months). 68 of 136 patients fully recovered (cure rate 50%). 59/136 (43.4%) were found to have some improvement resulting in an efficacy of 93.4% (127/136). 6.6% (9/136) found the treatment to be ineffective. 7/127 (5.51%) of the patients with improvement recurred. No severe side effects were found during treatment and no complications were observed during follow-up. The age, course of disease, and status of menopause were related to the efficacy (c2=21.017, P= 0.000; c2=26.591, P= 0.000; c2=8.199, P= 0.000). There was no significant difference in the efficacy of different pathological types (c2=1.635, P= 0.442).
Conclusions: Focused ultrasound is safe and effective for lichen simplex chronicus, lichen sclerosus, and lichen planus. The efficacy is correlated with age, menopause status, and course.

Cytolytic Vaginosis Does Not Have an Impact on Human Papilloma Virus (HPV) Infection and Cervical Dysplasia

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Objectives: Cytololytic vaginosis (CyV) is characterized by an increased number of lactobacilli in the vagina and epithelial cell lysis, most likely due to an excessively low pH. In this study, we examined the hypothesis of the existence of a relation between the premature and increased rupture of epitheliocytes and the risk of cervical dysplasia and HPV infection.

Methods: Consecutive women referred for consultation due to an abnormal Pap test or for family planning services were evaluated using wet mount microscopy, Pap test, HR-HPV test (cobas®, Roche), and biopsy of the transition zone according to current national guidelines.
Results: The prevalence of CyV among the 1.022 evaluated women was 3.1%. There were no differences in the prevalence between women with a normal and an abnormal Pap test (3.5% vs. 2.6%, p=0.4) nor between those with a normal Pap test or with minor abnormalities (ASC-US/LSIL) and more concerning abnormalities (3.2% vs. 2.8%, p=0.8). There were also no differences in the prevalence of CyV in women who were HR-HPV positive vs. those who were negative (2.7% vs. 3.5%, p=0.5); nor in those with or without HPV 16 (3.0% vs. 3.8%, p=0.6) or HPV 18 (3.0% vs. 5.7%, p=0.4). Amongst women who had a cervical biopsy (n=321), CyV was slightly more frequent in high-grade lesions than if histology was CIN1 or less (4.2% vs. 1.8%), but the difference was not statistically significant (p=0.3).
Conclusions: CyV is found in 3.1% in a random population. Opposed to the previously reported association with aerobic vaginitis, our data do not support a relation between CyV and HR-HPV infections or cervical dysplasia.

Multidisciplinary Management of Extramedullary Multiple Myeloma of The Vulva: A Case Report

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Objectives: Extramedullary plasmocytoma (EMP) is an uncommon condition. It rarely occurs on the vulva, with less than 10 cases described in literature.
Methods: A retrospective case report of a patient with a clitoral extramedullary plasmocytoma is presented.
Results: A 39-year-old woman with history of micromolecular multiple myeloma (diagnosed in 2014 and treated with systemic chemotherapy and autograph), was diagnosed in 2015 with an extramedullary relapse in two different sites 1) muscles of the left leg and 2) vulva. The patient was referred to our institution and the clinical examination showed a 2-cm mobile mostly exophytic nodule with a rounded, elastic, smooth surface which involved the lower third of the anterior/right vestibule (fig.1). Evaluation was completed including: i) groin ultrasound, negative for lymphadenopathy ii) pelvic MRI, negative for ureteral infiltration iii) 11C-Methionine PET/CT scan, positive for focal radiotracer uptake and iv) vulvar biopsy, positive for EMP. After multidisciplinary evaluation (gynecologic oncology surgeon, radiation oncologist and hematologist-oncologist) systemic chemotherapy was provided. After completion of the regimen, clinical/instrumental assessments showed a complete muscular response and a stable vulvar lesion. Vulvar surgery was considered feasible. The patient underwent an antero-lateral partial vulvectomy (fig.2). The pathological report confirmed an EMP with negative resection margins. Radiation was not indicated. Function was preserved and the patient was referred for regular follow up. Clinical visits at 3, 6, 9 and 12 months were negative for local-regional recurrence.

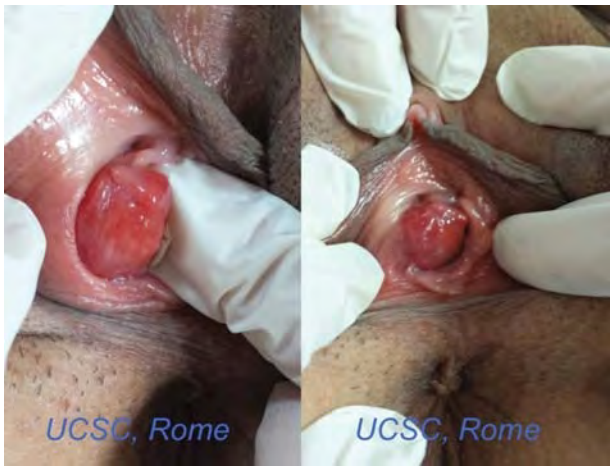

FIGURE 1. Clinical presentation.

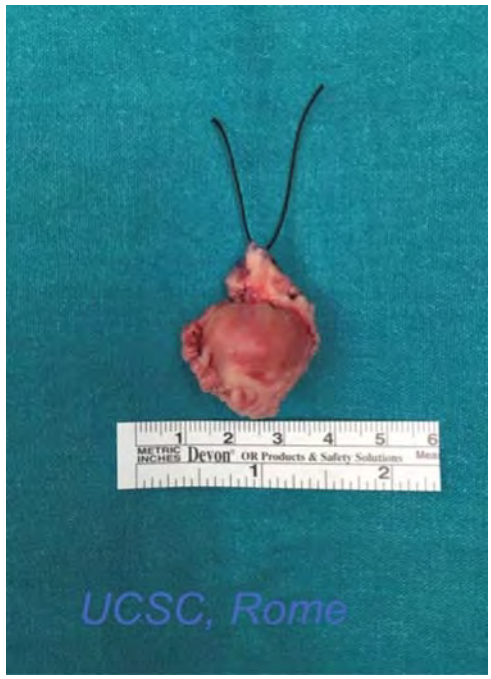

FIGURE 2. Vulvar resection.

Conclusions: The use of a multidisciplinary approach including a combination of chemotherapy and surgery resulted in adequate local and systemic control.

Vulvodynia: Item Development of a Self-Report Outcome Measure for Quality of Pain

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Objectives: The National Institutes of Health initiated the Patient-Reported Outcomes Measurement Information System (PROMIS®) network for uniformly assessing pain in research and patient care; however, a specific outcome measure for vulvodynia pain has not been developed to date. We describe item development of a self-report outcome measure for quality of vulvar pain in women with vulvodynia using the PROMIS® process for item selection.
Methods: The PROMIS® process for item selection includes conduction of a systematic literature review, item classification and selection, item review and revision, focus group input on domain coverage, cognitive interviews with individual items, and final revision before field-testing.
Results: 140 items were identified from widely accepted legacy measures in the literature, including the PROMIS® generic pain measures, McGill Pain Questionnaire, Brief Pain Inventory, and Vulvodynia Pain Assessment Questionnaire. Of those 140 items, a vulvodynia working group identified 51 items and four domains relevant to this condition, including burning pain, incisive pain, sensitivity pain, and emotional response. Five centers will conduct 1) two focus groups of six vulvodynia patients to confirm domain definitions and to add additional items and 2) four cognitive interviews to identify problematic items. Construct and discriminant validity will also be tested.
Conclusions: This measure will capture women's experiences of the quality of vulvar pain in a structured format, and add to the overall understanding of symptoms from affected women's perspectives. This work will advance patient-centered outcomes research and clinical care and guide future development of vulvodynia symptom measures.

The Utility of the Lidocaine Test in the Diagnosis of Localized Provoked Vulvodynia

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Objectives: The purpose of this study was to evaluate the lidocaine test as a means of clarifying the mucosal locus of pain in localized provoked vulvodynia (LPV).
Methods: Reproductive-age patients presenting with dyspareunia and clinical history consistent with LPV were recruited to participate in a prospective cohort study. Demographics and clinical data were recorded. Each participant had a standard cotton swab test (CST) of the vulvar vestibule at 6 points. Participants reported pain using a numeric rating scale 0-10 (NRS). Lidocaine 4% topical solution was then applied to the vestibule for three minutes and the CST repeated. Change in NRS at each point was analyzed by paired t-test.
Results: 16 patients completed the study. Mean age was 28.0 (+/- 4.6) years. Half of the participants (50%; n=8) had primary LPV and the rest had secondary. Participants reported symptoms for an average of 5.8 (+/-4.5) years. A history of abuse was reported by 25%. The majority (n=14) were nulligravid. All examinations were negative for vulvovaginitis and dermatoses. Participants reported highest pain at 4-8 o'clock. Lidocaine significantly reduced pain scores at all 6 points (see table 1.)
Conclusions: Lidocaine significantly reduced vestibular allodynia in paired cotton swab testing in participants with LPV. The finding that lidocaine extinguishes vestibular pain supports the theory that LPV is a superficial neuroproliferative condition of the mucosa. A successful lidocaine test can strengthen the diagnosis of LPV. This is the first detailed explication of this technique in premenopausal women with LPV.

Vulvar Myoepithelial Tumor

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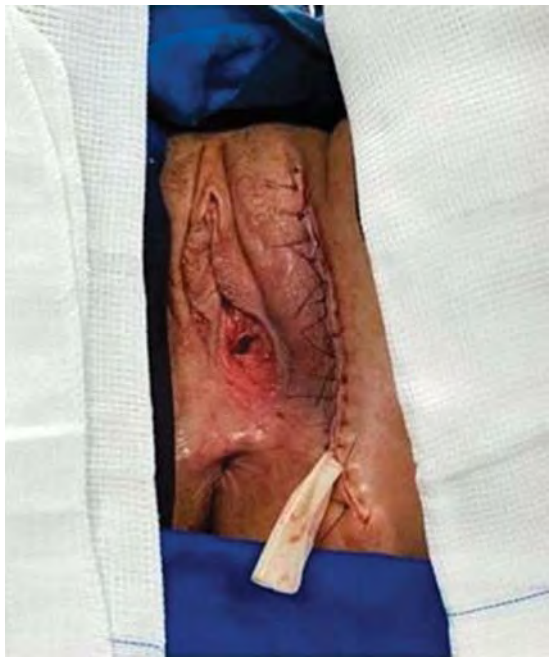
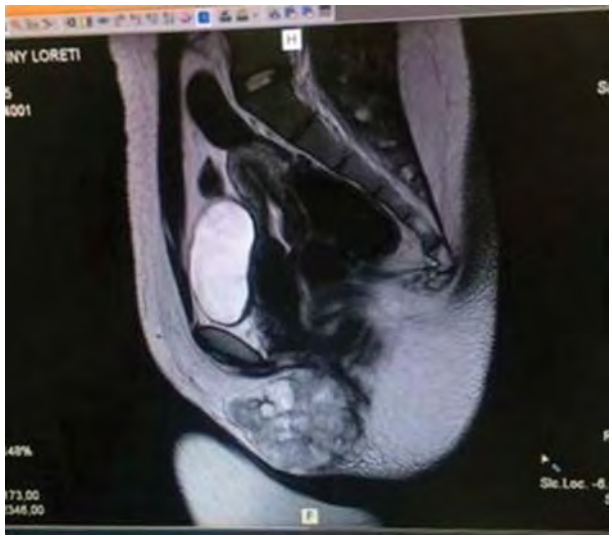
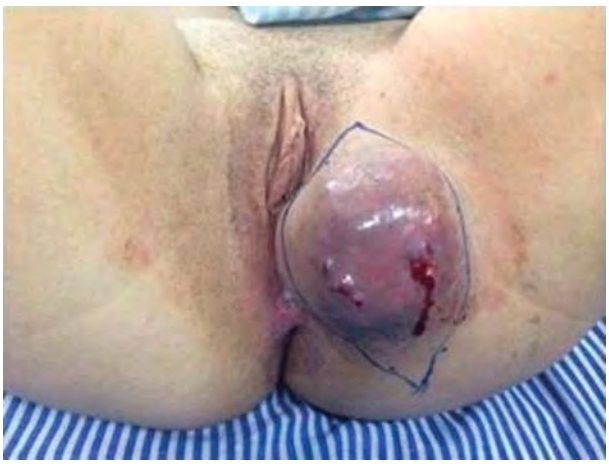
Objectives: A case report of a vulvar myoepithelial tumor.
Methods: Retrospective case report.
Results: A 20-year-old was referred to Antonio Pedro University Hospital (HUAP) with a 3-month history of an enlarging vulvar mass and severe pain. She reported continued/worsening of her symptoms despite antibiotic therapy. An ultrasound 2 months prior to her visit contained a 40.6 x 34.3 mm subcutaneous tumor in the left perineal region. Physical examination confirmed a solid and mobile tumor with inflammatory changes involving the left labium majus, perineum, and inguinal region. There was a central ulcerated area likely secondary to friction. A biopsy was nonspecific with chronic inflammatory changes and lymphedema. Magnetic resonance imaging confirmed a superficial, heterogeneous lesion. Excision was performed with histopathology demonstrating a myoepithelial soft tissue tumor with moderate pleomorphism, suggestive of a myoepithelial carcinoma. The patient was referred to oncology for continued care.
Conclusions: Myoepithelial carcinoma, defined in 1975, is a malignant tumor arising mainly from the parotid gland. Myoepithelial carcinoma of soft tissue is rare compared to its salivary gland homologue and shows a more aggressive behavior with recurrence and metastasis in up to 40-50% of the cases. Given the rarity of this disease and its uncertain prognosis, there are no clinical trials regarding the need for adjuvant therapy. Early diagnosis is essential to optimize treatment and outcome.

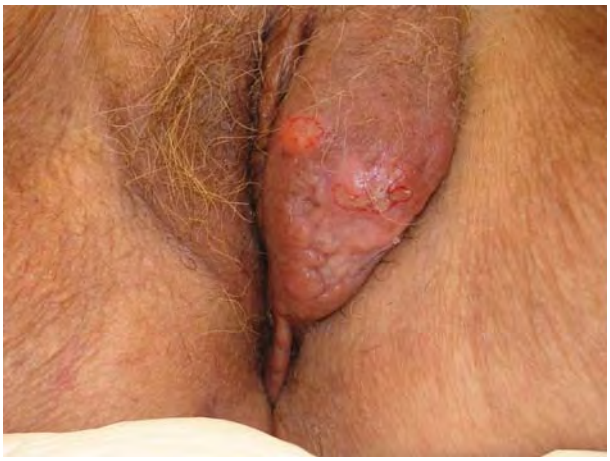


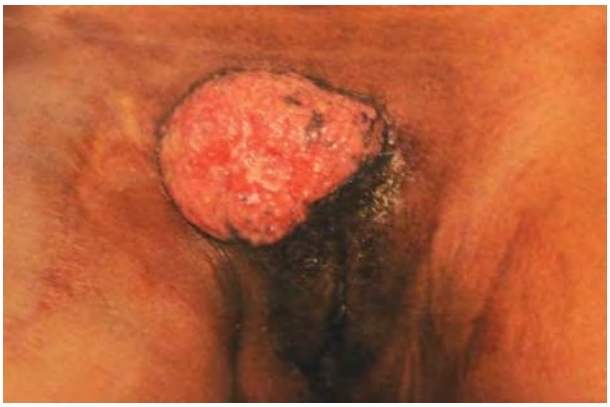


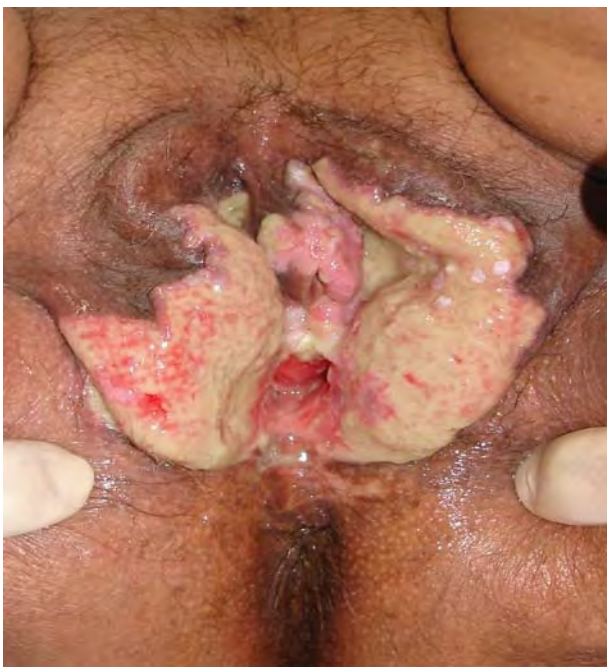


Atypical Manifestations of Genital Herpes in Immunosuppressed Patients

SCAV Fialho, PhD[1,2], ICCV Guimarães, PhD[1], DAS Monteiro[3], BO Vasconcellos[3], JL Xavier[3], and M Rochael[4]. [1]Department of Maternal and Child Health, Hospital Universitário Antônio Pedro, Universidade Federal Fluminense, Niterói, RJ, Brazil. [2]Candidate for Fellowship, Hospital Universitário Antônio Pedro, Universidade Federal Fluminense, Niterói, RJ, Brazil. [3]Residents in Obstetrics and Gynecology, Hospital Universitário Antônio Pedro, Universidade Federal Fluminense, Niterói, RJ, Brazil.







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Objectives: To report 3 cases of immunosuppressed patients with atypical manifestation of genital herpes (HSV) treated at the ambulatory of Vulvar Pathology of the University Hospital Antônio Pedro.

Methods: Retrospective review including 3 immunosuppressed patients with genital herpes treated at a single institution.

Results: Case 1: an 82 year old, with chronic lymphocytic leukemia, referred for evaluation of a vulvar lesion. Upon examination woody edema and painless ulcers with reddish edges and yellowish background, suggestive of fibrin were noted involving the left labium. Additionally a mobile inguinal lymph node was identified on the right. Case 2: a 37 year old, HIV positive patient, referred for evaluation of a vulvar lesion. Upon examination an approximately 8 cm painless, red, soft vegetative lesion with well delimited edges and serous secretion was noted. Additionally mobile and enlarged painful right inguinal lymph node adenopathy was present. Case 3: a 62 year old, with extensive ulcerated lesions on the vulva with purulent secretions and a fetid odor; bilaterally painful inguinal lymph nodes. In each case histopathology confirmed infection caused by herpes virus. In all three cases immunohistochemistry was positive for HSV-1 and HSV-2. All were treated with intravenous acyclovir. One case required intravenous foscarnet for acyclovir resistance.

Conclusions: The herpes simplex virus is the most common cause of genital ulcers in the world, being responsible 40% of the cases in Brazil. Immunosuppression influences the incidence, severity, and presentation of various opportunistic diseases. Resistance is caused by mutations in the viral thymidine kinase or DNA polymerase gene. Most cases of antiviral resistance are susceptible to other drugs that do not require the activation of thymidine kinase, such as foscarnet.

Profiling a Cohort of Vulvodynia Patients

S Johns[1], M Jantos[2], and E Baszak-Radomańska[3]. [1]BNurs, MMid, School of Nursing and Midwifery, University of South Australia. [2]Behavioural Medicine Institute of Australia & Department of Human Anatomy, Medical University of Lublin, Poland. [3]Terpa Clinic, Lublin Poland.

Objectives: To identify characteristics of a vulvodynia cohort, including, age related prevalence, comorbidities, description of pain, sexual history, and to determine differences between women of reproductive age (RA) and post-reproductive age (PRA) in relation to gynecological, urological and gastroenterological symptoms reported.

Methods: This is a retrospective study based on a database of 1143 women diagnosed with vulvodynia. The age ranged between 18-70 years. The study was approved by the University of South Australia's Human Research Ethics Committee.

Results: Vulvodynia prevalence peaked at age 25, with 76.6% of cohort under the age of 35 years. By age 36, prevalence decreased noticeably and plateaued from age 37 onwards as seen in Figure A. A comparison of gynecological, urological and gastroenterological symptoms between RA and PRA women showed a notable increase in incidence of cysts, dysuria, bladder pain syndrome (BPS)/interstitial cystitis, frequency, incontinence and gastro-intestinal

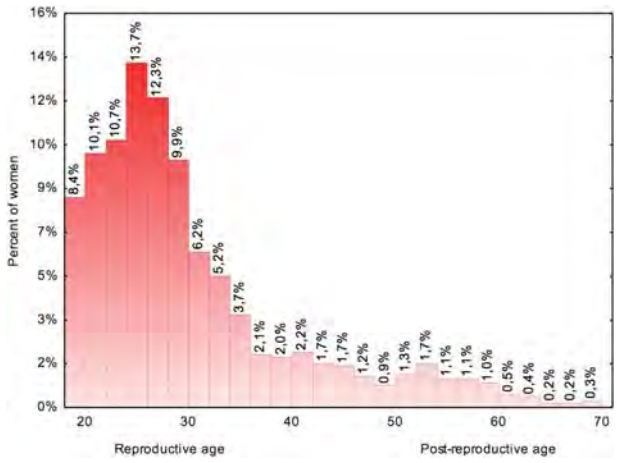

FIGURE A. Age related prevelance

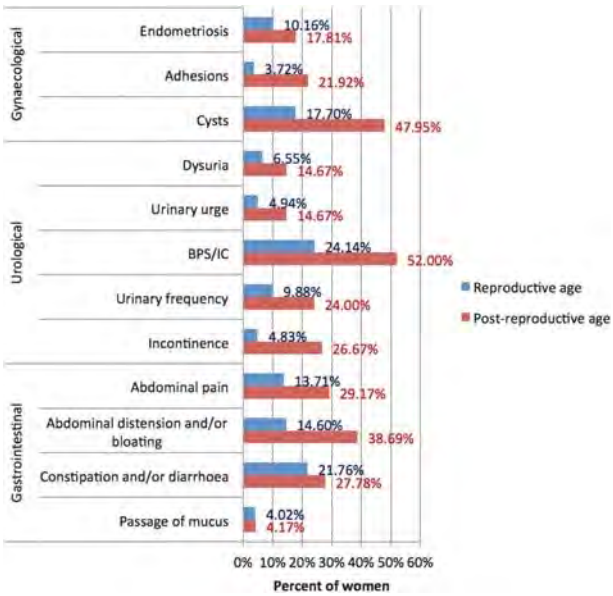

FIGURE B. Comparison of symptoms between reproductive and post-reproductive age women.

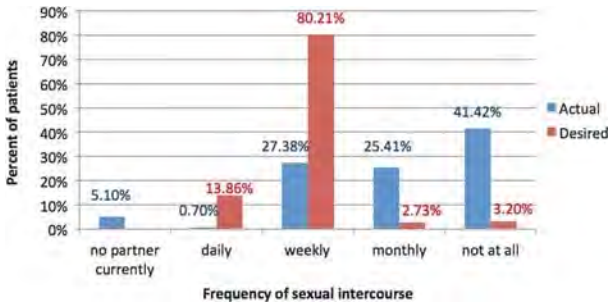

FIGURE C. Comparison of mean pain scores for Vd, BPS and controls

symptoms as shown in Figure B. Analysis of data showed the significant impact that vulvodynia has on quality of life and limitations it imposes on sexual activity as reflected in Figure C. Almost half of women with chronic urethral pain (CUP) were sexually abstinent (41.4%).

Conclusions: This study identifies age related prevalence, symptoms and changes in sexual function that impact vulvodynia sufferers. The findings provide insight into this complex pain syndrome.

Figure C: Difference between actual and desired frequency of intercourse.

Testing the Reliability of Q-tip Criteria in the Diagnosis of Vulvodynia

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Objectives: This study sought to establish the validity of the Q-tip test in the diagnosis of vulvodynia by comparing pain scores from multiple points in the vulvar, pelvic and paraurethral region and comparing them with vulvodynia, asymptomatic controls and general gynecology sample without pain.

Methods: A total of 320 pain maps were analyzed from 238 women with a diagnosis of vulvodynia [119 vulvodynia only, 119 vulvodynia + bladder pain syndrome (BPS)], 29 BPS, 32 asymptomatic controls and 21 general gynecology cases not presenting with pain. A total of 52 points were palpated. The study was approved by the University of South Australia's Human Research Ethics Committee.

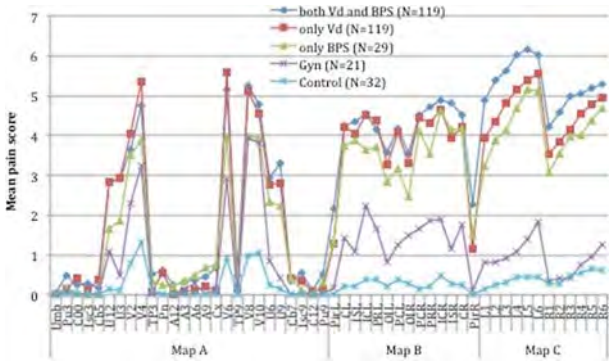

FIGURE 1. Comparison of mean pain scores for Vd, BPS and controls

Results: All groups were comparable in terms of age and parity. Pain scores were considered clinically significant if they were ≥2. A summary of pain scores for all groups is shown in Graph A. In vulvodynia women, the highest scores were recorded in the paraurethral area. The pain scores of the gynecology cases closely approximated those of vulvodynia women. Women in the control group reported pain on all vestibular points (V 2, 4, 6, 8, 10), but were significantly lower than the vestibular pain scores of vulvodynia and general gynecology cases. Regression analysis showed that for a reliable diagnosis of vulvodynia the following points need to examined; vestibule (V6), urethra (U9), pelvic ischial left (ISL) and puborectalis right (PRR), and two left paraurethral points (CL2 and CL5). Graph A. Pain mapping score comparisons for vulvodynia, vulvodynia + BPS, BPS, Gynecology group and Controls.

Conclusions: Past and present diagnostic criteria focus primarily on the mapping of pain in the vulvar vestibule and the inner thighs. Given that asymptomatic women also show hypersensitivity in the vestibule and that general gynecology cases present with significant pain in the vestibule, other points identified in this study must be added for a reliable diagnosis of vulvodynia as reliance of the Q-tip test in the vestibule is not reliable in differentiating between vulvodynia and non-vulvodynia cases.

Vulvodynia and the Validity of its Sub-Classifications: Evidence from Pain Mapping

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Objectives: Using pain mapping, this study seeks to examine the validity of the ISSVD sub-classification of vulvodynia as provoked, spontaneous and mixed.

