Year : 2023

Étude rétrospective de l'efficacité du PRP standardisé en fonction de son ratio avec la concentration plaquettaire dans le sang sur les tendinopathies du sportif, status actuel des exigences réglementaires en matière de production de PRP en Suisse et en Europe complété d'un guide d'implémentation pour l'utilisation de PRP dans les thérapies régénératives à destination des tissus musculo squelettiques

Sebbagh Patrick

Sebbagh Patrick, 2023, Étude rétrospective de l'efficacité du PRP standardisé en fonction de son ratio avec la concentration plaquettaire dans le sang sur les tendinopathies du sportif, status actuel des exigences réglementaires en matière de production de PRP en Suisse et en Europe complété d'un guide d'implémentation pour l'utilisation de PRP dans les thérapies régénératives à destination des tissus musculo squelettiques

Originally published at : Thesis, University of Lausanne

Posted at the University of Lausanne Open Archive http://serval.unil.ch
Document URN : urn:nbn:ch:serval-BIB_DECDB721A46C9

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

UNIVERSITÉ DE LAUSANNE - FACULTÉ DE BIOLOGIE ET DE MÉDECINE

Unité de thérapie régénérative

Service de chirurgie plastique et de la main

Étude rétrospective de l'efficacité du PRP standardisé en fonction de son ratio avec la concentration plaquettaire dans le sang sur les tendinopathies du sportif, status actuel des exigences réglementaires en matière de production de PRP en Suisse et en Europe complété d'un guide d'implémentation pour l'utilisation de PRP dans les thérapies régénératives à destination des tissus musculo squelettiques

THESE

Préparée sous la direction du Professeur Lee Ann Laurent-Applegate
avec la co-direction du Professeur Vincent Gremaux-Bader

et présentée à la Faculté de biologie et de médecine de
l'Université de Lausanne pour l'obtention du grade de

DOCTEUR EN MEDECINE

par

Patrick Sebbagh

Médecin diplômé de la Confédération Suisse
Originaire de Cheseaux sur Lausanne (Vaud)

Lausanne

2023

UNIL | Université de Lausanne
Faculté de biologie et de médecine
École doctorale

IMPRIMATUR

La Faculté de biologie et médecine de l'Université de Lausanne, sur proposition du jury, autorise l'impression de la thèse de doctorat rédigée par

Patrick Georges SEBBAGH

intitulée

Étude rétrospective de l'efficacité du PRP standardisé en fonction de son ratio avec la concentration plaquettaire dans le sang sur les tendinopathies du sportif, status actuel des exigences réglementaires en matière de production de PRP en Suisse et en Europe complété d'un guide d'implémentation pour l'utilisation de PRP dans les thérapies régénératives à destination des tissus musculo squelettiques

sans se prononcer sur les opinions exprimées dans cette thèse.

Directrice	Professeure Lee Laurent-applegate
Co-directeur	Professeur Vincent Gremeaux
Vice-directeur de l'Ecole doctorale	Professeur John Prior

Lausanne, le 10.10.2023

pour Le Doyen
de la Faculté de Biologie et de Médecine

Monsieur le Professeur John Prior
Vice-Directeur de l'Ecole doctorale

Résumé

Les préparations à base de plasma enrichi en plaquettes (PRP) sont largement utilisées en médecine du sport notamment pour le traitement des affections des ligaments et des tendons, que ce soit au sein des institutions ou des cabinets privés. Depuis 2013, les préparations de PRP sont employées au CHUV pour le traitement des tendinopathies (département de la médecine du sport).
La production du PRP consiste à prélever du sang veineux autologue, et à soumettre celui-ci a une série de centrifugations afin de concentrer les plaquettes qu'il contient. Au CHUV, il est fabriqué de manière standardisée tout en respectant les exigences édictées dans les Bonnes Pratiques de Fabrication selon un protocole mis au point par l'unité de thérapie régénérative.

Ce travail a consisté en une étude règlementaire et une étude rétrospective (2013-2020) qui ont donné lieu à la publication de deux articles. Le premier article de ce travail passe en revue la réglementation concernant l'utilisation des PRP aussi bien en Suisse qu'en Europe. Le second article est une étude rétrospective qui passe en revue les résultats thérapeutiques avec un PRP synthétisé de manière standardisée sur les tendinopathies au sein du département de médecine du sport du CHUV.

L'étude rétrospective a porté sur 48 patients (âgés de 18 à 86 ans, avec un âge moyen de 43,4 ans et différents niveaux d'activité physique) présentant des tendinopathies.

L'enjeu principal de cette étude rétrospective était de démontrer l'efficacité du PRP sur les tendinopathies produit de manière standardisée (reproductibilité du produit final) tout en respectant les exigences édictées dans les Bonnes Pratiques de Fabrication (Good Manufacturing Practices (GMP)). Cette double exigence permettrait alors de faire progresser la littérature dans le sens de la standardisation des protocoles pour obtenir une cible de concentration plaquettaire. La mise en place d'une infrastructure et d'une méthode standardisée de production ouvre la voie à la comparaison des résultats thérapeutiques de différentes études. Sans cela, toute méta analyse des données est rendue difficile tant il existe de paramètres qui interviennent entre la prise de sang et l'injection du PRP.

Conclusion du travail
Le suivi clinique a démontré que 61% des patients ont rapporté des résultats d'efficacité favorables à la suite d'une seule injection de PRP obtenu à partir de sang autologue, produit de manière standardisée selon les normes GMP et injectée de manière echo guidée par ultrasons au sein du département de médecine du sport du CHUV. L'examen des bilans sanguins des patients a mis en évidence un facteur de concentration plaquettaire compris pour la plupart des patients entre 2,0 et 2,5 permettant ainsi de réduire la variabilité du matériel biologique.

Bien que les résultats soient conformes aux études déjà publiées sur les tendinopathies en médecine du sport, dans lesquelles l'efficacité des interventions orthobiologiques à faible concentration ne semble pas liée aux niveaux d'activité sportive ou à l'âge du patient, ces résultats mettent en évidence l'intérêt de la standardisation du processus et sur l'importance du respect des normes standardisées. Cette standardisation et le respect des normes de production s'avèrent être une condition préalable à une évaluation solide, robuste et homogène de l'efficacité clinique obtenue dans différentes études (comparabilité de l'efficacité/amélioration du patient).

bioengineering

MDPI

Article

Current Status of PRP Manufacturing Requirements & European Regulatory Frameworks: Practical Tools for the Appropriate Implementation of PRP Therapies in Musculoskeletal Regenerative Medicine

Patrick Sebbagh [1], Alessandro Cannone [2], Gerald Gremion [1], Vincent Gremeaux [3], Wassim Raffoul [1,4], Nathalie Hirt-Burri [1], Murielle Michetti [1], Philippe Abdel-Sayed [1,5], Alexis Laurent [6], Nathalie Wardé [2,†] and Lee Ann Applegate [1,4,7,*,†]

1 Regenerative Therapy Unit, Plastic, Reconstructive & Hand Surgery Service, Lausanne University Hospital, University of Lausanne, CH-1066 Epalinges, Switzerland
2 NAD Consulting Sàrl, CH-1208 Geneva, Switzerland
3 Sport Medicine Unit, Division of Physical Medicine and Rehabilitation, Swiss Olympic Medical Center, Lausanne University Hospital, University of Lausanne, CH-1011 Lausanne, Switzerland
4 Lausanne Burn Center, Lausanne University Hospital, University of Lausanne, CH-1011 Lausanne, Switzerland
5 DLL Bioengineering, STI School of Engineering, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland
6 Manufacturing Department, LAM Biotechnologies SA, CH-1066 Epalinges, Switzerland
7 Center for Applied Biotechnology and Molecular Medicine, University of Zurich, CH-8057 Zurich, Switzerland
* Correspondence: lee.laurent-applegate@chuv.ch; Tel.: +41-21-314-35-10
† Considered as co-last authors.

Citation: Sebbagh, P.; Cannone, A.; Gremion, G.; Gremeaux, V.; Raffoul, W.; Hirt-Burri, N.; Michetti, M.; Abdel-Sayed, P.; Laurent, A.; Wardé, N.; et al. Current Status of PRP Manufacturing Requirements & European Regulatory Frameworks: Practical Tools for the Appropriate Implementation of PRP Therapies in Musculoskeletal Regenerative Medicine. *Bioengineering* **2023**, *10*, 292. https://doi.org/10.3390/bioengineering10030292

Academic Editor: Jeremy Magalon

Received: 28 January 2023
Revised: 17 February 2023
Accepted: 22 February 2023
Published: 24 February 2023

Abstract: Providing accurate and up-to-date practical tools enabling oversight of platelet-rich plasma (PRP) legislation and of the appropriate standards to be implemented for its manufacture and use in Europe is a demanding task. This is due to rapid medico-technological advancements, slowness and disparity in legislation updates and enforcement between member states, and many reported gray-zone practices, notably for autologous PRP use. The levels of risk associated with blood manipulation processes generally dictate the manufacturing requirements for PRP preparations, which have gradually shifted toward good manufacturing practices (GMP) for standardization and overall quality enhancement. This work firstly outlines Western European and Swiss legislation for PRP products/preparations, providing key simplified information and recommendations for medical doctors seeking to implement this biological-based therapy for safe use in hospital settings, clinics, or private offices. This work secondly shows the importance of PRP-based product manufacturing standardization, which subsequently enables sound clinical evaluation of therapeutic interventions. Although the applicable legal bases provide guidelines for GMP manufacturing infrastructure and basic process design, paramount importance is set on the definition of workflows, technical specifications, and key parameters for PRP preparation and delivery. Overall, the development of simple and robust technologies and processes for PRP preparation is critical for guaranteeing the high therapeutic quality of the intervention, in collaboration with qualified GMP manufacturing platforms. Importantly, this work aims to serve as a practical tool for clinicians based in Western Europe who are willing to appropriately (i.e., administratively and technically) implement autologous PRP treatments in musculoskeletal regenerative medicine workflows, to ensure they make informed and optimal regulatory or process-based decisions.

Keywords: biologicals; clinical cytotherapies; good manufacturing practices; musculoskeletal affections; orthobiologics; platelet-rich plasma; process standardization; regenerative medicine; regulatory frameworks; therapeutic products

1. Introduction

Regenerative medicine consists of the replacement or help in the regeneration of cells, tissues, or organs in the human body to establish or restore normal function, thus presenting tremendous curative and preventive potential [1]. It includes cell therapy, gene therapy, tissue engineering, biomedical engineering techniques, and treatments involving biological products, which encompass platelet-rich plasma (PRP) [2–4]. Although such techniques may not all be categorized as curative, many may provide strong ancillary therapeutic support and may significantly improve the quality of life of treated patients [2]. In common affections, such as articular lesions/inflammation or tissue scaring, regenerative medicine options may reduce the need for traditional medication administration and may provide a holistic solution in complex pathophysiological situations [5,6]. With highly versatile therapeutic approaches, which have been constantly improving for several decades, regenerative medicine can potentially address all systems and tissues of the human body [7–11].

Clinical results yielded by the application of cell-based therapies have been evolving rapidly since the 1980s, particularly in burn patient care and in orthopedics [2,6,9,12]. The use of biological products/cells has demonstrably improved clinical outcomes, such as those reported for PRP administration in burn patients and for cutaneous wound management [8,13]. PRP alone or in combination with rapidly isolated skin keratinocytes from severe burn patients have notably demonstrated that simple biological-based therapeutic approaches could procure significant advances in severe burn victim care [13]. As the time to initial treatment is a critical factor for these patients, the rapid use of such simple preparations has shown significantly increased healing rates and pain reduction, which represents major milestones [13]. From the technical standpoint, a recent study has shown that preparations based on rapidly isolated patient keratinocytes could be standardized and implemented for manufacture in a good manufacturing practice (GMP) facility, guaranteeing appropriate levels of process and environmental control [14].

The therapeutic use of PRP and similar orthobiologics for managing a variety of musculoskeletal conditions is rapidly increasing worldwide and was drastically enhanced by the availability of CE-marked or approved kits for ease of preparation of blood products [15–21]. This was made possible notably due to the absence of FDA regulations regarding the manufacture of the final orthobiologic product, while the focus was historically set on the used device [16,17,22]. Importantly, this lack of recommendations and guidelines potentially introduces unbearable iatrogenic risks (e.g., viral transmission), which are exacerbated for the use of PRP in non-medical settings [23]. Within appropriate clinical settings, the autologous local use of PRP has been shown to be safe, minimally invasive, and effective to promote tissue relief from inflammation and pain [8]. Notably, hundreds of thousands of athletes and patients are treated annually with PRP, with more than 80,000 athletes treated in the USA alone already a decade ago [24]. Interestingly, this treatment remains allowed by the World Anti-Doping Agency (WADA), while the use of growth factors remains prohibited [24,25].

Professional organizations have promoted the use of PRP, such as the American Academy of Orthopaedic Surgeons, which has included PRP in the 2021 practice guidelines for osteoarthritis (OA), or the American Medical Society for Sports Medicine, which supports advancements in the domain of clinical orthobiologics. In addition, the American Academy of Orthopaedic Surgeons has published a consensus paper aiming to improve and accelerate the clinical evaluation, clinical use, and multifactorial optimization of biological-based therapies for musculoskeletal diseases and affections [26]. From a therapeutic standpoint, PRP applications have frequently been used within tissues with low intrinsic healing potential and for indications such as tendinopathies, calcaneal and plantar fasciitis in the foot, muscle strains, ligament sprains, articular cartilage injuries and degeneration, or local preparation before hair transplants [7,27–36].

Accurately providing an appropriate and current oversight of PRP legislation in Europe is complex due to the rapidly evolving laws and regulations and the high number of individual member states or jurisdictions [3,34,37]. The same complexity applies to the

manufacturing standards for PRP and for its clinical use in regenerative medicine [34]. Multiple factors are interconnected and notably comprise the rapid medico-technological advancements, the slowness and disparity in legislation updates, and the inhomogeneous enforcement of the laws between member states. Additionally, many borderline or gray-zone practices have been reported in the field for autologous PRP injections [37]. From a technical standpoint, safety- and quality-oriented risk analyses for blood manipulation are used to determine the appropriate manufacturing process requirements for PRP-based products/preparations. In recent European regulations, these process requirements have gradually shifted toward good manufacturing practices (GMPs) for standardization purposes and with the objective of obtaining overall quality enhancements for the finished PRP products/preparations [37–48].

The background to the present study consists of the documented high heterogeneity in the application of legal and regulatory requirements for PRP treatment implementation in Western Europe [24,34,37,38]. A tangible need was thus identified to establish a summarized source of clear information on the legal and regulatory bases and key stakeholders, which should be preliminarily consulted by medical professionals willing to implement PRP treatments in regenerative medicine workflows. In parallel, high heterogeneity has been documented on the technical side for autologous PRP obtention and use, where the application of various and non-standardized methods and protocols results in high variability in the PRP products that are clinically administered [4,15,16,18–20]. Based on this background and on the extensive translational experience available in Lausanne for the GMP manufacture and clinical use of autologous PRP, process-based and quality-oriented considerations were set forth as an evidence-based source for the same professionals interested in the new implementation of PRP treatments in their practice [13,35].

This study firstly aims to outline and concisely summarize the key points of the current European and Swiss legislation for PRP products/preparations [39–47]. From a general technical standpoint, if the blood is manipulated in a closed system, most often in aseptic conditions using equipment that is a medical device (MD), some requirements (e.g., authorization from local authorities) might be not applicable/necessary for PRP preparation activities [39–44]. This is, however, not the case in Switzerland, where an authorization from Swissmedic (i.e., the national health authority for therapeutic products) is mandatory to manufacture blood products [35,46,47]. Importantly, most European countries consider PRP as a "non-standardized medicinal product", for which GMP standards must be applied [34,45]. Specifically, Directive 2005/62/EC clearly states that good practice guidelines (GPG) based on the principles of GMP must be implemented, and the guidelines clearly define the standards, which are very close to GMP [40,41]. Switzerland has adapted this same approach in the Therapeutic Products Act (TPA) and requires similar practices and standards [46,47]. This study secondly shows the importance of PRP-based product preparation (i.e., manufacturing process and controls) standardization, which is a prerequisite for sound clinical evaluation of high-quality therapeutic interventions [9]. Despite detailed descriptions of the necessary GMP manufacturing infrastructure in the applicable framework documents, few elements pertaining to process design are provided [39–41,49]. Therefore, the choice of the exact manufacturing process and protocol is the responsibility of the manufacturer, which should carefully consider and optimize the workflows, technical specifications, and key parameters of PRP preparation and clinical delivery [9,15,16,35].

