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REVIEW

AIRMESS – Academy of International Regenerative Medicine & Surgery Societies: recommendations in the use of platelet-rich plasma (PRP), autologous stem cell-based therapy (ASC-BT) in androgenetic alopecia and wound healing

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ABSTRACT

Introduction: Many investigations showed that platelet-rich plasma (PRP), human follicles stem cells (HFSCs), and adipose-derived stem cells (ASCs), considered autologous stem cell-based therapy (ASC-BT), are effective for hair regrowth (HR) in patients affected by androgenetic alopecia and for wound healing (WH). The aim of this article is to analyze the *in vitro* and *in vivo* impact of different PRP, HFSCs, and ASCs preparation methods on HR and in WH.

Areas Covered: The analyzed data intended to clarify the molecular mechanism in which PRP, HFSCs, and ASCs are involved, the clinical use and related indications, fully respecting the European rules. Comparative studies between different systems of PRP, HFSCs, and ASCs preparation revealed differences in terms of HR and WH.

Expert Opinion: Despite a lack of standardized protocols, there is convincing evidence with objective measurement modalities that display positive outcomes of ASC-BT in HR and WH.

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Plastic surgery; regenerative plastic surgery; platelet-rich plasma; PRP in androgenetic alopecia; PRP in hair loss; human follicle stem cells; HFSC; adipose-derived stem cells; ASCS; hair regrowth; wound healing

1. Introduction

The standardization of autologous therapies to promote innovative biotechnologies in plastic surgery, in particular in regenerative fields, for hair loss and wound healing, appears to be essential. Advances in autologous stem cell-based therapy (ASC-BT) and in autologous not-activated platelet-rich plasma (A-N-A-PRP) or autologous activated PRP (A-A-PRP) use, aiming to improve hair regrowth (HR) and wound healing (WH) by *in vitro* and *in vivo* regeneration, should be performed.

The effects of growth factors (GFs), released during ASC-BT and contained in A-N-A-PRP and A-A-PRP, represent these advances, through cell proliferation/differentiation, and neo-angiogenesis improvement, thereby aiding the WH process [1–3] and HR [4].

As is known, A-N-A-PRP and A-A-PRP contain many signaling proteins and after platelet activation discharge several major GFs such as PDGF, b-FGF, EGF, VEGF, TGF-β, and IGF-1 [4]. Every one of these is implicated in a specific biomolecular pathway during HR and WH. Manufacturing platelet recommendations and steering on their employment in regenerative fields have been performed by The Platelet Physiology Subcommittee of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) [5]. On the same line, the Academy of International Regenerative Medicine & Surgery Societies (AIRMESS) decided to describe and suggest in the present work, the ASC-BT and PRP's guidelines on the basis of the most recent European rules and on the scientific data published.

In fact, a significant article number on the different procedures for A-N-A-PRP, A-A-PRP preparation, and on ASC-BT use have been published, but the results are often contrasting, also due to a wide biological and temporal variation of GFs and platelets [6] and also for the different procedures of stem cell isolation such as enzymatic versus mechanic.

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Article highlights

- Platelet-rich plasma (PRP), human follicles stem cells (HFSCs), and adipose-derived stem cells (ASCs) in hair regrowth.
- Autologous stem cell-based therapy (ASC-BT) in hair regrowth.
- PRP, HFSCs, and ASCs, in wound healing (WH).
- ASC-BT in WH.
- Recommendations in androgenetic alopecia and WH.
- *In vitro* and *in vivo* impact of different PRP, HFSCs, and ASCs preparation methods on HR and in WH.

This box summarizes key points contained in the article.

For this reason, it appears to be complicated both to assess which method for PRP preparation is better and which is deficient [7] and that which kind of isolation stem cell procedure must be used; different PRP products and also different ASC-BT could be more or then less effective in the treatment of different kinds of tissues and pathologies. Although there is clear, well-known, and unequivocal scientific evidence, the efficacy of PRP, in some regenerative fields as HR, remains contested, while a standardized method has not yet been established [8]. Appropriate PRP preparation methods should be selected only after carefully considering their biomolecular specs and intended indications for use in patients [9]. The same concept should be applied to ASC-BT.

For the above-mentioned concepts, also the use of ASC-BT in HR and WH, prevalently represented by adipose-derived mesenchymal stem cells (AD-MSCs) and human follicle mesenchymal stem cells (HF-MSCs), appears to be contradictory.