Methods: A total of 238 women diagnosed with vulvodynia met the inclusion criteria, and were subdivided into provoked (VdP) (n=115), spontaneous (VdS) (n=111), and mixed (VdM) (n=12). Comparison of pain mapping scores with asymptomatic controls (n= 32) and general gynecology patients (n=21) was performed. A total of 52 points were assessed. The study was approved by the University of South Australia's Human Research Ethics Committee.

Results: Significant differences in pain scores between vulvodynia and control groups existed for 36/52 palpation points. When comparing VdP and VdS only two points, which have no diagnostic value, showed significant differences. Vulvodynia subgroups and controls were comparable in terms of age and parity. Graph 1: Comparison of mean pain scores for vulvodynia subgroups and controls

Conclusions: Pain mapping reliably differentiated between vulvodynia and asymptomatic controls. However, the sub-classification of VdP and VdS cannot be distinguished on the basis of pain mapping scores. Functionally, both VdP and VdS cases can identify specific triggers of pain during physical examination and intercourse, but the pain mapping profile of these subgroups is the same. While sub-classification is traditionally based on reported symptoms, pain mapping is based on physical examination. The lack of difference in pain scores between vulvodynia sub-groups makes the distinction between the two groups appear artificial and irrelevant.

Female Genital Mutilation as a Cause of Inclusion Cysts

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Objectives: To review a patient with a vulvar inclusion cyst in the setting of female genital mutilation.

Methods: Results: A 78-year-old multiparous and menopausal woman was brought by her daughter to emergency department, after she noticed that there was a restriction in her physical movement as well as abnormal walking. On examination, patient looked well, not in pain and all vital signs were within the normal ranges. The abdominal examination showed neither masses nor tenderness. Genital examination revealed a huge vulvar inclusion cyst (as demonstrated in the photo) measuring 13x13cm. There were no ulcers or erosions. Surgical excision was performed under spinal anesthesia with transverse rounded incision with confirmation of a vulvar inclusion cyst in the setting of female genital mutilation (FGM).

Conclusions: It is important to consider the multidisciplinary cultural and social complexity of traditional Sudanese community when addressing FGM. Efforts to fight FGM are, and have always been, criticized by conservative community and religious leaders who advocate for Sunna circumcision (excision of prepuce and the clitoris) as part of religion demands. The United Nations Children's Fund (UNICEF) Multiple Indictor Cluster Surveys report of 2014 demonstrated that the 86.6% of women in Sudan from the age group (15 – 49) have undergone a FGM, while 31.5% of girls from the age group (0-14) were victims of FGM.

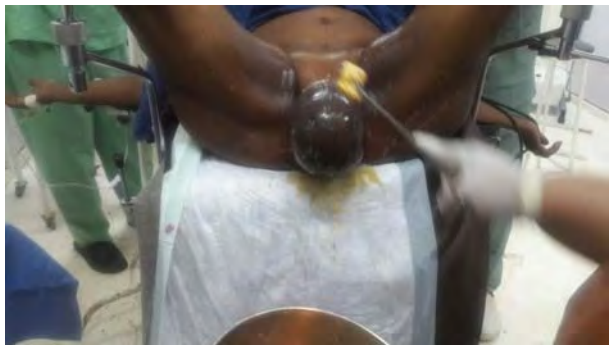

FIGURE 1. A patient with a vulvar inclusion cyst in the setting of female genital mutilation.

ORIGINAL ARTICLE

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Effect of platelet-rich plasma on polypropylene meshes implanted in the rabbit vagina: histological analysis

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ABSTRACT

Purpose: The polypropylene mesh (PPM) is used in many surgical interventions because of its good incorporation and accessibility. However, potential mesh-related complications are common. Platelet-rich plasma (PRP) improves the healing of wounds and is inexpensive. Thus, the purpose of this study was to analyze the effect of the PRP-gel coating of a PPM on inflammation, production of collagen, and smooth muscle in the rabbit vagina.

Materials and Methods: The intervention consisted of a 1.5cm incision and divulsion of the vaginal mucosa for the implantation of a PRP-coated PPM. The PRP-coated mesh was implanted in 15 rabbits, and in the second group, the same implant was used without the PRP coating. In the sham group, the intervention consisted of the incision, divulsion, and suture. The rabbits were euthanized at 7, 30 and 90 days, and full-thickness sagittal sections of the posterior vaginal wall and rectum were scored. The inflammatory infiltrate was evaluated using hematoxylin and eosin staining. The Sirius Red stain was used to examine deposition of collagen I and III, and Masson's trichrome staining was used to visualize the smooth muscle.

Results: The group with PRP-coated meshes had a lower inflammatory infiltrate count at 30 days. Deposition of collagen III increased with the use of PRP-coating at 90 days.

Conclusions: The area of inflammatory infiltrate was significantly increased in the group without the PRP-coated mesh at 30 days but not in the group with the PRP-coated mesh, indicating a less intense inflammatory response. In addition, a significant increase in collagen III occurred at 90 days.

ARTICLE INFO

Keywords:
Platelet-Rich Plasma; Collagen; Rabbits; Inflammation

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INTRODUCTION

The integration between meshes and vaginal tissue depends on the structure of the mesh and on factors such as tissue tropism, infection, and inflammation; these factors are also directly related to the risk of complications (1-4).

Rechberger et al. observed that the serum levels of cytokines are higher in patients with erosion slings than in those with proper healing (5). Similarly, Di Vita et al. demonstrated that the use of polypropylene for hernia repair is associated with high levels of interleukin-6 and interferon, when compared to traditional correction (6). Thus, chronic inflammation with a large foreign body reaction promotes the occurrence of complications.

Previous studies have evaluated coating meshes with materials known for their potential

to accelerate healing and attenuate the inflammatory response and downstream fibrosis and to modulate collagen deposition (7, 8). The composition of these coatings varies from synthetic materials including prophylactic antimicrobials and metal films to biologic materials such as collagen (7-10). Unfortunately, the results are inconsistent.

However, there are few studies on the use of PRP on meshes for the repair of hernias and in Urogynecology. An in vitro study performed using seven types of meshes with PRP showed a reduction in adhesions and improved biocompatibility after 6 weeks (11). In addition, in two experiments, using PRP in a biological loop resulted in less severe adhesions, increased angiogenesis, increased neovascularization, increased integrity of the fabric, and a decrease in the recurrence of hernia in the group with PRP (12).

Similarly, Gerullis et al. conducted an in vitro study comparing polypropylene-coated meshes coated with peripheral blood mononuclear cells, platelets, and plasma. They concluded that the autologous plasma promoted increased biocompatibility of fabrics, justifying in vivo studies (11).

Smooth muscle is often a minor contributor to resisting passive mechanical loading; however, it is extremely important in maintaining vaginal tone and actively resisting the forces of the surrounding connective tissues. Few studies have thoroughly investigated the impact of synthetic meshes on the smooth muscle, also known as functional properties (13).

To improve the understanding of the impact of meshes on the vagina, multiple mechanisms that could affect the properties of the vaginal tissue, like smooth muscle, should be investigated.

The PRP is acquired by centrifuging plasma in order to obtain a platelet and leukocyte concentration 2 to 3 times higher (on average) than the regular plasma and is a clinical option for accelerating the healing process in hernia correction with meshes (14, 15). PRP is easy to obtain at a low cost. Hence, we proposed coating the monofilament polypropylene mesh with PRP to study its effect on the inflammation process and collagen deposition in the vaginas of rabbits.

MATERIALS AND METHODS

The sample consisted of 45 sexually mature, pure-bred, female, white rabbits aged 40 weeks and weighing 4.5kg. A pilot project was performed to test the methods and define the sample numbers. Three groups of fifteen rabbits were randomly generated: sham, vaginal deployment of 1.0cm polypropylene mesh with pores of 1500µm, and the same mesh coated with PRP gel obtained after removal of a sample of 10mL of blood obtained by cardiac puncture (a large blood volume proportional to the animal's weight).

The blood sample was obtained during the implant procedure and was immediately taken to the laboratory for preparation using the same protocol as used for human samples (16). The specimens were anesthetized, and the blood was transferred to a sterile 1.8mL tube containing 0:10mL of citrate as an anticoagulant. The material was homogenized and centrifuged at 24ºC for 10 minutes. After centrifugation, it was possible to distinguish two distinct layers in the tube: the red blood cells at the bottom and the supernatant plasma. All plasma was removed with a pipette and placed in a sterile plastic tube. Additional centrifugation was performed at a speed of 1500rpm at 24°C. After centrifugation, the plasma around the top of the tube was removed, leaving only the portion to which 0.5mL 10% calcium gluconate was added. The solution was homogenized and allowed to stand for 30 minutes, acquiring a gel-like consistency.

Platelet counts were performed in 25% of the plasma samples chosen randomly before and after PRP preparation to confirm the increase in the number of platelets. The gel contained, on average, three times the platelet count of the peripheral blood. A 1–1.5cm vaginal incision was performed, and the implant was inserted, without fixation, to prevent tissue reactions. The mesh was inserted, in a standardized manner, between the vaginal epithelium and the rectovaginal fascia (17), and was coated with PRP gel so that the entire length and the interstice between the mesh pore was filled. The vaginal incision was closed with Vicryl. Penicillin was administered. The sham group underwent an operation consisting of the

same vaginal incision using the same protocol.

The animals were divided into three groups of fifteen animals per group and 5 were euthanized at 7, 30 and 90 days after implantation. All were anesthetized before lethal injection. The implant site was removed en bloc, including the vagina, mesh, and rectum. At each time point, the wounds were harvested and their histologic features were assessed in paraffin-embedded sections using hematoxylin and eosin staining. The Sirius Red stain was performed and samples were assessed using polarized light microscopy, a simple, sensitive, and specific method for quantification of collagen. It is particularly useful for examining the heterogeneity of collagen fibers in connective tissues, providing essential information in pathological studies (18).

One pathologist, who was blinded to the tissue type and time from wounding, evaluated all specimens. The slides were scanned under a microscope. Collagen I and III were assessed using polarized light, by density per micra. The inflammatory infiltrates (INI) and muscle tissue (micra^2) were counted in different fields. Four fragments of the material were placed on each slide.

All statistical analyses were carried out using the SPSS 20.0 system. We analyzed the hypothesis of normal distribution and homogeneity using the Shapiro and Levene tests. Because of a violation of normality, the data were analyzed using the Kruskal-Wallis test, followed by the Bonferroni test for comparisons between groups with and without PRP-coating and for comparisons between different time-points.

RESULTS

An extrusion of polypropylene mesh occurred in each of the groups (with and without PRP-coating); these animals were excluded from the study and replaced. None of the animals died during the observation period. Moreover, none of them presented signs of systemic compromise or procedure-related complications.

Table 1 shows the results (median and interquartile range) at the euthanasia times.

The amount of the inflammatory cells in the first seven days did not become elevated. However, at 30 days, the PRP-coated group had significantly lower levels of inflammatory cells than the group without PRP-coating (Figure-1). After 90 days, the inflammatory response between study groups was indistinguishable. The sham group had significantly lower levels of inflammatory cells at 30 and 90 days than the other groups.

In the group without PRP-coating, the concentration of collagen III did not vary between euthanasia times. In the group with PRP-coating, this value was significantly increased at 90 days (Figure-2).

The collagen I concentration did not vary with time and presence of PRP (Figure-3). The smooth muscle area showed a small increase; however, this was not significant (Figure-4).

DISCUSSION

To our knowledge, this is the first study coating meshes with PRP for vaginal implants. The local inflammatory reaction is an early event that occurs after mesh implantation, and a subsequent foreign body reaction caused by the implant was already established after 3 months and did not significantly change over a 24-month period (11).

In the present study, an acute inflammatory reaction occurred in rabbits implanted with meshes both with and without PRP-coating after seven days, suggesting that the use of PRP did not affect this initial inflammatory process. At 30 days, the PRP-coated group showed a significant reduction in inflammatory cells, suggesting that the PRP-coating shortened the time of the acute inflammatory response, leading to an early tissue repair proliferative phase.

In both in vitro and in vivo studies, Gerullis et al. also noted that the use of plasma on various materials did not influence the early inflammatory reaction (11, 19). However, three months after implantation, markers of tissue vascularization organization (invasion of myofibroblasts and endothelial cells) were detectable and there were differences between the three meshes investigated.

In addition, in the PRP-coated group, at 90 days post-implantation there was a significant increase in type III collagen fibers, the first to be produced in the presence of inflammatory cells.

Tabela 1 - The median and the interquartile range at the euthanasia times, groups with and without PRP. Quantification by cells number (INI) and micra² (collagen type I, type III and smooth muscle).

	Seven days		*p* Value
	Without PRP	**With PRP**	
INI	5.00 (1.00)	3.00 (3.00)	0.09
Ci	2874,01 (2140,42)	3455,19 (1040,62)	0.40
CIII	3060,48 (1094,56)	2398,66 (194,05)	0.26
Muscle	15522,15 (11707.01)	16829,06 (3085,59)	0.49
	30 Days		
INI	**141,00 (173,00) #**	**4,00 (0,00) #**	**0.0175#**
Ci	3463,84 (1836,11)	3846,56 (1614,01)	0.34
CIII	2613,64 (4687,18)	2543,25 (495,76)	0.17
Muscle	10216,80 (2361,56)	15085,41 (8758,95)	0.08
	90 days		
INI	20,00 (12,00)	19,00 (7,00)	0.6
C III	**2304,46 (1383,01) #**	**8617,72 (16671,74) #**	**0.022#**
CI	2247,62 (487,07)	1153,37 (18101,06)	0.098
Musc	6198,46 (1562,64)	10734,65 (9259,69)	0.061

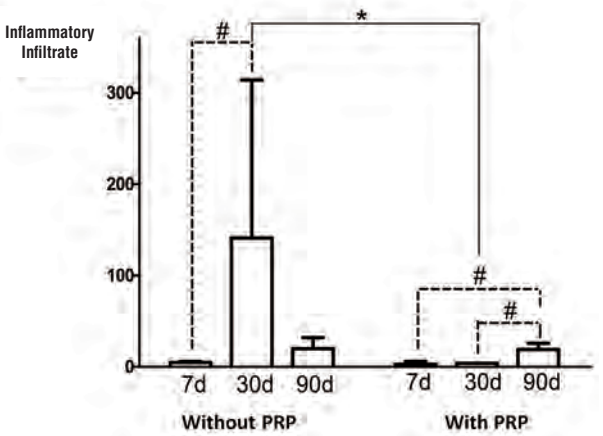
INI = Inflammatory infiltrate; **C III** = Colagen III; **C I** = Colagen I; **musc** = muscle
#*P*<0.05 without PRP x with PRP Mann-Whitney test;
**P*<0.05: Comparing days; Kruskall-Walis Test.

The higher presence of immature collagen (III) in the PRP-coated group at 90 days suggests that the wound was in the phase characterized by Schultz et al. as contraction and remodeling (20). It has been shown that premature type III collagen is predominantly synthesized in the early phases of wound healing and in the presence of inflammatory cells. Collagen III is then replaced by highly cross-linked and stable collagen type I later after implantation, and this slowly increases the tissue tensile strength.

Remodeling of the extracellular matrix is essential for implant integration, and the mesh-induced foreign body responses must be balanced to result in normal wound healing. Swift and adequate tissue ingrowth into the mesh results in superior biocompatibility and likely improves the clinical performance. Intense or prolonged inflammation and bad infiltration, resulting in scar plate formation, can be accompanied by shrinkage or deformation of the biomaterial, recurrence, adhesion, fistula, or erosion of nearby tissue (21).

The vagina is comprised of both passive (collagen) and functional (smooth muscle) components. To date, studies have thoroughly investigated the impact of synthetic meshes on the active properties of the vagina. Tissue degeneration was found to be in large part related to mesh stiffness (22). Liang et al. showed that following implantation with a stiffer mesh, the vagina demonstrated evidence of a maladaptive remodeling response (23). This is characterized by the thinning of the smooth muscle layer, increased cell apoptosis, increased collagenase activity, decreased collagen and elastin content, and increased glycosaminoglycan content. Furthermore, Jallah et al. obser-

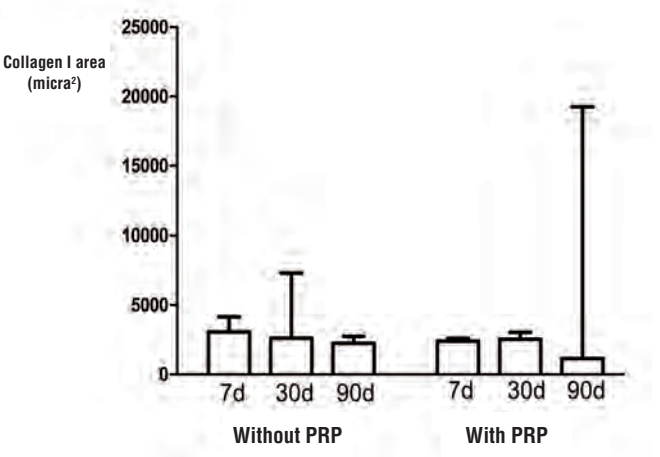
Figure 1 - Inflammatory infiltrate (INI) area at different time points in groups with and without PRP-coating.



*p<0.05 without PRP-coating versus with PRP-coating at 30 days (Mann-)

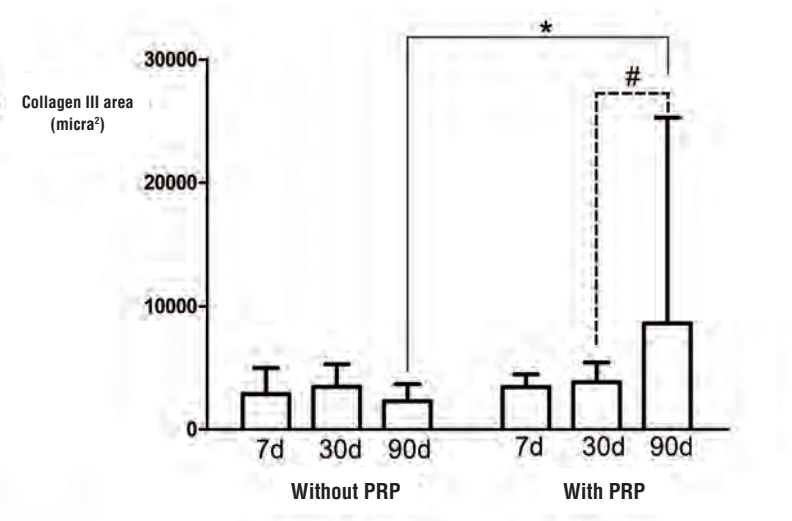
#p<0.05 without PRP-coating, comparisons between 7 and 30 days, Group with PRP-coating: comparisons between 7 and 30 days and 7 and 90 days (Kruskal)

Figure 3 - Comparisons of the median of collagen I area for the rabbits sacrificed at 7, 30 and 90 days.



PRP = Platelet-rich plasma.

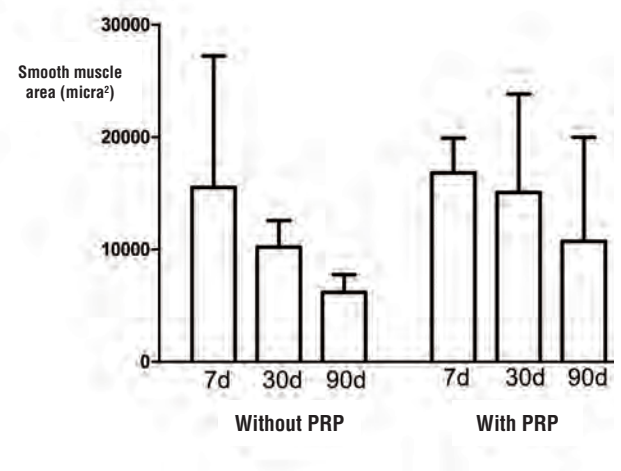
Figure 2 - Comparisons between the median collagen III areas in rabbits sacrificed at 7, 30 and 90 days.



ved that the mesh has an overall negative impact on vaginal smooth muscle function. In this study, unfortunately, the PRP-coating did not change the size of the vaginal smooth muscle area (13).

As with any animal research, the extrapolation of the results to clinical practice should be carefully considered, but the vaginal implant model is an advantage of this study (24). The inflammatory response and repair go far beyond the type of mesh deployed; each tissue and implant site responds differently to aggression. Abdominal implants, for example,

Figure 4 - Comparisons of the median of smooth muscle area for the rabbits sacrificed at 7, 30 and 90 days.



PRP = Platelet-rich plasma.

are placed in a sterile environment, with vastly different biomechanics than the vaginal implants, which are put into a potentially contaminated environment (25, 26). Thus, the use of implants in rabbits and vaginal mucosa are important points in our study.

The rabbit is considered a useful animal model for vaginal implants but is not a large primate mode. The rabbit's vagina has two portions; the inner is more akin to small intestinal histology, but the wider segment of the external vaginal wall makes a suitable model for histocompatibility studies (26).

A possible limitation in this study is the age of the rabbits; all were of reproductive age and had a good vaginal trophism. It is known that hipoestrogenic vaginal mucosa is less receptive to implantation meshes, increasing the rates of complications (27). Likewise, postoperative estrogen replacement for eight weeks in rabbits increased collagen deposition in the vaginal mesh implantation (28). However, there is data that suggests that the PRP would have an even more positive action in this type of animal. Abramov et al. observed in spayed rabbits that collagen production is diminished in the healing of the vaginal mucosa and there is increased inflammation (29).

Another limitation of this study was that only one mesh type was investigated. We chose a monofilament and macroporous polypropylene mesh because it is the most accepted design based on the literature and is used in surgeries (30). A study about the different structural weights and pore sizes is one possible continuation of this research. It is also necessary for better understanding the action of PRP on the enzymatic and immunological processes involved in mesh integration.

Moreover, before clinical implementation, it is necessary to conduct further studies evaluating the use of PRP-coating on mesh implants in vaginas of oophorectomized, older, and multiparous animals.

CONCLUSIONS

The inflammatory infiltrate area did not elevate in the group with platelet-rich plasma, at 30 days, indicating a less intense inflammatory response. Also, a significant increase of collagen type III occurred at 90 days of the study in the group with platelet-rich plasma.

CONFLICT OF INTEREST

None declared.

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medical sciences

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Review

A Review of Current and Emerging Therapeutic Options for Erectile Dysfunction

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Abstract: Contemporary treatment algorithms for erectile dysfunction (ED) involve the use of medical therapies such as phosphodiesterase type 5 (PDE5) inhibitors and intracavernosal injection therapy of vasoactive agents, as well as vacuum erection devices and penile prosthesis implants in medically refractory cases. However, the current therapeutic options only address the symptoms of ED and not the underlying pathogenesis that results in ED. Newer and novel ED therapies aspire to reverse ED conditions by preventing cavernosal fibrosis, promoting endothelial revascularization and modulating various neuro-hormonal pathways. Regenerative therapeutic strategies such as low-intensity shock wave, gene and cellular-based therapies, and penile transplants are designed to improve penile hemodynamics and revitalize the cavernosal smooth muscle to mitigate and/or reverse underlying ED. This state-of-art article evaluates current and emerging therapeutic options for ED.

Keywords: erectile dysfunction; phosphodiesterase type 5; intracavernosal injection; penile prosthesis implant; low-intensity shock wave; stem cell; gene therapy; platelet rich plasma; microvascular stent; penile transplant

1. Introduction

While the introduction of oral phosphodiesterase type 5 (PDE5) inhibitors revolutionized the management of erectile dysfunction (ED) since 1998, they are not always effective since the development and progression of ED is frequently attributable to both psychogenic factors and physiological alterations in various neural, vascular, hormonal and endothelial functions. Recent epidemiological studies have highlighted the correlation between ED and underlying cardiovascular and metabolic risk factors [1], and international guidelines advocate optimization of underlying medical comorbidities as the first line therapy for men presenting with ED [2,3].

In recent years, there have been significant advances made in the field of sexual medicine in terms of our understanding of the underlying molecular biology and neuro-humoral mechanisms governing male sexual function. In contrast to existing therapeutic approaches, newer and innovative ED therapies aspire to prevent underlying cavernosal fibrosis, promote endothelial revascularization and modulate the neuro-hormonal pathway with angiogenic and tissue growth factors (see Table 1). Gene and cellular-based therapies are designed to act on the molecular level to improve specific cellular and enzymatic functions to mitigate and/or reverse underlying ED. The following article evaluates the current standard treatment strategies as well as emerging novel and innovative therapeutic options in ED.

2. Current Standard Erectile Dysfunction Therapy

2.1. Oral Phosphodiesterase Type 5 Inhibitor (PDE5i) Therapy

The clinical efficacy and safety profile of PDE5i has been reported by numerous high-quality, well-designed, blinded, randomized controlled trials comparing PDE5i both to placebo and to other PDE5i drugs [3–6]. Overall, oral PDE5i in recommended doses is an effective medical therapy for ED with an excellent safety profile and is generally well tolerated. The underlying causative factor for ED such as radical prostatectomy [7] or radiation [8] and the presence of significant medical comorbidities with diabetes [9,10] can affect the success rate of PDE5i. Next-generation PDE5i drugs including avanafil (Stendra®), mirodenafil (Mvix®), lodenafil (Helleva®), and udenafil (Zydena®) work in a similar manner to increase nitric oxide (NO) concentration and have relatively similar side effect profiles.

It is important to note that PDE5i only works with sexual stimulation, and despite the initial success in 65–70% of patients, 30–40% do not respond to PDE5i alone, so alternative strategies must be considered to enhance the response rate [11]. Realistic expectations should be set, and patients should be encouraged to give the medications a chance to work and be aware of the dietary restrictions and latency time between ingestion and drug effect. In some patients with chronic ED, additional exposure of PDE5i drugs over several occasions might be required before a satisfactory response is achieved. Awareness of the patient and partner's sexual script will often be useful in determining both the choice of medication and the dosing strategy used [12].

2.2. Intracavernosal Injection

Intracavernosal vasoactive drug injection (ICI) therapy is an effective alternative with minimal systemic side effects compared to oral ED therapy [13]. Common vasoactive agents are prostaglandin E1 (PGE1), which stimulates cyclic adenosine monophosphate (cAMP); papaverine, a non-selective PDE5i; and phentolamine, which is a non-selective alpha-adrenergic antagonist that inhibits smooth muscle contraction. PGE1 can be used as a monotherapy or in combination with other vasoactive agents [3]. More recently aviptadil, a synthetic vasoactive intestinal polypeptide (VIP) that increases the activity of adenosine cyclase, was introduced and is available as a combination of aviptadil/phentolamine (Invicorp©) [14].