Importantly, an appropriate equilibrium must be found between the maintenance of the optimal biological properties of a given preparation and the safety-oriented requirements of the related manufacturing workflow, as well as feasibility in clinical routine [9,35]. Specifically, despite important regulatory and manufacturing constraints, the central aspect of successful PRP-based therapeutic approaches relies on the clinically-oriented and scientifically optimized product development process [49,50]. The continuous development of simple, yet robust technologies and processes for autologous PRP manufacture represents a key driver for delivering high therapeutic quality and cost-effective interventions in the clinic [51,52]. This may notably be performed in collaboration with qualified GMP

manufacturing contractors [15,35]. Indeed, recent evolutions in the applicable legal bases make it highly advisable to standardize PRP preparation protocols under GMPs, making it possible to adapt end-products to different clinical settings [3,37,49]. Most importantly, qualified personnel are required for the administration of PRP and biologicals to assure proper safety of the clinical intervention and to avoid iatrogenesis [3,12,17].

The major contributions and novelty of the present study consists of the availability (i.e., in a single source) of up-to-date legal and regulatory elements to be firstly considered by European clinicians in order to appropriately introduce PRP treatments in their practice from an administrative and legal standpoint. Secondly, a comparative discussion about the possibilities and limits existing in various Western European countries for autologous PRP preparation should provide important insights to the readers regarding the technical/quality aspects of product processing. In this context, the present work sets forth the significant advantages that may be procured by the use of GMP-accredited platforms for autologous PRP manufacture (i.e., process validation and standardization, insurance of traceability and consistency). Overall, the present study should significantly contribute to ensure that new physicians lay down the appropriate administrative and technical bases for PRP implementation in musculoskeletal regenerative medicine, which are prerequisites to providing safe and effective therapeutic interventions to their patients.

2. Aims, Scope, and Methodology of the Study

The first specific aim of the present study was to perform an updated analysis of the legal and regulatory frameworks that are currently applicable in Western European countries (i.e., Switzerland, Germany, France, Italy, and Spain) for autologous PRP manufacture and clinical human use. The objective of this part of the study was to provide a summary of the official sources and a currently valid catalogue of the applicable legal, regulatory, and guidance documents to physicians considering the implementation of autologous PRP treatments in their practice. From a methodological point-of-view, the compiled legal base documents were prospectively gathered from the official national websites of the Western European countries of interest and that of the European Union. This ensured that the considered documents were up-to-date and currently in force. These sources were cross-checked with the publicly available online records and registries of the national health authorities of the countries of interest.

The second aim of the present study was to perform an updated analysis of the responsible parties (i.e., health authorities, regulators, license or accreditation providers) in the same Western European countries. The objective of this part of the work was to provide a summary of the official sources and a currently valid catalogue of the parties who should be contacted by individual physicians interested in the clinical implementation of autologous PRP protocols. This aspect was considered as especially important for the readers due to the inhomogeneity of the requirements and practices across the analyzed jurisdictions. From a methodological point-of-view, the responsible parties were identified by a prospective analysis of the publicly available online records and registries of the national health authorities of the countries of interest.

Simplified and illustrated workflows are presented in Figures S1 and S2 for an optimal description of the general design of the study and presentation of the protocol used in Lausanne for the GMP preparation of autologous PRP. Overall, the present study and article were designed as practical tools for physicians based in Western Europe. Specifically, the present work was meant to ensure that the readers integrate the following notions and key messages:

(i) Basic scientific knowledge on the nature, growth factor contents, and known therapeutic effects of autologous PRP preparations in regenerative medicine;
(ii) Awareness of the currently applicable legal bases and regulatory frameworks in their respective jurisdictions;
(iii) Awareness of the currently competent authorities and entities that should be contacted in case of doubts on the requirements to fulfil before implementing autologous PRP treatments in their practice.

3. Biological-Based Therapeutics for Enhanced Patient Management: Overview of the Known PRP Attributes and Actions

Physiologically, platelets possess several functions, which include aggregation during primary haemostasis, release of inflammatory mediators, and stimulation of tissue healing [16,53,54]. Such effects are taken into account for the classification of platelet-based therapeutic preparations for human use (e.g., autologous PRP) as medicinal products [4,17,34]. In vivo, platelets become activated upon detection of the slightest damage to the endothelium [16,54]. In response to an injury, platelets release chemokines, growth factors, angiogenesis stimulators and inhibitors, bactericidal/fungicidal/virucidal proteins, immune mediators, coagulants, anticoagulants, and fibrinolytic proteins [55–57] (Figure 1).

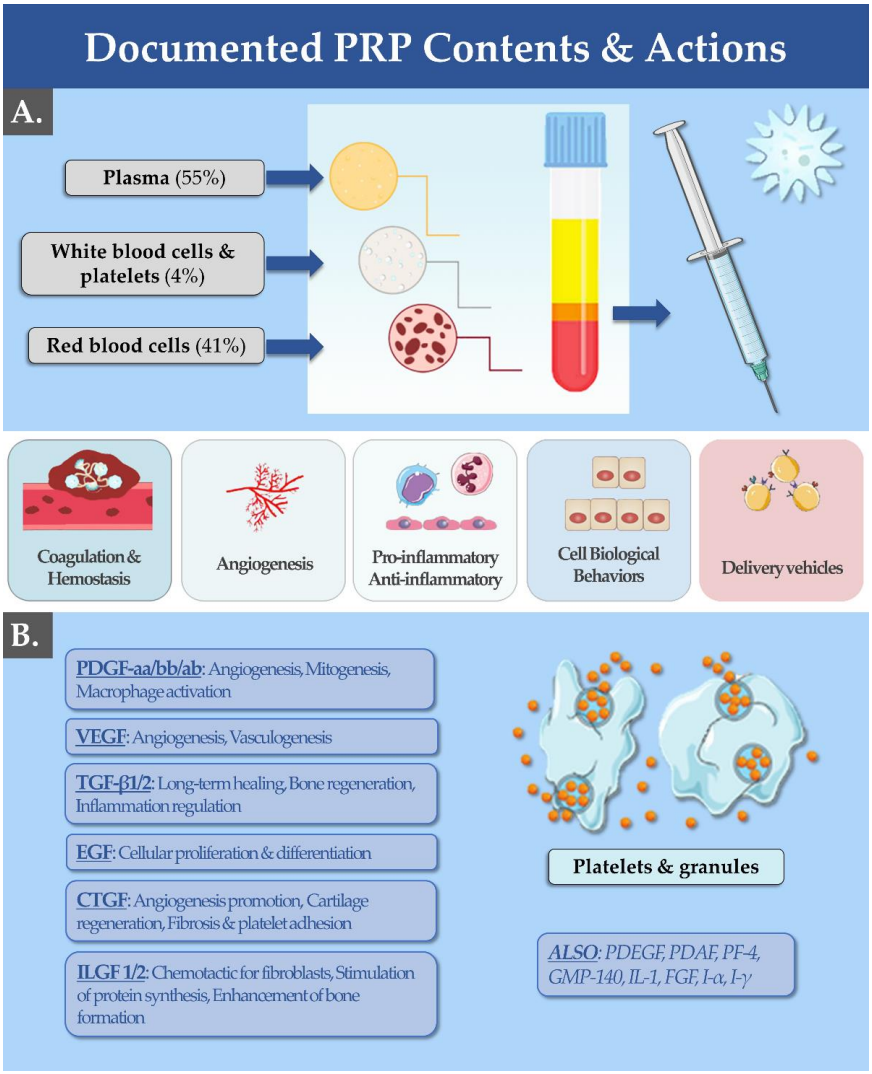

Figure 1. (**A**) PRP is isolated from a whole blood sample taken by venous access (i.e., ≥20 cc needed depending on the indication). Concentrated platelets are obtained by centrifugation of autologous blood in a 2-step procedure, aiming to eliminate RBC and to prepare a 2–5X concentrate of platelets in the isolated plasma volume. The mechanisms of action of PRP in regenerative medicine are implicated at all stages of wound healing (e.g., coagulation, angiogenesis, inflammation) due to the various growth factors available in the biological preparations. (**B**) Listing of the most common growth factors found in PRP, along with their documented effects. Cc, cubic centimeters; CTGF, connective tissue growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; GMP-140, platelet alpha-granule membrane protein; IL, interleukin; ILGF, insulin-like growth factor; PDAF, platelet-derived angiogenesis factor; PDGF, platelet-derived growth factor; PF, platelet factor; PRP, platelet-rich plasma; RBC, red blood cells; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

Platelets then express adhesion factors in order to adhere to the injured endothelium (Figure 1B). In the context of regenerative medicine, the platelet growth factors are of particular interest [54,57,58]. Platelets notably synthesize epidermal growth factor (EGF), hepatocyte growth factor (HGF), insulin-like growth factor, and transforming growth factor (TGF) (Figure 1B) [54,57]. These growth factors promote cell proliferation, cell migration, and collagen synthesis (Figure 1B). In addition, platelets contain vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), angiostatin, and endostatin, which help to improve the perfusion of damaged tissues and therefore contribute to rapid regeneration (Figure 1) [4,24]. These angiogenic factors allow the development of blood microcirculation and increase capillary permeability [24]. Both types of described factors (i.e., growth factors and angiogenic factors) are contained in the granules of platelets (Figure 1B) [57]. In PRP preparations, the method of extraction of these active agents consists of the centrifugation of whole blood to separate the plasma from the cells and to allow their release, making them freely available in the finished product form (Figure 1A) [50].

4. Updated Analysis of the Applicable European & Swiss Legal Frameworks and Requirements for the Manufacture of Clinical Grade PRP

As PRP constitutes a blood-derived product and is classified as a medicinal product, its use is strictly regulated in the European Union (EU) [34]. These stringent requirements were meant to ensure proper control of all the aspects related to preparations, storage, and distribution, and thereby guaranteeing the quality and safety of the finished PRP products [39–42]. In particular, the applicable regulatory framework concerning the quality and safety aspects surrounding blood is Directive 2002/98/EC (Table S1) [39]. Furthermore, Directive 2005/62/EC and its amending Directive 2016/1214 set out the rules in regards to the quality system standards and specifications for blood establishments (Table S2) [40,41]. This encompasses a broad range of necessary practices and controls, including technical requirements for the collection of blood and testing, license requirements, qualified personnel and responsible persons, quality system management, traceability of the products, and the guidelines to ensure that all of the steps are accomplished safely (Tables S1 and S2) [39–41]. The directives are acknowledged in the various EU member states with specific internal regulations [38]. This inhomogeneity in the EU regulatory ecosystem may lead to the community legislators to intervene rapidly in order to even out the "rules of engagement" of this particular class of biologicals [34,37].

In Switzerland, blood-derived products, including PRP, are considered as non-standardized medicines, therefore they are generally subject to authorization according to the law on therapeutic products [46,47]. The aim of the applicable documentation is to set forth the regulatory framework applicable to products obtained following the manipulation of whole blood (e.g., PRP) using means of separation such as medical devices [47]. However, the regulations concerning the accreditation and commercialization of the devices (i.e., as pertains to European Regulation 2017/746) are not specified [43,44,46,47]. Importantly, the medical devices used for PRP preparation often belong to various risk categories due to the heterogeneity of manufacture, assembly, and operating instructions, and therefore require specific and non-uniform regulatory pathways for obtaining the CE mark [44,59]. The scope of Directive 2002/98/EC is to ensure that blood and its components are of comparable quality and safety throughout the blood transfusion chain in all member states, bearing in mind the freedom of movement of citizens within the community territory (Table S1) [39].

In the Summary Report following the Meeting of the Competent Authorities on Blood and Blood Components concerning PRP (i.e., 11–12 October 2012, Brussels) the commission communicated that the considered procedure should be considered within the scope of the directives on blood [39–42]. Notwithstanding, the competent national authorities have subsequently provided the opinion that it is practically difficult to bring this procedure fully in line with what was set forth by the 2002 Directive [34,39]. Therefore, it was decided that

this procedure would constitute the object of ad hoc regulations by the EU in the future [37]. Some authorities have put more stringent authorization measures in place, resulting in divergent levels of safety and effectiveness for patients across member states [37,38]. However, the Directive 2002/98/EC states that "*The nature of autologous transfusion necessitates a specific consideration in respect of how and when to apply the different provisions of this Directive*", leaving some room for interpretation by hematological centers [39]. The margins for interpretation are partially filled by Directive 2005/62/EC and its amending Directive 2016/1214, which prepared rules with regard to quality system standards and specifications for blood establishments, defining additional details for the requirements already covered in Directive 2002/98/EC [39–41]. In particular, Directive 2005/62/EC states that good practice guidelines (GPG) shall be developed by the commission for ensuring alignment with the applicable standards and specifications [40]. Those guidelines are to be based on the principles of good manufacturing practices (GMP) [40].

Good manufacturing practices are a set of quality rules to be applied in the production and distribution of pharmaceutical products [49]. GMPs are used in Europe and in Switzerland and are similar or very similar in content to good manufacturing practices in the USA [35,49]. GPGs (and GMPs) are strict recommendations, whereas current good manufacturing practices (cGMPs) are fully-fledged legal texts [49]. GMPs define production, testing, and quality assurance objectives to ensure that pharmaceutical products are safe for consumption. The regulation uses the terms "pure, safe, and effective". GMPs set out rules to ensure that the basic principles detailed in Table S2 are met [49].

5. Updated Analysis of the Competent Health Authorities and Regulatory Structures/Entities for PRP in Switzerland and in Europe

In Switzerland, Swissmedic or the Swiss Agency for Therapeutic Products is responsible for monitoring the market for therapeutic products [49]. It was founded in 2002 following the merger of the Intercantonal Office for Drug Control (IOMC) and the Therapeutic Agents Unit of the Federal Office of Public Health (FOPH). Since its creation in 2002, Swissmedic has been responsible for the implementation of the law on therapeutic products [46].

In Europe, in addition to the national agencies that are able to authorize the marketing of a pharmaceutical product in their territory, there is a central agency, the European Medicines Agency (EMEA) [60]. This agency is an umbrella structure that allows parallel European registrations. Obtaining a marketing authorization issued by the European Medicines Agency allows the product to be marketed in all European countries [60].

5.1. Health Authorities in Switzerland with Competence over PRP

Switzerland is currently not an EU member state, yet similar Swiss regulations concerning blood-derived products exist, as compared to the principles expressed in the above-mentioned directives (Tables S1 and S2) [39–47]. In Switzerland, the law on therapeutic products (i.e., LPTh or TPA, RS 812.21) constitutes the legal basis for chemical and biotechnological drugs, medical devices, vaccines, implants, diagnostics, and blood products, for which the preparation process must follow the standards set forth for the preparation of medicinal drugs [46]. PRP is considered as a "non-standardized" medicinal product since it is not industrially produced, and it is a patient-specific preparation [50]. Indeed, patient-specific therapeutic preparations can seldom be standardized in a manner comparable to normal medicinal products, since their origin usually incurs high biological variability [49]. PRP preparations therefore cannot be defined as formula-related medicinal products. Although manufactured following a physician's prescription for his/her specific patients, PRP preparations are not usually produced according to a classical and standardized process [35]. They are usually not manufactured in public pharmacies or hospital pharmacy departments, which is the case for formula-based preparations [46].

However, since PRP preparations have historically been considered to be medicinal products, they are currently subjected to authorization following Art. 9, para. 1 of the Ther-

apeutic Products Act (TPA, RS 812.21) [46]. This is, however, not the case if the considered preparations fall under the exemption ruling stated in Art. 9, para. 2 TPA. Specifically, this exemption is applicable to formula-related medicinal products, preparations to be used in clinical trials, and non-standardized medicinal products [46]. The Therapeutic Products Act was revised in 2019 and the classification possibilities for non-standardized medicinal products were diversified. Consequently, some patient-specific preparations can currently be classified as non-standardized medicinal products following Art. 9, para. 2, let. E TPA, and such is the case for PRP [46]. Based on the fact that non-standardized medicinal products are distinct from formula-based preparations, the manufacture and distribution activities require a Swissmedic establishment license. Furthermore, such preparations are to be manufactured following the GMP requirements listed in Annex 1 of the Ordinance on Licensing in the Medicinal Products Sector (MPLO; RS 812.212.1) [47].