Currently, no standardized PRP, human follicle stem cells (HFSCs), and AD-MSCs preparation techniques exist, and additionally, their biomolecular mechanisms promoting HR and WH are poorly understood.

The aim of this paper is to discuss the *in vitro* and *in vivo* impact of different PRP and ASC-BT preparation methods on WH and HR, describing the related European rules and biomolecular pathway. The data analyzed intended to clarify any doubts regarding the molecular mechanism in which PRP and ASC-BT are involved, suggesting recommendations in WH and HR.

2. Methods

2.1. European laws and institutional rules related to the PRP use

The European laws (ELWs) on PRP use have been represented both by Decree of 9 November 2007, n. 207, 'Implementation of Decree 2005/61/EC in means of traceability of blood components intended for transfusion and the notification of adverse and severe reactions,' and by the Decree n. 208, 'Implementation of Directive 2005/62/EC relating to a quality system of blood.' Additionally, the regulatory framework concerning the blood system has been disciplined by Directive 2002/98/EC of the European Parliament and Council of 27 January 2003, which sets out quality and safety rules for collecting, controlling, processing, preserving, and distributing human blood and its components, acknowledged in the various states of the European Union with internal regulations. This lack of homogeneity in the European legal landscape will probably lead the community legislature to intervene in the near future to even out the 'rules of engagement' of this peculiar class of hemocomponents.

At the present time (February 2021), the PRP preparation's procedures must be realized respecting in Italy 'The Blood Law-Decree (BLD), 2 November 2015,' which incorporates European regulations. The European rules reported dispositions related to quality and safety parameters of blood and hemocomponents according to which all patients should receive detailed oral and written information on the PRP preparation procedures, modalities of PRP applications, including the risks, benefits, and alternative therapies. Currently, each European country follows different guidelines that may differ in many aspects. Every country has evaluated these guidelines based on their own criteria. The Italian guidelines established that each PRP procedure must take place in a structure authorized by the reference blood transfusion service by means of a specific agreement.

The BLD established

- the minimum level of platelets (PLTs) amount that need be obtained for every procedure (1×10^6 µL ± 20%)
- exclusion criteria (PLTs disorders, thrombocytopenia, anti-aggregating therapy, bone marrow aplasia, uncompensated diabetes, cancer and sepsis);
- clinical fields in which may use PRP only on the basis of available scientific evidence and guidelines of the national blood center;
- PRP preparation methods (kits and procedure);
- exclusively infiltrative or topical PRP use;
- quality and sterility checks on the sample obtained;
- blood amount to harvest (within 55 cm^3);
- the volume of A-N-A-PRP and A-A-PRP to obtain;
- labeling;
- extensive informed consent;
- side effects module; and
- data processing module.

In the light of the above-mentioned concepts, AIRMESS invites full respect of the ELWs for the PRP's preparations procedures, which must be conducted following the principles outlined in the Helsinki's Declaration and internationally consented ethics in clinical research [10]. A quality assessment based on the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist [11] is suggested.

For all clinicians that would like to start with the PRP's applications in regenerative fields, particularly in HR and WH, the AIRMESS suggests strictly respecting the European rules and own national guidelines.

2.2. Platelet-rich plasma preparations

There are different methods for A-N-A-PRP and A-A-PRP preparation, depending on the centrifugation's time, *g*-force, PLTs number, GFs, and chemokines availability, so obtaining

different PRP products by way of their cell content and fibrin architecture as followed identified:

(1) PRP's leukocyte-poor (PRP- LP). Absent leukocytes in the solution obtained with a low-density fibrin network.
(2) PRP's leukocyte-rich (PRP-LR). Present leukocytes in the solution obtained with a low-density fibrin network.
(3) Platelet-rich fibrin's leukocyte-poor (PRF-LP). A high-density fibrin network and absent leukocytes.
(4) PRF's leukocytes-rich (PRF-LR). A high-density fibrin network and leukocytes.

These different PRP products could be more or than less effective in WH and HR, and for this reason, only appropriate PRP preparations should be selected on the basis of their cellular and fibrin specs and intended indications in patients **[**4,9,12–14**]**.