Clinical guidelines advocate the use of combination intracavernosal therapy as an alternative to monotherapy due to its more favorable side-effect profile and higher efficacy [3,15]. ICI injections are a moderately invasive therapeutic option and require a degree of manual dexterity, from the patient or partner, with education to learn the mechanics of self-injection. It is recommended that patient counselling is pivotal due to high discontinuation rates and risk of priapism, and education on ICI administration techniques and regular patient follow-up are equally important. Discontinuation rates are typically greatest within 3–6 months of commencement and are usually due to factors such as pain, fibrosis, lack of a sexual partner, loss of spontaneity and anxiety [16].

2.3. Topical Drugs

Intraurethral alprostadil, often marketed as the Medicated Urethral System for Erection (MUSE), is a single-use pellet containing alprostadil suspended in polyethylene glycol administered using an applicator. Data from key clinical studies of intraurethral alprostadil show that it has a fast onset and a good safety profile, with no risk of penile priapism, fibrosis (as seen with intracavernosal injection) or other typical systemic effects observed with oral ED drugs [17]. While intraurethral alprostadil has been associated with good clinical efficacy and ease of use, it can cause urethral irritation and dysuria [18].

More recently, topical alprostadil cream has been tested and was reported to have similar clinical efficacy to that of intraurethral application [19]. Using a specialized dispenser, alprostadil cream

(Vitaros®) appeared to be more effective than the standard administration method and there was no difference in terms of local and systemic side effects [20].

2.4. Penile Prosthesis Implant

The advent of the modern penile prosthetic in 1973 offered a "real" treatment option for men with ED for the first time [21]. The non-inflatable or malleable penile prosthesis usually consists of a pair of rods which can be bent upright or downward depending on its use. The most common malleable prostheses are the American Medical Systems (AMS) 600, AMS 650 and Spectra (Boston Scientific, Minnetonka, MN, USA) and Coloplast Genesis (Coloplast, Minneapolis, MN, USA), while others such as the Silimed penile prosthesis (Brazil), Shah penile implant (India), Promedon Tube prosthesis (Argentina) and Zephyr ZSI 100 (Switzerland) have had limited commercial success [22]. The malleable penile prosthesis is an ideal option for those who are physically handicapped with poor hand dexterity or limited finger movement, complain of muscle fatigue (as in neurological disorders), or have limited reach or range of mobility (e.g., spinal patients). While malleable penile prosthesis implants have poor concealment, they are cheaper than inflatable penile prosthesis and have a lower mechanical failure rate due to their minimal components [23].

The Boston Scientific AMS 700 series (the AMS 700 LGX, AMS 700 CX and AMS 700 CXR) and Coloplast Titan device are the two main inflatable prostheses in the market, although the Zephyr ZSI 475 (Zephyr Surgical Implants SRAL, Geneva, Switzerland) is rapidly gaining popularity in certain parts of Europe [22]. The inflatable penile prosthesis implant is considered a superior option to the malleable prosthesis as it produces penile rigidity and flaccidity that closely replicates normal erectile function. Appropriate patient selection and counselling, strict adherence to antimicrobial prophylaxis, and safe surgical practice are paramount to ensure low complication and high patient satisfaction rates. Pre-operative patient counselling is essential to address any unrealistic expectations (including penile length loss associated with ED) and adequately inform patients of potential surgical complications in order to optimize post-operative satisfaction. Innovations in prosthetic technology and advances in surgical techniques have resulted in excellent clinical outcomes, mechanical reliability and high patient satisfaction [24].

2.5. Penile Revascularization

From the first penile revascularization surgery performed by Michal in 1972 [25] to microvascular anastomosis refinement by Virag a decade later [26], there have been numerous controversies due to the absence of large prospective and well-controlled studies and the fact that penile prosthesis implants appear to be a more effective solution. Nonetheless, penile revascularization may be offered to younger non-diabetic men (<55 years) with isolated arterial stenosis, usually in the setting of pelvic trauma and without generalized vascular disease. The surgical principle of penile revascularization includes anastomosis of the inferior epigastric artery to the dorsal penile arteries and/or the deep dorsal vein. In contrast, penile venous ligation has largely been abandoned due to poor long-term effect and high complication rates [27].

Extensive vascular evaluation is necessary to define the exact nature of vascular injury and stenosis since standardized criteria for patient selection, follow-up protocol and success definition have yet to be identified. Patients should be counselled regarding the future development of ED and serious complications such as priapism, glans hyperemia, vascular and harvest site complications are not uncommon and can be devastating. Where possible, physiological penile revascularization procedures are preferred and surgical technique should be individualized depending on the pathological findings in each case given that anatomical variations of the penile artery are common.

3. Emerging and Innovative Erectile Dysfunction Strategies

3.1. Novel Drugs and Drug Delivery Systems

Guanylate cyclase (GC) activators have been shown to increase NO levels and promote vasodilation [28]. In contrast to the classical NO donors, BAY 60-2770 is a soluble GC activator that increases cyclic guanosine monophosphate (cGMP) levels even when there is reduced NO bioavailability (e.g., following pelvic nerve injury) [29] and may offer advantages over conventional PDE5i drugs for treating patients with ED and extensive endothelial damage [30].

Several studies have highlighted important roles for GTPase RhoA and its effector, Rho-associated kinase (Rho-kinase) in the calcium-independent regulation of smooth muscle contraction and penile erection [31]. The literature shows that an elevated RhoA/Rho-kinase activity contributes to the pathogenesis of ED which is common in diabetes, ageing men and hypogonadism [32]. Several RhoA/Rho-kinase inhibitors have been tested in various animal models and showed promising outcomes in experimental models. Chronic treatment with fasudil, an oral RhoA/Rho-kinase inhibitor, prevents the development of both vasculogenic ED and pelvic atherosclerosis [33] while the Rho-kinase inhibitor Y27632 (unlike sildenafil) is largely independent of endothelial NO activity and may be a good option in diabetic and hypertensive patients where endothelial NO activity is usually impaired [34].

During the last decade, innovative drug delivery systems, including orally disintegrating formulations (ODF), have been developed as an alternative to conventional marketed dosage formulations to improve patient convenience and acceptability and enhance compliance [35]. In more recent years, ODFs have been developed with the aim of enhancing clinical efficacy and mechanical strength as well as improving patient compliance and acceptability over conventional solid dosing forms. Both oral dispersible sildenafil [36] and vardenafil [37] have been manufactured with some commercial success.

Solid lipid nanoparticles are lipid carriers that can greatly enhance drug solubility and bioavailability [38] and a variety of lipid vesicles such as ethosomes, transfersomes and penetration enhancing lipid vesicles have been designed with the objective of higher encapsulation rate and higher stability, such as solid lipid nanoparticles and nanostructured lipid nanocarriers [39]. The transdermal bioavailability of a vardenafil nanoethosome film was reported to be two-fold higher than the oral bioavailability from an aqueous suspension [40]. Papaverine with lyotropic liquid crystals has been shown to provide an effective alternative to injectable formulations both in vitro and ex vivo [41].

3.2. Low-Intensity Extracorporeal Shockwave Therapy (LIESWT)

The proposed mechanisms of action of low-intensity extracorporeal shock wave therapy (LIESWT) to promote neovascularization are thought to be related to the release of various angiogenic growth factors and activation tissue repair functions such as enhanced macrophage activity, alterations in cellular apoptosis, greater synthesis of cellular proteins and activation as well as enhanced recruitment and subsequent differentiation of stem/progenitor cells [42,43]. There is strong emerging literature to support the use of LIESWT, especially in vasculogenic ED, with many clinical studies reporting encouraging results in the use of LIESWT with improved erectile function, a good safety record and short-term durability [44,45]. The therapeutic efficacy of LIESWT in erectile function recovery appeared to last at least three months during the duration of study and patients with mild/moderate ED reported higher therapeutic efficacy than patients with more severe ED or those with multiple medical comorbidities.

While current clinical studies show that the stimulation effects and therapeutic mechanisms among LIESWT machines are similar, regardless of the physical differences and the treatment template, it remains unknown if one machine is superior to another [46]. Furthermore, there is a need to define the optimal LIESWT treatment protocol including the ideal treatment template, the modality of shock wave energy, the emission frequency and the total energy delivery. More stringent randomized controlled

trials with longer-term follow-ups are warranted before LIESWT technology is accepted as the standard of care in ED.

3.3. Cellular-Based Therapy

Scientific research into stem cell (SC) therapy has advanced rapidly in the last decade with an increased volume of publications resulting in the clinical translation of SC-based interventions, especially in the field of erectile dysfunction [47]. While a SC is a progenitor cell that has the capability to turn into any cell lineage, the likely mechanism for erectile recovery appears to involve neuronal preservation and cytoprotection by the inhibition of cellular apoptosis [48]. Adipose tissue-derived SC (ADSC) are the most widely used SC type in ED research given that they are easy to obtain from abundant tissue sources [49]. Proposed methods to enhance the therapeutic effects of SC including the use of certain growth factors (e.g., vascular endothelial growth factor (VEGF)-transfected ADSCs) [50], gene manipulation (e.g., the KCNMA1 gene in cellular excitement and neurotransmitter release) [51] and matrixen [52]. Clinical trials in human subjects showed that SC injection is well tolerated and appears to improve erectile function in men following radical prostatectomy [53] and diabetic ED [54] with no significant adverse reactions. In another study, in vitro preconditioning of ADSCs could accelerate functional and structural recovery in vivo, indicating that preconditioning by inhibition of PDE5 may improve ADSC therapy following diabetes-induced ED [55].

On the other hand, platelet-rich plasma (PRP) is an autologous product obtained from whole blood, containing high concentrations of platelet growth factors [56], and provides a fibrin framework over platelets that has the potential to support a regenerative matrix [57]. While existing human clinical trials showed that PRP is well-tolerated, safe, and provides a feasible treatment modality in patients with ED [58,59], there is a need for standardization of PRP processing methods [60] and higher quality randomized controlled trials with larger patient samples and longer-term follow up [61]. Co-administration of mesenchymal SC and platelet lysate in men with ED appears promising and safe [62].

While rapid advances of SC clinical trials are paving the clinical pathway for an emergent new medicine that may one day replace current medical and surgical interventions, there are serious considerations with regards to longer-term clinical efficacy and safety profile as well as the quality of published SC trials. Even when SCs are administered directly, they might not always migrate or differentiate as desired, either of which could create a risk of harm, in addition to compromising the effectiveness of the treatment. Safety concerns such as the risk of malignant degeneration and proliferation of transplanted stem cells (including possible genomic or epigenetic changes) in the longer term, and infection (including zoonotic infections with virus integration), as well as potential immune reactions, need to be identified in more stringent clinical trials. Appropriate government regulation of these regenerative therapies is essential given scientific and clinical analysis for the safe development and implementation of the rapidly expanding landscape of local bioactive agents and regenerative cell therapy.

3.4. Gene Therapy

Gene therapy involves the replacement or upregulation of specific genes to repair tissue damage and regenerate nerves. At present, gene therapy for ED can be divided into three main components: activators of the nitrergic–neural system, endothelial growth factors (GFs) promoters, and modulators of ion channels in smooth muscle cells [31]. Various growth factors have been explored as gene therapies for ED including brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF), usually delivered via a vector such as adeno-associated and herpes simplex viruses [63]. While various preclinical investigations have been conducted on gene therapy to treat various causes of ED such as ageing, diabetes and cavernous nerve injury (CNI) animal models with relatively good outcomes, it remains far from reality whether gene therapy can be adopted as the standard of care in humans. So far, the only human phase 1 trial was published more than 15 years ago [64].

While the outcomes of these neuromodulatory GFs as a possible therapeutic option in the management of ED in various animal models have been promising, numerous unanswered concerns remain especially regarding long term outcomes and safety issues such as the risk of an excessive inflammatory response, transgenic infection and carcinogenesis. In addition to the vector delivery system, various tissue promoters and enhancers are required to improve the efficacy of gene therapy and potentially minimize adverse effects. Future studies need to be conducted to examine the efficacy of one GF agent over another, proper dosage of the GF agent, the exact mechanism of delivery and ideal time course for therapy delivery.

3.5. Vascular Stent

Coronary artery stents have revolutionized the treatment of men with ischemic heart disease and this minimally invasive endovascular intervention has offered many patients who are frail and not suitable for open cardiac bypass a renewed hope. Given the strong correlation between the presence of angiographic coronary artery and internal pudendal artery disease in men with ED [65], attempts have been made to perform microvascular stenting to treat men with arteriogenic ED. Clinical trials such as the Pelvic Angiography in Non-responders to PDE5 Inhibitors (PANPI trial), the Zotarolimus–Eluting Peripheral stent system for the treatment of erectile dysfunction in males with suboptimal response to PDE5 inhibitors (ZEN trial) and the Incidence of Male Pudendal Artery Stenosis in Suboptimal Erections Study (IMPASSE trial) are designed to evaluate the angiographic patterns of atherosclerosis in erectile-related arteries in men with suspected or known coronary artery disease or peripheral artery disease with the added potential aim of microvascular stenting [31].

A more recent balloon angioplasty study for men with isolated arteriogenic ED reported a reasonable and acceptable sustained clinical success [66]. While penile artery angioplasty is clinically feasible and safe, there is a need for a more durable treatment strategy for penile artery stenotic disease, especially as many of these men will invariably develop a generalized vascular disease in the future. More studies are warranted to define the role of endovascular procedures in this ED subpopulation and potential complications, long term safety and efficacy outcomes will need to be addressed before it can be embraced as the standard of care.

3.6. Tissue Engineering and Penile Transplant

The notion of replacing end organ damage with newly engineered tissue is exciting and reconstruction of normal erectile tissue using autologous cells, derived from the patient's own body, has far-reaching implications not just in the field of ED but also in other reconstructive cases. While Atala and his group have been at the forefront of tissue engineering [67], many issues remain unresolved including clinical translation to human subjects (especially the safety and efficacy of these engineered materials). Furthermore, the creation of custom-made bioengineered organs using acellular scaffolds and matrices in regenerative medicine is still far from ideal and further research to develop novel biomaterials and cellular sources, coupled with improvements in tissue engineering techniques, will no doubt assist in this "bench-to-bedside" translational research.

On another hand, penile transplantation using vascularized composite allografts is an emerging technique to treat genital loss [68,69]. Penile transplantation allows for restoring both urinary and sexual function by providing a highly functional conduit for urination and a "normal"-appearing and functional organ for sexual intimacy. This complex reconstructive surgery would surpass many of the pitfalls of current penile reconstruction, particularly neophalloplasty. Conventional genital reconstruction is often associated with complications such as suboptimal cosmetic appearance, urethral fistula and/or stricture development, and the inability to restore "normal" erectile function as well as the need for multiple complex procedures [70].

However, the process of selecting the appropriate candidate for genitourinary vascularized composite allograft surgery is rigorous with extensive clinical visits, laboratory testing, imaging and psychological evaluations [71]. Side effects of high dose immunosuppression, including infection and

renal toxicity, should be discussed and recipients are screened for possible rejection of the graft with multiple tissue biopsies and regular blood test monitoring for the rest of their life. Pertinent questions such as donor/recipient programs, psychological implications, cost modelling and ethical concerns will warrant further discussion [72].

4. Conclusions

There have been considerable scientific advances made in the field of ED in recent years. Existing ED therapies are far from ideal and likely unable to address the growing medical needs of our ageing patient population. Innovative and novel ED therapies currently under development may be able to reverse, regenerate and replace underlying diseased endothelial, neural and penile vascular smooth muscle cells in the very near future. If proven to be safe and effective in the longer-term, these state-of-the-art therapeutic agents will transform the way ED is managed and perhaps cure ED once and for all.

Table 1. Current and emerging therapeutic options for erectile dysfunction.

Therapeutic Agents	Mechanism(s) of Action	Additional Description	References
PDE5i	Inhibits PDE5 enzyme to increase cGMP and NO release	1st generation: Sildenafil, Vardenafil, Tadalafil 2nd generation: Avanafil, Mirodenafil, lodenafil Udenafil	[3–12]
Intracavernosal agents	1. Prostaglandin E1 (Alprostadil) stimulates cAMP release 2. Papaverine is a non-selective PDE5i 3. Phentolamine is a non-selective alpha-adrenergic antagonist 4. Aviptadil is a synthetic VIP	Available as single agent or in combination as Bimix (2 agents), Trimix (3 agents) or Quadmix (including atropine).	[13–16]
Penile prosthesis implant	1. Non-inflatable (malleable) implant consists of a pair of rods 2. Inflatable implant (usually 3-piece) consists of a reservoir, pair of cylinders and a pump	Boston Scientific AMS 700 series and Coloplast Titan series are the dominant 3-piece inflatable penile prostheses in the market	[22–24]
Penile revascularisation surgery	Anastomosis of the inferior epigastric artery to the dorsal penile arteries and/or the deep dorsal vein	Should only be offered to younger non-diabetic men (<55 years) with proven isolated arterial stenosis, usually in the setting of pelvic trauma and without generalized vascular disease	[25–27]
Novel drug or drug delivery systems	1. Guanylate cyclase (GC) activators (e.g., BAY 60-2770) 2. RhoA/Rho-kinase inhibitor (e.g., Fasudil)	Drug delivery systems using oral disintegrating formulations and nanotechnology (e.g., nanoethosomes, transfersomes and penetration enhancing lipid vesicles)	[28–41]
Low intensity shockwave therapy	Promotes neovascularization through release of various angiogenic growth factors and activation tissue repair functions (e.g., enhanced macrophage activity, alteration in cellular apoptosis, greater synthesis of cellular proteins and activation as well as enhanced recruitment and subsequent differentiation of stem/progenitor cells)	Various machines, treatment templates and protocols	[42–46]
Cellular-based therapy	1. Stem-cell therapy 2. Platelet-rich plasma therapy	Methods to enhance the therapeutic effects with the use of growth factors, gene manipulation and matrixen	[47–61]
Gene therapy	1. Activator of nitrergic–neural system 2. Endothelial GFs promoters 3. Modulator of ion channels in smooth muscle cells	Concurrent use of growth factors and vector delivery system	[31,63,64]
Vascular stents	Provides microvascular stenting and balloon angioplasty	Requires angiographic evaluation for patterns of atherosclerosis in erectile-related arteries in men with suspected or known coronary artery disease or peripheral artery disease	[31,65,66]
Tissue engineering and penile transplant	Tissue engineering using biomaterials, acellular scaffolds and matrices Penile transplant using vascularized composite allografts	Issues relating to cost, intensive programs, immunosuppression, psychological, safety and ethical concerns	[67–72]

PDE5—phosphodiesterase type 5; cGMP—cyclic guanosine monophosphate; NO—nitric oxide; cAMP—cyclic adenosine monophosphate; VIP—Vasoactive Intestinal Peptide; GF—growth factor.

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INVESTIGATIVE AND CLINICAL UROLOGY
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Safety and feasibility of platelet rich fibrin matrix injections for treatment of common urologic conditions

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Purpose: Autologous platelet rich plasma (PRP) is used increasingly in a variety of settings. PRP injections have been used for decades to improve angiogenesis and wound healing. They have also been offered commercially in urology with little to no data on safety or efficacy. PRP could theoretically improve multiple urologic conditions, such as erectile dysfunction (ED), Peyronie's disease (PD), and stress urinary incontinence (SUI). A concern with PRP, however, is early washout, a situation potentially avoided by conversion to platelet rich fibrin matrix (PRFM). Before clinical trials can be performed, safety analysis is desirable. We reviewed an initial series of patients receiving PRFM for urologic pathology to assess safety and feasibility.

Materials and Methods: Data were reviewed for patients treated with PRFM at our center from November 2012 to July 2017. Patients were observed immediately post-injection and at follow-up for complications and tolerability. Where applicable, International Index of Erectile Function (IIEF-5) scores were reviewed before and after injections for ED and/or PD. Pad use data was collected pre/post injection for SUI.

Results: Seventeen patients were identified, with a mean receipt of 2.1 injections per patient. Post-procedural minor adverse events were seen in 3 men, consisting of mild pain at injection site and mild penile bruising. No patients experienced complications at follow-up. No decline was observed in men completing pre/post IIEF-5 evaluations.

Conclusions: PRFM appears to be a safe and feasible treatment modality in patients with urologic disease. Further placebo-controlled trials are warranted.

Keywords: Erectile dysfunction; Penile induration; Platelet-rich fibrin; Platelet-rich plasma; Urinary incontinence, stress

INTRODUCTION

Platelet-derived therapies are a growing trend across multiple medical and surgical specialties [1-5]. Evidence suggests that platelets play an important role in tissue repair, vascular remodeling and inflammatory and immune responses through secretion of growth factors, cytokines and chemokines [6,7]. These biologically active proteins include transforming growth factor-β, platelet-derived growth factor, platelet-derived epithelial growth factor, insulin-like growth factor, vascular endothelial growth factor, basic fibroblast growth factor, as well as many others [8]. These

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growth factors are implicated in many aspects of natural wound healing, including chemotaxis, cell proliferation, cell differentiation and angiogenesis. They also control and conduct synthesis, modification and degeneration of extracellular matrix proteins. Coordination of these cellular and molecular processes is integral to proper wound healing and tissue regeneration [9]. The key role of platelets in these processes makes them an attractive candidate for therapies aimed at accelerating natural healing.

One of the most well described platelet-based therapies is autologous platelet-rich plasma (PRP) [10]. PRP is derived from the centrifugation of whole blood with a separator gel to remove the red and white blood cells. The resulting supernatant has a greater than four-fold increase in platelets and other plasma proteins [11]. This concentrate is then administered via injection. Newer strategies to prolong the anti-inflammatory and wound healing properties of platelets have focused on creating a fibrin matrix (platelet rich fibrin matrix, PRFM) to bind the platelets and prevent extravasation from the site of injection, thereby addressing the concern of early washout with PRP [12]. In addition, PRFM offers a potential scaffold for tissue ingrowth and may allow continued release of platelet-related factors for a longer duration.

Autologous blood-based biomaterials are promising therapeutic options for varied pathology. Rapid generation of therapeutic material following collection allows for point-of-care therapy [13]. Furthermore, an autologous therapy avoids the need for immunosuppression and eliminates concern of rejection. Within urology, as with many other specialties, there are numerous conditions where tissue regeneration is desirable. In a prior rodent model, Wu et al. [14,15] performed intracavernosal injection of PRP after cavernous nerve crush injury and noted increased myelinated axons and improved recovery of erectile function. Currently, there are no reports of PRP or PRFM for the treatment of urologic conditions in humans, and thus, no assessment of safety. The aim of this study was to evaluate the safety and feasibility of PRFM injections in a subset of patients treated for erectile dysfunction (ED), Peyronie's disease (PD), or stress urinary incontinence (SUI).

MATERIALS AND METHODS

The study was approved by the Institutional Review Board of Wake Forest School of Medicine (approval number: IRB00042919). Data was prospectively collected and retrospectively reviewed for patients treated with PRFM for ED, PD, or SUI by a single surgeon from November 2012 to July 2017 as part of our novel therapeutics program. Informed consent was obtained and patients were aware of off-label use. Demographic data, clinical pathology, procedural details, outcomes data, and pre- and post-procedural International Index of Erectile Function (IIEF-5) questionnaires (for male patients) were collected. Each participant was injected with autologous PRFM using a proprietary system (Selphyl, Aesthetic Factors Inc., Wayne, NJ, USA).

1. Preparation and injection process

Venipuncture was performed in the clinic. Two separate collection tubes were filled with 9 mL of whole blood. The samples were centrifuged at 6,000 RPMS for six minutes, and the supernatant was separated from the remaining blood sample using a proprietary system. Ten percent calcium chloride solution was then added to the PRP in a 1:10 ratio, converting fibrinogen to fibrin. This process would generally yield approximately 5.5 mL of injectable PRFM per tube with patients receiving either 1 or 2 tubes. PRFM, referred to some as 'activated PRP' was chosen so as to allow better local retention of product and thus avoid early washout. Administration was performed within ten minutes of final preparation.

Injections were performed based on the targeted genitourinary pathology. Between 4 and 9 mL of PRFM was injected per treatment session. Intracavernosal injection was performed for ED. For patients with PD, an artificial erection was induced with 20 μg of alprostadil to assess curvature, and injections were placed directly into tunical plaques under ultrasound guidance. After a thorough discussion of potential risks and benefits, three patients elected needle fracture of plaque(s) with concomitant 10 mL saline injection prior to PRFM injections. For SUI, a pediatric cystoscope and transurethral injection needle were used to inject PRFM into the urethral submucosa, distal to the bladder neck.

Patients were observed in the clinic for 20–30 minutes post-procedurally for potential complications or side effects. Clinical information, safety related questions, survey data, and IIEF-5 questionnaires were collected at the time of clinical follow-up and telephone calls were used to evaluate for possible adverse events for which no medical attention was sought.

RESULTS

Seventeen patients underwent injections for the treatment of organic ED (4), PD (11), coexisting ED with

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Table 1. Demographic breakdown (n=17)

Demographic	Value
Male	16
Female	1
Mean age (y)	46 (27–61)
Mean body mass index (kg/m^2)	25.5
Urologic diseases treated	
ED	4
PD	11
ED+PD	1
Stress urinary incontinence	1
Mean of injections	2.1 (1–8)

Values are presented as number only or mean (range).
ED, erectile dysfunction; PD, Peyronie's disease.

Table 2. Demonstrates the minor adverse effect rate (n=17)

Adverse event	No. (%)
Minor	
Overall	4 (23.5)
Mild pain	4 (23.5)
Bruising	1 (5.9)
Major	
Overall	0
Bleeding	0
Infection	0
Compartment syndrome	0

PD (1), and female SUI (1) (Table 1). Cited reasons for ED included vasculogenic, penile fracture, medication-related and electrical injury to the genitalia. Mean patient age at time of first injection was 46 years (range, 27–61 years). Patients received an average of 2.1 (range, 1–8) injection procedures during the study period. Additional injections were provided upon patient request. Injections were well tolerated in all cases. Three patients reported mild pain at the injection site, one of whom also noted mild penile bruising after the injection (Table 2). All patients who noted bruising were PD patients who were given intracavernosal alprostadil were also given planned injection of 250 μg of phenylephrine at the conclusion of the procedure to detumescence. No systemic complications were noted initially or during follow-up. Mean follow-up was 15.5 months.