Specifically, in order to protect the intended recipients, mandatory authorizations are now required for certain non-standardized medicinal products, including PRP [47]. However, the Therapeutic Products Act describes some settings in which non-standardized medicinal products may conditionally continue to be manufactured and used without regard to the new provisions (Table 1) [46]. These exceptions encompass association with particular equipment and environments, patient management during blood processing, assuring product sterility and rapid use, and overall patient safety.

Table 1. Exceptions to new rulings on the authorization requirements for PRP manufacture in Switzerland, in the case where the criteria are respected (e.g., the physician must ensure the safety, quality, and efficacy of the treatment). PRP, platelet-rich plasma.

Exception ID	Description of the Exceptions
1.	The preparation of autologous products fully complies with the equipment manufacturers' instructions.
2.	The patient does not leave the room where the initial blood material is withdrawn until final administration, including during the preparation.
3.	The blood is prepared in the same room under the direct supervision of the patient's physician.
4.	The preparation is performed in a closed system if the product must be sterile.
5.	The administration to the patient happens immediately after the preparation is performed and under the supervision of the patient's physician or personnel under her/his direct responsibility following her/his detailed instructions.
6.	The product cannot be used for the treatment of other patients.
7.	The product cannot be distributed to the patients.
8.	The procedure and the relevant documentation must be in writing and part of the quality system.

In scenarios not covered by the new provisions, the person performing the treatment remains subject to the basic and general medical duty of care and is responsible for guaranteeing the safety, quality, and efficacy of the administered treatment [46,47]. There are activities that may be allowed only with proper authorization and these conditions are related to timing of the preparation and the product administration [47]. Specifically, these provisions apply when the sterility of the product could be a factor of concern and when the preparations are not destined to the same patient, as presented more specifically in Table 2 below.

Table 2. PRP manufacturing activities allowed only with an authorization from Swissmedic (i.e., the physician must ensure the safety, quality, and efficacy of the treatment). PRP, platelet-rich plasma.

Activity ID	Description of the Activities
1.	Preparation and/or administration after a period of storage or not immediately.
2.	Additional manipulation of the obtained fractions out of the closed system.
3.	Addition of active ingredients or substances that are not part of the production process, according to the instructions from the equipment vendor.
4.	Preparation of multiple doses at any stage of the manipulation.
5.	Allogenic preparation (i.e., preparation destined to a patient different than the donor).

As stated for Switzerland, the national regulatory body that oversees the domain of PRP manufacture and use is Swissmedic. In accordance with Article 58 of the Swiss Law on Therapeutic Products, Swissmedic is responsible for hemovigilance [46]. Therefore, all institutions that are duly authorized to handle blood and blood products (e.g., blood transfusion services) must implement a reporting procedure and must employ a responsible person for hemovigilance, who appropriately reports adverse incidents related to production and distribution [46,47]. The Swiss approach is therefore similar to the one adopted in Germany, notably with the implementation of GMP standards and accreditations/authorizations issued by regulatory authorities.

5.2. Health Authorities in Germany with Competence over PRP

In Germany, the law governing blood components is the Decree of 20 December 2007 (i.e., number 261) associated with the Health Ministry Decree of 3 March 2005, which constitutes the key document regulating the preparation and use of blood components. The law classifies blood components as medicinal products and an authorization is therefore necessary for marketing, according to the German Medicinal Products Act (i.e., AMG, Arzneimittelgesetz). Consequently, GMP and good distribution practices (GDP) apply to the manufacturing/manipulation and distribution phases.

The national organization that issues the required authorizations is the Paul Ehrlich Institute (PEI) within the German ministry of health. In Germany, transfusion of blood components is specifically regulated by the Transfusion Act (i.e., TFG Transfusionsgesetz). Within this legal framework, section 18 of the TFG must be considered with great care, as the hemotherapy guidelines are particularly important.

5.3. Health Authorities in France with Competence over PRP

In France, implementation of the "Blood System" is the responsibility of the Direction Générale de la Santé (DGS). Hemovigilance and the related systems are the responsibility of the ANSM (Agence nationale de sécurité du médicament et des produits de santé/National Agency for Drug Security and Health Products). Practically, the French blood system relies on a network of over 800 blood transfusion centers. Transfusion control procedures and systems mainly cover the "Produits Sanguins Labiles" (PSL, labile blood products), which are obtained from donors and are intended to be transfused to a patient for therapeutic purposes (article L1221–8 of the Code de la Santé Publique (Code of Public Health), amended by the law 2011–2012 of 29 December 2011, Article 5). These processes usually involve whole blood, plasma, and human blood cells. The corresponding list and PSL attributes are specified by the ANSM, which consults the Etablissement français du sang (EFS, French Blood Establishment). The EFS is a French public institution purposed with an array of advisory functions and activities, notably those related to the therapeutic use of human blood. Accordingly, whereas stable preparations for medical blood transfusions are considered as drugs, PSLs (i.e., including all blood components for topical application) are not regulated in a similar manner and are instead assessed and authorized case-by-case.

5.4. Health Authorities in Italy with Competence over PRP

Italian legislation for "blood components for non-infusion/transfusion" (e.g., topical preparations) use encompasses all the components used in all therapeutic scenarios in which blood components are not transfused into the patient. Instead, the blood components must be used directly in the pathologic area and may be administered locally by various means (e.g., intra-articular/deep tissue injection in orthopedics, cutaneous use in dermatology, subcutaneous use in plastic surgery, etc.). The preparation of platelet concentrates or platelet gels is not considered to fall under the category of blood derivative production due to simple processing by physically fractionating the blood into blood components. Once authorized by the competent/specific authorities and even in an outpatient setting, it is technically possible for any orthopedic surgeon to prepare concentrated platelets by using appropriate machines. Such machines normally possess a CE-marked closed circuit and

make it possible to centrifuge and/or further filter the autologous blood, separating the plasma portion (i.e., portion rich in platelets) for therapeutic use.

In Italy, the notion of "minimum manipulation" is applicable to the processing of blood components that are not combined with other drugs and that are prepared rapidly. A major risk, in the case of autologous donors, would be the dysfunction of the preparation apparatus or system. Additionally, in the case where the blood components are to be used in a topical therapeutic indication, this would deviate from the classic infusion/transfusion administration route. In order to abide by the rules of minimal manipulation, concentrated platelets are to be prepared by simple physical means (e.g., centrifugation, separation), and must not be used for experimental purposes or for somatic cell therapy.

Of note, the Committee for Advanced Therapies (CAT) has identified (i.e., on 13 November 2009) "fresh and freeze-dried thrombocytes isolated from autologous or allogeneic blood" as "products intended for wound healing in orthopedic and dental surgery". Consequently, such products were not considered as advanced therapy medicinal products at that time. Currently, the blood system regulatory framework is specified by the decree of 21 October 2005 (i.e., no. 219). While this law and the decree of 20 December 2007 (i.e., no. 261) generally refer to blood systems, the decree of 3 March 2005, specifically defines the applicable rules for blood component preparation.

In Italy, the manipulation of blood is, by law (i.e., no. 219/2005), restricted to transfusion services. The transfusion services are public institutions subject to accreditation and are usually located in hospitals. Such services are competent for all processes encompassing the collection, storage, handling, and distribution of blood and blood components. Nevertheless, it has been legally recognized that structures supplied by the transfusion services may be authorized for the "remote" outpatient preparation of blood components for topical autologous use if ad hoc agreements are established and specify the following:

(a) The transfusion service must keep full control over the remote processing of blood components, including all traceability aspects, meaning registration of the procedure;

(b) The medical professionals must provide their patients with a rapid and effective service, safe products, and tangible logistical advantages (i.e., no need for pre-donation, use of a fresh product, use of a non-frozen product, reduced need for blood harvesting).

Therefore, agreements between medical doctors and transfusion services may allow the physician to accomplish the outpatient preparation of blood components for topical use, provided that appropriate oversight is ensured by the competent transfusion service. Despite the Italian regulation being relatively lenient, all manipulation of blood components performed without the appropriate authorization is constitutive of a criminal offense. The unauthorized ambulatory use of PRP preparations without an agreement with a transfusion center is never legal, which can make this practice more heterogeneous and complex than in other European countries.

5.5. Health Authorities in Spain with Competence over PRP

Spain has implemented the European Directive 2002/98/EC in 2005, further specifying the technical requirements for the donation, processing, and use of whole blood and blood components. As regards the PRP manufacturing process, the platelet separation step must be performed in a closed system or in a circuit or must at least be performed in sterile/aseptic systems. Notably, all the procedures governing blood manipulation for products that are intended for transfusion are placed under the authority of blood transfusion centers (i.e., Center de Transfusión Sanguínea). However, for special procedures (e.g., autologous, intraoperative use, with small amounts for specific protocols of treatment), the Comité Cientifico para la Seguritad Transfusional (CCST, Committee for Transfusion Security) has declared that regulations for hemotherapy practices (i.e., CCST opinion in response to a request for clarification of 10 May 2004) would not apply.

In May of 2013, the Spanish Agency of Medicines and Medical Devices (AEMPS) together with different experts in the field of PRP set forth a resolution. This resolution established the classification of non-replacement therapeutic use of autologous plasma

and associated fractions, components, or derivatives as a medicinal product for human use to meet specialized needs. Therein, PRP was classified as a non-industrial biological medicine intended for human clinical application. Similarly, the AEMPS has stated that in Spain, PRP therapy could not be classified as an ATMP. Thereby and with this new framework, the AEMPS aimed to ensure the quality, efficacy, traceability, information, and pharmacovigilance for PRP preparations in a similar manner to classical medicinal products. PRP preparations must therefore be considered as medicines, which incurs strict requirements for manufacturing, traceability, efficacy and safety, and patient information, implicating GMP and GDP application.

6. Importance of Manufacturing Process Standardization for Therapeutic Biologicals, Such as Autologous PRP

Overall, there seems to be a consensus to standardize therapeutic biological preparation manufacture, in addition to basic monitoring of the environment and infrastructure used in their preparation [16,26]. Based both on past in-house experience in the Lausanne University Hospital and on the literature, key parameters and recommendations are provided hereafter for the optimization of GMP preparation processes of PRP (i.e., open processes under grade A working environments, Figure S2, Table 3) [15,16,35,50].

Table 3. Key manufacturing and control parameters influencing PRP quality, as well as recommendations and possibilities for PRP quality optimization. PPP, platelet-poor plasma; PRP, platelet-rich plasma; RBC, red blood cell.

Parameter	Definition of Critical Process Items, Parameters, and Quality Optimization Options
Blood Draw Devices and Anticoagulants	Anticoagulant vacutainer tubes and blood collection needles of the appropriate size and medical device grade quality. Anticoagulant choice for the preservation of platelet function, integrity, and morphology (e.g., citrate and sodium citrate dextrose are recommended).
Blood Draw Homogenization	Blood collections in tubes or bags should be thoroughly homogenized by gentle inversion at least 5–10 times, depending on the size and the volume, for mixing of the anticoagulant throughout. This needs to be assured to avoid any small fibrin clot formation, which would influence the total platelet count.
Blood Centrifugation-Step N°1	Validation of the equipment/method for the initial centrifugation to assure optimal separation of platelets from the whole blood. A platelet count in the RBCs and within the supernatant would allow this step. Altering the centrifugation time and rotor speed would be indicated if the parameters have not yet been optimized. In addition, the temperature within the centrifuge should be standardized.
Blood Centrifugation-Step N°2	The second centrifugation step should be optimized based on the determination of the optimal platelet counts in PPP and PRP. A higher concentration of platelets in the upper layer (PPP) would indicate that the process parameters are not optimal.
Controls of PPP and PRP	PRP should be separated from PPP rapidly following the secondary centrifugation step, as concentrated platelets diffuse into the PPP over time and reduce the PRP preparation yield. The concentrated final PRP preparation should be appropriately re-suspended to allow homogenization before the platelet counting procedure.

When working with biologicals destined for medical treatments, the defined parameters prepared specifically based on the needs of the prescribing clinician are very important [9]. Autologous PRP products are complex and naturally variable in their composition and the need for thorough quality controls in view of clinical applications makes it crucial to demonstrate consistency in the manufacturing process [4,15,51,52]. General protocols for PRP preparation consist of blood collection and a first centrifugation step to separate the red blood cells (RBC), which is then followed by a subsequent second centrifugation step to concentrate the platelets and other components and to provide activation of the biological product (i.e., mechanical action of injection, Figure S2) [61–66].

The main parameters that may be optimized comprise the type of medical devices chosen for blood draw, variation of the relative centrifugal force, as well as temperature and time brackets for platelet isolation (Table 3) [16,59,62,66]. Even though commercially marketed PRP kits offer the possibility of rapid obtention of ready-to-use PRP suspensions,

which should be sterile, several disadvantages have been identified [16,59]. Indeed, such kits often come with a high purchasing cost, limited blood draw possibilities, and variable guidelines for centrifugation steps, which are related to large variability in the final platelet concentration/purity of the PRP delivered to the patient [16]. Therefore, a variety of technical aspects make it difficult for the clinician to practically decide which commercial system would be most adequate for the considered clinical application.

7. Discussion

7.1. Current Inhomogeneity in Practices for PRP Preparation and Clinical Use in Europe

The indications for PRP use are varied and are found in the fields of plastic surgery, hand surgery, septic conditions and sports medicine, anaesthesiology, radiology, and for many specific indications such as epicondylitis, patellar tendinopathy, Achilles tendinopathy, osteoarthritis, burns, wounds, grafts, scars, and hair loss [17,67–73]. Therefore, guidelines to aid the safe and correct use of PRP will potentially benefit many healthcare providers within diverse environments, whether in hospitals, clinics, or private practices [3,12,16,26,33]. Specifically, such guidelines may help to assure that the proper treatment protocol is provided for the patient and that PRP preparation is performed within registered and licensed laboratories [16,37].

Careful consideration of the applicable European and national regulations regarding the manufacture and use of PRP enable us to draw several conclusions on the use of such blood products (Tables S1 and S2) [39–42]. In general, the operational principle is that, based on the type of blood component to be used, authorizations may be required from the national authorities responsible for transfusion activities or for therapeutic products [39,46,47]. In Italy, exceptions are not allowed, and blood components prepared for topical use (i.e., considered as blood products) must always be placed under the responsibility of an accredited blood transfusion service. This oversight structure is always necessary, regardless of the type of product, the amount of blood and blood-derived product, and the retained processing protocol for clinical use [37].

In Europe, and depending on the country, specific cases or settings make it possible for blood components to be considered either as blood products or, alternatively, as medicines [37]. Therein, and depending on the quantity of retrieved blood, the manufacturing process and the applied clinical protocol, PRP preparations may be used by the physician under his/her responsibility in a less restrictive manner (Table 1) [47]. Therefore, the medical professional should submit the considered protocol to the competent regulatory authority in order to receive advice on the appropriate use of specific blood components. Specifically, the physician should determine if an authorization is required or if the considered form of therapy may be classified as an act to be performed freely under his/her control and responsibility.

The reported high inhomogeneity in the EU legislative ecosystem regarding the management of products obtained from whole blood processing will probably lead the legislators to soon work toward harmonization of the "rules of engagement" of this particular class of biologicals/cell therapy [34,37]. Indeed, PRP technologies are being promoted to European medical doctors based on the technical aspects of separation devices that have a CE mark [16,59]. However, the different EU member states are not regulating the use of such devices in a homogenous manner. For instance, the Italian framework allows the physical and contractual delegation of product manufacture and administration to physicians, yet the responsibility remains that of the blood transfusion service. In other European states, ambulatory use under the direct responsibility of the physician is possible. Therein, high variability exists between the procedures, which depend on the protocol used by the physician, the specific therapeutic application, the amount of blood, the manufacturing system, etc. [37].