2.3. European and institutional rules related to the ASC-BT

ASC-BT must be performed in full agreement with the current ELWs. The reference law is 1394/2007 of the European Parliament for advanced therapies, where the definition of '*bioprocess engineering products*' is given. Cells and tissues are to be considered bioprocess engineering products (BEPs) only if they are undergoing a *'considerable manipulation'* and therefore to a cellular expansion/culture. Included among the advanced therapy pharmaceutical products are those used for gene and somatic cell therapy (2001/83 directives of the European Parliament, Annex I). The other way around, cells and non-vital human or animal tissues and that do not have immunological, pharmacological, or metabolic action are not considered BEPs.

At the same time, *'minimal manipulations'* like centrifugations, filtrations, cuttings, separations, grindings, shaping, concentrations or purifications, sterilizations, soaking in antimicrobial and/or in antibiotic suspensions, sterilizations, irradiations, lyophilization, freezing, cryopreservation, and nitrification are not considered as BEPs.

In line with the above-mentioned concepts, the European Medical Agency (EMA) and also the Food and Drug Administration (FDA) consider in fact two different kinds of cell products:

– Cellular products obtained via minimal manipulation.
– Cellular products obtained via extensive manipulation. This must be considered a drug, and therefore an Advanced Therapy Medicinal Products (ATMPs) requiring the rule's application of good manufacturing practice (GMP) for preparation.

In the last case, following the Article 17 of 1394/2007 law (the ATMPs Regulation), applicants are required to approach the Committee for Advanced Therapies (CAT) with a proposal for the arrangement of ATMPs. This procedure must be not applied for minimal manipulations.

Indeed, ATMPs are implicated in gene therapy medicinal products (GTMPs), tissue-engineered products (TEPs), and somatic cell therapy medicinal products (sCTPMPs). The proposal also looks at whether the cellular product can live up to a consolidated ATMP's definition.

In full agreement with the reflection paper EMA/CAT/600,280/2010 Rev 1, 20 June 2014, by the CAT, Line 10, according to which *'a similar basic capacity for a cell populace implies that the cells, when expelled from their unique condition in the human body are used to maintain the original capacity in a similar anatomical or histological condition'* it appears possible to affirm that autologous applications in a one-step surgery, minimal and omo-functional applications *'used for an indistinguishable fundamental capacity in the beneficiary as in the donor,'* and procedures performed via devices in aseptic conditions (operatory room) would be conditions that do not require the rule's application of GMP for preparation or ethical committee underwriting.

For the above-mentioned concepts, it is possible to consider the ASC-BT as minimal manipulation when it is prepared through centrifugation, filtration, and purification procedures. Additionally, the isolation and related use of AD-MSCs, SVFs, and HFSCs, it may be considered a minimal manipulation when they are obtained through minimal manipulation procedure, including enzymatic digestion when it is respected the omo-functional application. In this last case, only GCPs for clinical applications and a largely shared protocol are recommended.

3. Discussion

A lack of rigorous investigations done to analyzing and comparing the final product obtained by all available procedures of PRP and ASC-BT preparation (kits and methods) presents a challenge for physicians seeking to identify an appropriate procedure for PRP and ASC-BT preparation. The outcomes evaluated report that comparative investigations between different PRP's preparation system can produce a different impact in terms of WH [1–3] or HR [4]. Regarding HR, different hair count (HC) and hair density (HD) outcomes have been described *in vivo* [4]. *In vitro*, the antiapoptotic effect of PRP (both A-N-A-PRP and A-A-PRP) appears the most important contributing factor stimulating HR via the activation of the Bcl-2 protein (antiapoptotic regulator) and Akt signaling, improving the survival of dermal papilla cells (DPCs) during the hair growth cycle (HGC). In detail, the upregulation of (FGF-7)/b-catenin signaling pathways, produced by A-N-A-PRP injection, seems to stimulate HR by inducing HFSCs differentiation, prolonging the anagen phase of the HGC [4].

Additionally, an improvement of the perifollicular vascular plexus via the increase of VEGF and PDGF levels, which have the angiogenic potential, was correlated to the PRP [4].