Among ED and/or PD patients queried with IIEF-5 (7), no patient reported a worsening of overall score or of any individual domain score. IIEF-5 scores improved by an average of 4.14 points after PRFM therapy. In patients with PD with subsequent follow-up, 80% (4/5) initially reported subjective improvement in their degree of curvature. One female patient underwent transurethral injection for SUI with 50% reduction in pad usage. When asked whether they would be likely to undergo further PRFM injections, 80% of patients answered affirmatively.

DISCUSSION

Platelet based therapies are being increasingly utilized in multiple medical settings, including dermatology, ophthalmology, cardiology, colorectal surgery, and plastic surgery [1,11]. PRP has been frequently used for orthopedic conditions such as bone and soft tissue trauma, inflammatory conditions, and chronic pain syndromes [1,7,10]. Across multiple disciplines, PRP has been used both as a primary treatment modality and as a supplement to other therapies in hopes of supplementing wound healing, tissue regeneration, and angiogenesis. Although most of the studies focusing on PRP injections have been relatively small and heterogenous, they largely support safety and efficacy. Additionally, the concept of autologous therapy may be particularly attractive to some patients [16].

ED affects as many as 1 in 4 men, and evidence indicates the incidence is rising [17,18]. The pathophysiology is multifactorial, but a significant proportion results from endothelial dysfunction secondary to inflammation [19]. The most common treatments for ED aim to improve endothelial function through augmentation of the nitric oxide pathway [20]. To date, there are no treatments that address the underlying cause of endothelial dysfunction. Platelet-derived therapies targeting inflammation and promoting tissue regeneration may represent a potential treatment option.

PD, while less common than ED, affects roughly 1%–8% of men [21]. The pathophysiology appears to involve increased inflammation from tissue disruption, followed by aberrant wound healing resulting in fibrotic plaques [22]. Current treatment regimens include plaque injection, plication, grafting, or insertion of penile prosthesis to restore appropriate form and function. Currently there are no therapies targeting either the inflammatory processes or the aberrant wound healing that causes PD. Furthermore, therapies focusing on disrupting the fibrotic plaques through mechanical manipulation, or more recently, collagenase injection, do not address appropriate wound healing or regeneration of the damaged tissue [23]. Theoretically, injection of PRFM could combine mechanical disruption of the plaque, via needle fracture, while simultaneously neutralizing destructive inflammatory processes in an effort to promote a better wound-healing response and stabilize the disrupted plaque.

Biologic materials have been used for decades in the

treatment of SUI. Multiple products have been used as bulking agents to supplement urethral coaptation. While generally less efficacious than surgical repairs, injectable agents remain attractive given their relative ease of administration and lack of need for implantable mesh-based materials. When it was previously available, glutaraldehyde cross-linked bovine collagen was the most commonly injected biomaterial used to treat female SUI and was associated with a cure rate of 53% [24]. Theoretically, injection of autologous PRFM could provide both urethral bulking and potential regenerative effects to a damaged female urethra.

Investigations of PRFM for the urologic conditions noted in this report have not been previously reported. Wu et al. [15] investigated the effects of several different preparations of PRP injections in rat models with bilateral cavernous nerve crush injuries. Their data suggest that an "optimized" PRP formulation with a high level of growth factors was more stable than other preparations of PRP. Rats receiving this formulation showed significantly greater increases in intracavernosal pressure, higher mean arterial pressure, higher levels of nitric oxide synthase, and greater recovery of erectile function than those receiving saline injections or other formulations of PRP. Tang et al. [25] also showed that PRP injections at the site of cavernous nerve crush injuries helped facilitate nerve regeneration and erectile function in a rat model. More recently, Shirvan et al. [26] described injection of PRP and interposition platelet rich fibrin glue into the fistulous tracts of 12 patients with vesicovaginal fistulas (most <5 mm). All patients showed significant improvement with 11 patients cured at six-month follow-up, both subjectively and by examination.

We recognize that a variety of preparations, delivery modalities, and dosing schedules are available for PRP/PRFM therapies. A mean of 2.1 injection procedures per patient were performed during the study period. In our study, the PRP was added to a calcium chloride preparation to create PRFM. This was done to theoretically prevent rapid washout of the PRP from the corpora. One potential safety concern about using a colloid/hydrogel type of material in the corpora was the possibility of interrupting corporal blood flow, creating the possibility of a 'penile compartment syndrome,' akin to priapism. This did not occur in any of the ED or PD patients in our study, as each of these injections was well tolerated.

Data from this report regarding functional assessments must be interpreted with caution. This was not a prospective study, and we believe a significant placebo effect exists for research involving male sexual health. Objective improvements in the IIEF-5 score (4.14 points, 9.1%) were seen in patients receiving PRFM therapy for ED and PD. This level of improvement was similar to the average IIEF score increase (4.45 points, [3.42, 5.29]) seen in patients using PDE5Is after nerve sparing prostatectomy in a recent meta-analysis [20]. At follow-up interviews, patients expressed specific improvements in the rigidity of erections and improvements in satisfaction due to increased confidence. Of PD patients available for follow-up, 80% noticed an initial subjective improvement in their degree of curvature. Additionally, the one patient who received PRFM injections for SUI noted a 50% decrement in pad usage. Patients injected with silicone polymers (Macroplastique, Cogentix, Minnetonka, MN, USA) reported a 77% subjective cure rate but only a 9% objective cure rate on urodynamic testing [27]. No conclusions can be drawn from a single patient, but a 50% objective improvement from a transurethral injection procedure using an autologous product seems promising. With regards to feasibility of the procedure, there were no concerns related to the preparation of the PRFM or the injection process itself into the corpora cavernosa, tunical plaques, or urethral submucosa for patients with ED, PD, or SUI, respectively.

While this study attests to safety in this selected population, it has multiple limitations. This was a retrospective review of a small cohort of patients with a spectrum of pathology that may not be representative of the general population. As an autologous product, we expect that reabsorption rates are high, such that repetitive therapy will be required. This raises the possibility of treatment-related fibrosis from injection site trauma. As mentioned, although there was no detriment in IIEF score, the lack of a placebo arm prevents a detailed context. Future work will involve placebo control, with structured assessments for efficacy.

CONCLUSIONS

Our initial experience suggests that PRFM injections for ED, PD, and female SUI are feasible and safe. Although the limited data is suggestive of efficacy, a placebo control will be required in subsequent efforts for confirmation. Future studies evaluating efficacy of PRFM injections for genitourinary pathology appear warranted.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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ORIGINAL RESEARCH & REVIEWS

ERECTILE FUNCTION

Platelet-Rich Plasma (PRP) Improves Erectile Function: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial

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ABSTRACT

Background: Animal studies postulate that platelet-rich plasma (PRP) injections improve key elements of the pathophysiologic mechanisms leading to erectile dysfunction (ED).

Aim: To conduct the first double-blind, randomized, placebo-controlled trial assessing the efficacy and safety of PRP injections in patients with mild and moderate ED.

Methods: Sixty sexually active patients with mild and moderate ED were randomly assigned to two sessions, with a one-month difference, of 10 mL PRP (n = 30) or placebo (n = 30) intracavernosal injections. An FDA-approved separation system was used. Patients were evaluated at 1, 3 and 6 months after completion of the treatment protocol. A per-protocol analysis was applied. All participants withheld any ED treatment during the trial.

Outcomes: The achievement of minimal clinically important difference (MCID) in the International Index of Erectile Function − Erectile Domain (IIEF-EF) from baseline to 6 months after final treatment. Erectile function at all time points, as well as safety of PRP injections, were also evaluated.

Results: At 6 months, a MCID was achieved by 20/29 (69%) patients in the PRP group compared to 7/26 (27%) in the placebo group. The risk difference between the two groups was 42% (95%CI: 18−66), $P < 0.001$ and the baseline-adjusted mean between-group-difference in the IIEF-EF score was 3.9 points (95%CI: 1.8−5.9). Similarly, a statistically significant difference of both the number of participants attaining a MCID and the IIEF-EF score was also observed at the 1- and 3-month evaluation between the two groups. Accordingly, patients receiving PRP were more satisfied with the treatment. No adverse events were observed during the study period.

Clinical implications: Intracavernosal PRP injection therapy used as outlined in this trial appears to be a safe and effective short-term treatment for the management of mild to moderate ED.

Strengths & Limitations: We conducted the first clinical trial exploring the role of PRP in the management of ED. Conversely, our findings lack external validity due to single-center design. Furthermore, our results cannot be extrapolated to other PRP separation systems.

Conclusions: PRP intracavernosal injections may be a promising addition to the urologist's armamentarium for the management of ED. Still, further high-quality studies are warranted to corroborate our findings. **Evangelos P, Mykoniatis I, Pyrgidis N, et al. Platelet-Rich Plasma (PRP) Improves Erectile Function: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial. J Sex Med 2021;18:926−935.**

Key Words: Platelet-rich plasma; PRP; Erectile function; Erectile dysfunction; Randomized controlled trial

INTRODUCTION

Vasculogenic erectile dysfunction (ED) is a complex, multidimensional disorder that predominantly manifests due to reduced penile blood flow, arterial insufficiency or stenosis, as well as endothelial dysfunction.[1] Most recommended treatments improve erectile function by enhancing penile hemodynamics without reversing the pathophysiologic mechanisms leading to ED.[2]

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Platelet-rich plasma (PRP) is an autologous plasma fraction produced from the centrifugation of whole blood that contains a 3- to 7-times higher mean platelet concentration compared to whole blood.[3] Due to the beneficial properties of growth factors contained in high concentrations in this fraction, numerous medical specialties have included PRP injections in the quiver of their offered treatment options.[4–9] Recently, PRP intracavernosal injections emerged as a promising, angiogenic, vasculogenic and regenerative treatment modality for ED.[10] Animal studies postulate that PRP injections may improve key elements of the pathophysiologic mechanisms leading to ED through anti-inflammatory, reparative, neuroprotective and neurotrophic effects.[11–14] Still, these mechanisms are yet neither adequately explored nor completely understood.

Despite the favorable outcomes of PRP and the exploding interest in regenerative medicine, limited data support its use as part of the established ED therapeutic algorithm.[15–17] Given the paucity of human clinical trials, there is currently an unmet need for high-quality studies exploring the use of PRP for the management of ED.[18] In this scope, we conducted the first double-blind, randomized, placebo-controlled clinical trial aiming to assess the efficacy and safety of PRP injections versus placebo in patients with non-severe ED.

METHODS

Study Design

This study was a prospective, double-blind, randomized, placebo-controlled clinical trial performed at the outpatient clinic of the First Department of Urology, Aristotle University of Thessaloniki, Greece. The study protocol was approved by our institutional review board (protocol number: 15538/8-10-18) and registered at ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT04050020). This study was performed in accordance with the Consolidated Standards of Reporting Trials (CONSORT) statement and all participants provided written informed consent before enrollment.[19] The study was supported by a research grant from the European Society for Sexual Medicine (ESSM). Patients were recruited from October 2019 to April 2020 and the final results were obtained in October 2020.

Selection Criteria

The predefined inclusion criteria were: (i) Sexually active male patients 40−70 years old in a stable, heterosexual relationship for more than 3 months; (ii) Use of any phosphodiesterase type 5 inhibitor (PDE5i) intake during the month before screening; (iii) Presence of mild or moderate ED after washout from PDE5i or any other ED treatment, documented with a score of 11−25 in the International Index of Erectile Function-Erectile Function (IIEF-EF) domain; (iv) Agreement to suspend all ED treatments for the duration of the study and; (v) Agreement to attempt sexual intercourse at least four times every month for the duration of the study, without being under the influence of alcohol or recreational drugs, and document the outcome using the Sexual Encounter Profile (SEP) diaries.

The predefined exclusion criteria were: (i) Previous major pelvic surgery or trauma; (ii) Previous major penile surgery or radiation; (iii) History of priapism, penile fracture, Peyronie's disease, penile curvature or any other anatomical disorder affecting erectile function; (iv) Abnormal morning serum testosterone levels (lower than 300 ng/dL or greater than 1197 ng/dL); (v) Psychogenic ED; (vi) History of any severe medical and psychiatric condition impairing participation in the study and; (vii) Subjects having partners that reported during the study period sexual dysfunction or any other major medical condition limiting sexual activity as well as those who presented with age less than 18 years, breastfeeding or pregnancy.

Study Protocol

At initial screening, all eligible patients underwent detailed medical history by two experienced physicians, extensive physical examination and appropriate medical tests. Subsequently, a 1-month washout from PDE5i or any other ED treatment was applied while patients were asked to attempt sexual intercourse at least four times and record outcomes in the SEP diaries. After this 1-month period, the SEP dairies were evaluated, and all patients completed the IIEF-EF questionnaire.[20] If patients were still eligible, they signed a written informed consent and were randomized in a 1:1 ratio to two sessions, with a one-month interval, of 10 mL PRP or normal saline injections. Randomization was performed according to a computer-generated sequence developed by the study coordinating team. To ensure allocation concealment and minimize selection bias, assignment to groups was communicated by the coordinating center (DK and PK) via a web-based registration system to a member of the research team (AF). One research team member (FZ) was only responsible for blood sampling and preparation of the injections. To ensure the double-blind character of our study, all injections were concealed by tinfoil to make their content invisible to both the participants and the investigators. Subsequently, the prepared injections were delivered by two experienced urologists of our research team (EP or IM) who were responsible for the administration of treatment.

All included patients underwent the first session of PRP or placebo injections within the same visit. An additional administration was performed one month after the initial session. Accordingly, participants were assessed at 1, 3 and 6 months after completion of the treatment protocol. PDE5i intake or other ED treatments were prohibited throughout the whole duration of the study. Treatment-induced pain was evaluated after the end of each visit with a Visual Analogue Scale (VAS) ranging from 0 (no pain) to 10 (intolerable pain). To assess the effect of PRP on erectile function, participants returned, at each visit, the completed SEP diaries for the last month and filled out the IIEF-EF. The number of patients attaining minimal clinically important difference (MCID) was measured. MCID was considered as an improvement in the IIEF-EF of 2 or more points in patients

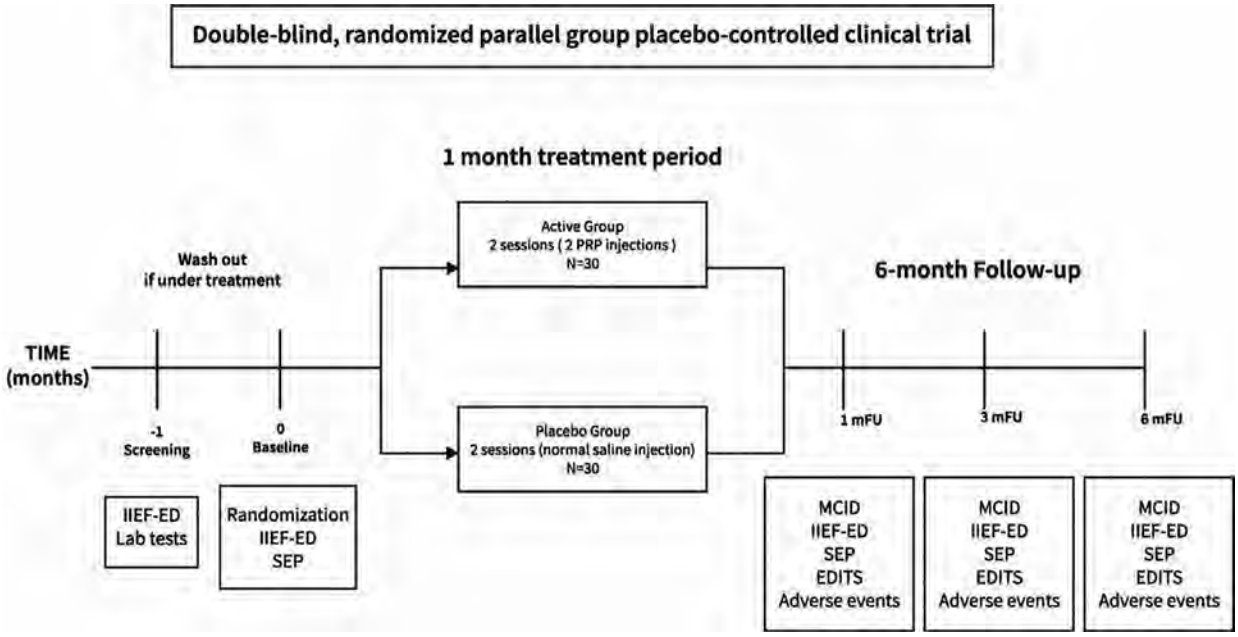

Figure 1. Study protocol. EDITS = Erectile Dysfunction Inventory of Treatment Satisfaction; FU = Follow-up; IIEF-EF = International Index of Erectile Function-Erectile Domain; MCID = Minimal Clinically Important Difference; PRP = Platelet-Rich Plasma; SEP = Sexual Encounter Profile.

with mild or mild to moderate ED (IIEF-EF score: 17−25) or 5 or more points in patients with moderate ED (IIEF-EF score: 11−16) after treatment.[21] Additionally, to measure treatment satisfaction, all subjects completed the Erectile Dysfunction Inventory of Treatment Satisfaction (EDITS) questionnaire.[22] At each follow-up visit, any adverse events were documented. The applied study protocol is depicted in Figure 1.

PRP Preparation and Administration

All patients underwent blood sampling in a 60 ml syringe containing 8 mL of anticoagulant. Preparation of PRP and placebo injections was performed in a separate room. Samples of patients randomized to placebo were dismissed and samples of patients randomized to PRP were processed by an FDA-approved autologous platelet separator (Magellan Autologous Platelet Separator; Arteriocyte Medical Systems, Hopkinton, MA) to yield approximately 10 mL of PRP. The Magellan Separator is a fully automated closed-loop processing system that requires limited intervention during processing. In particular, PRP is automatically separated from anticoagulated whole blood in approximately 15 minutes and dispensed into a separate sterile syringe. A comparative study among available commercial PRP separation systems has shown that the Magellan system offers high quality PRP.[23] One mL of PRP is used for a quality control analysis, while the remaining aliquot is ready for intracavernosal administration.

After preparation of the injection, patients were placed in a supine position and a penile tourniquet was clipped around the base of the penis. A total of 5 mL was infused in each corpus cavernosum − slowly retracting the needle for better distribution of PRP into the erectile tissue - over a 2-minute period to minimize platelet cell injury. The whole procedure was performed under sterile conditions without anesthesia. Following administration, additional compression of the penis was performed with a dressing placed around the penile shaft. The penile tourniquet was removed 20 minutes after the injections and patients were released. All patients were instructed to remove the compression bandage at home, 4 hours after the injection.

Outcomes

The primary outcome of our study was the proportion of patients in each group attaining MCID in the IIEF-EF domain from baseline to 6 months after the final treatment. Secondary outcomes included: (i) The proportion of patients in each group attaining MCID in the IIEF-EF domain from baseline to 1 and 3 months after final treatment; (ii) The mean change from baseline of the IIEF-EF between the two groups at 1, 3 and 6 months after final treatment; (iii) The mean change from baseline of positive responses to the question 3 of SEP between the two groups at 1, 3 and 6 months after final treatment; (iv) Treatment-induced pain and safety and possible side effects after PRP vs placebo injections.

Sample size calculation

Due to the lack of RCTs evaluating the role of PRP on ED, we, initially, performed a pilot study with 30 patients (15 in each group) to determine the appropriate sample size. This double-blind, placebo-controlled pilot study was conducted to compare the proportion of patients in each group attaining MCID in the IIEF-EF from

baseline to 6 months after the final treatment. In particular, at the 6-month evaluation, the proportion of subjects with MCID in the PRP group was 66.7% and in the placebo group 26.7%. Considering 80% statistical power and a 5% margin of error, we estimated a sample size of 23 participants per group. Assuming a 20% dropout rate, we recruited a total of 60 patients.

Statistical Analysis

We applied a per-protocol analysis. Categorical variables were estimated as frequencies with proportions, while continuous variables as mean ± standard deviation (SD) or median and interquartile range (IQR). We compared the categorical variables between the two treatment groups using the chi-squared (χ^2) and calculated their absolute risk difference with the 95% confidence intervals (CIs). Accordingly, we compared the continuous variables using the two-sample t-test or the Mann-Whitney test and estimated their mean differences with the corresponding CIs. Moreover, for continuous outcomes, the analysis of covariance (ANCOVA) was applied to assess the change from baseline between the two treatment groups, adjusting for the baseline value of each variable. Normality was evaluated both statistically with the Shapiro-Wilk test and visually with histograms, P-P and Q-Q plots. All statistical analyses were performed with the R statistical software (version 3.6.3) and two-sided *P*-values lower than 0.05 were considered statistically significant.

RESULTS

Patient Selection and Baseline Characteristics

We enrolled 60 patients that were allocated to either PRP (n = 30) or placebo (n = 30) injections and presented a median age of 58 (IQR: 51.5, 62) and 59 (IQR: 53.5, 61) years, respectively. The median ED duration was 78 (IQR: 48, 120) months in the PRP and 60 (IQR: 39, 117) months in the placebo arm. A total of 20 patients reported mild ED (PRP = 13, placebo = 7), 32 mild to moderate ED (PRP = 14, placebo = 18) and 8 moderate ED (PRP = 3, placebo = 5). No statistically significant differences were detected in the baseline characteristics between the two groups (Table 1). All participants underwent two sessions of PRP or placebo injections. Five participants, four in the placebo group and one in the PRP group, did not proceed for the follow-up evaluations due to the COVID-19 pandemic (dropouts not related to the study). The step-by-step study flow chart as well as the exact timepoint of each dropout are illustrated in Figure 2.

Minimal Clinically Important Difference in the IIEF-EF

Among participants presenting to the follow-up evaluations, 22/29 (76%) patients attained a MCID in the PRP group compared to 7/28 (25%) in the placebo group ($p < 0.001$) at 1 month. At this time point, 51% (95% CI: 29 to 73) more patients treated with PRP injections developed a MCID in the IIEF-EF scale compared to placebo. At 3 months, 20/29 (69%) patients achieved a MCID in the IIEF-EF scale after PRP injections versus 10/26 (39%) after placebo ($P = 0.018$). Therefore, 30 per 100 (95% CI: 5.3 to 56) additional subjects treated with PRP injections attained a MCID in the IIEF-EF scale compared to placebo. At 6 months, 20/29 (69%) patients reported a MCID in the IIEF-EF scale with PRP injections versus 7/26 (27%) with placebo ($P < 0.001$) and the risk difference between the two groups was 42% (95% CI: 18 to 66). All relevant statistical analyses are available in Table 2 and the raw data of all participants in Appendix 1.1.

Table 1. Baseline characteristics of the study participants

Baseline characteristics	Overall, n = 60	PRP, n = 30	Placebo, n = 30	*P-value*
Age (years)	58.5 (52.8, 62)	58 (51.5, 62)	59 (53.5, 61)	*0.92*
BMI (Kg/m^2)	28.6 (26.4, 31.6)	29.4 (26.6, 32.1)	28.5 (26, 30.4)	*0.26*
Smoking	35 (58%)	16 (53%)	19 (63%)	*0.60*
Hypertension	18 (30%)	10 (33%)	8 (27%)	*0.78*
Diabetes	15 (25%)	11 (37%)	4 (13%)	*0.074*
Hyperlipidemia	21 (35%)	12 (40%)	9 (30%)	*0.59*
CHD	7 (12%)	5 (17%)	2 (6.7%)	*0.42*
Testosterone (ng/dl)	507.5 (367.8, 581.8)	510.5 (428, 608.8)	448.5 (360.9, 563)	*0.21*
ED duration (months)	66 (48, 120)	78 (48, 120)	60 (39, 117)	*0.68*
ED severity				*0.25*
Mild	20 (33%)	13 (43%)	7 (23%)	
Mild to moderate	32 (54%)	14 (47%)	18 (60%)	
Moderate	8 (13%)	3 (10%)	5 (17%)	
IIEF-EF	19.9 ± 3.3	20.4 ± 2.9	19.4 ± 3.7	*0.28*
SEP Question 3 (Yes %)	46.7 ± 21.3	47.5 ± 20.1	45.8 ± 22.8	*0.77*

Statistics presented as mean ± SD or median (IQR).
BMI = Body Mass Index; CHD = Coronary Heart Disease; ED = Erectile Dysfunction; IIEF-EF = International Index of Erectile Function-Erectile Function; IQR = Interquartile Range; PRP = Platelet-Rich Plasma; SEP = Sexual Encounter Profile.

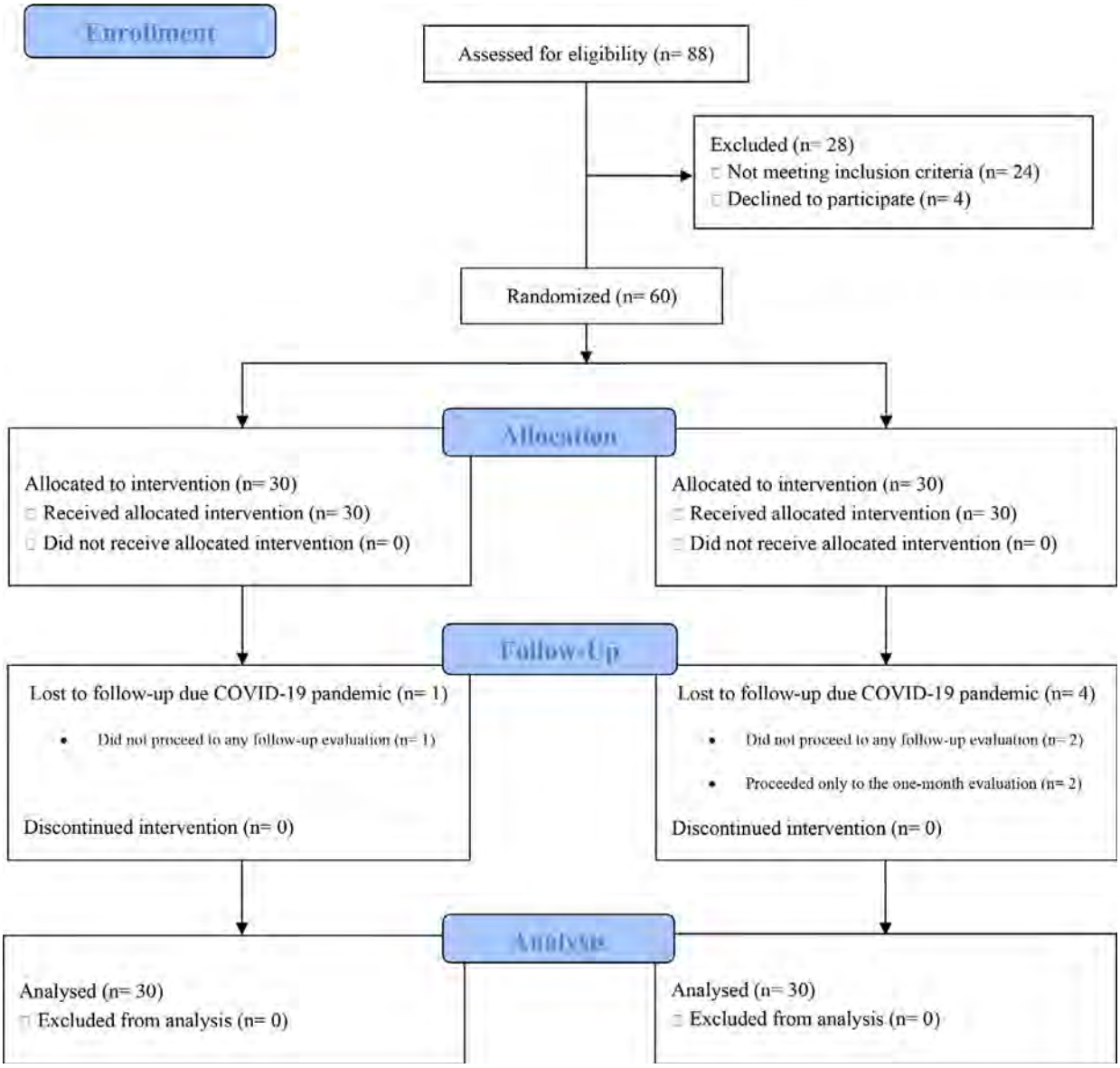

Figure 2. Study flow chart. [Figure 2 is available in color online at www.jsm.jsexmed.org.]