Therefore, throughout European countries, there is a general rule based on the same directive, yet in each country, the "legal" way to use PRP depends on the retained manufacturing and clinical protocols [37–39]. Because of this, the regulation tends to be het-

erogeneous and quite complex to correctly assimilate by individual healthcare providers. These factors sometimes lead to requirements for specific authorizations or the need for other clinicians or structures (i.e., transfusion services) to be involved in the PRP processing steps. Furthermore, these requirements make it somewhat difficult for clinicians in certain countries to use autologous PRP within their operative and clinical practices [37,38]. In this sense, it would certainly be helpful for all the countries that accept a CE mark for a device to subsequently function similarly with respect to processing specifications/standards, simplifying, and therefore favoring the use of PRP [59]. This concerted approach bares the potential of harmonizing the practices in clinical settings, providing tangible advantages for patient care [16].

7.2. PRP Preparation and Use in Switzerland: Toward Harmonization and Standardization under GMPs

In Switzerland, the Therapeutic Products Act includes possible scenarios in which, under certain conditions, non-standardized medicinal products may continue to be manufactured and used by physicians without regard to the new provisions (Table 1) [46,47]. In scenarios not covered by the new provisions, the person performing the PRP treatment remains responsible for guaranteeing the safety, quality, and efficacy of the administered treatment. Focusing specifically on the Swiss legislation, it can be concluded that Switzerland is interpreting and implementing elements of Directive 2002/98/EC following a similar approach to the one introduced in Germany, which considers blood components as medicinal products, therefore requiring GMP standards [39,46].

7.3. PRP Manufacturing Devices and Ready-to-Use Kits: Technical Focus of Regulators

From a technical standpoint, the currently marketed medical devices for PRP preparation claim to have different concentration possibilities for platelets, yet it is not clinically confirmed that a higher concentration of platelets correlates with enhanced efficacy [16,59,74,75]. Indeed, the literature covering clinical work suggests that the main consensus is to have a 2–3-fold platelet concentration factor and that the technique used to produce the final PRP product should be gentle enough to avoid any damage to the biological constituents [16,50,76]. In practice, platelet concentration factors in PRP can vary from 2–12 times and some studies have even reported a 0.52 value from the baseline, even though evoking standardization methods [15–17]. It is not clear from available clinical studies that higher platelet doses or concentrations provide an increased clinical benefit [27,75].

Based on the commercial availability of over 40 systems for the preparation of PRP, all claiming safe production and the potential to promote healing, there seems to be a necessity for specific regulation and oversight of their use [16,59]. Indeed, despite its simple concept and rapid manufacturing technique, the use of PRP injections is invasive and bears a certain level of risk [24,36]. Medically speaking, there are several contraindications for PRP use, which include hypofibrinogenemia, anticoagulation, hemodynamic instability, sepsis, infection, chronic liver problems, platelet dysfunction and critical thrombocytopenia, and the use of corticosteroids or nonsteroidal anti-inflammatory drugs, for example [36]. In addition, if PRP is used for facial rejuvenation, there could be a risk of a viral flare-up due to tissue manipulation and inflammatory reactions [15,17,36]. Therefore, the therapeutic use of PRP should be delegated to medically-associated facilities, whether private practices, private clinics, or hospitals, and not within basic esthetic centers, which can be seen in countries with lenient regulation (e.g., USA) [22,23].

Since PRP final products themselves are not regulated, it is only the medical devices, such as the centrifuge and the preparation kits, which are regulated and these do not require clinical data for registration [16,59]. Specifically, this is possible due to the fact that these systems are intended for autologous use with minimal sample manipulation and are considered as a low-risk biological product [43,44]. Therefore, PRP and the individual components of platelets and plasma are often exempt from quality control measures in practice [51,52]. Although the use of these systems is not regulated to date, there are

several recent events which may change the relevant regulatory ecosystem in the future, as unobstructed use may have severe consequences on patient safety [37]. A notable example of iatrogenesis cause by the inappropriate use of PRP has been documented in an esthetic spa, with the transmission of HIV in New Mexico [23]. There are also significant implications from the financial side of healthcare systems, as these PRP preparations can be very expensive (i.e., 500–2500 US dollars per treatment) and are mainly not covered by insurance [22,59].

7.4. Transitioning toward GMP-Accredited Platforms for PRP Preparation: Enhanced Traceability and Quality of Orthobiological Care

For the provision of the highest available quality of autologous PRP, it is important that the laboratory tasked with PRP preparation operates under the requirements of GMP for small quantities of medicines [35]. The main burdens lie in the ability to show that the laboratory premises and staff meet GMP requirements, that the entity has a quality assurance system, and that the centrifuge settings, temperature, and equipment remain unchanged so that the product specifications are also standardized [49]. For private clinics or private practices wishing to produce their own PRP, it is always possible to acquire equipment that has been validated for this purpose [59]. Indeed, the centrifuge and preparation kits should be simple to use with as little handling as possible, be a "closed" system (i.e., the sample is never exposed to air in order to avoid any contamination of the PRP that will be injected into the patient), be sterile, and have the medical CE mark as a guarantee of safety [43,59].

Overall, the competent supervisory authorities are entitled to request the user (i.e., physician and/or manufacturer) to submit data and evidence concerning the quality, safety, and efficacy of the preparation under consideration [37,38]. In addition, the competent authorities may prohibit the manufacture of these preparations and the practice of PRP injections if these conditions are not found to be met [47]. From a technical point-of-view, high attention should be paid to the standardization of PRP-based product preparation methods and final product attributes, which enables the sound clinical evaluation of treatment efficacy [16]. Despite the relatively elevated fixed costs of PRP manufacturing under GMPs as compared to the use of kits, the increasing regulatory pressure is orienting healthcare providers toward the augmentation of quality standards [16,37,49]. Therein, the appropriate and widespread use of standardized GMP processing workflows in musculoskeletal regenerative medicine applications bears the potential of lowering healthcare costs by systematically enhancing the therapeutic quality of orthobiologic clinical interventions [50].

7.5. Comparative Aspects of PRP Manufacture under GMPs in Lausanne or Using PRP Kits and Closed Systems

A main technical difference between the standardized protocol adopted in Lausanne for the GMP preparation of PRP and the use of alternative systems consists in the fact that the GMP process is not "closed" (Figure S2) [50]. Indeed, several steps of open-container manipulation and liquid handling are performed, which increase the product contamination (i.e., particulate and/or microbiological) risk level as compared to the use of a "closed" system (i.e., where the blood products are always contained during processing). However, the contamination risks are brought down to negligible levels in the validated GMP process, as all open-container operations are carried out in a class A environment under a laminar flow (Figure S2) [49]. Specifically, this enables more flexibility and ease of processing of the fluids as compared to closed systems, as the manipulator retains better direct control over the process [50].

Several reports and studies have described high rates of clinical success for PRP treatment in a number of indications, yet it is now well-known that both the preparation method and the clinical administration modalities play critical roles in the efficacy of the intervention [77–80]. Therefore, many calls for the standardization of PRP preparation methods have been documented in order to globally enhance the related clinical success rates [16]. Within this objective, the use of a standardized GMP manufacturing workflow

for autologous PRP (i.e., as described for the Lausanne University Hospital) presents multifactorial advantages and technical superiority, as detailed hereafter (Figure S2). Specifically, best-in-class reproducibility may be obtained by the use of an accredited GMP platform, which is inherently qualified and validated to perform the manufacturing process [49]. This is due to the fact that the equipment is appropriately designed and maintained for the needs of the operations, and that such operations are carried out by trained and qualified personnel [9]. This aspect is drastically different from the use of PRP kits by individual physicians at the bedside of patients, where numerous variability factors may come into play and affect the attributes of the final product [16,59].

Furthermore, maximal traceability and safety can be demonstrably achieved with GMP production of PRP due to the documentary requirements for manufacturing and testing activities [9]. Therefore, the use of a standardized GMP process leads to the generation of exhaustive batch records that contain valuable data for further process optimization or for efficacy-related studies [9]. Furthermore, the control requirements of GMP processes enable us to set forth a higher level of safety of the finished products as compared to a PRP kit. Therein, in-process and post-process sampling and testing of the materials enable us to document and demonstrate the safety and quality of the administered preparations [49,50]. Overall, the proposed solution for the GMP preparation of autologous PRP is in line with current reflections and recommendations regarding the standardization of processes in the field of cell-based regenerative medicine [35,49,50].

7.6. Study Significance and Identified Limitations

Overall, this study was meant to significantly contribute to the existing professional body of knowledge around autologous PRP, notably to ensure that new physicians dispose of simple tools to proceed with the appropriate administrative and technical bases prior to PRP implementation. Such preliminary elements are considered to be of the utmost importance for the provision of legal, safe, and effective therapeutic interventions. Importantly, this study also aimed to set forth several process-based and quality-oriented considerations for the same medical professionals with the demonstration and the discussion of the various advantages of using a GMP platform instead of CE-marked PRP kits for product preparation.

The main identified limitations of this study are related to the highly dynamic nature of the legal and regulatory ecosystems for autologous PRP in Europe [37]. Namely, it is probable that several elements referenced in the present study will be outdated in a matter of years or even months due to iterative updates in the respective laws, frameworks, and guidelines.

8. Conclusions and Perspectives

The present study has strongly emphasized the current disparities (i.e., legal, administrative, technical) that exist in the field of autologous PRP use for human clinical practices in Western Europe. Specifically, the diversity in the applicable legal and regulatory frameworks was described for Switzerland, Germany, France, Italy, and Spain, along with the diversity in the documented medical practices that may be encountered in each country. Importantly, it was discussed that the harmonization of practices or the use of appropriate infrastructure and quality systems are essential in the domain of autologous PRP for the assurance of optimal safety and efficacy of the orthobiologic interventions. Overall, appropriate and systematic consideration of the available technical, regulatory, and clinical hindsight available for autologous PRP use needs to be integrated by clinicians willing to implement such treatments. These elements are considered to be central in view of maintaining and enhancing the quality level of the available personalized regenerative medicine healthcare. This objective may be further attained tangibly through the holistic consideration of specific clinical cases, the effective dialogue between attending physicians and PRP manufacturers, and the use of standardized autologous PRP preparation workflows.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/bioengineering10030292/s1, Table S1: Scope of Directive 2002/98/EC: Blood Component Quality and Safety; Table S2: Scope of Directive 2005/62/EC: Good Practice Guidelines (GPG) based on Good Manufacturing Practices (GMP); Figure S1: Schematic illustration of the design of the study; Figure S2: Schematic illustration of the PRP manufacturing process performed under GMP in the Lausanne University Hospital.

Author Contributions: Conceptualization, P.S., A.C., G.G., V.G., N.W. and L.A.A.; methodology, P.S., G.G., V.G., N.H.-B., N.W. and L.A.A.; software, P.S., G.G., V.G., N.H.-B., A.L. and L.A.A.; validation, P.S., A.C., G.G., V.G., W.R., N.H.-B., M.M., P.A.-S., A.L., N.W. and L.A.A.; formal analysis, P.S., G.G., V.G., N.H.-B., N.W. and L.A.A.; investigation, P.S., A.C., G.G., V.G., N.W. and L.A.A.; resources, P.S., V.G. and L.A.A.; data curation, P.S., N.H.-B., P.A.-S., A.L., N.W. and L.A.A.; writing—original draft preparation, P.S., A.C., A.L., N.W. and L.A.A.; writing—review and editing, P.S., A.C., G.G., V.G., W.R., N.H.-B., M.M., P.A.-S., A.L., N.W. and L.A.A.; visualization, M.M., A.L. and L.A.A.; supervision, V.G., N.W. and L.A.A.; project administration, L.A.A.; funding acquisition, L.A.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available upon reasonable request made in writing to the corresponding author.

Acknowledgments: The authors wish to thank the S.A.N.T.E and the Sandoz Family foundations, as well as the Lausanne Orthopedics Research Foundation (LORF) for their continuous support of the Regenerative Therapy Unit (CHUV-DAL-CPR-UTR), especially for the procurement of materials and consumables. Artwork templates for the figures of the present study were partly created with www.biorender.com, accessed on 8 February 2023.

Conflicts of Interest: Authors A.C. and N.W. were employed by NAD Consulting Sàrl during the completion of this work. Author A.L. was employed by LAM Biotechnologies SA during the completion of this work. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. The remaining authors declare no conflicts of interest.

Abbreviations

AEMPS	Spanish Agency of Medicines and Medical Devices
AMG	Arzneimittelgesetz, German medicinal products act
ANSM	French National Agency for Drug Security and Health Products
ATMP	advanced therapy medicinal product
CAT	Committee for Advanced Therapies
cc	cubic centimeter
CCST	Spanish Committee for transfusion security
CE	EU medical device conformity evaluation symbol
cGMP	current good manufacturing practices
CTGF	connective tissue growth factor
DGS	French Direction Générale de la Santé
EC	European Commission
EFS	French Blood Establishment
EGF	epidermal growth factor
EMEA	European medicines agency
EU	European Union
FDA	US Food and Drug Administration
FGF	fibroblast growth factor
FOPH	federal office of public health

GDP	good distribution practices
GMP	good manufacturing practices
GMP-140	platelet alpha-granule membrane protein
GPG	good practice guidelines
HIV	human immunodeficiency virus
IL	interleukin
ILGF	insulin-like growth factor
IOMC	Swiss intercantonal office for drug control
LPTh	Swiss law on therapeutic products
MD	medical device
MPLO	Swiss ordinance on licensing in the medicinal products sector
OA	osteoarthritis
PDAF	platelet-derived angiogenesis factor
PDGF	platelet-derived growth factor
PEI	Paul Ehrlich Institute
PF	platelet factor
PPP	platelet-poor plasma
PRP	platelet-rich plasma
PSL	labile blood products
RBC	red blood cells
TFG	Transfusionsgesetz, German transfusion act
TGF	transforming growth factor
TPA	therapeutic products act
USA	United States of America
VEGF	vascular endothelial growth factor
WADA	World Anti Doping Agency

References

1. Vacanti, J.P.; Langer, R. Tissue engineering: The design and fabrication of living replacement devices for surgical reconstruction and transplantation. *Lancet* **1999**, *354*, SI32–SI34. [CrossRef] [PubMed]
2. Ramezankhani, R.; Torabi, S.; Minaei, N.; Madani, H.; Rezaeiani, S.; Hassani, S.N.; Gee, A.P.; Dominici, M.; Silva, D.N.; Baharvand, H.; et al. Two decades of global progress in authorized advanced therapy medicinal products: An emerging revolution in therapeutic strategies. *Front. Cell Develop. Biol.* **2020**, *8*, 547653. [CrossRef] [PubMed]
3. Gentile, P.; D'angiolino, A.; Giusti, D.; Bocchini, I.; Di Pasquali, C.; Curcio, B.C.; Cervelli, V. Platelet-rich plasma and blood components for non-transfusion use: Technical and medicolegal aspects. *Med. Sci. Law* **2012**, *52*, 234–239. [CrossRef] [PubMed]
4. Amable, P.R.; Carias, R.B.; Teixeira, M.V.; da Cruz Pacheco, I.; Corrêa do Amaral, R.J.; Granjeiro, J.M.; Borojevic, R. Platelet-rich plasma preparation for regenerative medicine: Optimization and quantification of cytokines and growth factors. *Stem Cell Res. Ther.* **2013**, *4*, 67. [CrossRef] [PubMed]
5. Sax, O.C.; Chen, Z.; Mont, M.A.; Delanois, R.E. The efficacy of platelet-rich plasma for the treatment of knee osteoarthritis symptoms and structural changes: A systematic review and meta-analysis. *J. Arthroplast.* **2022**, *37*, 2282–2290. [CrossRef]
6. Al-Dourobi, K.; Laurent, A.; Deghayli, L.; Flahaut, M.; Abdel-Sayed, P.; Scaletta, C.; Michetti, M.; Waselle, L.; Simon, J.P.; Ezzi, O.E.; et al. Retrospective evaluation of progenitor biological bandage use: A complementary and safe therapeutic management option for prevention of hypertrophic scarring in pediatric burn care. *Pharmaceuticals* **2021**, *14*, 201. [CrossRef]
7. Cash, C.; Scott, L.; Lane Walden, R.; Kuhn, A.; Bowman, E. Bibliometric analysis of the top 50 highly cited articles on platelet-rich plasma in osteoarthritis and tendinopathy. *Regen. Med.* **2022**, *17*, 491–506. [CrossRef]
8. Gentile, P.; Garcovich, S. Systematic review: Adipose-derived mesenchymal stem cells, platelet-rich plasma and biomaterials as new regenerative strategies in chronic skin wounds and soft tissue defects. *Int. J. Mol. Sci.* **2021**, *22*, 1538. [CrossRef]
9. Philippe, V.; Laurent, A.; Hirt-Burri, N.; Abdel-Sayed, P.; Scaletta, C.; Schneebeli, V.; Michetti, M.; Brunet, J.-F.; Applegate, L.A.; Martin, R. Retrospective analysis of autologous chondrocyte-based cytotherapy production for clinical use: GMP process-based manufacturing optimization in a Swiss university hospital. *Cells* **2022**, *11*, 1016. [CrossRef]
10. Laurent, A.; Abdel-Sayed, P.; Grognuz, A.; Scaletta, C.; Hirt-Burri, N.; Michetti, M.; de Buys Roessingh, A.S.; Raffoul, W.; Kronen, P.; Nuss, K.; et al. Industrial development of standardized fetal progenitor cell therapy for tendon regenerative medicine: Preliminary safety in xenogeneic transplantation. *Biomedicines* **2021**, *9*, 380. [CrossRef]
11. Petrou, I.G.; Grognuz, A.; Hirt-Burri, N.; Raffoul, W.; Applegate, L.A. Cell therapies for tendons: Old cell choice for modern innovation. *Swiss Med. Wkly* **2014**, *144*, w13989. [CrossRef] [PubMed]
12. Hsu, W.K.; Mishra, A.; Rodeo, S.R.; Fu, F.; Terry, M.A.; Randelli, P.; Canale, S.T.; Kelly, F.B. Platelet-rich plasma in orthopaedic applications: Evidence-based recommendations for treatment. *J. Am. Acad. Orthop. Surg.* **2013**, *21*, 739–748. [CrossRef] [PubMed]