To better describe the different *in vivo* outcomes observed in HR using A-N-A-PRP and A-A-PRP, it appears useful to analyze the most recent results in HD and HC obtained for these procedures and to compare the results with those, obtained by alternative autologous therapies, like stem cell therapy represented by HFSCs and HF-MSCs infiltration. In detail, 3 months after the last infiltration (A-N-A-PRP and A-A-PRP have been infiltered every 30 days, three times), HD measurements for patients suffering from androgenetic alopecia (AGA) treated with A-N-A-PRP and A-A-PRP were 65.0 ± 5.0

and 28.0 ± 4.0 hairs/cm^2, respectively. These outcomes represent a 31.0% ± 2.0% increase in HD when A-N-A-PRP is administered versus a 19.0% ± 3.0% increase in HD when A-A-PRP is administered, with a statistically significant difference in HR (p = 0. 0029) [4]. Differences between the 3 months follow-up counts and the baseline count for these HG parameters were higher in the A-N-A-PRP group than in the A-A-PRP group as described in the previous study published by Gentile et al. [4]. Average 6 months after the last infiltration, HD measurements for patients treated with A-N-A-PRP and A-A-PRP were 28.0 ± 2.0 and 15.0 ± 3.0 hairs/cm^2, respectively [4].

The major increase of HD and HC for A-N-A-PRP over A-A-PRP can reflect the greater efficiency of *in vivo* thrombin to activate PLTs compared with *in vitro* Ca^{2+} activation. Moreover, delivery of A-N-A-PRP can enable the thromboxane A2 (TXA2) release by the PLTs once they are activated *in vivo*, which would, in turn, activate additional platelets and amplify PLTs aggregation [15].

In vitro, both AGA patient groups treated with A-N-A-PRP and A-A-PRP, respectively, displayed an increase in the follicular bulge cell number and follicles, epidermal thickening improvement, increased vascularization, and a higher number of Ki67+ basal keratinocytes in PRP-treated scalp tissue compared with placebo (saline solution) [4]. Microscopic analysis of A-N-A-PRP and A-A-PRP-treated scalp tissue from the authors' previous article [4] confirms such *in vitro* evidence.

The *in vivo* outcomes of A-N-A-PRP and A-A-PRP administration in AGA patients may be considered similar to those of HFSCs/HF-MSCs procedure [16]. In fact, a recent investigation by Gentile et al. [16] displayed an HD improvement for HFSCs treatment of 29.0 ± 5.0% hairs/cm^2, 6 months average after the second infiltration (one infiltration was performed every 60 days for two injection cycles) compared with 28.0 ± 2.0 hairs/cm^2 when A-N-A-PRP was used. In this investigation, autologous HFSCs solution, obtained by centrifugation of a 2 mm punch biopsy of the scalp, via commercial CE kit, was infiltrated in AGA patients [16]. In 2019, using the 'Gentile protocol' [17] based on mechanical and controlled infiltrations of autologous micro-grafts containing human intra and extra dermal adipose tissue-derived hair follicle stem cells obtained by cutting, disaggregation, fragmentation, and centrifugation of 2 mm scalp's punch biopsy, without any commercial kit or device, a 33.0% ± 7.5% increase of HD increments, after 6 months average, has been described [17].

As introduced, the GFs concentration in PRP appears to be different depending on the procedure used (A-N-A-PRP or A-A-PRP), stimulating both HR and tissue regeneration, positively influencing the WH. Each one of the above-mentioned GFs is implicated in a specific biomolecular pathway during HR and WH.

In vitro, regarding HR, EGF stimulates migration and growth of follicle ORS cells by activation of Wnt/β-catenin signaling; b-FGF stimulates hairs' follicles growing; VEGF improves perifollicular angiogenesis; TGF-β acts and promotes the biomolecular pathway regulating hair cycle; IGF-1 stimulates multiplication, moving, and survival of hair follicle (HA-F) cells; IL-6 is involved in WIHN via STAT3 activation; IGFBP-1 to -6 regulates IGF-1 function and its interplay with extracellular matrix (ECM) proteins at the HA-F level; PDGF and PDGFR-β/-α64 upregulate the genes involved in HA-F differentiation; PDGF promotes follicular development; Wnt3a is involved in HA-F growth and development through β-catenin signaling; PGE2 stimulates anagen in HA-F; PGF2α and analogs stimulates the transition from telogen to anagen; BIO (GSK-3 inhibitor); PGE2 or inhibition of PGD2 or PGD2 receptor D2/GPR4477 stimulates HA-F regeneration; BMP keeps DPC phenotype; BMPR1a keeps the proper identity of DPCs; and M-CSF and M-CSFR are implicated in wound-induced HR [10]. The list of GFs present in PRP and their *in vitro* mechanism and biomolecular pathway in HR are reported in Table 1.