Erectile Function

At the baseline evaluation, the score of the IIEF-EF questionnaire and the proportion of "yes" responses to question 3 of SEP diaries did not differ between the two groups. PRP injections resulted in a statistically significant improvement of both the IIEF-EF and "yes" responses to SEP question 3 at all follow-up evaluations compared to placebo. The scores of the two questionnaires at all time points are presented in Figures 3 and 4. In particular, adjusting for the baseline value, IIEF-EF domain score improved by 2.7 points (95% CI: 0.9 to 4.5, P = 0.004) at 1

Table 2. Comparative data of the two groups about patients attaining MCID in the IIEF-EF at the follow-up evaluations

Patients with MCID in the IIEF-EF	PRP	Placebo	RD (95% CI)	*Between-group p-value*
1month	22 / 29 (76%)	7 / 28 (25%)	51% (29, 73)	*<0.001*
3 months	20 / 29 (69%)	10 / 26 (39%)	30% (5.3, 56)	*0.018*
6 months	20 / 29 (69%)	7 / 26 (27%)	42% (18, 66)	*<0.001*

The bold cells indicate statistically significant p-values.
CI = Confidence Interval; ED = Erectile Dysfunction; IIEF-EF = International Index of Erectile Function - Erectile Function; MCID = Minimal Clinically Important Difference; PRP = Platelet-Rich Plasma; RD = Risk Difference.

month, 2.8 points (95% CI: 0.4 to 5.2, $P = 0.023$) at 3 months and 3.9 (95% CI: 1.8 to 5.9, $P = < 0.001$) at 6 months in patients treated with PRP compared to placebo. Similarly, the proportion of positive answers to question 3 of SEP improved by 19.4% (7.3 to 31.6, $P = 0.002$) at 1 month, 17.9% (2.1 to 33.6, $P = 0.028$) at 3 months and 28.6% (14.4 to 42.8, $P < 0.001$) at 6 months. All measures and comparisons can be seen in Table 3 and the corresponding raw data of all participants in Appendix 1.1 and 1.2.

Satisfaction and Safety

Patients receiving PRP injections were more satisfied with treatment and outcomes compared to placebo. In particular, the EDITS score after PRP compared to placebo was 62.7 ± 27.7 vs 34.5 ± 17, $P < 0.001$ at 1 month, 62.2 ± 27.4 vs 38.5 ± 24.3, $P < 0.001$ at 3 months and 63.2 ± 24.6 vs 32.8 ± 24, $P < 0.001$ at 6 months. Regarding treatment-induced pain, the mean VAS score of the two sessions was higher in patients undergoing placebo injections compared to PRP (2.6 ± 0.4 vs 2.2 ± 0.6, respectively, $P = 0.008$). No transient hemorrhagic adverse events (hematuria, local petechial bleeding or ecchymosis) or other side effects were reported during the injection and follow-up period in both groups. All relevant raw data are illustrated in Appendix 1.2.

DISCUSSION

Our findings demonstrate that intracavernosal injections with PRP are a safe and effective treatment modality for the management of non-severe ED. Based on our results, two sessions of PRP led to a statistically significant improvement of the erectile function compared to placebo and this effect was maintained for 6 months. More than two-thirds of participants in the active arm presented a MCID in the IIEF-EF scale at all follow-up evaluations, demonstrating that the improvement in erectile function may be clinically important. Furthermore, no major or minor adverse events occurred during the treatment and follow-up period. Of note, subjects receiving PRP displayed higher satisfaction rates compared to placebo, while subjects receiving placebo injections reported more pain during treatment.

To our knowledge, the present study is the first prospective, double-blind, randomized, placebo-controlled clinical trial evaluating the efficacy and safety of PRP intracavernosal treatment for ED. The beneficial effect of PRP injections on erectile function was demonstrated based on the measures of the three most established questionnaires in the literature: the IIEF-EF domain, the SEP diaries and the EDITS. Similarly, this beneficial effect remained significant compared to placebo or to baseline across multiple analyses at the short- and long-term evaluations. In terms of satisfaction assessed with the EDITS index score, the significant difference of PRP treatment versus placebo exceeded ten points, which is considered the benchmark for achieving a MCID.[24] Accordingly, the mean VAS pain score after each PRP session was relatively low indicating that PRP intracavernosal injections represent a patient-friendly ED treatment modality. Besides, the increased VAS score after normal saline injections versus PRP may rather be clinically irrelevant.

Still, the findings of the present study should be interpreted with caution in the context of some limitations. First of all, our results lack external validity as we performed a single-center clinical trial with strict eligibility criteria, relatively small number of participants and rather short follow-up. It should be stressed that the five dropouts, although not related to the study, might still have affected our findings. Since we could not handle missing data by applying the last-observation-carried-forward method due to the early time point of most dropouts or by performing a multiple imputation method due to the relatively small number of participants, we undertook a per-protocol analysis. Of interest, given that the sample size of our study was estimated based on the total number of participants with non-severe ED expected to attain a MCID in the IIEF-EF, our study was underpowered to perform any comparisons in patients with different degrees of ED (mild, mild to moderate, moderate). Moreover, it should be highlighted that the concentration of platelets and growth factors in a PRP fraction is predominantly based on the system used for its preparation.[23] Since we performed all PRP preparations with the Magellan Autologous Platelet Separator, our results cannot be extrapolated to other PRP separation systems. Accordingly, even though we performed a quality control analysis of all PRP samples, we did not evaluate the qualitative or quantitative composition of growth factors, cytokines or other molecules with regenerative properties. Therefore, the exact mechanism through which PRP improves erectile function remains unknown.

Indeed, to date, no consensus exists regarding the optimal platelet concentration in the PRP.[25] Some studies report that the therapeutic effect of PRP requires platelet concentrations greater than 200,000/ μL, while others greater than 1,000,000/ μL.[26] Based on the previous notion, PRP separation systems are divided into high- (platelet concentrations about 750,000/ μL) and low- (platelet concentrations about 500,000/ μL) yielding devices.[27] The Magellan Autologous Platelet Separator used in our trial is considered a high-yielding device and, therefore, produces higher concentrations of platelets and, in turn, molecules with regenerative properties.[23]

The beneficial effect of PRP in the regenerative and wound healing process is predominantly exerted through high concentrations of platelets and growth factors.[28,29] In particular, platelets contain multiple regenerative molecules such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor (IGF-1) and fibroblast growth factor (FGF), that improve angiogenesis stimulation, stem cell recruitment and inflammatory.[30] Regarding ED, in rat models with cavernosal nerve injury, PRP seems to improve erectile function by regenerating cavernosal nerves and by increasing nitric oxide synthesis, indicating that PRP may be effective for neurogenic ED.[11,12,31,32] Hence,

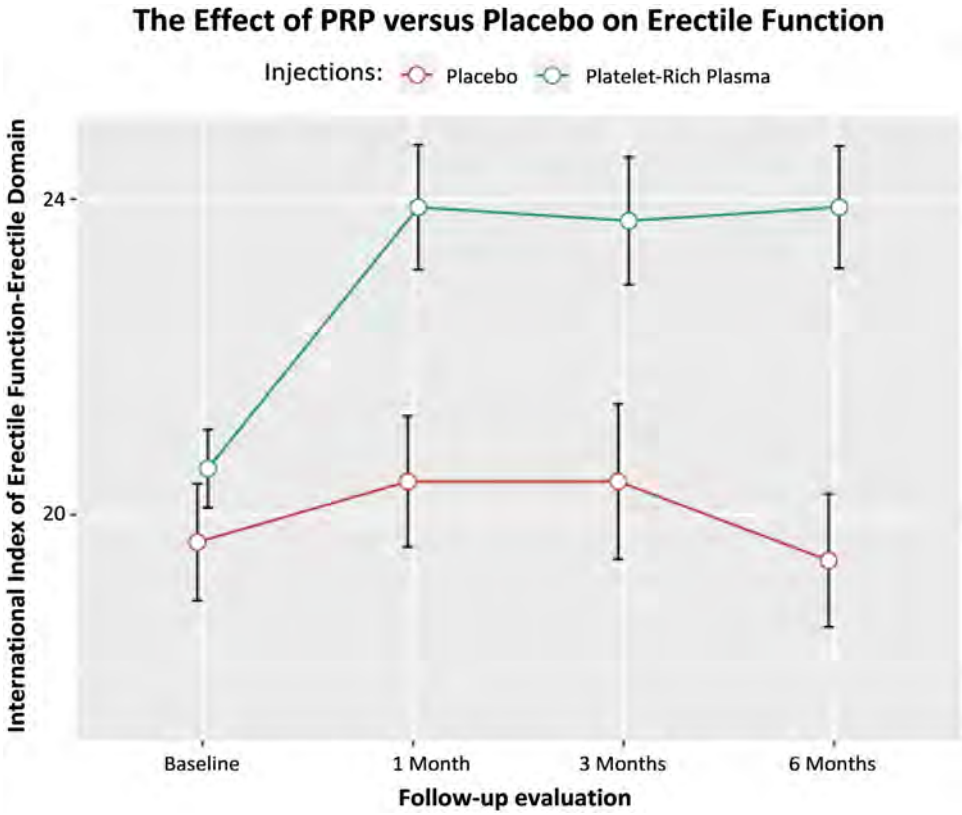

Figure 3. The effect of PRP versus placebo on IIEF-EF. PRP = Platelet-Rich Plasma.; IIEF-EF = International Index of Erectile Function-Erectile Domain. [Figure 3 is available in color online at www.jsm.jsexmed.org.]

even though it is suggested that PRP might restore penile blood flow and regenerate smooth muscle cells, to date, no basic research study has evaluated the effect of PRP on vasculogenic ED.[15]

Despite the accumulating evidence from molecular and animal studies, limited data suggest the use of PRP in everyday clinical practice.[10] In two previous studies from a center in Russia, patients with ED were randomized to (i) three sessions of activated with 10% $CaCl_2$ intracavernosal PRP injections once weekly (Group 1, 30 patients); (ii) the same regime of PRP combined with PDE5i (Group 2, 30 patients) or; (iii) inactivated PRP once weekly for three weeks (Group 3, 15 patients). Across all groups and time points, a significant improvement in the erectile function compared to baseline was demonstrated and no adverse events were reported.[33] Moreover, the authors concluded that PRP contains the necessary concentration of growth factors for a therapeutic effect.[17] Nevertheless, in these studies, no placebo arm existed, and no long-term evaluations were performed.

Matz et al. examined retrospectively the safety and feasibility of the platelet-rich fibrin matrix (PRFM) in four patients with ED, eleven with Peyronie's disease and one with concomitant Peyronie's disease and ED. Among seven patients evaluated with the IIEF-5, the IIEF-5 increased by a mean of 4.14 points, while no major adverse events were reported in all patients.[16] Still, the absence of a comparator and the methodological concerns of the study limited the extrapolation of its findings.

Ruffo et al. assessed in two trials published as conference abstracts the effect of PRP combined with low-intensity shockwave therapy (LiST). In the first study, 100 patients received LiST twice weekly for 6 weeks alone (Group 1, 58 patients) or in combination with PRP injections once weekly for 6 weeks (Group 2, 55 patients).[34] In the other study, 112 patients received LiST once weekly for 6 weeks alone (Group 1, 53 patients) or in combination with PRP injections once every 2 weeks for 6 weeks (Group 2, 59 patients).[35] In both trials, at 12 and 24 weeks, combination treatment significantly improved erectile function compared to baseline or LiST monotherapy. Nevertheless, despite the fact that the mechanism of actions of shockwaves and PRP seem complimentary, well-designed placebo-control trials are needed to reach conclusions on such combination treatment.

In the post-PDE5i era, regenerative treatment modalities such as PRP, LiST as well as gene and cellular-based therapies have emerged as promising options for the management of ED.[36] Even though ten review articles stress the potential benefits of PRP on ED,[10,15,28,37–43] limited, human and translational

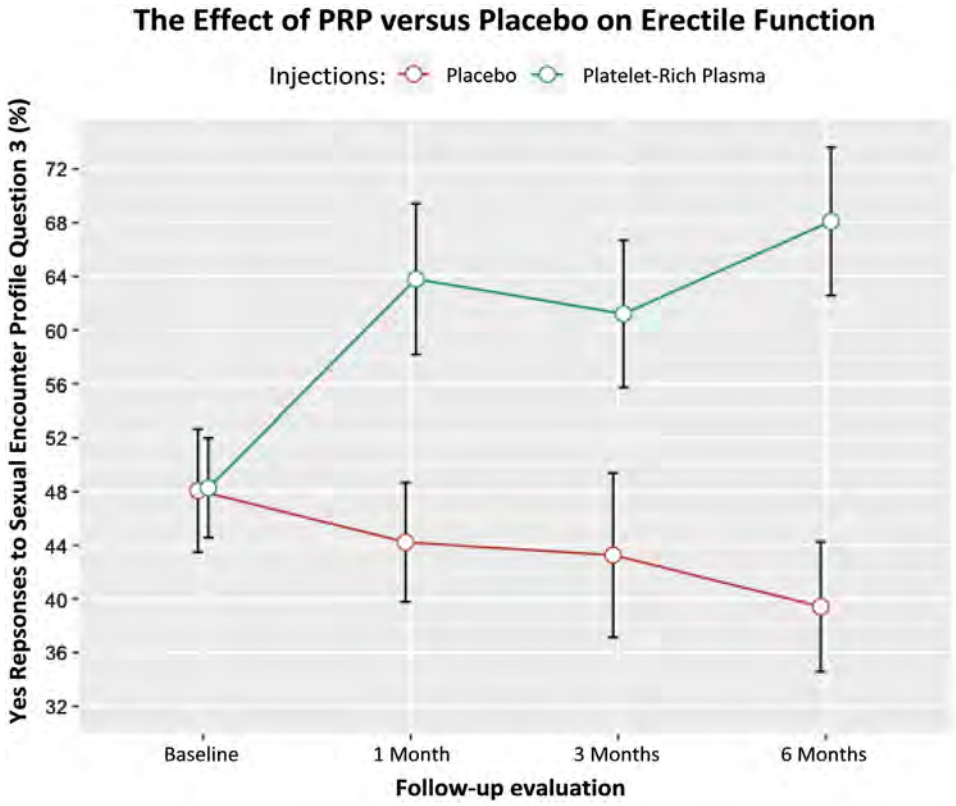

Figure 4. The effect of PRP versus placebo on SEP Question 3 "Yes" response rate (%). PRP = Platelet-Rich Plasma.; SEP = Sexual Encounter Profile. [Figure 4 is available in color online at www.jsm.jsexmed.org.]

studies exist. However, there is an increasing number of relevant ongoing clinical trials and their outcomes are expected with great interest. The design of all registered clinical trials is summarized in Appendix 2. Nevertheless, future studies should produce evidence on PRP systems, preparation, composition, administration and frequency for the management of ED. Additionally, well-conducted molecular and animal studies may further elucidate the pathophysiological mechanisms of PRP leading to erectile function improvement in models with vasculogenic ED. Accordingly, trials assessing the efficacy of PRP as part of monotherapy or combination treatment for ED are deemed necessary. In particular, trials comparing PRP to PDE5i, LiST or other recommended ED treatments, as well as trials assessing the synergic effect of PRP with such recommended treatments are needed to determine the ideal therapeutic approach in patients with ED.

Table 3. Comparison of changes from baseline in the IIEF-EF and SEP Question 3 after PRP injections versus placebo unadjusted and adjusted for the baseline evaluation

Parameter		PRP Mean ± SD	Placebo Mean ± SD	Unadjusted mean difference (95% CI)	Adjusted mean difference (95% CI)	*Adjusted between-group p-value*
IIEF-EF	Baseline – 1 month	3.3 ± 3.8	0.8 ± 3	2.5 (0.7 to 4.3)	2.7 (0.9 – 4.5)	*0.004*
	Baseline – 3 months	3.1 ± 4.1	0.8 ± 5	2.4 (-0.1 to 4.9)	2.8 (0.4 – 5.2)	*0.023*
	Baseline – 6 months	3.3 ± 4	-0.2 ± 3.8	3.5 (1.4 to 5.7)	3.9 (1.8 – 5.9)	*<0.001*
SEP Question 3 (Yes %)	Baseline – 1 month	15.5 ± 28.7	-3.6 ± 17.6	19.1 (6.5 to 31.7)	19.4 (7.3 – 31.6)	*0.002*
	Baseline – 3 months	12.9 ± 28.3	-4.8 ± 33.9	17.7 (0.6 to 34.9)	17.9 (2.1 – 33.6)	*0.028*
	Baseline – 6 months	19.8 ± 28.6	-8.7 ± 29.1	28.5 (12.8 to 44.1)	28.6 (14.4 – 42.8)	*<0.001*

The bold cells indicate statistically significant p-values.
CI: Confidence Interval; IIEF-EF: International Index of Erectile Function - Erectile Function; SD: Standard deviation; SEP: Sexual Encounter Profile.

CONCLUSION

Our findings demonstrate that two PRP intracavernosal injections within a one-month interval were safe and effective for the improvement of erectile function in patients with mild and moderate ED. Overall, PRP intracavernosal injection treatment, as a new representative of the flourishing field of regenerative medicine, seems to be a promising addition to the urologist's armamentarium. Nevertheless, before it is accepted as part of the ED algorithm, further high-quality studies are warranted to corroborate our findings.

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STATEMENT OF AUTHORSHIP

Poulios Evangelos: Methodology, Investigation, Writing – Original Draft, Writing – Review & Editing; Ioannis Mykoniatis: Conceptualization, Methodology, Writing – Original Draft, Writing – Review & Editing, Funding Acquisition; Nikolaos Pyrgidis: Methodology, Writing – Review & Editing; Filimon Zilotis: Investigation, Data Curation; Paraskeui Kapoteli: Data Curation, Project Administration; Dimitrios Kotsiris: Investigation; Dimitrios Kalyvianakis: Investigation, Writing – Review & Editing; Dimitrios Hatzichristou: Conceptualization, Funding Acquisition, Supervision.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jsxm.2021.03.008.

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Platelet-rich fibrin matrix for improvement of deep nasolabial folds

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Affiliations + expand

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Abstract

Background: Dermal augmentation continues to grow as an aesthetic facial procedure. Many exogenous filler materials rely on an autologous fibrotic response for volume augmentation.

Aims: To evaluate the efficacy of a single injection of autologous platelet-rich fibrin matrix (PRFM) for the correction of deep nasolabial folds (NLFs).

Patients/methods: Whole blood was obtained from 15 adults, and an activated autologous PRFM produced using a proprietary system (Selphyl; Aesthetic Factors, Inc., Wayne, NJ, USA) was then injected into the dermis and immediate subdermis below the NLFs. Subjects were photographed before and after treatment; NLFs were rated by the treating physician before and after treatment using the Wrinkle Assessment Scale (WAS) and patients rated their appearance at each post-treatment visit using the Global Aesthetic Improvement Scale. Patients were evaluated at 1, 2, 6, and 12 weeks after treatment.

Results: All patients were treated to maximal (no over-) correction, with a mean reduction in WAS score of 2.12 +/- 0.56. At 1 week after treatment, this difference was 0.65 +/- 0.68, but rose to 0.97 +/- 0.75, 1.08 +/- 0.59, and 1.13 +/- 0.72 at 2, 6, and 12 weeks after treatment, respectively ($P < 0.001$). No patient noted any fibrosis, irregularity, hardness, restricted movement, or lumpiness.

Conclusions: PRFM can provide significant long-term diminution of deep NLFs without the use of foreign materials. PRFM holds significant potential for stimulated dermal augmentation.

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Research Article

Platelet-Rich Plasma (PRP) in Breast Cancer Patients: An Application Analysis of 163 Sentinel Lymph Node Biopsies

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Introduction. Literature shows platelet-rich plasma (PRP) to improve overall outcomes in orthopedics, dermatology, ophthalmology, gynecology, and plastic surgery. Data on oncological patients is very limited. Only one publication is available on PRP in breast cancer patients. This work evaluated PRP in sentinel node biopsy procedures for breast cancer patients in terms of complication rates and oncological short-term follow-up. *Methods*. The evaluated PRP was ACP®, i.e., autologous conditioned plasma by Arthrex®. Between 2015 and 2018, 163 patients were offered to receive an ACP®/PRP injection in their lymph node biopsy site. Recruitment resulted in an approximate one-to-one ratio for analysis. Endpoints were major (revision) and minor (seroma, hematoma, and infection) complications rates as well as distant metastases, local recurrence, and overall survival. Median follow-up was 30 months. *Results*. Complication rates and oncological follow-up showed PRP to be applicable to use in a sentinel node biopsy scenario in breast cancer patients. There were 0 revisions in the ACP®/PRP group and 1.2% revisions in the control group (not significant). Oncological follow-up showed zero (0) distant metastases and local recurrences as well as a 100% 30-month overall survival. *Conclusions*. This is the first analysis of ACP®/PRP used in breast cancer patients in a sentinel node biopsy setting worldwide. PRP does not seem to increase rates of local recurrence within this 30-month follow-up time frame. Also, trend towards decreasing complication rates could be shown.

1. Introduction

Oncoplastic surgery is always aimed at improving overall surgical outcome. This research group recently published data regarding significantly improved patient satisfaction, postsurgical outcome, and complication rates on subcutaneous access devices in oncological patients when treated with platelet-rich plasma (PRP) [1]. In addition, literature shows improvement in overall outcomes in orthopedic surgery [2], conservative orthopedics [3–5], dermatology [6, 7], ophthalmology [8], gynecology [9], and plastic surgery. Complex wound management also found this product to be beneficial [10]. Especially the numerous positive results from the conservative PRP treatment of joints, justify a continuous investigation of the PRP issue. The overwhelming amount of available data is retrospective; this, however, leads to some meta-analyses on this topic comparing PRP to corticosteroids for mostly orthopedic procedures [11–13], again showing some benefit. Currently, there is no other data, apart from our previous work, towards a PRP application in breast cancer patients. This work will thus add a new area of interest, i.e., breast cancer patients that received PRP after a sentinel lymph node biopsy in the axilla.

Literature states PRP to not only contain platelets, but also growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), basic fibroblast growth factor (bFGF), endothelial growth factor (EGF), and vascular endothelial growth factor (VEGF). These in turn improve wound healing although their possible oncological effect is entirely unclear [14, 15]. In order to decrease patient morbidity, we aimed to improve wound healing by instilling this PRP product subcutaneously after performing

a single-incision sentinel lymph node biopsy in the axilla. Postoperative complication rates were to be evaluated. Major complications, such as revision surgeries, or minor complications, such as seroma and hematoma, may lead to subsequent interventions and increased patient morbidity. In addition, revision surgeries, seroma aspiration, etc. increase the financial burden on any health care system and should thus be avoided.

As a formal approach to the improvement of surgical technique, we require an evidence base. As randomized trials are difficult to fund, we nonetheless favor the adherence to the IDEAL framework for surgical innovation [16]. Three of the 4 stages were completed prior to this publication with the final stage, long-term study, being addressed within this work.

A fundamental problem in evaluating PRP products is patient subjectivity. Almost all patients subjectively feel better after receiving a PRP injection and or have knowledge of a PRP application. Thus, there will always be a subjective bias towards a positive outcome in a PRP treated collective when evaluated by questionnaire (i.e., pain and range of motion). This was shown in our previous work. Therefore, this evaluation focused on objective outcomes such as complication rates and recurrence only. The following questions were asked:

(1) Is ACP®/PRP safe to use in a sentinel node biopsy scenario?

(2) Is ACP®/PRP able to improve complication rates?

(3) Is there an oncological risk for ACP®/PRP application regarding the short-term follow-up in oncological patients?

2. Patients and Methods

The PRP product evaluated in this trial was the autologous conditioned plasma system (ACP®—double syringe system) by Arthrex®. Thus, the terms PRP and ACP become interchangeable throughout this paper. The study was performed retrospectively at the Municipal Hospital Holweide, Breast Cancer Center, Cologne, Germany. Between these 2015 and 2018, patients were offered to receive ACP®/PRP injections in their lymph node biopsy site should the sentinel lymph node not be involved. As this choice in itself would introduce a bias, subjective outcome evaluations were omitted in this trial. All patients were offered the ACP®/PRP injections free of charge. This resulted in two cohorts of a total of 163 patients for retrospective analysis. The ACP®/PRP cohort included 82 patients, and the control cohort (no ACP®/PRP) included 81 patients. The one-to-one ratio was coincidental. No patients were excluded from this consecutive, retrospective analysis. There was no intent to form a one-to-one ratio. The initial overall goal was to stop recruitment when approximately 80 patients had received the ACP®/PRP product due to application cost.