13. Guerid, S.; Darwiche, S.E.; Berger, M.M.; Applegate, L.A.; Benathan, M.; Raffoul, W. Autologous keratinocyte suspension in platelet concentrate accelerates and enhances wound healing—A prospective randomized clinical trial on skin graft donor sites: Platelet concentrate and keratinocytes on donor sites. *Fibrogen. Tissue Rep.* **2013**, *6*, 1–8. [CrossRef] [PubMed]
14. Nuutila, K.; Katayama, S.; Laitinen, A.; Siltanen, A.; Patrikoski, M.; Valtonen, J.; Kankainen, M.; Kerkelä, E.; Kaartinen, T.; Juteau, S.; et al. ATMP-classified, scalable, autologous cell spray for the treatment of skin wounds and assessment of its effects on wound healing clinically and on a molecular level. *Burns* **2022**, in press. [CrossRef] [PubMed]
15. Dhurat, R.; Sukesh, M.S. Principles and methods of preparation of platelet-rich plasm: A review and author's perspective. *J. Cutan. Aesthet. Surg.* **2014**, *7*, 189–197. [CrossRef] [PubMed]
16. Fadadu, P.P.; Mazzola, A.J.; Hunter, C.W.; Davis, T.T. Review of concentration yields in commercially available platelet-rich plasma (PRP) systems: A call for PRP standardization. *Reg. Anesth. Pain Med.* **2019**, *44*, 652–659. [CrossRef]
17. Samadi, P.; Sheykhhasan, M.; Khoshinani, H.M. The use of platelet-rich plasma in aesthetic and regenerative medicine: A comprehensive review. *Aesth. Plast. Surg.* **2019**, *43*, 803–814. [CrossRef]
18. Klatte-Schulz, F.; Schmidt, T.; Uckert, M.; Scheffler, S.; Kalus, U.; Rojewski, M.; Schrezenmeier, H.; Pruss, A.; Wildemann, B. Comparative analysis of different platelet lysates and platelet rich preparations to stimulate tendon cell biology: An in vitro study. *Int. J. Mol. Sci.* **2018**, *19*, 212. [CrossRef]
19. Bernardi, M.; Agostini, F.; Chieregato, K.; Amati, E.; Durante, C.; Rassu, M.; Ruggeri, M.; Sella, S.; Lombardi, E.; Mazzucato, M.; et al. The production method affects the efficacy of platelet derivatives to expand mesenchymal stromal cells in vitro. *J. Transl. Med.* **2017**, *15*, 90. [CrossRef]
20. Harrison, T.E.; Bowler, J.; Levins, T.N.; Reeves, K.D.; Cheng, A.L. Platelet-rich plasma centrifugation changes leukocyte ratios. *Cureus* **2021**, *13*, e14470. [CrossRef]
21. Machado, E.S.; Leite, R.; Dos Santos, C.C.; Artuso, G.L.; Gluszczak, F.; de Jesus, L.G.; Caldas, J.M.P.; Bredemeier, M. Turn down—Turn up: A simple and low-cost protocol for preparing platelet-rich plasma. *Clinics* **2019**, *74*, e1132. [CrossRef] [PubMed]
22. Beitzel, K.; Allen, D.; Apostolakos, J.; Russell, R.P.; McCarthy, M.B.; Gallo, G.J.; Cote, M.P.; Mazzocca, A.D. US definitions, current use, and FDA stance on use of platelet-rich plasma in sports medicine. *J. Knee Surg.* **2015**, *28*, 29–34. [CrossRef] [PubMed]
23. PRP Therapies—Avoiding the Risk of Disease Transmission. Available online: https://cpsa.ca/news/prp-therapies-avoiding-the-risk-of-disease-transmission/ (accessed on 12 December 2022).
24. Wasterlain, A.S.; Braun, H.J.; Harris, A.H.S.; Kim, H.J.; Dragoo, J.L. The systemic effects of platelet-rich plasma injection. *Am. J. Sports Med.* **2013**, *41*, 186–193. [CrossRef] [PubMed]
25. Dhillon, M.S.; Behera, P.; Patel, S.; Shetty, V. Orthobiologics and platelet rich plasma. *Indian J. Orthop.* **2014**, *48*, 1–9. [CrossRef]
26. Chu, C.R.; Rodeo, S.; Bhutani, N.; Goodrich, L.R.; Huard, J.; Irrgang, J.; LaPrade, R.F.; Lattermann, C.; Lu, Y.; Mandelbaum, B.; et al. Optimizing clinical use of biologics in orthopaedic surgery: Consensus recommendations from the 2018 AAOS/NIH U-13 conference. *J. Am. Acad. Orthop. Surg.* **2019**, *27*, e50–e63. [CrossRef]
27. Yaman, R.; Kinard, T.N. Platelet rich plasma: Hope or hype? *Ann. Blood* **2022**, *7*, 1–7. [CrossRef]
28. Bigby, M.; Grimalt, R. Platelet-rich plasma lacks evidence of clinically significant improvement in androgenetic alopecia. *J. Am. Acad. Dermatol.* **2021**, *84*, 1183–1185. [CrossRef]
29. Gupta, A.K.; Cole, J.; Deutsch, D.P.; Everts, P.A.; Niedbalski, R.P.; Panchaprateep, R.; Rinaldi, F.; Rose, P.T.; Sinclair, R.; Vogel, J.E.; et al. Platelet-rich plasma as a treatment for androgenetic alopecia. *Dermatol. Surg.* **2019**, *45*, 1262–1273. [CrossRef]
30. Lamplot, J.D.; Rodeo, S.A.; Brophy, R.H. A practical guide for the current use of biologic therapies in sports medicine. *Am. J. Sports Med.* **2019**, *48*, 488–503. [CrossRef]
31. Chan, G.K.L.; Guo, M.S.; Dai, D.K.; Lai, Q.W.S.; Fung, K.W.C.F.; Zheng, B.Z.; Wu, K.Q.; Man, B.K.K.; Dong, T.T.; Tsim, K.W.K. An optimized extract, named self-growth colony, from platelet-rich plasma shows robust skin rejuvenation and anti-ageing properties: A novel technology in development of cosmetics. *Skin Pharmacol. Physiol.* **2021**, *39*, 1–12. [CrossRef]
32. Chamata, E.S.; Bartlett, E.L.; Weir, D.; Rohrich, R.J. Platelet-rich plasma: Evolving role in plastic surgery. *Plast. Reconstr. Surg.* **2021**, *147*, 219–230. [CrossRef] [PubMed]
33. Gentile, P.; Alves, R.; Cole, J.; Andjelkov, K. 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. *Exp. Opin. Biol. Ther.* **2021**, *21*, 1443–1449. [CrossRef]
34. Fiorentino, S.; Roffi, A.A.L.A.; Filardo, B.G.; Marcacci, M.; Kon, E. European definitions, current use, and EMA stance of platelet-rich plasma in sports medicine. *J. Knee Surg.* **2015**, *1*, 51–54. [CrossRef] [PubMed]
35. Chemali, M.; Laurent, A.; Scaletta, C.; Waselle, L.; Simon, J.-P.; Michetti, M.; Brunet, J.-F.; Flahaut, M.; Hirt-Burri, N.; Raffoul, W.; et al. Burn center organization and cellular therapy integration: Managing risks and costs. *J. Burn Care Res.* **2021**, *42*, 911–924. [CrossRef]
36. Peng, G.L. Platelet-rich plasma for skin rejuvenation: Facts, fiction, and pearls for practice. *Facial Plast. Surg. Clin. N. Am.* **2019**, *27*, 405–411. [CrossRef]
37. Anitua, E.; Prado, R.; Orive, G. Closing regulatory gaps: New ground rules for platelet-rich plasma. *Trends Biotech.* **2015**, *33*, 492–495. [CrossRef]
38. Anitur, E.; Prado, R.; Orive, G. A new regulatory framework for platelet-rich plasma in Spain. *J. Knee Surg.* **2015**, *28*, 355–356. [CrossRef] [PubMed]

39. European Parliament and Council. Directive 2002/98/EC setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending Directive 2001/83/EC. *Off. J. Eur. Union* **2003**, *33*, 30–40.
40. European Parliament and Council. Directive 2005/62/EC implementing Directive 2002/98/EC of the European Parliament and of the Council as regards Community standards and specifications relating to a quality system for blood establishments. *Off. J. Eur. Union* **2005**, *256*, 41–48.
41. European Parliament and Council. Directive 2016/1214 amending Directive 2005/62/EC as regards quality system standards and specifications for blood establishments. *Off. J. Eur. Union* **2016**, *199*, 14–15.
42. European Parliament and Council. Directive 2004/33/EC implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components. *Off. J. Eur. Union* **2004**, *91*, 25–39.
43. European Parliament and Council. Regulation (EU) 2017/745 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC. *Off. J. Eur. Union* **2017**, *117*, 1–175.
44. European Parliament and Council. Regulation (EU) 2017/746 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU. *Off. J. Eur. Union* **2017**, *117*, 176–332.
45. European Parliament and Council. Regulation (EC) No 1394/2007 on Advanced Therapy Medicinal Products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004. *Off. J. Eur. Union* **2007**, *324*, 121–137.
46. Federal Assembly of the Swiss Confederation. *Federal Law on Medication and Medical Devices (Law on Therapeutic Products) RS 812.21*; Switzerland's Federal Council: Bern, Switzerland, 2000; Available online: https://fedlex.data.admin.ch/eli/cc/2001/422 (accessed on 1 November 2022).
47. The Swiss Federal Council. *Ordinance on Licensing in the Medicinal Products Sector (Medicinal Products Licensing Ordinance) RS 812.212.1*; Switzerland's Federal Council: Bern, Switzerland, 2018; Available online: https://www.fedlex.admin.ch/eli/cc/2018/786/en (accessed on 1 November 2022).
48. *BS EN 16844:2017+A2:2019*; European Standards. Aesthetic Medicine Services. Non-Surgical Medical Treatments. UK HealthCare: London, UK, 2019; ISBN 978 0 539 06159 8.
49. Dimitropoulos, G.; Jafari, P.; de Buys Roessingh, A.; Hirt-Burri, N.; Raffoul, W.; Applegate, L.A. Burn patient care lost in good manufacturing practices? *Ann. Burns Fire Dis.* **2016**, *29*, 111–115.
50. Akhundov, K.; Pietramaggiori, G.; Waselle, L.; Darwiche, S.; Guerid, S.; Scaletta, C.; Hirt-Burri, N.; Applegate, L.A.; Raffoul, W. Development of a cost-effective method for platelet-rich plasma (PRP) preparation from topical wound healing. *Ann. Burns Fire Dis.* **2012**, *4*, 207–213.
51. Popescu, M.N.; Iliescu, M.G.; Beiu, C.; Popa, L.G.; Mihai, M.M.; Berteanu, M.; Ionescu, A.M. Autologous platelet-rich plasma efficacy in the field of regenerative medicine: Product and quality control. *BioMed Res. Int.* **2021**, *2021*, 4672959. [CrossRef]
52. Kawase, T.; Okuda, K. Comprehensive quality control of the regenerative therapy using platelet concentrates: The current situation and prospects in Japan. *BioMed Res. Int.* **2018**, *2018*, 6389157. [CrossRef]
53. Qian, Y.; Han, Q.; Chen, W.; Song, J.; Zhao, X.; Ouyang, Y.; Yuan, W.; Fan, C. Platelet-rich plasma derived growth factors contribute to stem cell differentiation in musculoskeletal regeneration. *Front. Chem.* **2017**, *5*, 89. [CrossRef]
54. Alsousou, J.; Thompson, M.; Hulley, P.; Noble, A.; Willett, K. The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery. *J. Bone Joint Surg.* **2009**, *91-B*, 987–996. [CrossRef]
55. Martinez, C.E.; Smith, P.C.; Palma Alvarado, V.A. The influence of platelet-derived products on angiogenesis and tissue repair: A concise update. *Front. Physiol.* **2015**, *6*, 290. [CrossRef] [PubMed]
56. Pavlovic, V.; Ciric, M.; Jovanovic, V.; Stojanovic, P. Platelet rich plasma: A short overview of certain bioactive components. *Open Med.* **2016**, *11*, 242–247. [CrossRef] [PubMed]
57. Boswell, S.G.; Cole, B.J.; Sundman, E.A.; Karas, V.; Fortier, L.A. Platelet-rich plasma: A milieu of bioactive factors. *Arthroscopy* **2012**, *28*, 429–439. [CrossRef] [PubMed]
58. Galán, V.; Iñigo-Dendariarena, I.; Galán, I.; Prado, R.; Padilla, S.; Anitua, E. The effectiveness of plasma rich in growth factors (PRGF) in the treatment of nerve compression syndromes of the upper extremity: A retrospective observational clinical study. *J. Clin. Med.* **2022**, *11*, 4789. [CrossRef] [PubMed]
59. Magalon, J.; Brandin, T.; Francois, P.; Degioanni, C.; De Maria, L.; Grimaud, F.; Veran, J.; Dignat-George, F.; Sabatier, F. Technical and biological review of authorized medical devices for platelets-rich plasma preparation in the field of regenerative medicine. *Platelets* **2021**, *32*, 200–208. [CrossRef] [PubMed]
60. European Parliament and Council. Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency. *Off. J. Eur. Union* **2004**, *136*, 1–33.
61. Coulange Zavarro, A.; De Girolamo, L.; Laver, L.; Sánchez, M.; Tischer, T.; Filardo, G.; Sabatier, F.; Magalon, J. The top 100 most cited articles on platelet-rich plasma use in regenerative medicine–A bibliometric analysis–from the ESSKA orthobiologic initiative. *Bioengineering* **2022**, *9*, 580. [CrossRef]
62. Ojea-Pérez, A.M.; Acebes-Huerta, A.; Arias-Fernández, T.; Gutiérrez, L.; Muñoz-Turrillas, M.C. Implementation of a closed platelet-rich-plasma preparation method using the local blood bank infrastructure at the Principality of Asturias (Spain): Back to basic methodology and a demographics perspective after 1 year. *Transfus. Apheres. Sci.* **2019**, *58*, 701–704. [CrossRef]