In WH, GFs may act by reducing bleeding and accelerating healing time [13]. In detail, several GFs represented by TGF-β,

Table 1. List of GFs identified in PRP and their suggested biomolecular pathway in HR.

Growth factors	Biomolecular pathway in hair regrowth
VEGF	Improves perifollicular angiogenesis; elevated expression in dermal papilla cells during anagen phase; endothelial cell-specific mitogen; microvascular permeability and perifollicular vascularization;
EGF	Improves the activity and growth of follicle outer-root sheath cells by activation of Wnt/β-catenin signaling; cell growth modulator during follicular differentiation; proliferation and migration of follicular outer root sheath cells;
FGF	Improves the advancement of hair follicles; anagen phase induction via B-catenin expression; angiogenesis; dermal fibroblast and hair follicle mitogen;
PDGF	Upregulate the genes associated with HF separation, induction, and control of anagen; angiogenesis and vascularization; hair follicle dermal stem cell proliferation; mesenchymal stem cell mitogen;
IGF-1	Improves the migration, survival, and proliferation of HF cells; hair follicle proliferation during development; increase hair density and inhibit apoptosis;
HGF	Enhance the proliferation of follicular epithelial cells; hair follicle elongation; inhibits catagen phase induction;
TGF-ß	Stimulates the signaling pathways that manage the hair cycle; extracellular matrix synthesis; fibroblast and mesenchymal stem cell proliferation; hair folliculogenesis and maturation;
IL-6	Involved in WIHN through STAT3 enactment
IGFBP-1 to −6	Manages the IGF-1 effect and its connection with extracellular matrix proteins at the hair follicle level
BMP	Maintains the DPC phenotype (fundamental for stimulation of HFSCs)
BMPR1	Maintains the proper identity of the DPCs (basic for explicit DPC work)
M-CSF	Involved in wound-induced hair growth
M-CSFR	Involved in wound-induced hair growth
Wnt3a	Involved in HF advancement through β-catenin signaling
PGE2	Stimulates anagen in HF
PGF2α	Enhance change from telogen to anagen
BIO	GSK-3 inhibitor
PGD2	Enhances follicle regeneration
Iron and l-lysine95	Still under examination

DPCs = dermal papilla cells; HFSCs = human follicles stem cells.
VEGF: vascular endothelial growth factor; EGF: epidermal growth factor; FGF: fibroblast growth factor; PDGF: platelet-derived growth factor; IGF: insulin like growth factor; HGF: hepatocyte growth factor; TGF: transforming growth factor; IL: interleukin; IGFBP: Insulin Like Growth Factor Binding Protein; BMP: Bone morphogenetic proteins; M-CSF: macrophage colony- stimulating factor; PGF: Placental growth factor

EGF, FGF, GM-CSF, VEGF, PDGF, CTGF, IL, and the TNF-α family may improve the neo-angiogenesis, stimulate cell growth, morphogenesis, and recruitment, constituting a three-dimensional matrix that allows cellular arrangement into a correct three-dimensional organization [13,18]. For all the above-mentioned reasons, PRP is identified as a biostimulator. Therefore, PRP's activation promotes platelets' degranulation where the secretory proteins changed to a bioactive state [14,19] going to bind transmembrane receptors of fibroblasts, MSCs, osteoblasts, and endothelial and epidermal cells promoting tissue regeneration [20–22].

The list of GFs present in PRP and their *in vitro* mechanism and biomolecular pathway in WH are reported in Table 2.

During the WH process, constituted by four stages – hemostasis, inflammation, proliferation, remodeling – platelet GFs serve as messengers to regulate a well-orchestrated and complex series of events involving cell–cell and cell–matrix interactions and promoting the proliferation of cell at the wound site. Autologous PLTs-derived GFs were proposed to modulate and regulate WH by promoting the formation of granulation tissue in the early healing stage.

In addition to their tissue forming and proliferative effects, GFs exhibit chemotactic effect that causes the migration of macrophages and neutrophils, adding an antimicrobial component to the wound site.

GFs involved in each of the WH stages are shown in Table 3.

The PRP healing properties have been reported in many *in vivo* studies [2,3,6,8,12,13]. PLTs play a fundamental role in the WH process thanks to hemostatic functions and concentrated levels of GFs and cytokines [13]. A higher concentration of GFs stimulates the endothelial and epithelial cells regeneration, improving neo-angiogenesis, collagen deposition, and accelerating the healing process [13].