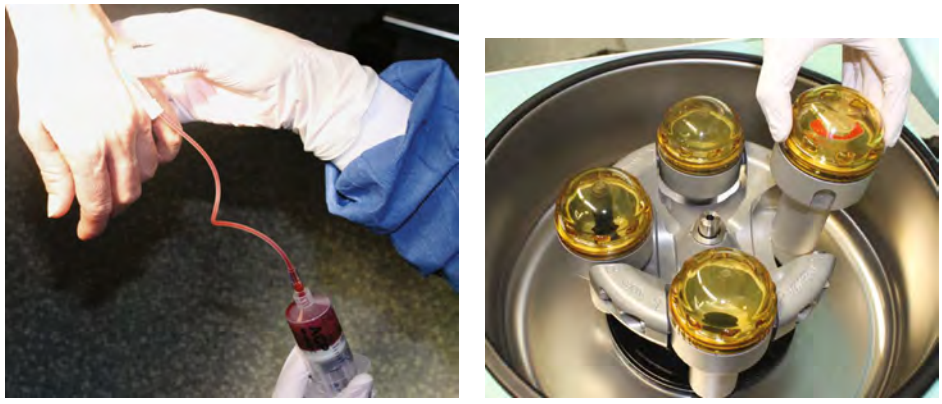
In order to match both cohorts, group comparability had to be established with respect to the commonly known risk-factors for decreased wound healing such as age, BMI, type of surgery, prior chemotherapy, and smoking habits [17–19]. Once group comparability was established, the clinical outcome of this head-to-head analysis could be compared. Complication rates were divided into major and minor complication rates. Major complication rates, i.e., revision surgeries, were considered a severe adverse event as a significant increase in patient morbidity as well as patient discomfort and a financial burden to the medical system is introduced [17, 20, 21]. Minor complication rates include seroma, requiring and not requiring aspiration, hematoma, and infection. This type of complication rate analysis is commonly used in analyses of medical products in oncoplastic surgery. It allows a preliminary evaluation of the usefulness of any new product. As complication rates are generally very low, trial participation needs to be high in order to elucidate more than a trend and establish significance. This issue is often problematic as any product application and evaluation is limited by product costs.

2.1. Breast Surgery and Lymphadenectomy. All surgeries were performed by experienced breast surgeons, and gold standards were adhered to during all surgeries. All sentinel lymph node (SNL) biopsies were performed via a single incision. Preparation and identification of sentinel lymph nodes were done atraumatically, and a handheld gamma-detection probe was used to identify the target lymph node. Sentinel lymph node intraoperative frozen sections were performed for all patients in the ACP®/PRP only. All patients were node negative, i.e., no tumor cells were found in the lymph node, during surgery. After a full pathologic workup, some patients showed nodal involvement (12.2% ACP®/PRP vs. 17.3% control). Patients received either breast-conserving surgery (BCS) or mastectomy (MRM). The wound areas did not come into contact with the separate sentinel node biopsy area for any of the patients.

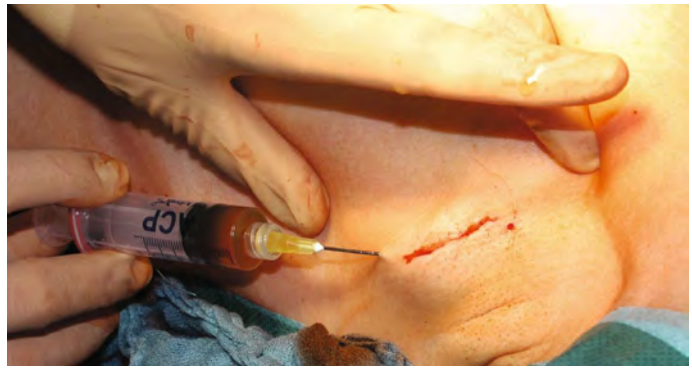
2.2. PRP Preparation and Application. The ACP® double syringe system (Arthrex, Naples, Florida, USA) was used during surgery. Procedures were followed as mandated by the manufacturer. Patient blood was extracted under sterile conditions during surgery via the port system or via a peripheral vein (see Figure 1). After centrifuge treatment, the double syringe system allowed the sterile transfer of the ACP®/PRP. It was then injected into the sentinel biopsy wound area subcutaneously, before a sterile dressing was applied. Figure 2 shows several different separation stages of the ACP®/PRP.

2.3. Follow-Up. This low-risk cohort analysis was associated with a short-term follow-up. This is the first follow-up of any kind for ACP®/PRP in a low-risk oncological cohort. Evaluation included the endpoints: overall survival, local recurrence, and metastasis-free survival for a median follow-up of 30 months. Kaplan-Meier plots were not possible as zero events occurred.

2.4. Ethics Committee. Written informed consent was obtained from all patients. A copy of the written consent is available for review by the editor-in-chief of this journal. This study was conducted in accordance with institutional review



(a) (b)



(c)

Figure 1: Shown are the collection of peripheral blood into the double syringe system (a) as well as the placement of the double syringe system into a centrifuge (b). (c) Shows the subcutaneous application of the ACP/PRP product after SNL biopsy wound closure in the patient's left axilla.

board standard operating procedures. The application and production of a patient blood product were listed with Bezirksregierung Koeln, Dezernat 24: Öffentliche Gesundheit, Medizinische und Pharmazeutische Angelegenheiten.

An ethics committee approval/vote was obtained at the University of Cologne, Cologne, Germany, with ethics case number #20-1058.

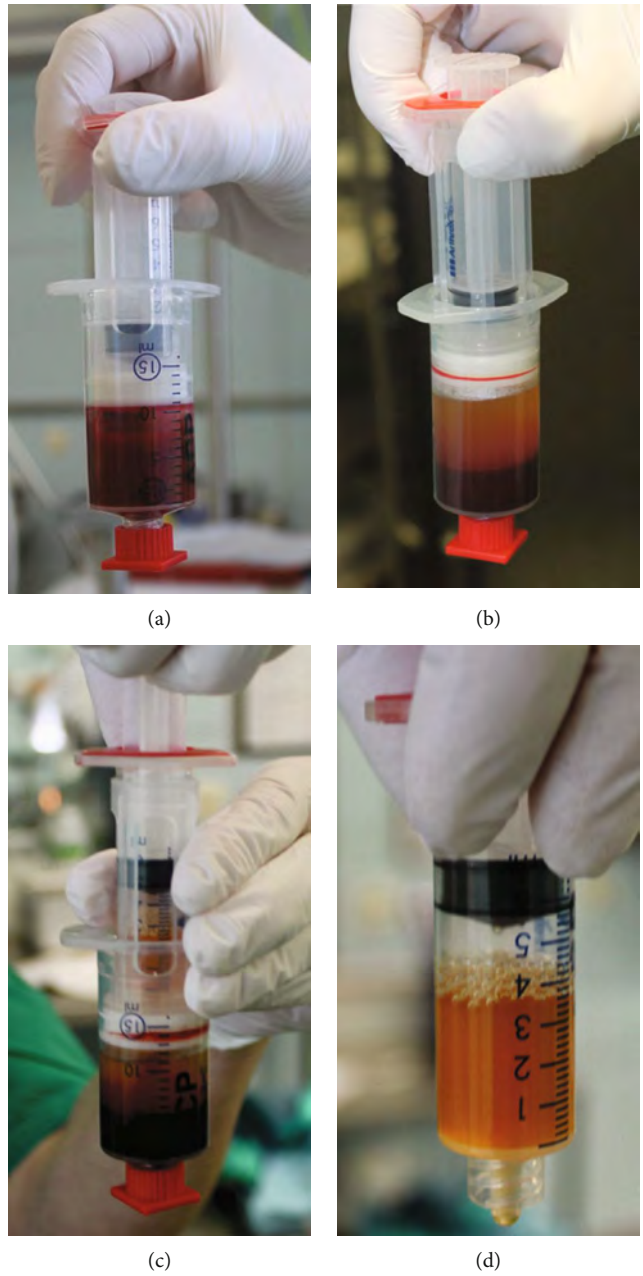
2.5. Statistics. Statistical analysis was performed using the VassarStats® (Vassar College, Poughkeepsie, NY, USA) statistics program. Pearson's chi-squared tests and t-tests were used in order to evaluate significances when appropriate.

3. Results

In order to allow for intercohort comparability, it was important to establish equal distribution of risk factors.

The average age of the ACP®/PRP cohort was 59.7 ± 9.9 years and 62.5 ± 12 years in the control cohort ($p = 0.13$). The average BMI was $23.4 \pm 3.4\,kg/m^2$ for the ACP®/PRP cohort and $25.1 \pm 5.2\,kg/m^2$ for the control group. This difference is in slight, but significant, favor of the control cohort ($p = 0.03$). Results are shown in Table 1. 81.7% ($n = 67$) in the ACP®/PRP cohort received a breast-conserving surgery compared to 71.6% ($n = 58$) in the control cohort. The remaining patients had a mastectomy with or without implant reconstructions. All patients received separate, noncommunication incisions for the SNL biopsies. The differences in surgical procedures were not significant ($p = 0.18$). Smoking, prior chemotherapy, and prior or concomitant antihormone therapy did also not differ significantly. Note that prior radiation was omitted in the analysis since prior radiotherapy of the axilla would automatically prohibit a SNL biopsy. Thus, all above mentioned complication-associated risk factors were not significant ($p = 0.69$). Differences in grading, tumor size, hormone receptor status, and Her2 status were also not observed (see Table 2). Note that despite having a negative lymph node, i.e., no metastasis in the lymph node during intraoperative frozen section, 12.2% ($n = 10$, ACP®/PRP) and 17.2% ($n = 14$, control) of all patients showed some sort of nodal involvement when the complete pathological workup was complete. This data included micrometastases. Nodal involvement did not exceed pN1a. Both cohorts do not differ. Therefore, regarding the oncological quality of the tumor, both cohorts were considered comparable.

3.1. Oncological Characteristics. All patients were early breast cancer patients. None of the patients had metastases. Table 2 shows a summary of patient characteristics. Cohorts did not differ in grading, immunohistochemistry, and/or involved lymph nodes. Due to this fact, administered chemotherapy and or radiation therapy did also not differ between these cohorts (see Table 1). There was no difference in BIRADS (Breast Imaging Reporting and Data System) classification



(a) (b) (c) (d)

Figure 2: Shown are the different stages of PRP preparation. The Arthrex ACP® double syringe system with whole blood (a) and after centrifugation (b). The double syringe allows the syphoning off of the PRP (c) which yields pure PRP, i.e., ACP (d). This product may now be injected.

regarding the initial tumor (all BIRADS 6). Regarding the lymph nodes, all ultrasound findings were negative; thus a sentinel node biopsy was required.

The key endpoints of this analysis, as seen in Table 3, show overall complications to occur in 21.9% ($n = 18$, ACP®/PRP) and 23.4% ($n = 19$, control) of all cases. Severe complications, i.e., revision surgeries did not occur in ACP®/PRP cohort and 1.2% ($n = 1$) needed a revision in the control cohort. Regarding minor complications, seroma requiring aspiration was found in 2.4% ($n = 2$, ACP®/PRP) and 3.7% ($n = 3$, control) of the cases. Seroma not requiring an aspiration was found in 19.5% ($n = 16$, ACP®/PRP) and 16% ($n = 13$, control) of the cases. Infections did not occur in either group. A trend favoring ACP®/PRP was seen for postsurgical hematoma with 0% (ACP®/PRP) vs. 2.5% ($n = 2$, control) of all cases ($p = 0.25$). Significance could not be reached. A 30-month follow-up for overall survival, local recurrence, and metastasis-free survival showed zero ($n = 0$) cases for both cohorts.

4. Discussion

After establishing initial comparability, we evaluated major and minor complication rates. As seen in Tables 1 and 2,

Table 1: Shown are the patient characteristics for both cohorts.

	ACP/PRP		Control (no ACP/PRP)		p value
	No.	%	No.	%	
Patients (total $n = 163$)	82		81		
Breast-conserving therapy	67	81.7	58	71.6	
Mastectomy	15	18.3	23	28.4	0.18
Smoking*	11	13.4	9	11.1	
Radiation*	61	74.4	33	40.7	
Chemotherapy*	18	22.0	22	27.2	
Hormone therapy*	72	87.8	71	87.7	
Average age	59.7 ± 9.9		62.5 ± 12		0.13
Range	37-79		36-82		
Average BMI (kg/m^2)*	23.4 ± 3.4		25.1 ± 5.2		0.03
Range	17.6-35		17.9-40.5		
Postmenopausal	53	64.6	56	69.1	

*All percentage data was calculated excluding the missing data (smoking: ACP $n = 67$, no ACP $n = 71$; Rtx: ACP $n = 73$, no ACP $n = 73$; Ctx: ACP $n = 78$, no ACP $n = 74$; hormone therapy: ACP $n = 80$, no ACP $n = 79$; BMI: ACP $n = 66$, no ACP $n = 71$).

Table 2: Documentation on tumor size and grading could not be procured for all cases due to the retrospective nature of this work. No patients were excluded.

	ACP/PRP		Control (no ACP/PRP)		p value
	No.	%	No.	%	
Patients	82		81		
Metastatis in SNL	10	12.2	14	17.3	0.3594
Tumor size*					
Tis	3	3.7	4	4.9	
T1	57	69.5	41	50.6	
T2	21	25.6	30	37.0	
T3	0		3	3.7	0.607
Grading*					
G1	15	18.3	17	21.0	
G2	49	59.8	46	56.8	
G3	11	13.4	18	22.2	0.7943
Hormone receptor status					
Positive	72	87.8	72	88.9	
Negative	10	12.2	9	11.1	0.0037
HER2/neu					
Positive	54	65.9	35	43.2	
Negative	28	34.1	46	56.8	0.0037

*All percentage data was calculated excluding the missing data (tumor size: ACP $n = 81$, no ACP $n = 67$; grading: ACP $n = 75$, no ACP $n = 81$).

comparability for both cohorts was given as risk factors were equal for both cohorts. Therefore, the subsequent endpoint analysis of major and minor complications could be performed. Overall, we found ACP®/PRP to have no disadvantages when applied.

As discussed in the introduction section, platelets contain a variety of growth factors, coagulation factors, adhesion molecules, cytokines, chemokines, and integrins. When activated, these entities are released causing an increase in concentration which is significantly higher than the baseline blood levels. This was thought to improve wound healing. However, a significant benefit could also not be established. Major complication rates in the control cohort, i.e., revision rates, were very low (1.2%, control) which means that in order to produce a significant difference, a study would have to vastly increase participant

Table 3: Shown are the major and minor complication rates for both cohorts.

	ACP/PRP		Control (no ACP/PRP)		p value
Patients	82		81		
SLN removal via separate incision***	75	91.5	45	55.6	
SNL removal via existing incision***	7	8.5	36	44.4	<.0001
Total*	18	21.9	19	23.4	
Major*					
Revision surgery	0		1	1.2	0.5
Minor*					
Seroma requiring aspiration	2	2.4	3	3.7	0.68
Seroma not requiring aspiration	16	19.5	13	16.0	0.68
Hematoma	0		2	2.5	0.25
Infection requiring antibiotics	0		0		

*All percentage data was calculated excluding the missing data (ACP, $n = 67$ and no ACP, $n = 73$). ***SNL: sentinel lymph node.

numbers. This is currently being done as ACP®/PRP application is continuously offered to all SNL patients at the investigation site. A follow-up publication will be attempted in the future. Minor complications, such as aspirated seroma and hematoma, numerically favor ACP®/PRP application although significance was also not reached. This could again be attributed to low patient numbers. Summarizing the complication data, it can be stated that ACP®/PRP was not able to produce a significant advantage in a SNL-biopsy scenario. Advantageous trends were observed; they do however require a follow-up trial.

In addition, this is the first short-term follow-up analysis for any ACP®/PRP data in oncological, specifically breast cancer, patients. Although the 30-month follow-up interval may be considered short, it is an important step towards establishing that ACP®/PRP in oncological patient does not seem to cause any concern. A 50-month (long-term) follow-up for the analysis of the treatment of subcutaneous venous access device is currently also being evaluated. Both of these results may begin to solidify our confidence in the safety of PRP in oncological patients.

Financially, the burden to the health care system is minimal. At approximately $50 cost for the ACP® double syringe system, costs are negligible. The subjective benefit of increased patient satisfaction, shown in our prior publication, itself should be enough to consider ACP® application. However, as objective complication rate analysis currently does not show a significant benefit, these authors consider ACP®/PRP application in a SNL scenario a possible and safe option, although we do not consider it a mandatory recommendation.

(1) Is ACP®/PRP safe to use in a sentinel node biopsy scenario?

Preliminary data is promising. We were able to establish some data for ACP®/PRP use in oncological patients yielding no negative side effects.

(2) Is ACP®/PRP able to improve complication rates?

This remains somewhat unclear as the data is immature. We were able to show a slight trend towards improving hematoma and seroma rate. Significance could not be established.

(3) Is there an oncological risk for ACP®/PRP application regarding the short-term follow-up in oncological patients?

No. There were zero cases of recurrence and/or death in the 30-month follow-up. This product seems to be oncologically benign.

5. Trial Limitations

This work can be interpreted as hypothesis generating—a prospective and randomized trial would be needed to evaluate product impact onto overall short-term and long-term efficacy and safety. Within the scope of such a trial, a very homogenous patient group would have to be evaluated over a time period of ten to 15 years in order to evaluate short-term local and long-term distant recurrence risk.

6. Conclusion

This is the first analysis of ACP®/PRP used in breast cancer patients in a SNL biopsy setting worldwide. ACP®/PRP seems to be oncologically inert while displaying a trend towards decreasing complication rates. A zero cancer event risk in a 30-month follow-up was documented.

Data Availability

All data is available upon request from the author.

Conflicts of Interest

This is an investigator driven study. The authors have no conflicts of interest.

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Platelet-Rich Plasma for Skin Rejuvenation

Facts, Fiction, and Pearls for Practice

Grace Lee Peng, MD

KEYWORDS

• Platelet-rich plasma • Microneedling • Collagen induction • Platelet gel • Skin rejuvenation • Facial rejuvenation • Wrinkles • Acne scars

KEY POINTS

- Use of platelet-rich plasma in plastic surgery.
- Facial plastic surgery and platelet-rich plasma use.
- Microneedling and platelet-rich plasma.
- Microneedling for acne scars.

INTRODUCTION

Platelet Function in Hemostasis and Wound Healing

Platelets are an important part of hemostasis as well as the process of wound healing. There are 3 stages to wound healing: inflammatory, proliferative, and remodeling.[1–4] During inflammation, the goal is for hemostasis and the initiation of the wound-healing process.

With tissue injury, platelets come in direct contact with and aggregate at the site of damaged blood vessels.[5] Tissue injury also leads to platelet activation, which in turn leads platelets to release biologically active proteins and growth factors that promote wound healing. This includes platelet-derived growth factor, transforming growth factor-β, fibroblast growth factor, epidermal growth factor, keratinocyte growth factor, and vascular endothelium growth factor, among others.[6,7] In addition, adhesion molecules, such as fibronectin and vitronectin, scaffolding proteins, such as fibrinogen, and other molecules responsible for intercellular binding and communication, are stimulated. Together, they promote connective tissue healing, epithelial development, angiogenesis, and deposition of collagen matrix[6,7] (**Table 1**).

Platelet-Rich Plasma

Platelet-rich plasma (PRP) is autologous blood plasma with a concentration of platelets well above baseline.[8–10] The usual concentration of platelets in the blood is approximately 150,000 to 400,000 platelets per cubic microliter.[1,8] PRP contains 4 to 7 times the physiologic concentration of platelets.[11,12] PRP is prepared by centrifugation of blood drawn from the patient before any procedure or surgery.[11]

The whole blood, once drawn, needs to have an anticoagulant to prevent clotting. Most PRP kits come with venipuncture tubes that already contain an anticoagulant. This anticoagulant is most often citrate, which will bind to the calcium ions, thus disrupting the coagulation cascade. In the anticoagulated state, the blood is stable for up to 8 hours.[9,13]

The next step in the processing of the PRP is centrifugation and subsequent separation of blood components (red blood cells, white blood cells and platelet-poor plasma, and PRP). In most cases, manufacturers will have their own centrifuges, which lead to differential centrifugation and yield a higher concentration of platelets and a cleaner separation of blood components.[8] To enhance this, many venipuncture tubes come

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Table 1
Platelet growth factors and their mechanism of action

Growth Factors Released by Platelets	Mechanism
Platelet-derived growth factor	Initiates connective tissue healing Increases mitogenesis and angiogenesis Enhances collagen synthesis
Transforming growth factor-β	Increases chemotaxis Stimulates deposition of collagen matrix
Fibroblast growth factor	Stimulates angiogenesis Stimulate proliferation of myoblasts Promotes migration of fibroblasts
Epidermal growth factor	Increases proliferation of mesenchymal cells Increases proliferation of epithelial cells Enhances potentiation of other growth factors Stimulates differentiation of epithelial cells
Keratinocyte growth factor	Stimulates epithelialization
Vascular endothelium growth factor	Increases vascular permeability Enhances endothelial cell migration/proliferation Promotes collagen deposition

with various types of gel separators, which provide a gradient and allows for ease of separation during centrifugation.[14,15] After centrifugation, the platelet concentration in the plasma is considered PRP. However, there is no evidence the concentration of platelets is proportional to efficacy[16,17] (**Fig. 1**).

At this stage of preparation, PRP can be used immediately. Because the various components of commercial collecting venipuncture tubes and systems differ in their concentrations of platelets as well as the active nature of the platelets, there may or may not be a need for further activation.[18–20] Some studies show that calcium-based activation is needed because the initial calcium was inhibited with the anticoagulant.[6,21,22]

Regardless of the concentration of platelets in the PRP, or the activation of the PRP after processing, the concentration of growth factors and biologically active proteins secreted by each individual will undoubtedly vary. This is due to not only each patient's own responses but also the number of platelets that are active. Currently, there are not many studies measuring the amount of growth factors as related to the concentration of PRP.

Clinical Use

PRP has been used for orthopedic indications, wound healing, facial skin rejuvenation, and hair restoration.[23,24] In studies for facial skin rejuvenation, PRP has been shown to improve texture, wrinkles, and facial volume.[24]

Application for Skin Rejuvenation

Use of platelet-rich plasma in conjunction with microneedling

Microneedling is the result of multiple, often oscillating needles causing damage to the skin, which then induces the skin itself to repair by collagen stimulation. The needles in microneedling devices are extremely fine and can penetrate up to a depth of 3 mm.[25] They reach the papillary and reticular dermis in a purely mechanical way. Therefore, there is preservation of stratum corneum and the epidermal barrier function, leading to the lack of scarring.[26,27] Microneedling also does not carry with it thermal injury and necrosis, thus making it safe for patients of all skin types and Fitzpatrick classification[10,26] (**Table 2**).

However, this trauma alone can activate the healing process. Immediately following the injury, fibroblasts inundate the region for wound healing, stimulating endothelial cells and starting the process of neoangiogenesis, and elastin and collagen production.[27–29] Commonly termed collagen induction therapy, microneedling has been used to improve the appearance of acne scarring, skin discoloration, melasma, fine lines, wrinkles, and facial scars.[29,30] When used in conjunction with PRP, its effects can be potentiated and can help improve skin elasticity.[26]

Immediately after the procedure, there will be redness from pinpoint areas of minimal bleeding. Redness usually does not last long, with most patients having minimal redness after the first 1 to 2 days. Some patients with more sensitive skin

Fig. 1. After centrifugation, the blood separates into its various layers with the bottom layer being the red blood cells, the middle layer being the white blood cells, and the upper layer being the PRP.

may have more prolonged redness. Of note, areas of thinner skin, such as periorbital regions, over the upper nasal dorsum, and areas of the forehead may have some increased chances of localized bruising.

Use of platelet-rich plasma as facial injection

PRP is commonly injected to the face and neck to help increase facial volume through collagen stimulation.[18,31,32] This injection can be either intradermal or subdermal, or a combination of the two. PRP injections have been shown to improve the skin color and texture as well as the depth of fine lines and wrinkles through an increase of dermal collagen.[18,27,31] However, given the depth of injection, there is no ability for topical numbing, and there are higher reports of pain and discomfort during the procedure. In addition, there may be increased downtime from bruising and some swelling.[33,34]

In general, these treatments are done at 4- to 6-week intervals and repeated at least 3 to 5 times, or until the desired result is achieved. Additional procedures for maintenance are performed in a timeline that is spaced out for maintenance (**Figs. 2** and **3**C).

Table 2
Microneedling devices and their depth of penetration

Name of Pen	Depth (mm)
Collagen PIN	3.0
Cosmo Pen	2.5
CytoPen	2.5
DermaPen	2.5
MD Needle Pen	2.5
MD Pen	2.8
MesopenMD	2.0
Micropen	2.5
Rejuvapen	2.5

PEARLS FOR PRACTICE

Numbing the patient before the procedure is important, because it allows for a pleasant experience. Topical numbing cream should be comprehensive, extending to all areas where microneedling will be performed. For example, the topical numbing should extend all the way to the edge of the hairline as well as to the edge of the tragus, if it is to be treated. Treated areas can also include the neck and décolleté. Topical numbing cream comes in a variety of forms,

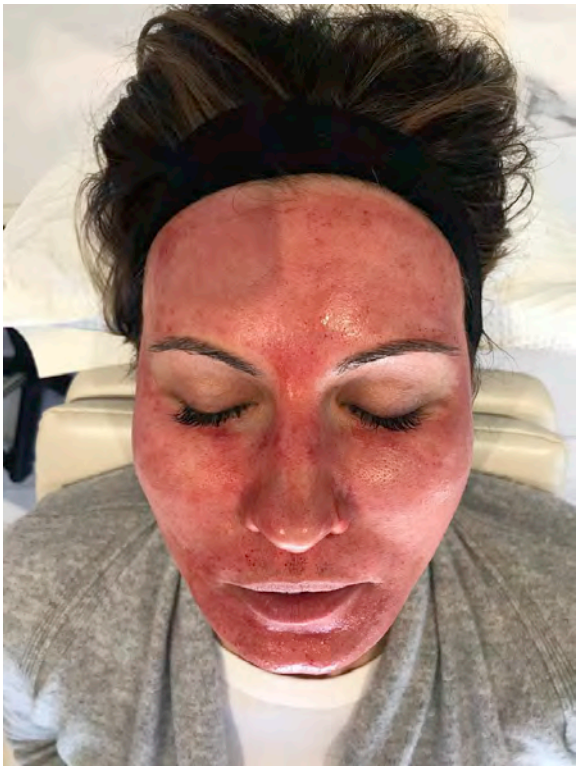

Fig. 2. Redness immediately after procedure is similar to that of a medium sunburn.