63. Božič, D.; Vozel, D.; Hočevar, M.; Jeran, M.; Jan, Z.; Pajnič, M.; Pađen, L.; Iglič, A.; Battelino, S.; Kralj-Iglič, V. Enrichment of plasma in platelets and extracellular vesicles by the counterflow to erythrocyte settling. *Platelets* **2022**, *33*, 592–602. [CrossRef]
64. Borghese, C.; Agostini, F.; Durante, C.; Colombatti, A.; Mazzucato, M.; Aldinucci, D. Clinical-grade quality platelet-rich plasma releasate (PRP-R/SRGF) from $CaCl_2$ -activated platelet concentrates promoted expansion of mesenchymal stromal cells. *Vox Sang.* **2016**, *111*, 197–205. [CrossRef]
65. Huang, L.H.; Rau, C.S.; Zeng, W.H.; Lu, T.H.; Wu, Y.C.; Chiu, Y.H.; Wu, K.M.; Tsai, C.W.; Lin, C.W.; Wu, C.J.; et al. A new technique for separating platelet-rich plasma by a copolymer device—Without a centrifugation process. *Biomed. Pharmacother.* **2022**, *153*, 113481. [CrossRef] [PubMed]
66. Machado, E.S.; Soares, F.P.; Yamaguchi, R.S.; Felipone, W.K.; Meves, R.; Souza, T.A.C.; Topolniak, R.; Caldas, J.P.; Abreu, E.V.; Rabelo Neto, L.S.; et al. A simple double-spin closed method for preparing platelet-rich plasma. *Cureus* **2022**, *14*, e20899. [CrossRef] [PubMed]
67. Nazaroff, J.; Oyadomari, S.; Brown, N.; Wang, D. Reporting in clinical studies on platelet-rich plasma therapy among all medical specialties: A systematic review of Level I and II studies. *PloS ONE* **2021**, *16*, e0250007. [CrossRef] [PubMed]
68. Romero, A.; Barrachina, L.; Ranera, B.; Remacha, A.R.; Moreno, B.; de Blas, I.; Sanz, A.; Vasquez, F.J.; Vitoria, A.; Junquera, C.; et al. Comparison of autologous bone marrow and adipose tissue derived mesenchymal stem cells, and platelet-rich plasma, for treating surgically induced lesions of the equine superficial digital flexor tendon. *Vet. J.* **2017**, *224*, 76–84. [CrossRef]
69. Van der Vlist, A.C.; Winters, M.; Weir, A.; Ardern, C.L.; Welton, N.J.; Caldwell, D.M.; Verhaar, J.A.; de Vos, R.J. Which treatment is most effective for patients with Achilles tendinopathy? A living systematic review with network meta-analysis of 29 randomised controlled trials. *Br. J. Sports Med.* **2021**, *55*, 249–256. [CrossRef]
70. Dong, C.; Sun, Y.; Qi, Y.; Zhu, Y.; Wei, H.; Wu, D.; Li, C. Effect of platelet-rich plasma injection on mild or moderate carpal tunnel syndrome: An updated systematic review and meta-analysis of randomized controlled trials. *BioMed Res. Int.* **2020**, *2020*, 5089378. [CrossRef]
71. Andriolo, L.; Altamura, S.A.; Reale, D.; Candrian, C.; Zaffagnini, S.; Filardo, G. Nonsurgical treatments of patellar tendinopathy: Multiple injections of platelet-rich plasma are a suitable option: A systematic review and meta-analysis. *Am. J. Sports Med.* **2019**, *47*, 1001–1018. [CrossRef]
72. Gupta, A.K.; Renaud, H.J.; Bamimore, M. Platelet-rich plasma for androgenetic alopecia: Efficacy differences between men and women. *Dermat. Ther.* **2020**, *33*, e14143. [CrossRef]
73. Bansal, H.; Leon, J.; Pont, J.L.; Wilson, D.A.; Bansal, A.; Agarwal, D.; Preoteasa, I. Platelet-rich plasma (PRP) in osteoarthritis (OA) knee: Correct dose critical for long term clinical efficacy. *Sci. Rep.* **2021**, *11*, 3971. [CrossRef]
74. Weibrich, G.; Hansen, T.; Kleis, W.; Buch, R.; Hitzler, W.E. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. *Bone* **2004**, *34*, 665–671. [CrossRef]
75. Cole, B.J.; Seroyer, S.T.; Filardo, G.; Bajaj, S.; Fortier, L.A. Platelet-rich plasma: Where are we now and where are we going? *Sports Health* **2010**, *2*, 203–210. [CrossRef] [PubMed]
76. Marx, R.E. Platelet-rich plasma (PRP): What is PRP and what is not PRP? *Implant. Dent.* **2001**, *10*, 225–228. [CrossRef] [PubMed]
77. Cavallo, C.; Roffi, A.; Grigolo, B.; Mariani, E.; Pratelli, L.; Merli, G.; Kon, E.; Marcacci, M.; Filardo, G. Platelet-rich plasma: The choice of activation method affects the release of bioactive molecules. *BioMed Res. Int.* **2016**, *2016*, 6591717. [CrossRef] [PubMed]
78. Larsson, A.; Carlsson, L.; Gordh, T.; Lind, A.L.; Thulin, M.; Kamali-Moghaddam, M. The effects of age and gender on plasma levels of 63 cytokines. *J. Immunol. Methods* **2015**, *425*, 58–61. [CrossRef]
79. O'Donnell, C.; Migliore, E.; Grandi, F.C.; Koltsov, J.; Lingampalli, N.; Cisar, C.; Indelli, P.F.; Sebastiano, V.; Robinson, W.H.; Bhutani, N.; et al. Platelet-rich plasma (PRP) from older males with knee osteoarthritis depresses chondrocyte metabolism and upregulates inflammation. *J. Orthop. Res.* **2019**, *37*, 1760–1770. [CrossRef]
80. Graiet, H.; Lokchine, A.; Francois, P.; Velier, M.; Grimaud, F.; Loyens, M.; Berda-Haddad, Y.; Veran, J.; Dignat-George, F.; Sabatier, F.; et al. Use of platelet-rich plasma in regenerative medicine: Technical tools for correct quality control. *BMJ Open Sport Exerc. Med.* **2018**, *4*, e000442. [CrossRef]

Supplementary Tables

Table S1. Scope of Directive 2002/98/EC: Blood Component Quality and Safety. These regulatory framework elements are of specific importance for the appropriate setup and operation of manufacture and control activities for clinical grade PRP products/preparations. EC, European Commission; GMP, good manufacturing practices; PRP, platelet-rich plasma.

Parameter or Nomenclature Term	Nomenclature Term Definition and Specification of Directive Scope
Technical Requirements	Collection and testing of blood and blood components, including starting materials for medicinal products.
License Requirements	Insurance that appropriate mechanisms for designating, authorizing, accrediting, or licensing hematological centers exist. Insurance that the Health Authority inspections for the activities of blood establishments are performed within the scope of the Directive.
Head Transfusion Service Designation	Responsible for ensuring that each unit of blood or blood product is collected, controlled, processed, preserved, distributed, and assigned in compliance with the Directive.
Qualified Personnel	Appropriately qualified personnel involved in the collection, testing, processing, storage, and distribution of blood and blood components is necessary. Relevant training should be periodically updated and recorded.
Quality System	Quality systems involve all activities that determine the quality policy objectives and responsibilities and are implemented by such means as quality planning, quality control, quality assurance, and quality improvement within the quality system, taking into account the principles of good manufacturing practice (GMP) as well as the EC conformity assessment system.
Traceability System	Traceability of whole blood and blood components should be enforced through accurate donor, patient, and laboratory identification procedures, through record maintenance, appropriate identification, and labelling systems. 1. Autologous blood and blood components must be clearly identified as such and stored, transported, and distributed separately from allogeneic blood and blood components. 2. Autologous blood and blood components must be labelled as required by Directive 2002/98/EC and in addition the label must include the identification of the donor and the warning '*FOR AUTOLOGOUS TRANSFUSION ONLY*', ensuring that they will not be used for transfusion to other patients.
Surveillance Procedures	Information collection and evaluation on adverse or unexpected events or reactions resulting from the collection of blood or blood components must be performed, in order to prevent similar or equivalent events or reactions. Common systems of notification of serious adverse events and reactions linked to the collection, processing, testing, storage, and distribution of blood and blood components must be established.
Counselling	Insurance about when and if abnormal findings have to be reported to a particular donor.

Table S2. Scope of Directive 2005/62/EC: Good Practice Guidelines (GPG) based on Good Manufacturing Practices (GMP). These regulatory framework elements are of central importance for the appropriate manufacture and control activities for clinical grade PRP products/preparations. CAPA, corrective and preventive actions; CE, conformity evaluation; EC, European Commission; GMP, good manufacturing practices; GPG, good practice guidelines; PRP, platelet-rich plasma; QA, quality assurance.

Parameter or Nomenclature Term	Nomenclature Term Definition and Specification of Directive Scope
Quality	Responsibility of all employees involved in the process. Management is responsible for ensuring a systematic approach, implementation, and maintenance of a Quality System.
Critical Process	Instructions which are carried out in accordance with predefined standards and specifications.
Management Review	Performed at regular intervals to verify the effectiveness of the Quality System and to identify potential improvements, if needed.
Independent Quality Assurance	Support by a Quality Assurance (QA) function. Involved in all of the quality-related matters and responsible for approval of all of the appropriate quality-related documents. Independent from the processing management.
Validation Process	Procedures, premises, and equipment that have an influence on the quality and safety of blood and blood components need to be validated prior to introduction and be re-validated at regular intervals, determined as a result of these activities.
Personnel	Available in sufficient numbers to carry out the activities related to the collection, testing, processing, storage, and distribution of blood and blood components and be trained and assessed to be competent to perform their tasks. All personnel shall have up-to-date job descriptions which clearly set out their tasks and responsibilities. All personnel shall receive initial and continued training appropriate to their specific tasks. Training records shall be maintained. Training programs shall be in place and shall include good practice with content periodically assessed, and the competence of the personnel evaluated regularly. There shall be written safety and hygiene instructions in place and adapted to the activities to be carried out.
Premises	Premises, including mobile sites, shall be adapted and maintained to suit the activities. They shall enable the work to proceed in a logical sequence so as to minimize the risk of errors and shall allow for effective cleaning and maintenance in order to minimize the risk of contamination. Blood collection shall be carried out in an area intended for the safe withdrawal of blood from donors, appropriately equipped for the initial treatment of donors experiencing adverse reactions or injuries from events associated with blood donation and organized in such a way as to ensure the safety of both donors and personnel, as well as to avoid errors in the collection procedure. Dedicated laboratory areas exist for testing that are separate from blood donor and blood component processing areas, with access restricted to authorized personnel. Storage areas shall provide for properly secure and segregated storage including quarantined and released materials and units of blood or blood components collected under special criteria (e.g., autologous donation). Provisions shall be in place in the event of equipment or power failure in the main storage facility. An area shall be designated for safe disposal of waste, for disposable items used during the collection, testing, and processing, and for rejected blood or blood components.
Equipment & Materials	Equipment shall be validated, calibrated, and maintained. Operating instructions shall be available and appropriate records kept. Equipment shall be selected to minimize any hazard to donors, personnel, or blood components. Only reagents and materials from approved suppliers that meet the documented requirements and specifications shall be used. Critical materials shall be released by a person qualified to perform this task. Where relevant, materials, reagents, and equipment shall meet the requirements of European Regulation 2017/745 for medical

	devices and European Regulation 2017/746 for in vitro diagnostic medical devices or comply with equivalent standards in the case of collection in third countries. Inventory records shall be retained for a period acceptable to and agreed upon with the competent authority.
Computerized Systems	Software, hardware, and backup procedures must be checked regularly to ensure reliability, be validated before use, and be maintained in a validated state. Hardware and software shall be protected against unauthorized use or unauthorized changes. Back-up procedures shall prevent the loss of or damage to data at expected and unexpected down times or function failures.
Documentation	Documents setting out specifications, procedures, and records covering each activity performed by the blood establishment shall be in place and kept up-to-date. Records shall be legible and may be handwritten, transferred to another medium and documented in a computerized system. All significant changes to documents shall be acted upon promptly and shall be reviewed, dated, and signed by a person authorized to perform this task.
Donor	Procedures for safe donor identification, suitability interview, and eligibility assessment shall be implemented and maintained. The donor interview shall be conducted in such a way as to ensure confidentiality. The donor suitability records and the final assessment shall be signed by a qualified health professional.
Traceability	Blood collection procedures shall be designed to ensure that the identity of the donor is verified and securely recorded and that the link between the donor and the blood, blood components, and blood samples is clearly established. There shall be a system in place to ensure that each donation can be linked to the collection and processing system into which it was collected and/or processed. The sterile blood bag systems/tubes used for the collection of blood and blood components and their processing shall be CE-marked or comply with equivalent standards if the blood and blood components are collected in third countries. The batch number of the blood bag/tube shall be traceable for each blood component.
Blood Collection Procedures	Procedures shall minimize the risk of microbial contamination. Laboratory samples shall be taken at the time of donation and appropriately stored prior to testing/use. The procedure used for the labelling of records, blood bags/tubes, and laboratory samples with donation numbers shall be designed to avoid any risk of identification error and mix-up. After blood collection, the blood bags/tubes shall be handled in a way that maintains the quality of the blood and at a storage and transport temperature appropriate to further processing requirements and patient application.
Laboratory Testing	Laboratory testing procedures shall be validated before use. Each donation shall be tested in conformity with the requirements laid down.
Validation Devices	Equipment and technical devices shall be used in accordance with validated procedures, including measures to avoid the risk of contamination and microbial growth in the prepared blood components.
Labelling	At all stages, all containers shall be labelled with relevant information of their identity. In the absence of a validated computerized system for status control, the labelling shall clearly distinguish released from non-released units of blood and blood components. The labelling system for the collected blood, intermediate, and finished blood components and samples must unmistakably identify the type of content and comply with the labelling and traceability requirements. For autologous blood and blood components, the label also shall comply with Article 7 of Directive 2004/33/EC and the additional requirements for autologous donations specified in Annex IV to that Directive.

Release	There shall be a safe and secure system to prevent each single blood and blood component from being released until all mandatory requirements are fulfilled. Each blood establishment shall be able to demonstrate that each blood or blood component has been formally released by an authorized person. Records shall demonstrate that before a blood component is released, all current declaration forms, relevant medical records and test results meet all acceptance criteria. Before release, blood and blood components shall be kept administratively and physically segregated from released blood and blood components. In the absence of a validated computerized system for status control, the label of a unit of blood or blood component shall identify the release status.
Storage & Distribution	The quality system of the blood establishment shall ensure that, for blood and blood components intended for the manufacture of medicinal products, the storage and distribution requirements shall comply with Directive 2003/94/EC. Procedures for storage and distribution shall be validated to ensure blood and blood component quality during the entire storage period and to exclude mix-ups of blood components. All transportation and storage actions, including receipt and distribution, shall be defined by written procedures and specifications. Autologous blood and blood components as well as blood components collected and prepared for specific purposes shall be stored separately. Appropriate records of inventory and distribution shall be kept.
Packaging	Packaging shall maintain the integrity and storage temperature of blood or blood components during distribution and transportation.
Return	Return of blood and blood components into inventory for subsequent re-issue shall only be accepted when all quality requirements and procedures laid down by the blood establishment to ensure blood component integrity are fulfilled.
External Contractors	Tasks that are performed externally shall be defined in a specific written contract.
Non-Conformity/Deviations	Blood components deviating from the required and set out standards shall be released for transfusion only in exceptional circumstances and with the recorded agreement of the prescribing physician and the blood establishment physician.
Complaints & Adverse Events	Complaints and other information, including serious adverse reactions and serious adverse events, which may suggest that defective blood components have been issued, shall be documented, carefully investigated for causative factors of the defect and, where necessary, followed by recall and the implementation of corrective actions to prevent recurrence. Procedures shall be in place to ensure that the competent authorities are notified as appropriate of serious adverse reactions or serious adverse events in accordance with regulatory requirements.
Recall	There shall be personnel authorized within the blood establishment to assess the need for blood and blood component recall and to initiate and coordinate the necessary actions. An effective recall procedure shall be in place, including a description of the responsibilities and actions to be taken. This shall include notification to the competent authority. Actions shall be taken within pre-defined periods of time and shall include tracing all relevant blood components and, where applicable, shall include trace-back. The purpose of the investigation is to identify any donor who might have contributed to causing the transfusion reaction and to retrieve available blood components from that donor, as well as to notify consignees and recipients of components collected from the same donor in the event that they might have been put at risk.
Corrective & Preventive Actions	A system to ensure corrective and preventive actions (CAPA) on blood component non-conformity and quality problems shall be in place. Data shall be routinely analyzed to identify quality problems that may require corrective action or to identify unfavorable trends that may require preventive action. All errors and accidents shall be documented and investigated to identify system problems for correction.