The main *in vivo* use of PRP was related to chronic wound conditions, such as diabetic ulcers, characterized by persistent inflammation due to an imbalance between pro-inflammatory and anti-inflammatory cytokines and also by low GFs concentration or even due to excess reactive oxygen species. In this sense, GFs and cytokines contained in PRP may play a fundamental role in controlling oxidative damage [13].

An additional factor promoting WH appears to be the acceleration of hyaluronic acid (HA) production [13]. HA is a biopolymer detected in the ECM of cartilage, skin, bone, and brain, among other tissues [23]. It may regulate and hydrate the cellular microenvironment, while its cell surface receptor bindings induce cellular migrations and proliferation, cell–substrate adhesions, and cell-to-cell adhesions. Therefore, HA appears to be a useful and effective scaffold contributing to the orientation of the ECM [24] and fibrous component [25] facilitating the entry of many cells to the wound site. For these reasons, HA may act as a perfect scaffold for PRP promoting tissue-remodeling and restoring, stimulating cell renovation, with the aim to improve the healing.

PRP as a bio-stimulator and HA as a scaffold both engaged in a bio-functionalized scaffold could be considered, in chronic ulcer treatment, the 'gold standard' *in vivo* practice, especially where autologous/allogenic tissues might not be available in sufficient amount for the repair [13]. Many investigations showing the combined use of HA and PRP in WH has been reported [2,13].

In vivo, as published by De Angelis et al. [13], the patients affected by chronic ulcers who underwent combined treatment (A-N-A-PRP + HA) had 96.8% ± 1.5% of reepithelization compared to 78.4% ± 4.4% in patients (treated with HA alone; $p < 0.01$) 30 days later the last treatment. No local recurrence has been described during the follow-up period [13].

The use of autologous A-N-A-PRP/A-A-PRP in regenerative therapy contributes to the damaged tissue's regeneration through both their use in isolated suspensions and/or in combination with biomaterials.

Additionally, several *in vivo/in vitro* studies were published on the use of PRP mixed with fat graft and AD-MSCs with the aim to promote and accelerate the WH [26,27], in particular in soft tissue defects [28–31]. From the data analyzed above, it

Table 2. List of GFs identified in PRP and their suggested biomolecular pathway in WH.

Growth factors	Biomolecular pathway in wound healing
VEGF	Improves angiogenesis; microvascular permeability; strong paracrine effect on endothelial cells; promotes and supports the wound angiogenesis process; initiate the angiogenesis process in granulation tissue;
EGF	Improve skin re-epithelialization and angiogenesis; cell growth modulator; epithelial and keratinocyte cell proliferation, differentiation, growth, and migration
FGF	Re-epithelialization, and granulation tissue formation; improves angiogenesis; cell mitogen
PDGF	Angiogenesis and vascularization; stimulates protein and collagen synthesis chemotaxis of fibroblast; proliferation and migration of endothelial cells; mesenchymal stem cell mitogen
IGF-1	Improves the migration, survival, and proliferation of cells; promoting migration of keratinocytes and enhancing tissue repair
HGF	Regulating cell growth, motility, and morphogenesis in epithelial and endothelial cells; epithelial repair, granulation tissue formation, and neovascularization
TGF-ß	Extracellular matrix synthesis; fibroblast and mesenchymal stem cell proliferation; (TGFβ1 and -β2 are associated with scarring and fibrosis while TGFβ3 is related to angiogenesis)
TNF-alpha	Regulate diverse cell functions, including immune response and inflammation, but also proliferation, differentiation, apoptosis, and embryogenesis
GM-CSF	Stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes; signals via signal transducer and activator of transcription, STAT5; facilitates development of the immune system and promotes defense against infections
CTGF	Binds growth factors and extracellular matrix proteins; induce sustained fibrosis

Table 3. GFs involved in different phases of WH.

Growth factors	Wound healing stages
G-CSF, TGF-ß1, TGF-ß2	Inflammatory
PDGF, FGF, VEGF,	Proliferative
EGF, GM-CSF	Epithelialization
TGF-ß3	Remodeling

GFs = growth factors; WH = wound healing.

appears possible to highlight several common points between the biomolecular pathway of WH and HR. In particular, the stimulation of Wnt signaling appears to be present both in WH and HR especially when PRP and ASC-BT are performed.