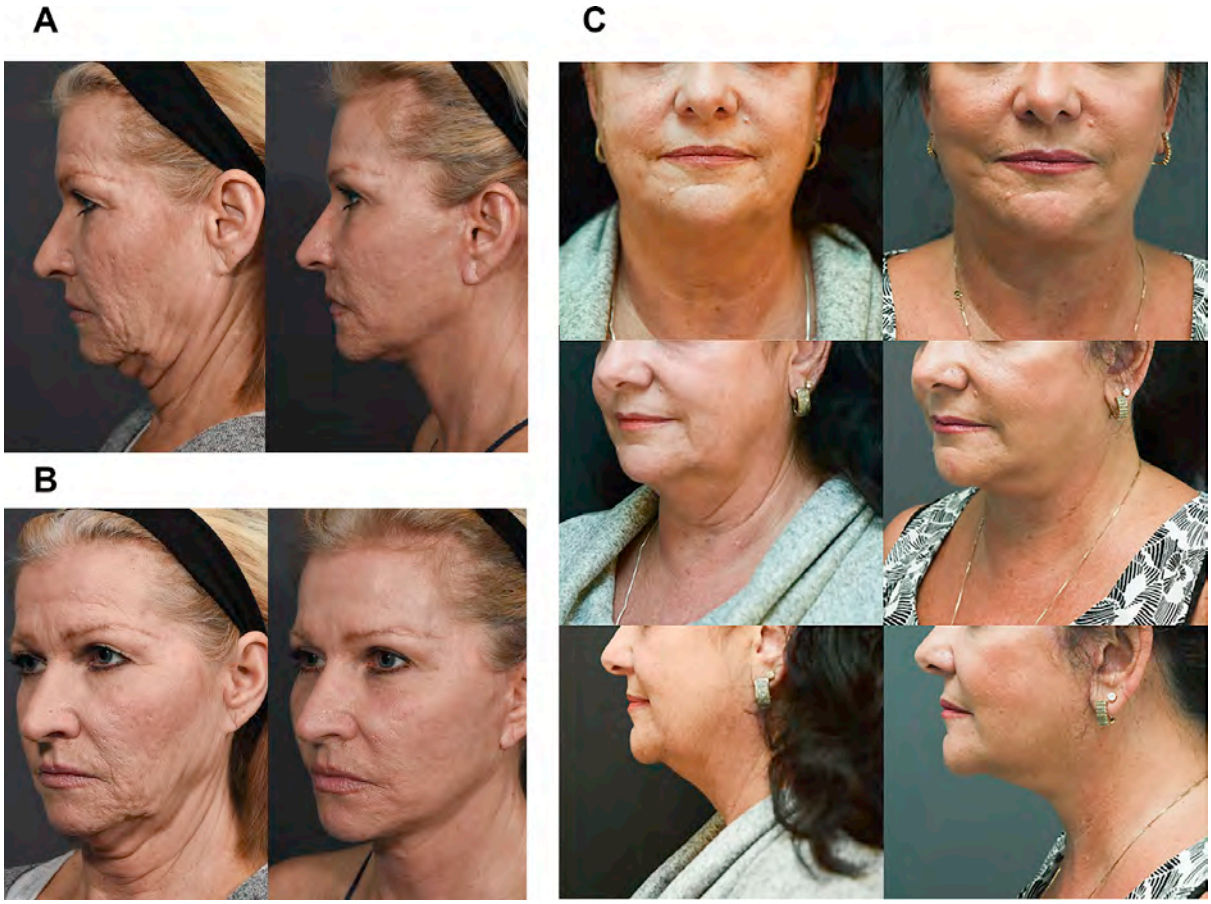

Fig. 3. (*A*) Microneedling performed 3 times spaced 1 month apart improved the appearance of ice pick acne scars, fine lines, and pores in this 60-year-old woman. (*B*) Microneedling performed 4 times spaced 1 month apart improved the active cystic acne as well as acne scars, and pore size in this 40-year-old woman. (*C*) Facial PRP injections in the midface performed 3 times spaced over 6 months as well as microneedling performed 2 times during those 6 months helped to improve the skin discoloration, fine lines, and the midfacial volume. This patient had also had a facelift, although the midface region was not entered during the facelift.

although the author has found a compounded ointment of lidocaine (10%), prilocaine (10%), and phenylephrine (0.25%) to be the most efficacious. Patients can be topically numbed immediately on arrival to the office for a duration of 45 to 60 minutes. During this time, they can also have their blood drawn in order to save time. These procedures add to office efficiency, because numbing can be done concurrently while waiting for the blood to be spun and processed.

Microneedling for various areas should be done at different depths, because the skin of the face, neck, and chest varies in its thickness. Each individual, their skin texture, and sebaceous quality should be taken into account, with patients with thicker skin able to have microneedling performed at deeper depths (**Fig. 4**).

Typically, to treat acne scars and other scars, the depth should range from 2.5 to 3 mm. However, areas of facial skin, such as the periorbital, upper nasal dorsal, and forehead areas, should be treated with depths of 0.5 to 1.5 mm, because of its thinner and more sensitive skin nature. Multiple passes in various directions should be used to ensure even treatment. As the needles oscillate, they will automatically puncture the skin to the desired depth, and additional pressure does not need to be used while using microneedling devices. It is important to avoid additional pressure and dragging the device along the skin, because that can cause deeper line injuries, irregularity, and unevenness.

Platelet-Rich Plasma Injection

Microneedling with PRP and injections of PRP can be combined during the same treatment. For most patients, microneedling will help the overall skin texture, whereas injections will further help collagen stimulation from the deeper tissues, helping to improve volume (**Fig. 5**).

Use of small needles such as a 32- or a 34-gauge needle will help with discomfort during

Fig. 4. Microneedling around different areas of the face should be performed at different depths and depends on the skin texture of each patient. Forehead: 1 to 1.5 mm; periorbital: 0.5 to 1 mm; face: 1.5 to greater than 2 mm; nasal skin, especially over the upper dorsum: 0.5 to 1 mm; neck and décolleté: 0.5 to 1.5 mm.

the procedure. Slow injection is recommended during the procedure, because this helps to overcome the mild burning sensation that may be experienced. Also, the use of an ice roller or vibration device can improve patient comfort during the procedure. In general, given that there is a higher chance of bruising with injections of PRP, as opposed to microneedling with topical application, it is recommended that patients refrain from taking any blood thinners a few days before the procedure.

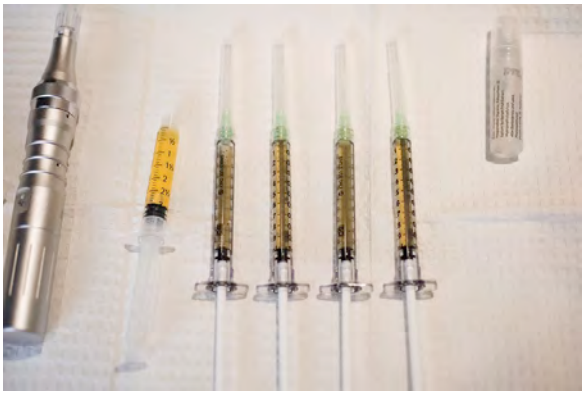

Fig. 5. Setup of a microneedling tray (from left to right): microneedling pen and single-use tip; PRP in a 3-cc syringe to be used while microneedling; PRP in 1-cc syringes with a 32-gauge needle for injection into the deeper dermis; hyaluronic acid for the patient to use at postprocedure day 1.

Reactivation of Herpes Simplex Virus and Prophylaxis

Patients with a history of cold sores should take prophylaxis with an antiviral before the procedure to prevent a flare-up after microneedling and PRP injections. Any procedure that causes trauma to the skin carries with it the potential of reactivation of the herpes simplex virus (HSV). With microneedling and PRP injections, it is possible that the tissue manipulation and the inflammatory reaction can reactivate the HSV. Although this is more likely after ablative procedures, it can be devastating to a patient coming in for what they expected to be a minimal-downtime, elective, skin rejuvenation procedure.[35,36] Recommendations for prophylaxis include any patient with the following:

1. Previous outbreak after a procedure
2. Multiple herpetic outbreaks a year
3. Lip augmentation and subsequent outbreak
4. Facial resurfacing procedures (medium or deep peels, fractional lasers, microneedling)
5. Immunocompromised state[37]

There are no evidence-based studies comparing the prophylactic efficacy of acyclovir, valacyclovir, and famciclovir for these aesthetic patients. However, there are commonly recommended dosages, including acyclovir 400 mg twice daily (or 3 times for those at high risk) or valacyclovir 500 mg daily (or twice daily if high risk).[37] Antiviral medication is usually started the day before or the day of the procedure and for an additional 5 to 7 days.[38]

Despite the potentially beneficial properties of PRP, there are currently no studies that show whether PRP use in conjunction with a procedure, such as microneedling, will affect HSV reactivation. Awareness of cold sore reoccurrence and minimizing the HSV reactivation are important to prevent postprocedure complications.

POSTPROCEDURAL RECOMMENDATIONS

Patients should always avoid direct sunlight immediately after and for at least 2 weeks following the procedure. Sun protection should always be used. Hydration of the skin is also important and can be achieved with a variety of hyaluronic acid serums.

In addition, pairing the microneedling with microdermabrasion 7 to 10 days after the procedure can help to remove some of the dead skin cells and with the efficacy of cellular turnover. Intense hydration, such as with a Hydrafacial, at the same time, can also help to enhance results.

Contraindications to Use of Platelet-Rich Plasma

Although the use of PRP has a relatively low side-effect profile, there are some contraindications for its use.[39] Absolute contraindications include platelet dysfunction syndrome, hemodynamic instability, chronic liver disease, local infection at the site of the procedure, septicemia, hypofibrinogenemia, and anticoagulant use.[19,39]

Relative contraindications include use of nonsteroidal anti-inflammatory drugs within 48 hours of the procedure, corticosteroids injection in the area of treatment within a month before the procedure, systemic corticosteroids, tobacco use, recent illness and fever, cancer hemoglobin less than 10 g/dL, or thrombocytopenia with a platelet count less than $10^5/\mu L$, and autoimmune conditions not associated with thrombocytopenia.[39]

In general, in an outpatient setting, most of these contraindications are rare due to the overall health of patients seeking elective skin rejuvenation procedures. However, asking about platelet dysfunction, bleeding issues, recent illnesses, and localized infections is important.

SUMMARY

Being a product of autologous blood, PRP is relatively safe for use in facial rejuvenation procedures. It is always recommended that handling patient blood products be done in a safe and sterile manner to prevent contamination, especially in situations wherein there are multiple patients all receiving treatments at the same time.

PRP appears to have efficacy in the management of acne scarring, and when combined with microneedling, has improved results as compared with microneedling alone and injection alone.

Currently, there are no set guidelines about obtaining and processing PRP to yield the most efficacious plasma solution or platelet concentration. Variables that may affect the efficacy of PPR include the volume of blood obtained, anticoagulant used, the speed and time for centrifugation, and activating agents.

Clinical studies that compare these various forms will help provide better insight as to which PRP is better suited for various aesthetic purposes. Well-controlled, split-side treatments will be able to better define efficacy and minimize the inevitable intersubject variability.

Incorporating the use of PRP into any clinic and practice can help with patient retention, as well as conversion into other procedures, be it surgical or nonsurgical.

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Review

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The evidence behind the use of platelet-rich plasma (PRP) in scar management: a literature review

Osaid H Alser and Ioannis Goutos

Abstract

Introduction: Autologous platelet-based concentrates represent increasingly popular adjuncts to a variety of medical, surgical and aesthetic interventions. Their beneficial potential rests on the ability to deliver a high concentration of growth factors to the target tissues. There are currently no reports in the literature appraising the evidence behind the use of platelet-rich plasma (PRP) in scar management.

Methods: A detailed English literature review was conducted using PubMed Medline, Embase and Web of Science; the manuscripts were appraised and classified according to the Joanna Briggs Institute Levels of evidence. The results are presented in descending order of evidence separately for atrophic, keloid, surgical and traumatic scars.

Discussion: On the basis of level 1 evidence currently available, it appears that PRP can improve the quality of atrophic acne scars treated with ablative fractional CO2 laser and decrease the duration of laser-related side effects including oedema and erythema. Regarding surgical scars, the current data suggest that PRP may improve wound healing and early scar quality; furthermore, incorporation of PRP in fat-grafting procedures undertaken in conjunction with non-ablative, fractional laser can contribute to better wound healing as well as a significant improvement in texture, colour and contour in traumatic scar resurfacing. There are no high level studies at present to support the incorporation of autologous platelet-based concentrates in the management of keloid scars.

Conclusion: PRP is a promising adjunct in scar management practice. Further research with long-term follow-up is warranted to delineate the value of this modality in different subtypes of scars.

Keywords
Atrophic, concentrate, keloid, management, platelet, platelet-rich plasma, PRP, scar, traumatic

Lay summary

Platelet-rich plasma (PRP) is an increasingly popular product used in a variety of medical, surgical and aesthetic interventions; it is derived by spinning down a patient's own blood and applying it back to an area of the body undergoing an intervention. We undertook this study to find out whether the use of PRP can have a beneficial effect on scars. We conclude that at present there is some evidence that it may improve the quality of depressed acne scars and ameliorate the duration of side effects associated with

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fractional laser treatment. Furthermore, PRP can improve healing parameters and early scar quality following a Caeserean section as well as enhance outcomes if used in combination with fat grafting and fractional laser for the revision of traumatic scars. The evidence behind the role of PRP for the management of keloid scars is low at present. Most studies do not assess long-term results, so further research is needed before PRP is widely adopted in scar management protocols.

Introduction

Platelet-rich plasma (PRP) is an autologous blood-derived product enriched in platelets, growth factors and chemo/cytokines delivered in a concentrated volume of plasma. Since the 1970s, PRP has received significant attention as applied to tissue repair and regeneration.[1,2] Initial studies focused predominantly on applications within the musculoskeletal and maxillofacial fields; however, in recent years, it has been used for a range of dermatological indications including wound healing, fat grafting, alopecia, scar management as well as soft-tissue volume augmentation.[3]

PRP has the potential to deliver a high concentration of growth factors to target tissues by virtue of the contents within the alpha and dense granules.

a) Alpha granules contain seven fundamental growth factors: platelet-derived growth factors (PDGFaa, PDGFbb and PDGFab); transforming growth factor beta (isoforms TGFβ1 and 2); epithelial growth factor (EGF); and vascular endothelial growth factor (VEGF). These modulate cell proliferation, differentiation, angiogenesis and chemotaxis;[4,5]

b) The dense granules contain bioactive agents including serotonin, histamine, dopamine, calcium and adenosine; these can increase membrane permeability and modulate inflammatory processes.[6,7]

Degranulation of these organelles results in the release of pre-packaged growth factors, many of which have short half-lives; therefore, greater effectiveness may result if they are activated at or just before application. PRP has 3–5 times the concentration of platelets normally found in wounds and the resulting growth factor release following activation can further stimulate cell proliferation and differentiation towards tissue regeneration.[8]

PRP is prepared either manually or using automated devices or kits. In the manual method, blood is withdrawn from the patient, an anticoagulant is added and the mixture is centrifuged. In the double spin-method, blood is separated into three layers: platelet-poor plasma (PPP) at the top of the tube; PRP in the middle; and red blood cells (RBCs) at the bottom (image 1). The RBCs are discarded and following the second centrifugation, the PPP is discarded. The end product consists predominantly of PRP and thrombin or calcium chloride is subsequently added as a platelet activator.[7,8] The value of the second centrifugation relates to the concept that after a single spin, the RBCs interfere with the fine separation of the platelets regardless of the rate or the time of centrifugation, hence producing a preparation with less percentage of PRP.[9,10]

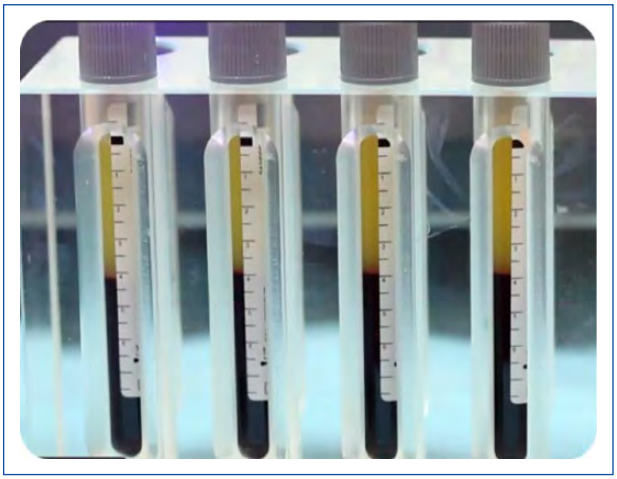

Image 1. Appearance of whole blood centrifugates illustrating the separation of platelet (top) and red blood cell (bottom) portions before the platelet rich plasma (PRP) is extracted for clinical use.

Various automated devices and kits have been manufactured in the last few years in order to facilitate the delivery of ready-to-apply PRP without the need for manual separation. The limitation associated with the wide range of available automated devices/kits is that their products are likely to have different growth factor concentrations; this poses challenges in comparing clinical efficacy. Some of them have been approved by the U.S. Food and Drug Association (FDA), e.g. Smart PReP® (Harvest Technologies Inc, Plymouth, MA, USA), PCCS® (3i Implant Innovations Inc, West Palm Beach, FL, USA), BioMet GPSII®, etc.[11]

The current consensus classification system for the four main categories of autologous platelet concentrates is based on their fibrin architecture and cell content:

1. Pure PRP (P-PRP) or leukocyte-poor PRP: this is by far the most common category used in clinical practice in either liquid or gel form;
2. Leukocyte- and PRP (L-PRP): this can be delivered in either liquid or gel form;
3. Pure platelet-rich fibrin (P-PRF) or leukocyte-poor PRF: this is a solid preparation and therefore has limited clinical applications;
4. Leukocyte- and PRF (L-PRF) available in gel form only.

PRP can be applied as an isolated modality or as an adjunct to minimally invasive and surgical interventions given its potential to improve repair and regeneration.[13–15] Scar management is an expanding field encroaching a number of disciplines including plastic surgery, dermatology and physical therapy to mention a few.

A number of animal models have been used over the last few years to assess the effectiveness of PRP in the treatment of different types of scars. There is preliminary evidence in a canine model[16] that application of autologous PRP using the subcutaneous infiltration route at the wound margins can produce a significant enhancement of wound re-epithelisation and a reduction of scar formation at weeks 1 and 3 in comparison to controls. Malondialdehyde (MDA) concentration, which is a marker of oxidative damage, was also significantly decreased in PRP-treated wounds in this work; furthermore, the activity of matrix metalloproteinase 9 (MMP-9) reached its peak at the second week and was significantly higher in the PRP-treated group.

In another experimental rat uterine horn adhesion model, the inflammatory cytokine tumour growth factor 1 beta (TGF-1beta) expression was significantly reduced in the PRP-treated group compared to the control and hyaluronic acid groups; these findings prompted the authors to conclude that PRP is a promising and effective adjunct in the prevention of postoperative adhesion development.[17] The aim of this article is to review the current available evidence on the efficacy of platelet preparations in the management of scars and present future directions for research in the field.

Methodology

A detailed English literature review was conducted of the following databases: PubMed Medline; Embase; and Web of Science. The following keywords were used: 'platelet-rich plasma AND scar'; 'PRP AND scar'; 'PRP AND keloid'; 'hypertroph* AND platelet-rich AND plasma'; 'platelet-rich plasma AND hypertroph* AND scar'. Our search retrieved 52 citations; all abstracts were screened by both authors to ensure relevance. A total of 36 articles were selected for inclusion into our work; following full detailed manuscript review, 20 were deemed relevant to be presented in our analysis. The selected papers were appraised and classified according to the Joanna Briggs Institute Levels of evidence with the help of an independent research consultant in evidence synthesis.[18]

We present our findings based on a categorisation system according to the type of scars platelet-rich preparations were applied to, namely atrophic, keloid, surgical and traumatic scars.

Results

Atrophic acne scars

Atrophic scars are the most common type of acne scars; they can be further subdivided into ice pick, boxcar and rolling subtypes.[16] A variety of management modalities have been employed including laser resurfacing, chemical peeling, dermal fillers, dermabrasion, needling, subcision and punch excision.[17] PRP is a recently introduced adjunct and has been used predominantly in combination with other modalities.

Level 1

Lee et al.[19] carried out a split face study in 14 Korean patients who underwent ablative fractional laser resurfacing and were randomly assigned to receive either injectable PRP or normal saline following the treatment episode. The PRP was extracted with a double spin-method and produced 6 mL for each patient; injection points were spaced 1.5–2 cm apart.

The laser settings were: pulse energy 25 mJ per fixed 150-μm diameter microbeam and a density of 400 MTZ/cm^2 with concurrent forced air cooling for epidermal protection; assessment was performed with the help of a standardised photographic method by blinded dermatologists. Erythema on the PRP side improved faster compared to controls and was significantly less at day 4 as confirmed by chromometer readings (P = 0.01 and 0.047, respectively). Duration of oedema as well as crusting were significantly shorter in the PRP group (6.1 ± 1.1 vs 7.1 ± 1.5 days and 5.9 ± 1.1 vs. 6.8 ± 1.0 days, P = 0.04) and the overall clinical improvement four months after

treatment was superior on the PRP arm (2.7 ± 0.7 vs. 2.3 ± 0.5, $P = 0.03$).

In another study, Gawdat et al.[20] compared the efficacy of topical versus intradermal PRP (double-spin method) after ablative fractional CO2 laser (FCL) using a single-blind randomised split-face study design in 30 patients with facial atrophic acne scars (Fitzpatrick skin types III to V). Group 1 underwent ablative fractional CO2 laser followed by intradermal PRP on one side and intradermal saline on the other; Group 2 had FCL followed by intradermal on one side and topical PRP on the other side. Each patient received three treatment sessions at monthly intervals. The settings for the laser were as follows: 15 W, dwell time 600 ms, spacing 700 μm, smart stack level 2. The injectable PRP and saline were administered in 0.2-mL volumes and injected at 1.5 cm apart; topical PRP was applied in 2-mL volumes. Photographic assessment comparison by a blinded physician using a four-point scale (excellent to poor) at three months after the last session showed that the combination of ablative fractional CO2 laser and PRP (topical and intradermal administration) showed significantly better results than ablative fractional CO2 laser alone ($P = 0.03$). Interestingly, no significant difference was observed between topical and intradermal PRP adjuvant administration ($P = 0.10$). Results based on a patient clinical satisfaction scale showed a similar trend to the above physician-reported outcomes. Side effects including erythema, oedema, post-inflammatory hyperpigmentation and acneiform reaction were all of a significantly shorter duration in the PRP-treated areas ($P = 0.02$) leading to significantly shorter downtime. Results regarding scar depth using optical coherence tomography showed that the PRP-treated areas were improved in a statistically significant manner ($P = 0.01$). In conclusion, this study showed that the combination of PRP and ablative fractional CO2 laser produced a better resurfacing response, fewer side effects and quicker recovery than laser alone; additionally, given that there was no statistically significant difference between the results obtained with topical versus intradermal PRP, the authors advocate the topical administration in order to minimise discomfort associated with the injectable route.

Level 2

Nofal et al.[8] conducted a quasi-experimental prospective controlled study on 45 patients with atrophic acne scars of varying severity. The cohort was divided into three groups of 15 patients each undergoing one of the following treatments: (1) intradermal injection of PRP; (2) application of 100% trichloroacetic acid (TCA)-CROSS; and (3) combination therapy of skin microneedling and topical PRP. Each patient underwent three sessions at two-weekly intervals. Results were assessed using the qualitative global acne scarring grading system (QGSGS) by two blinded dermatologists using photographs before and two weeks after the last treatment. Patient satisfaction ratings were also obtained. QGSGS findings suggested that a highly significant improvement in scar severity was seen after all modalities ($P < 0.001$); namely, an excellent to very good rating was found in 46.7% of the PRP group, 26.7% of the TCA CROSS group and 60% of the PRP with microneedling group. Nevertheless, none of the three treatments were significantly superior in comparison ($P = 0.87$) with regards to the quartile grading scale as well as patient satisfaction ratings. Limitations of this study include apart from the small cohort size, the large number of variables (including the number of treatment sessions used, the depth of needling as well as the mode of PRP administration), which make any valid conclusions challenging.

Similarly, Ibrahim et al.[21] compared microneedling alone to combined microneedling and PRP for post-acne atrophic scars as part of a split-face comparative study involving 35 patients. All patients (Fitzpatrick skin types I–IV) were treated with four sequential microneedling sessions using a 1.5 mm dermaroller to pinpoint bleeding alone on the right side of the face, and a combination of microneedling and topical PRP on the left side with an interval of three weeks between sessions. The follow-up period was three months following which, two blinded dermatologists performed photographic evaluation using the Goodman and Baron grading system. Patients also graded their response as poor, good, very good or excellent. Both treatment modalities produced a significant improvement in the global acne scoring system, namely from 3.2 ± 0.7 to 1.8 ± 0.6 for the right side and 2.1 ± 1.1 for the left side ($P < 0.001$); nevertheless, the difference between both modalities were not statistically significant ($P = 0.73$). A similar trend was observed with regards to the patient satisfaction scores for both modalities. Interestingly, there was a statistically significant difference in the post-procedural erythema and oedema in favour of the PRP-treated side ($P < 0.001$); hence the authors concluded that PRP can minimise side effects of microneedling in acne management.

Another split-face randomised controlled study was performed by Faghihi et al.[22] in which 16 patients (Fitzpatrick skin type II–IV) with atrophic acne scars received ablative fractional CO2 laser combined with intradermal PRP treatment on one half of their face and laser with intradermal normal saline (NS) on the other half. The PRP was prepared using a two-stage centrifugation process and was injected intradermally within 2-cm intervals to an overall volume of 0.2-mL following ablative CO2 laser session (settings: power 25 W; duration of 3; energy 30 mJ; pixel pitch of 1; and ablation depth of 600 μm). Participants received two treatment cycles one month apart. Serial digital photography at baseline, one month after the first session and four months after the second session were obtained and a quartile improvement grading scale was used by two blinded dermatologists to evaluate the overall clinical improvement. Participants were asked to grade overall satisfaction based on a range between 0–3 (slightly to very satisfied) and a 0–10 visual analogue scale was used to record adverse effects (erythema and oedema). The overall clinical improvement of acne scars was higher on the PRP-laser treated side, but the difference was not statistically significant either at one month following the first session or four months following the final session ($P = 0.15$ and 0.23). Moreover, the adverse effects including erythema and oedema were more severe and of longer duration in the PRP-laser treated side in a statistically significant manner. In this work, PRP addition to the laser modality appears to produce more severe side effects and longer downtime.