Self-Inspection/Audits	Systems shall be in place for all parts of the operations to verify compliance with the defined standards. They shall be carried out regularly by trained and competent persons in an independent way according to approved procedures. All results shall be documented, and appropriate corrective and preventive actions shall be taken in a timely and effective manner.

Supplementary Figures

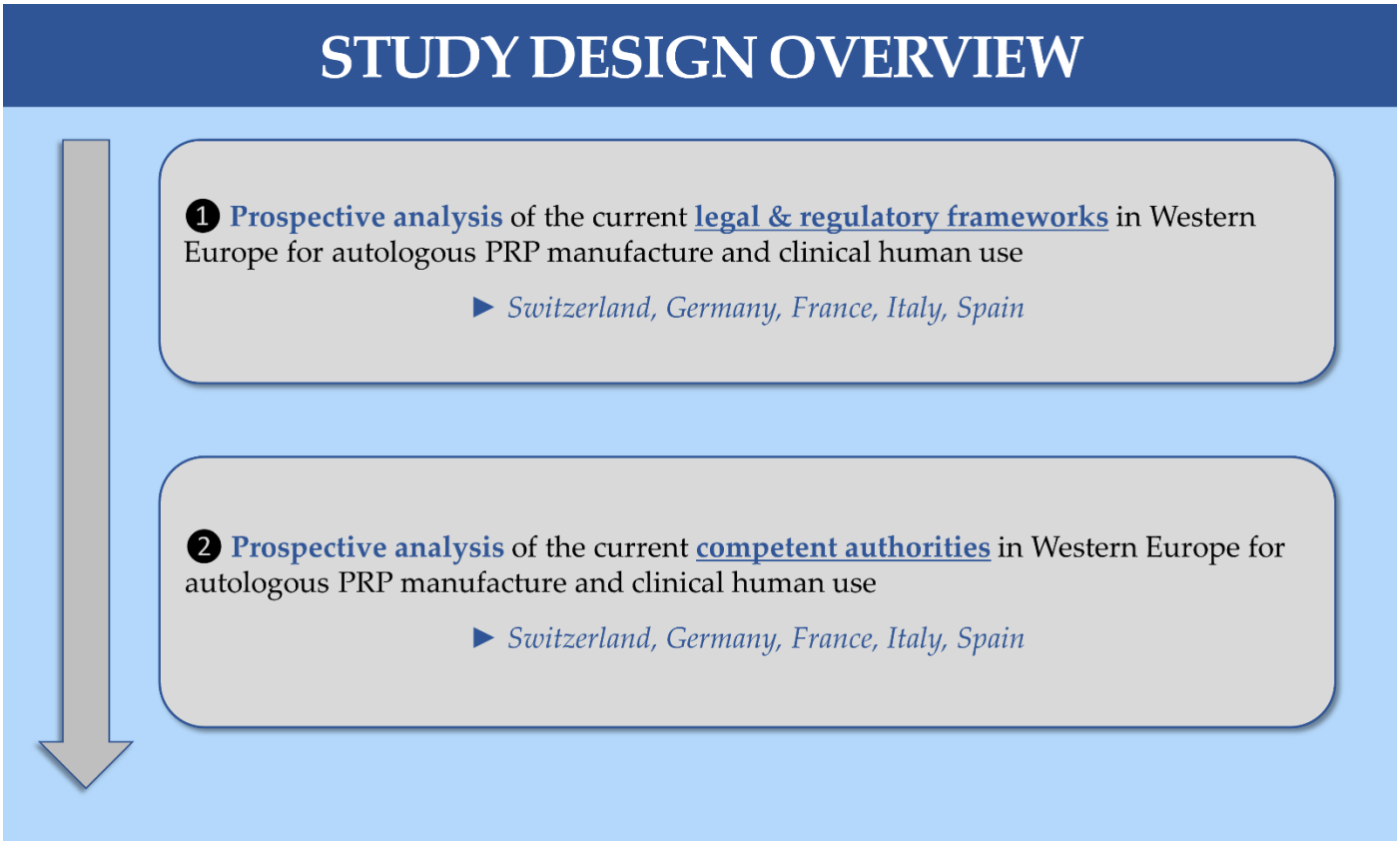

Figure S1. Schematic illustration of the design of the study. GMP, good manufacturing practices; PRP, platelet-rich plasma.

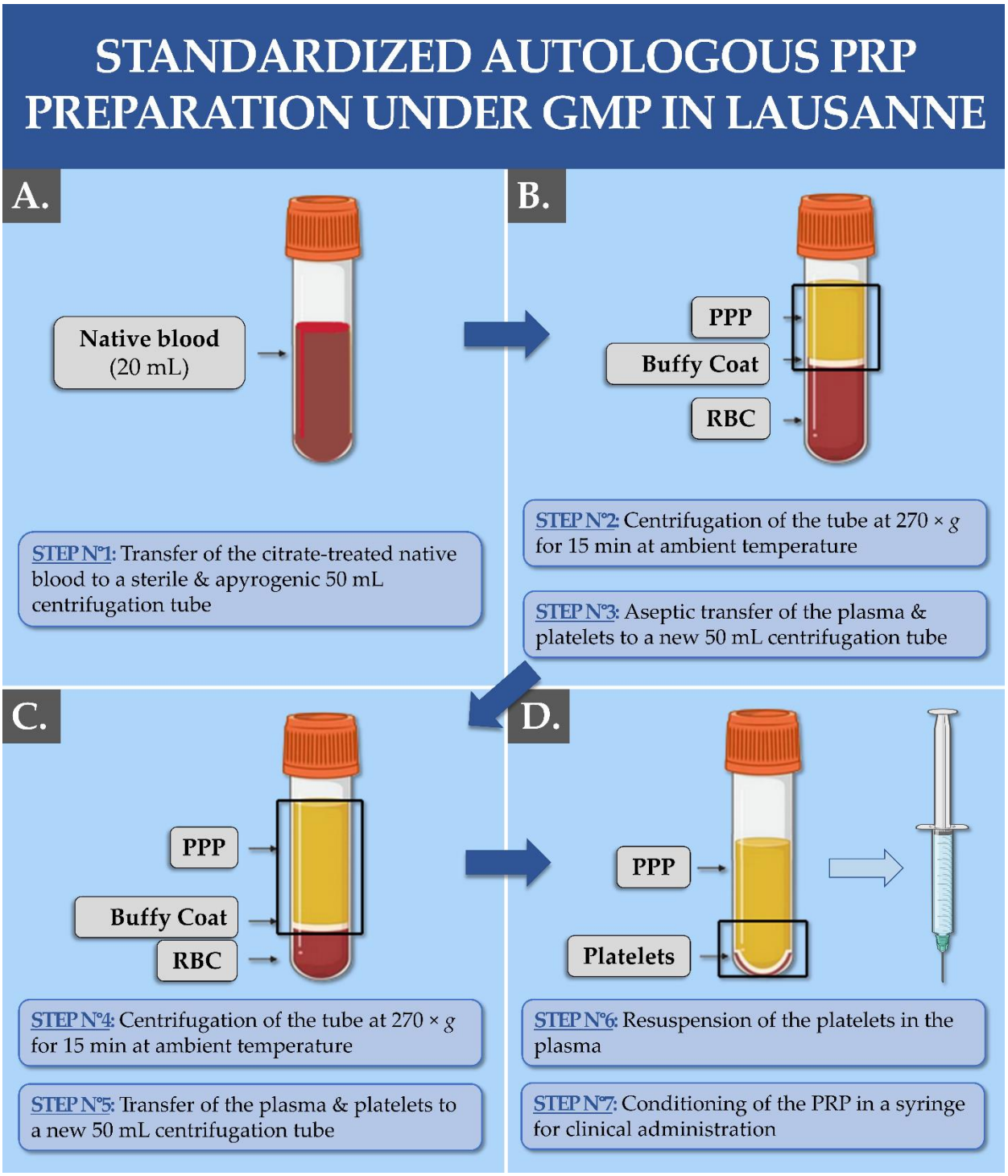

Figure S2. Schematic stepwise illustration of the standardized autologous PRP manufacturing process performed under GMP in the Lausanne University Hospital. All open-container manipulations are performed by trained and qualified personnel in a grade A GMP environment. (**A**) A small volume of whole blood (i.e., ~ 20 mL) is drawn from the patient and is prepared for centrifugation. (**B**) Following the first centrifugation step, a large amount of the RBCs is discarded. (**C**) Following the second centrifugation step, the remaining RBCs are discarded. (**D**) The obtained platelets are resuspended in the appropriate volume of autologous plasma to constitute the PRP. The PRP is then conditioned for clinical administration. Appropriate retention samples and environment samples are isolated for quality controls. GMP, good manufacturing practices; PPP, platelet-poor plasma; PRP, platelet-rich plasma; RBC, red blood cells.

bioengineering

MDPI

Article

Process Optimization and Efficacy Assessment of Standardized PRP for Tendinopathies in Sports Medicine: Retrospective Study of Clinical Files and GMP Manufacturing Records in a Swiss University Hospital

Patrick Sebbagh [1], Nathalie Hirt-Burri [1,2], Corinne Scaletta [1,2], Philippe Abdel-Sayed [1,2,3], Wassim Raffoul [1,2], Vincent Gremeaux [4], Alexis Laurent [1,5], Lee Ann Applegate [1,2,6,*,†] and Gerald Gremion [1,4,†]

1 Regenerative Therapy Unit, Plastic, Reconstructive & Hand Surgery Service, Lausanne University Hospital, University of Lausanne, CH-1066 Epalinges, Switzerland; patrick.sebbagh@gmail.com (P.S.); nathalie.burri@chuv.ch (N.H.-B.); corinne.scaletta@chuv.ch (C.S.); philippe.abdel-sayed@chuv.ch (P.A.-S.); wassim.raffoul@chuv.ch (W.R.); alexis.laurent@lambiotechnologies.com (A.L.); gerald.gremion@bluewin.ch (G.G.)
2 Lausanne Burn Center, Lausanne University Hospital, University of Lausanne, CH-1011 Lausanne, Switzerland
3 DLL Bioengineering, STI School of Engineering, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland
4 Sport Medicine Unit, Division of Physical Medicine and Rehabilitation, Swiss Olympic Medical Center, Lausanne University Hospital, University of Lausanne, CH-1011 Lausanne, Switzerland; vincent.gremeaux@chuv.ch
5 Manufacturing Department, LAM Biotechnologies SA, CH-1066 Epalinges, Switzerland
6 Center for Applied Biotechnology and Molecular Medicine, University of Zurich, CH-8057 Zurich, Switzerland
* Correspondence: lee.laurent-applegate@chuv.ch; Tel.: +41-21-314-35-10
† These authors contributed equally to this paper.

Abstract: Platelet-rich plasma (PRP) preparations have recently become widely available in sports medicine, facilitating their use in regenerative therapy for ligament and tendon affections. Quality-oriented regulatory constraints for PRP manufacturing and available clinical experiences have underlined the critical importance of process-based standardization, a pre-requisite for sound and homogeneous clinical efficacy evaluation. This retrospective study (2013–2020) considered the standardized GMP manufacturing and sports medicine-related clinical use of autologous PRP for tendinopathies at the Lausanne University Hospital (Lausanne, Switzerland). This study included 48 patients (18–86 years of age, with a mean age of 43.4 years, and various physical activity levels), and the related PRP manufacturing records indicated a platelet concentration factor most frequently in the range of 2.0–2.5. The clinical follow-up showed that 61% of the patients reported favorable efficacy outcomes (full return to activity, with pain disappearance) following a single ultrasound-guided autologous PRP injection, whereas 36% of the patients required two PRP injections. No significant relationship was found between platelet concentration factor values in PRP preparations and clinical efficacy endpoints of the intervention. The results were in line with published reports on tendinopathy management in sports medicine, wherein the efficacy of low-concentration orthobiologic interventions appears to be unrelated to sport activity levels or to patient age and gender. Overall, this study confirmed the effectiveness of standardized autologous PRP preparations for tendinopathies in sports medicine. The results were discussed in light of the critical importance of protocol standardization for both PRP manufacturing and clinical administration to reduce biological material variability (platelet concentrations) and to enhance the robustness of clinical interventions (comparability of efficacy/patient improvement).

Keywords: cell therapies; good manufacturing practices; musculoskeletal regenerative medicine; optimized manufacturing; orthobiologics; platelet-rich plasma; process standardization; sports medicine; tendinopathies; transfusion medicine

Citation: Sebbagh, P.; Hirt-Burri, N.; Scaletta, C.; Abdel-Sayed, P.; Raffoul, W.; Gremeaux, V.; Laurent, A.; Applegate, L.A.; Gremion, G. Process Optimization and Efficacy Assessment of Standardized PRP for Tendinopathies in Sports Medicine: Retrospective Study of Clinical Files and GMP Manufacturing Records in a Swiss University Hospital. *Bioengineering* **2023**, *10*, 409. https://doi.org/10.3390/bioengineering10040409

Academic Editor: Redha Taiar

Received: 13 February 2023
Revised: 17 March 2023
Accepted: 23 March 2023
Published: 25 March 2023

1. Introduction

Tendinopathies are debilitating and painful affections, which often have severe consequences on the daily activities (e.g., temporary or permanent interruption of work) of patients and on healthcare systems [1–4]. Frequently developed following overuse/overload injuries (i.e., professional or sports-related), tendinopathies mostly affect the rotator cuff, Achilles tendon, tibialis posterior, common wrist extensor, and patellar tendons [1,3,5]. Within the aging populations, sports-related (i.e., recreational or elite levels) tendon overuse drastically increases the risks and prevalence of injury [1,2]. Due to tissular hypocellularity and hypovascularity, the intrinsic and physiological healing ability of tendons is slow and inefficient [1]. This process becomes even slower with increasing patient age and can be disturbed by physical activity, especially in cases when sports-related practices exceed tissue recovery abilities. Lengthy recuperation results in direct (i.e., healthcare costs) and indirect (e.g., productivity loss) financial burdens on society [3,4]. Chronic tendon lesions are often degenerative in nature, yet they do not necessarily implicate inflammatory components [2,5].

If the tendon tissue's extracellular matrix (ECM) structure is damaged, physiological repair processes may result in the synthesis of disorganized matrix, potentially leading to fibrin strand breakage (e.g., fibrillar slippage, breakage of cross-linking, and fibrillary rupture) [5]. The resulting structural anomalies may lead to a reduction in the axial strength and elasticity of the affected tissue, thus favoring the recurrence of lesions. Standard therapeutic management of sub-critical tendon defects rely on well-managed analgesia (e.g., prescription of NSAIDs) and physical therapy (e.g., eccentric muscle work) [3–5]. In case of important structural damage and low probability of spontaneous healing, invasive therapeutic interventions may be considered (e.g., tendon suture, autografts, allografts, and synthetic prostheses) [1,5]. As an intermediate approach, injectable hyaluronan-based hydrogels have demonstrated multiple therapeutic benefits in tendon-related affections [6]. Furthermore, diverse regenerative medicine approaches have recently been investigated to improve tendinopathy management, including stem cell therapies, tissue graft bioengineering, and the use of orthobiologics [7–14]. Notably, recent regulatory shifts and quality-oriented constraints related to cytotherapies have prompted the deployment of important efforts toward the development of cell-derived and cell-free therapeutic management options for tendinopathies [15–22]. Parallelly, the medical use of autologous platelet-rich plasma (PRP) preparations in orthopedics and in sports medicine for tendon and ligament affections has drastically increased over the past two decades [23–27].