More recently, the use of AD-MSCs and ASC-BT has been reported in COVID-19 patient's treatment thanks to their immunomodulatory and anti-inflammatory activities promoting the WH in damaged tissue by a cytokine storm [32–34].

When the choice of the PRP procedure must be performed, it is necessary to consider all the data available and not rely purely on the information provided by the manufacturer.

At the same time, attention must be given in the evaluation of PRP- LP, PRP-LR, PRF-LP, and PRF-LR use. In particular, the PRF is considered the second generation of platelet concentrates, and it may have some merits over PRP. First, it does not require the addition of anticoagulants. Second, PRF platelets and leukocytes entrapped inside fibrin gel release growth factors sustained for a long time. Third, immune cells and cytokines trapped in a PRF clot counteract infection, offering the possibility to suture the gel PRF clot directly into the bed of bone defect [35].

The most recent studies [36–39] have been focused on cutting-edge strategies to meet the requirements for tissue restoration by improving the properties of autologous platelet concentrates. In particular, Ding et al. [36] have reported in a recent review several aspects of these strategies, such as the advantages of lyophilized platelet concentrates and the combination of platelet concentrates with biomaterials, stem cells, or drugs, are discussed, with the aim to improve the outcomes of wound healing, as also confirmed by Gentile et al. [37] in a recent systematic review. A pivot role seems to be determined by the centrifugation force, freeze–thaw, sonication, and inclusion of calcium chelator as reported by Chan et al. [38]. Despite a lack of standardized protocols for PRP preparation, there is convincing evidence with objective measurement modalities that display positive outcomes after treatment for skin rejuvenation, HR, WH, and fat graft take, as stated by Chamata et al. [39].

4. Recommendations suggested by AIRMESS

On the basis of European rules and the analyzed investigations (retrospective, prospective, case report, case series, RCTs), including several systematic reviews of randomized clinical trials, related to the PRP and ASC-BT uses in WH and HR, it is possible to suggest the following recommendations:

- The A-N-A-PRP and A-A-PRP can be indicated in the AGA treatment in selected patients (grade I–IV according to Norwood–Hamilton scale for males and grade I–II according to Ludwig scale for females).
- The ASC-BT, regarding the AD-MSCs, SVFs, HFSCs, and HF-MSCs, can be indicated in WH and HR when they are obtained in a one-step medical procedure, via minimal manipulation and when they are involved for omofunctional use *'used for an indistinguishable fundamental capacity in the beneficiary as in the donor.'*

5. Expert opinion

The data analyzed highlights the positive effects of PRP and ASC-BT on HR in AGA patients and on WH, as showed by *in vivo* and *in vitro* results representing a safe and effective treatment.

Appropriate PRP preparation methods should be selected only after carefully considering their biomolecular specs and intended indications for use in patients. The upregulation of (FGF-7)/b-catenin signaling pathways, produced by A-N-A-PRP injection, stimulates HR by inducing HFSCs differentiation, prolonging the anagen phase of the HGC, improving the HD better than A-A-PRP. Regarding WH, both A-N-A-PRP and A-A-PRP may improve the neo-angiogenesis, stimulate cell growth, morphogenesis, and recruitment, constituting a three-dimensional matrix that allows cellular arrangement into a correct three-dimensional organization promoting tissue regeneration and repair, without any significant difference, between PRP-LP and/or PRP-LR in soft tissue defects. For bone defects, PRF-LP and/or PRF-LR appear to be more indicated compared with PRP, showing a faster healing.

The authors believe that the future will be based on regenerative-based therapies, and for this reason, invite all the international scientific audience to improve the publication's level in this field by focusing prevalently on Evidence-Based Medicine level 1 studies.

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Declaration of interest

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Author contributions

P.G. was the leader and principal investigator of this paper, performing the methodology, formal analysis, conceptualization, data curation, investigation, validation, writing—original draft preparation, writing—review and editing, acquisition funding and resources and project administration, analysis of European and Italian rules; R.A., J.P.C, K.A., T.V.H, J.F., A.T., L. G., A.V., P.T., J.M., and G.M. contributed resources and were involved in data curation and visualization; S.M. contributed resources, data curation, validation, review, and editing. All authors have agreed to the final version to be published and agree to be accountable for all aspects of the work.

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