Abdel et al.[23] conducted a similar study in which he treated 30 patients suffering from post-acne scars with ablative fractional CO2 laser (settings: 15 W, 600 ms dwell time, spading 700 μm, smart stack level 3); Only the right side of the face received intradermally injected autologous PRP (0.1 mL per point separated by 1–1.5cm). The laser was applied in two separate sessions (every 3–4 weeks) and patient's follow-up was completed six months after the final laser session. Assessment was done by two blinded dermatologists based on digital photographs; additionally patients filled out a questionnaire to grade their improvement. The overall improvement of the right side based on the Qualitative Global Grading System was better than on the left side ($P < 0.001$). The resolution of erythema following the laser was faster on the PRP-treated side ($P = 0.0052$) and post-inflammatory pigmentation did not occur on the treated side; the occurrence of acneiform eruption was also significantly lower on the treated side. In addition, patient satisfaction was also higher on the PRP-treated side ($P < 0.001$).

Level 3

Chawla[24] performed a study to investigate the efficacy of PRP versus vitamin C as adjuncts to microneedling for the treatment of atrophic post-acne scars as part of a split-face prospective study. Four sessions separated by a four-week interval were offered to 30 patients (Goodman and Baron grades II–IV), 23 of which completed the study. A double-spin method was used for the PRP and 1.5-mm needling rollers were used. At the end of the four treatments, photographic assessment was undertaken by the patient and treating physician and improvement was graded on a scale from poor to excellent. Results suggest that PRP compared favourably as contributing to an excellent outcome by the physician (18.5% vs. 7%) and also to those who had a poor response (37% vs. 22.2%); additionally, patient scores indicated that patients were more satisfied with the PRP adjunct ($P = 0.01$).

Level 4

Zhu et al.[25] examined the combination of topical PRP with erbium fractional laser for the treatment of 22 patients (Fitzpatrick type III or IV) with facial atrophic acne scars. The settings for the laser treatment were: pulse duration 300–600 ms; pulse energy 600–1200 mJ; microbeam diameter 2–7 mm; and penetration depth 18–24 μm. At 1–3 month follow up, digital photography assessed by two blinded dermatologists showed difference of 2.77 ± 0.39 corresponding to moderate improvement; additionally, self-evaluation using a quartile grading scale was shown to have improved by 3.3 ± 0.36 and 91% of the patients were 'very satisfied'.

Nita et al.[26] treated 64 patients suffering from 43 atrophic and 21 'contractile' scars involving different body parts with combination of ablative fractional CO2 laser, PRP and autologous fat grafting. Standard fat harvesting and the Coleman technique were employed for the lipofilling technique and fractional CO2 laser settings (power 9–12 W, time 4 ms, medium density) were matched to skin type. The PRP was obtained after two centrifugations (GLOFIN, Salo, Finland kit) and injected in the mid to deep dermis. At six-month follow-up, the overall patient satisfaction rate was $> 50\%$ (55.81% for atrophic

and 52.38% for contractile scars). The authors proposed that the combination of the three modalities seems to be an effective approach for scars. Table 1 summarises the salient literature reports relating to the use of PRP and atrophic acne scars.

Keloid scars

Level 4. Keloids represent a particularly challenging subset of scars for which a variety of therapeutic approaches have been described including surgical excision and radiotherapy, intralesional steroid injection and cryotherapy.[27] Jones et al.[28] recruited 40 patients (with 44 keloid scars) and treated them using a combination of:

1. Extralesional surgical excision;
2. PRP (2–3 mL) applied to the excision wound bed and incision site; and
3. Postoperative superficial photon X-ray radiation therapy within 72 h of excision (cumulative dose of 13–18 Gy delivered as 2–3 fractions).

All patients were advised to use a corticosteroid-based cream (0.5% hydrocortisone) twice daily for the first three months; additionally, triamcinolone injections were administered to four patients who were scored 'poor' on the Kyoto Scar Assessment Scale (KSAS); the latter was employed to assess scar outcomes in the study. This novel protocol approach achieved a recurrence rate of 4.5% (defined as induration, hypertrophy or extraordinary erythema beyond the site of excision) at a minimum three-month follow-up (range = 3–11 months). Assessment using the KSAS revealed that 61% achieved an excellent rating, 24% good, 3% fair and 12% poor. Radiation-induced hyperpigmentation was noted in all patients in the study. Limitations of the study included the short follow-up period, the non-standardised radiation protocol used as well as the variable use of triamcinolone; these factors make it very challenging to identify the exact contribution of PRP in the final recurrence rate. The same author[29] reported results of a retrospective study on 49 patients with 50 ear keloids treated with the same combination therapy as described above and achieved a 6% recurrence rate with a two-year follow-up period.

Currently, there is an ongoing randomised controlled trial (RCT) to assess the efficacy of autologous PRP administered immediately after complete surgical excision and then subsequently within the first month postoperatively on three occasions.[30] Given the limitations of the reported studies so far, results are eagerly awaited to assess the role of PRP in keloid scar management. Table 2 summarises the salient literature reports relating to the use of PRP and keloid scars.

Surgical scars

Surgical or postoperative scars have received special attention in the literature in the last few years and a limited number of studies have investigated the role of PRP in optimising final scar quality.

Level 1. Tehranian, et al.[31] conducted a RCT involving 140 patients undergoing elective Caesarean delivery. They were randomly allocated into two groups; the intervention group received PRP applied to the subcutaneous tissues of the wound before closure, whereas the control group received the usual care (i.e irrigation of the wound with saline before closure). Patients were examined by blinded physicians on days 1 and 5 as well as eight weeks after the procedure using a visual analogue scale for postoperative pain (VAS) and the Redness, Oedema, Ecchymosis, Discharge, Approximation (REEDA) scale to assess wound-healing progress; the Vancouver Scar Scale (VSS) was additionally used to grade the quality of scar formation. The authors identified that patients who were treated with topical PRP had a significant reduction in the REEDA score (85.5% for PRP vs. 72% for control group, $P < 0.0001$) implying better healing progress. Regarding the VSS, treatment with PRP had a significant effect on reducing the score beginning on the fifth day and continuing with a stable trend at the end of the eight weeks (54% vs. 18% reduction, $P < 0.001$). Furthermore, based on the VAS score for pain, PRP contributed to statistically significant reduction of pain experienced at the end of the follow-up period (93% vs. 79%, $P < 0.001$). Limitations of this study include the very short follow-up, which did not extent into the remodelling phase of scar maturation.

Level 4. Azzena et al.[32] reported a case of a painful adherent postoperative scar following a shoulder replacement surgery in which they injected a gel mixture of autologous adipose tissue combined with PRP into a subcutaneous pocket using a novel in vivo adipocyte delivery system. The patient reported complete remission of pain and ultrasonography performed six months and one year after treatment showed enhanced fat survival and resolution of the adhesion with the underlying fascia. Table 3 summarises the salient

Table 1. A summary of the different studies investigating the role of PRP in the management of atrophic acne scars.

Author, reference	Level of evidence	Patient clinical criteria	Study design	Follow-up	Outcomes
Lee et al.[19]	Split-face RCT (1c)	N = 14 Patients with post-acne atrophic scars	Patients underwent ablative fractional laser (pulse energy 25 mJ per fixed 150-μm diameter microbeam and a density of 400 MTZ/cm^2) resurfacing and were randomly assigned to receive either injectable PRP or normal saline following the treatment episode	Assessment was performed on days 0, 2, 4, 6, 8, 15 and 30 through standardised photographic assessment by blinded dermatologists	The overall clinical improvement four months after treatment was better on the PRP arm (2.7 ± 0.7 vs. 2.3 ± 0.5, $P = 0.03$). Erythema on the PRP side improved faster compared to controls and was significantly less at day 4 and confirmed by chromometer ($P = 0.01$ and 0.047, respectively). Duration of oedema as well as crusting were significantly shorter in the PRP groups (6.1 ± 1.1 vs. 7.1 ± 1.5 days and 5.9 ± 1.1 vs. 6.8 ± 1.0 days, $P = 0.04$)
Gawdat HI et al.[20]	RCT (1c)	N = 30 Patients with post-acne atrophic scars	30 patients were randomly divided into two groups. Group 1 was administered fractional carbon dioxide laser (15 W, dwell time 600 ms, spacing 700 μm, smart stack level 2) followed by intradermal PRP on one side and fractional carbon dioxide laser followed by intradermal saline on the other. In group 2, one cheek was treated with fractional CO2 laser followed by intradermal PRP, and the other received fractional CO2 laser followed by topical PRP	Each patient received three treatment sessions at monthly intervals. Photographic assessment comparison by a blinded physician using a four-point scale (excellent to poor) at 3 months after the last session	The combination of ablative fractional CO2 laser and PRP (topical and intradermal) showed significantly better results than ablative fractional CO2 laser alone ($P = 0.03$); nevertheless, no significant difference was observed between topical and intradermal PRP adjuvant administration ($P = 0.10$)
Nofal E et al.[8]	Quasi-experimental prospectively controlled study (2c)	N = 45 Patients with atrophic post-acne scars	Patients were randomly assigned to three equal groups: group A (intra-dermal PRP); group B (100% TCA peel); group C (combined skin needling and topical PRP). Results were assessed using the qualitative global acne scarring grading system (QGSGS) and patient satisfaction ratings.	Digital colour facial photographs were taken at baseline, and at the end of follow-up (2 months after the last session)	QGSGS findings suggested that a highly significant improvement in scar severity was seen after all modalities ($P < 0.001$), the highest was 60% with the PRP with microneedling group; nevertheless, none of the three treatments were significantly superior in comparison ($P = 0.87$) with regards to both outcomes from the quartile grading scale and patient satisfaction

(Continued)

Table 1. (Continued)

Author, reference	Level of evidence	Patient clinical criteria	Study design	Follow-up	Outcomes
Ibrahim MK et al.[21]	Quasi-experimental prospectively controlled study (2c)	N = 35 Patients with post-acne atrophic scars	All patients were treated with four sequential microneedling sessions using a 1.5mm dermaroller alone on the right side of the face, and combination of microneedling and topical PRP (double-spin method) on the left side with an interval of 3 weeks	The follow-up period was 3 months following which, two blinded dermatologists performed photographic evaluation using the Goodman & Baron grading system	Both treatment modalities produced a significant improvement in the global acne scoring system, namely from 3.2 $\pm$ 0.7 to 1.8 $\pm$ 0.6 for the right side and 2.1 $\pm$ 1.1 for the left side ($P < 0.001$); nevertheless, the difference between both modalities were not statistically significant ($P = 0.73$). Interestingly, there was statistically significant erythema and oedema in the PRP-treated side ($P < 0.001$)
Faghihi G et al.[22]	Quasi-experimental prospectively controlled study (2c)	N = 16 Patients with post-acne atrophic scars	Patients received ablative fractional CO2 laser (power 25 W, duration of 3, energy 30 mJ, pixel pitch of 1 and ablation depth 600 μm) combined with intradermal PRP treatment on one half of their face and laser with intradermal normal saline (NS) on the other half	Serial digital photography at baseline, 1 month after the first session and 4 months after the second session were obtained and a quartile improvement grading scale was used by two blinded dermatologists to evaluate the overall clinical improvement	The overall clinical improvement of acne scars was higher on the PRP-laser treated side, but the difference was not statistically significant either one month after the first session or four months after the final session ($P = 0.15$ and 0.23). Moreover, the adverse effects including erythema and oedema were more severe and of longer duration than the NS-laser-treated side in a statistically significant manner
Abdel Aal AM et al.[23]	Quasi-experimental prospectively controlled study (2c)	N = 30 Patients with post-acne scars	Patients were treated with ablative fractional CO2 laser (15 W, 600 ms dwell time, spading 700 μm, smart stack level 3) on both sides of the face and then only the right side received intradermally injected autologous PRP (0.1ml per point separated by 1–1.5cm. The laser was applied in two separate sessions (every 3–4 weeks)	Follow-up was completed 6 months after the final laser session. Assessment was done by two blinded dermatologists based on digital photographs; additionally patients filled out a questionnaire to grade their improvement	The overall improvement of the right side based on the QGSGS was better than on the left side ($P < 0.001$). The resolution of erythema following the laser was faster on the PRP-treated side ($P = 0.0052$) and post-inflammatory pigmentation did not occur on the treated side ; the occurrence of acneiform eruption was also significantly lower on the treated side. Patient satisfaction was also higher on the PRP treated side ($P < 0.001$)

Table 1. (Continued)

Author, reference	Level of evidence	Patient clinical criteria	Study design	Follow-up	Outcomes
Chawla[24]	Observational split-face study (3)	N = 30 (23 completed the study) Patients with post-acne atrophic scars	Patients were offered four sessions of microneedling with PRP on one side and microneedling with vitamin C on other side of the face with an interval of 4 weeks between sessions.	At the end of the four treatment sessions, photograph assessment was undertaken by the patient and treating physician and improvement was graded on a scale from poor to excellent	PRP compared favourably as contributing to an excellent outcome by the physician (18.5% vs. 7%) and also to those who had a poor response (37% vs. 22.2%); additionally, patient scores indicated that patients were more satisfied with the PRP adjunct ($P = 0.01$)
Zhu JT et al.[25]	Case series (4c)	N = 22 Patients with post-acne scars	PRP combined with erbium fractional laser therapy (300–600 ms, pulse energy 600–1200 mJ, microbeam diameter 2–7 mm, penetration depth 18–24 μm)	Follow-up after 1–3-month interval, based on digital photographs assessed by two blinded dermatologists	The magnitude of difference was found to be 2.77 ± 0.39 corresponding to moderate improvement. Self-evaluation using a quartile grading scale improved by 3.3 ± 0.36 and 91% of the patients were 'very satisfied'
Nita AC et al.[26]	Case series (4c)	N = 64 Patients with atrophic and 'contractile' scars	Patients were treated with combination of ablative fractional CO2 laser (power 9–12 W, time 4 ms, medium density) with PRP and autologous fat graft	The follow-up took place after 1 week, 1, 3 and 6 months, using digital photographs	At six-month follow-up, the overall patient satisfaction rate was > 50% (55.81% for atrophic and 52.38% for 'contractile' scars).

Table 2. A summary of the different studies investigating the role of PRP in the management of keloid scars.

Author, reference	Level of evidence	Patient clinical criteria	Study design	Follow-up	Outcomes
Jones ME et al.[16]	Case series (4c)	N = 40 Patients with 44 keloid scars	Patients with keloid scars were treated using surgical excision, PRP and postoperative superficial photon X-ray radiation therapy within 72 h of excision (cumulative dose of 13–18 Gy delivered as 2–3 fractions)	Assessment was performed on day 10 and at 1, 3, 6 and 9 months; recurrence was determined by examination and photo documentation	This approach achieved a 4.5% recurrence rate at a minimum of 3 months follow-up. Assessment using the Kyoto Scar Assessment Scale (KSAS) revealed that 61% achieved an excellent rating, 24% good, 3% fair and 12% poor
Jones ME et al.[17]	Case series (4c)	N = 49 Patients with 50 ear keloids	Patients were treated with extralesional surgical excision of keloids localised to the ear followed by the application of autologous PRP to wound site and postoperative superficial photon X-ray radiation therapy within 72 h of excision (cumulative dose of 13–18 Gy delivered as 2–3 fractions)	On completion of initial protocol, patients are instructed to follow-up on day 10 and 1, 3, 6, 9 and 12 months postoperatively	This approach achieved a 6% recurrence rate on follow-up over a 2-year period

literature reports relating to the use of PRP and surgical scars.

Traumatic scars

Scarring following trauma can lead to both aesthetic and functional sequelae for patients;[33] a small number of studies have investigated the role of PRP in this subset of scars.

Level 1. Cervelli et al.[33] recruited 60 patients (Fitzpatrick skin types II–IV) affected by traumatic scars of varying aetiology in different bodily parts. They were randomly allocated to one of three groups (20 patients each): group A was treated with fat grafts mixed with PRP at one and three months; group B underwent four sessions of 1540-nm non-ablative laser alone (settings: 20–40 J/cm^2 using a 10-mm fractional handpiece); and group C was treated with a combination of both procedures (laser at one and three months delivered seven days after the graft/PRP). The PRP was extracted with a single-spin preparation and was added to the fat (harvested using the Coleman technique) before injection at volumes in the range of 5–50 mL depending on the defect. The combined modality group showed greater overall clinical improvement in comparison to the other groups as assessed using the Manchester Scar Scale (MSS). The most effective scar treatment was the combination of the fat graft-PRP and non-ablative laser resurfacing in group C, which had increases in wound healing of 22% and 11% compared with groups A and B, with significant improvement in texture, colour and scar contours on MSS. The authors concluded that the addition of PRP to a combination of fat grafting and non-ablative 1540-nm laser increases the efficacy of the combined scar management strategy.

Level 2. Gentile et al.[34] conducted a comparative study on 20 patients with burn and traumatic scars using either stromal vascular fraction (SVF)-enhanced autologous fat grafts or 1 mL of Coleman-based fat grafting mixed with 0.5 mL of PRP; in this study a control group of 10 patients were treated with centrifuged fat without PRP addition. The fat re-implantation was performed following scar subcision with 1.5-mm diameter cannulas. In both groups (study and control), one operation was required in six cases and two in four cases. Evaluation was performed using photographic team evaluation and radiological assessment as well as patient self-evaluation. In patients treated with PRP-enriched fat, 69% maintenance of contour and three-dimensional volume after one year was observed in comparison to the control group. Magnetic resonance imaging (MRI) additionally showed that patients treated with PRP as well as SVF-enriched fat showed lower fat absorption. The authors concluded that use of PRP during fat grafting improves adipose tissue maintenance and survival.

Level 4. Majani et al.[35] evaluated the results of lipografting in 28 patients with different types of scars (including burn cicatricial, traumatic and postoperative scars). Eleven patients (group 1) received lipografting only and 11 (group 2) were treated with PRP 7–10 days before the surgery; six patients (group 3), who had symmetrical scars, were treated on the left side only with lipografting and on the right side with a combination therapy of PRP and lipografting. The PRP was obtained using a single-spin technique and infiltrated into the scars in volumes in the range of 1–8 mL and fat was injected in volumes in the range of 8–37 mL. Patients were photographed at 30, 90 and 180 days postoperatively. Thirty days following lipografting, all patients showed better scar elasticity and the treated area showed evidence of aesthetic improvement. Ninety days after surgery, in three patients from group 1 and one patient from group 2, there was absorption of the injected fat. In patients from group 3, the increase was most evident on the right side. The authors concluded that a suitable preparation of the treated areas with the combination therapy of PRP and lipografting resulted in more durable corrections, particularly in situations where vascularization is more impaired. Table 4 summarises the salient literature reports relating to the use of PRP and traumatic scars.

Discussion

Based on the studies available in the field of atrophic acne scarring, there is level 1 evidence that PRP in conjunction with ablative fractional CO2 laser treatment may improve the overall clinical response/quality of scars attained and decrease the duration of laser related side effects including erythema and duration of oedema.[19,20] Additionally, there appears to be no statistically significant difference between the intradermal and topical application of PRP after fractional CO2 laser treatment in terms of efficacy.[20] Level

Table 3. A summary of the different studies investigating the role of PRP in the management of surgical scars.

Author (ref.)	Level of evidence	Patient clinical criteria	Study design	Follow-up	Outcomes
Tehranian A et al.[31]	RCT (1c)	N = 140 Patients who underwent elective Caesarean delivery	Patients were randomly allocated into two groups; the intervention group received PRP applied to the subcutaneous tissues of the wound before closure, whereas the control group received the usual care (irrigation of the wound with saline before closure)	Patients were examined by blinded physicians on days 1 and 5 as well as 8 weeks following the procedure using a visual analogue scale (VAS) for postoperative pain, the Redness, Oedema, Ecchymosis, Discharge, Approximation (REEDA) scale to assess wound healing progress and the Vancouver Scar Scale (VSS) for the quality of scar formation	Topical PRP had a significant effect on reducing the REEDA score (85.5% for PRP vs. 72% for control group, $P < 0.0001$), implying better healing progress. Additionally, PRP significantly reduced the VSS score beginning on day 5 and continued with a stable trend at the end of week 8 (54% vs. 18% reduction, $P < 0.001$). Furthermore, pain was significantly reduced at the end of the follow-up period in the PRP group. (93% vs. 79%, $P < 0.001$)
Azzena B et al.[32]	Case study (4d)	N = 1 Patient with postoperative scar following shoulder replacement surgery	A single painful adherent postoperative scar treated with a gel mixture of autologous adipose tissue combined with PRP using an in vivo adipocyte delivery system	6 months and 12 months after treatment using pain assessment and ultrasound imaging	Complete remission of pain; ultrasonography performed 6 months and 12 months after treatment showed fat survival and resolution of the adhesions

Table 4. A summary of the different studies investigating the role of PRP in the management of traumatic scars.

Author, reference	Level of evidence	Patient clinical criteria	Study design	Follow-up	Outcomes
Cervelli V et al.[33]	RCT (1c)	N = 60 Patients with traumatic scars	Patients were randomly allocated to one of three groups (20 patients each): group A was treated with fat grafts mixed with PRP at months 1 and 3; group B was treated with four sessions treatment with 1540-nm non-ablative laser (20–40 J/cm^2 using a 10-mm fractional handpiece) alone; and group C was treated with both procedures (laser at 1 and 3 months delivered 7 days after the graft/PRP)	The overall degree of patient satisfaction was assessed at 6-month follow-up, using a structured questionnaire grading the aesthetic and functional quality of the scar as excellent, good, fair or poor. Postoperative follow-up examinations were performed at weeks 1, 2, 4 and 8, and months 3 and 6	The most effective scar treatment was the combination of the fat graft, PRP and non-ablative laser resurfacing in group C, which produced increases in wound healing of 22% and 11% compared with groups A and B, with significant improvement in texture, colour and scar contours on Manchester Scar Scale (MSS). At 6 months, 84% patients evaluated the scar appearance as good to excellent and 16% as poor to fair
Gentile P et al.[34]	Quasi-experimental prospectively controlled study (2c)	N = 20 Patients with burn and traumatic scars	Patients were treated using either stromal vascular fraction (SVF)-enhanced autologous fat grafts or 1 mL of Coleman-based fat grafting mixed with 0.5 mL of PRP. There was a control group of 10 patients who were treated with centrifuged fat without PRP addition. The fat re-implantation was performed following scar subcision with 1.5-mm diameter cannulas	Evaluation was performed via photographic team evaluation, radiological assessment (MRI and ultrasound) as well as patient self-evaluation	In patients treated with PRP-enriched fat, a 69% maintenance of contour and 3D volume after 1 year was seen compared to the control group. Additionally, MRI showed that patients treated with PRP as well as SVF-enriched fat showed lower fat absorption
Majani et al.[35]	Case series (4c)	N = 28 Patients with different types of scars (6 cicatricial burn scars, 12 scars from previous plastic surgery, 2 post-Caesarean section, 4 post-general surgery and 4 patients traumatic scars)	All patients were treated using lipografting: 11 patients (group 1) without PRP; 11 patients (group 2) were treated with PRP (7–10 days before); 6 patients (group 3), with symmetrical scars, were treated on the left side with lipografting and on the right side with a combination of PRP and lipografting	Patients were followed-up and photographed at 30, 90 and 180 days postoperatively	30 days following lipografting, better scar elasticity and evidence of aesthetic improvement was observed. 90 days after surgery, in three patients from group 1 and one patient from group 2, there was absorption of the injected fat. In patients from group 3, the increase was most evident on the right side (i.e PRP and lipografting).

2 studies suggest that PRP can decrease erythema and oedema following 1.5-mm microneedling in a statistically significant manner.[21] Another study in this category of evidence failed to show statistical superiority of PRP and skin microneedling compared with TCA CROSS and isolated intradermal PRP administration on the basis of QGSGS ratings.[8] Furthermore, two other level 2 studies provided contradictory results around the role of PRP in ameliorating post-fractional CO2 side effects including erythema resolution.[22,23] Some of the main limitations of the studies available in this arena include the small cohort sizes as well as their short follow-up period (range = 2–6 months); this is very limited given that the timescale of scar remodelling is considered to span over at least 12 months. Furthermore, the heterogeneity of treatment parameters (volume/concentration of PRP, laser settings, Fitzpatrick skin types) may render generalised conclusions challenging.

There is weak evidence (level 4) for the inclusion of PRP in the management of keloid scars employing surgery and radiotherapy. The non-comparative design of the two available studies, the selective use of steroids to a significant percentage of patients as well as the short follow-up period in one report (deviating significantly from the widely accepted minimum two-year period to assess long-term efficacy reliably) make valid conclusions challenging to draw.[27,28] The results of the currently conducted RCT are eagerly awaited to appraise the value of PRP in keloid scar management.

Regarding surgical scars, there is level 1 evidence that autologous platelet preparations may improve wound healing and scar quality at eight weeks as well as mediate a reduction in postoperative pain following a Caesarean section; nevertheless, the short follow-up in the study does not provide an indication of the possible contribution of PRP towards better long term scar quality.[31]

Reviewing studies in resurfacing of traumatic scars, the addition of PRP in fat-grafting procedures combined with non-ablative, fractional 1540-nm laser appears to contribute to better wound healing compared to isolated modalities (PRP-enriched fat grafting and laser alone). Furthermore, MSS scores indicated a significant improvement in texture, colour and scar contours at six-month follow-up.[33] Another level 2 study showed that PRP enrichment of autologous fat grafts can improve adipose tissue survival and maintenance during fat grafting at 12-month follow-up.[34]

Concluding remarks

This work appraised the literature concerning the use of PRP in scar management. The use of autologous plasma derived adjuncts has a number of potential advantages by virtue of the ability to deliver a high concentration of growth factors to target tissues and potentially improve wound healing and scarring parameters. The majority of studies currently available focus on the adjunctive use of PRP in the management of atrophic acne scars; nevertheless, there are significant shortcomings in this arena including the small cohort sizes appraised, the short follow-up periods as well as the heterogeneity of treatment parameters employed, which render generalised conclusions challenging. Further high-quality studies are eagerly awaited in order to further delineate to role of autologous platelet-derived adjuncts in scar management protocols.

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