Autologous PRP therapy for tendon affections requires a local ultrasound-guided injection of a processed blood extract, which has been obtained by minimal manipulation (e.g., differential centrifugation) from patients' blood. Such extracts are composed of concentrated platelets in autologous plasma following the removal of erythrocytes and lymphocytes [28–30]. Standard native blood sample volumes to be drawn from patients for PRP therapies in sports medicine range from 20 mL to 40 mL, with average blood platelet counts of 200,000 $\pm$ 75,000 platelets/μL. Following processing, the plasma, inflammatory agents, platelets, and plasma proteins are conserved in the PRP preparation [25,28,31]. Delivery of supra-physiological concentrations of growth factors and cytokines found in PRP (i.e., following platelet breakdown) often stimulates an accelerated and physiological regenerative response at the site of the tissular injury. Currently, PRP is commonly used in surgery and regenerative medicine to improve the recovery potential of soft tissues (e.g., ligaments, tendons, cartilage, and nerves) and bones [32–37]. Its clinical use has also been extensively reported for cutaneous wounds, burns, and skin donor sites, as well as for esthetic applications (e.g., cutaneous and capillary) [28,38–43]. Finally, PRP applications in sports medicine have been shown to enhance recovery following tendon lesions and, in particular, result in shortened overall recovery periods for athletes [7,8,33].

Importantly, highly contradictory reports and analyses of PRP clinical efficacy are available in current scientific literature sources, when considering all therapeutic indications and manufacturing methods [12,14,24,25,27,38,40,44,45]. The critical importance of

standardizing sample processing workflows and therapeutic protocols has been identified in order to obtain maximal benefits from orthobiologic treatments. However, a vast array of PRP production protocols is available on the market, which can potentially explain several aspects of the observed variability in clinical efficacy outcomes [44,46,47]. Previous literature reviews have shown that actual PRP platelet concentration factors vary from 2 to 12 times in value, wherein extreme values are reported to be as low as 0.52 times the baseline ratio of platelets to whole blood [23,24,44]. Such diversity in PRP product attributes may be linked to the specific manufacturing systems used (e.g., commercial kits), the volumes of drawn blood, the starting cellular concentrations, the anti-coagulation techniques, and patient-related factors (e.g., comorbidities, age, circulatory and nutritional status, and drug use) [44]. While PRP manufacturing standardization has become a main clinical-oriented focus point, regulatory scrutiny is directed at the PRP manufacturing systems, rather than at the exact manufacturing process or the final PRP product attributes [48–55]. Importantly, it should be noted that higher platelet concentrations in the final PRP preparation do not systematically correlate with enhanced clinical efficacy as it seems that saturation can occur, and the timing of administration may also have an impact on overall tissue healing parameters [24,44]. Finally, there is no widespread consensus regarding post-injection management for PRP in sports medicine, wherein rehabilitation plays a crucial role [56].

This retrospective study (i.e., covering the 2013–2020 period) investigated the standardized GMP manufacturing and sports medicine-related clinical use of autologous PRP for tendinopathies at the Lausanne University Hospital (CHUV, Lausanne, Switzerland). The primary objective of the study was to evaluate PRP injection efficacy in patients treated at the Sports Medicine Unit for tendon-related affections based on the clinical files and GMP manufacturing records of PRP preparations. As previously reported, the in-house GMP manufacturing of PRP preparations at CHUV has enabled rapid clinical management of tendinopathies, cutaneous burns, and arthrosis lesions in multiple institutional departments [31]. From a technical point-of-view, the developed and standardized PRP manufacturing protocol increases the platelet concentration by a factor of 2–3 times from 20 mL of native blood, using a two-step centrifugation method within a GMP platform [31]. A secondary objective of this study was to determine if a relationship could be identified between individual PRP concentration factors and patient-reported efficacy of the therapeutic intervention. Therefore, individual patient follow-up data were analyzed for the determination of the number of received PRP injections and for the assessment of patient clinical conditions at two, three, four, and five weeks post-PRP administration. Overall, the presented information outlines the key steps of PRP protocol standardization (i.e., manufacturing process and clinical administration), which were designed to reduce variability in final platelet concentrations and to enhance the quality of clinical interventions.

2. Materials and Methods

2.1. Study Design and Ethics Committee Approval of the Retrospective Study

The present retrospective study was reviewed and approved by the Vaud State Ethics Committee (i.e., authorization N°CER-VD-ID#2022-00305, 2022) and was conducted following the principles of the Declaration of Helsinki and applicable Swiss laws [57]. In this study, patient data from clinical cases of tendinopathy were used to assess the effectiveness of autologous PRP injections. The primary objective of the study was to determine the level of efficacy (i.e., categorized as "positive" or "non-positive" evolution) of PRP injections for the therapeutic management of tendinopathies. The secondary objective of the study was to determine if there was a relationship between the concentration factor of platelets in the administered PRP preparations and patient-reported efficacy of the intervention, which was assessed based on the patients' clinical status at the time of the 2-, 3-, 4-, and 5-week follow-up contacts after the PRP injection. Specifically, the data were analyzed for the following purposes:

(i) Discriminate the success rate according to the age of the patients at the time of the PRP treatment.

(ii) Highlight whether PRP could help in resuming physical activity faster in younger versus older patients.
(iii) Determine how many PRP injections were necessary on average.
(iv) Highlight the nature and volume of the resumed physical activity in the studied patient population.

In order to obtain appropriate data and information within the scope of this retrospective study, the specific elements of the request submitted to the local ethics committee comprised the following:

(i) Number of PRP applications required for healing.
(ii) Ratio of platelet concentration in the PRP injection to platelet concentration in native blood (i.e., platelet concentration factor) for each patient.
(iii) Possible relationship between the number of PRP injections required and the platelet concentration factor.
(iv) Potential treatment-related adverse events as detected.
(v) Patient clinical evolution following the PRP treatment.

Given the exploratory nature of this retrospective study, the sample size calculation was not based on formal statistical methods. The sample size was evaluated according to an internal feasibility study, which showed that 75 patients with diagnosed tendinopathies were treated with autologous PRP injections since the implementation of the procedure in 2013 at the CHUV and would be eligible for the retrospective study.

2.2. Clinical Data Gathering and Processing

Patient clinical files were investigated at a preliminary level for cases of tendinopathy treatment by autologous PRP injection at the Sports Medicine Unit, since the implementation of the procedure at the CHUV in 2013 and until December 2020. The inclusion criteria restricted the search to the adult patient population (i.e., $\geq$18 years of age) that received a PRP injection treatment for tendinopathy. The exclusion criteria disqualified pediatric patients (i.e., <18 years of age) and patients who refused to be included in any research or have their data anonymously analyzed. Overall, 75 patients were initially included in the study. Further investigations led to the exclusion of several cases based on the absence or refusal of consent provision or on the presence of documented epicondylalgia. Finally, a total of 48 patients were retained for an in-depth analysis within this retrospective study.

Demographic, radiological, biological, and clinical data were collected and analyzed from computerized patient records (i.e., Soarian, Archimedes, PACS). The patient data notably included clinical and anamnestic histories, clinical and health observations, ultrasound image reports, choice of treatment, application of treatment, and ambulatory follow-up. In order to investigate the potential relationship between platelet concentration factors and PRP treatment efficacy, the patients were classified according to the qualitative assessment of their clinical state and the ultrasound appearance of their tendon tissular lesion.

2.3. GMP Manufacturing Process for Autologous PRP at the CHUV

All patients included in the present retrospective study received final autologous PRP products prepared by an in-house GMP platform, following a standardized CHUV-UTR protocol [31]. The in-house GMP facilities at the CHUV were accredited by Swissmedic (i.e., Swiss national therapeutic products regulator) for the production and storage of biologicals, including PRP. The specific manufacturing method comprised aseptic open-container manipulation, a two-step serial centrifugation workflow, and conditioning of the final PRP products in patient-specific syringes. The step-by-step instructions for PRP manufacture following the CHUV-UTR protocol are further provided in the following section. The final PRP products were typically characterized by a 2–3-fold platelet concentration factor (i.e., compared to native blood) using a low starting blood volume (i.e., approximatively 20 mL). The CHUV-UTR protocol for GMP manufacturing of PRP was validated and qualified as simple, inexpensive, and rapid (i.e., short production time) [28]. Furthermore, the protocol

offered reproducibility of quality and limited risks for patients, with low volumes of blood draw, and was, thus, considered an asset for autologous biological treatments at the CHUV.

2.4. Medical Method for Orthobiological Management of Tendinopathies in Sports Medicine at the CHUV

The use of autologous blood for PRP preparations significantly reduces the risk of cross-contamination compared to the use of third-party blood. This approach was, therefore, retained by the attending physicians at the Sports Medicine Unit in the CHUV. During patient anamnesis and diagnostic investigation, an ultrasound scan was performed on all the patients included in this study. The purpose of the ultrasound was to determine the precise site of the tissular injury and to qualify the type of tendon injury. Notably, several patients did not present any ultrasound-detectable lesions. Then, a native blood sample was drawn and was sent to the GMP production unit, along with a medical prescription for an autologous PRP preparation. Following the manufacture, the final PRP products were returned to the attending physicians within 2 h of blood collection.

The attending physicians excluded the presence of contra-indications for PRP treatment (i.e., presence of fever, infection, allergy, and other contra-indications) for each patient. For outpatient PRP local administration by injection, ultrasound guidance was chosen to ensure accurate positioning of the needle on the tendon tissue lesion. Ultrasound guidance (GE LOGIQ e, GE Medical Systems, Milwaukee, WI, USA) of the needle also ensured that sensitive tissues, such as nerves, vessels, and healthy tendons, were avoided. For the few patients who did not present any ultrasound-detectable tendon lesions, the available clinical information was used to determine the PRP injection site. For the administration of the PRP injection, the patients lied on an examination table in the dorsal, lateral, or ventral position depending on the area to be treated. Particularly, the most comfortable position for the patients was sought in order to avoid any movement during the procedure. The injection area was then locally disinfected using topical chlorhexidine, and the PRP preparation was injected. Standard antalgics were prescribed, and follow-up visits were specified as appropriate.

2.5. Statistical Assessment of Data

Quantitative variables that followed a Gaussian/normal distribution were analyzed and visualized using means and standard deviations. Categorical (i.e., qualitative) variables were represented by percentages and frequencies. The statistical calculations and/or data presentation were performed using Microsoft Excel and Microsoft PowerPoint (Microsoft Corporation, Redmond, WA, USA).

3. Results

3.1. Retrospective Study Workflow and PRP GMP Manufacture

The methodological overview of the present retrospective study is presented in Figure 1, with a description of the patient inclusion/exclusion criteria and the processing of clinical files within the study workflow.

For all patients included in this retrospective study (i.e., 48 patients, Figure 1), a complementary analysis of available PRP manufacturing records was performed. The exact method of autologous PRP manufacture by the in-house GMP platform is presented in Figure 2, with an illustrated and step-by-step process description.

3.2. Patient-Related Parameters and PRP Clinical Administration for Tendinopathies

The methodological aspects of the ultrasound-guided PRP administration for therapeutic tendinopathy management are presented in Figure 3, with anatomical description of the injured tendons and ultrasound records for a managed case of Achilles tendon fissure.

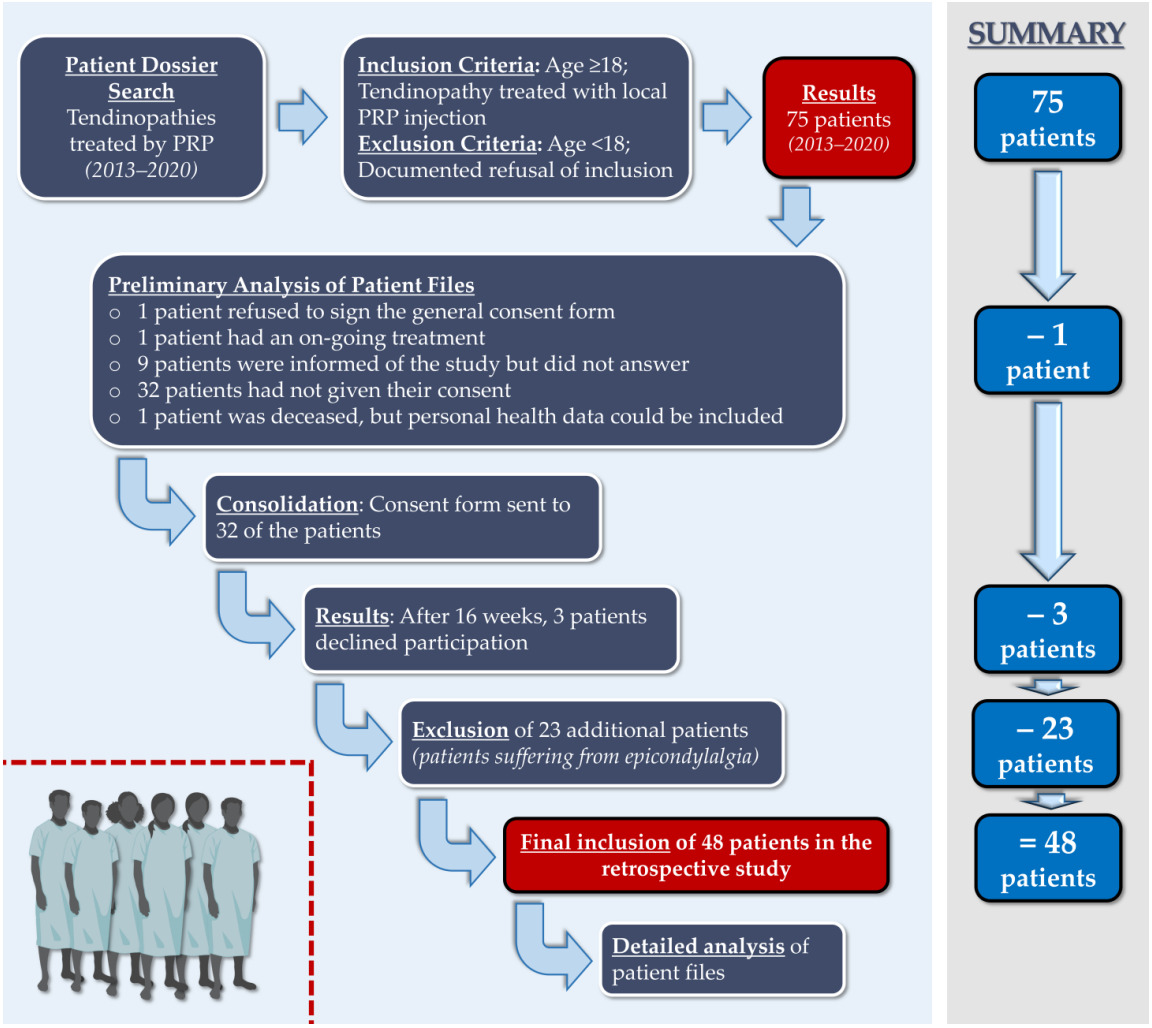

Figure 1. Overview of the retrospective study protocol (i.e., study workflow) for patient selection and inclusion for analysis. Data procurement was based on a retrospective review of patient dossiers for clinical cases of tendinopathies treated with local autologous PRP injections between 2013 and 2020 at the Sports Medicine Unit of the Lausanne University Hospital. A summary of the number of included patients during the selection procedure is presented in the column on the far right. PRP, platelet-rich plasma.

The demographic characteristics of the patients included in the present retrospective study are presented in Table 1.

Table 1. Demographic characteristics of the patients treated with local autologous PRP injection for tendinopathies at the Sports Medicine Unit of the Lausanne University Hospital and included in this retrospective study. The patient age data follow a normal distribution. PRP, platelet-rich plasma.

Year	2013	2014	2015	2016	2017	2018	2019	2020	Total
Patients (n)	1	7	17	4	4	5	5	5	48
Age of patients (years)	Average of 43.4 ± 16.6 years								
Patient age distribution (n) [1]	≤45 years old: 26 patients (54%) 46–65 years old: 16 patients (33%) >65 years old: 6 patients (13%)								

[1] The results indicate that approximately half of the included clinical cases in this study are likely to be unaffected by menopause or andropause, while the remaining patients are likely to be already affected by steroidal decline.

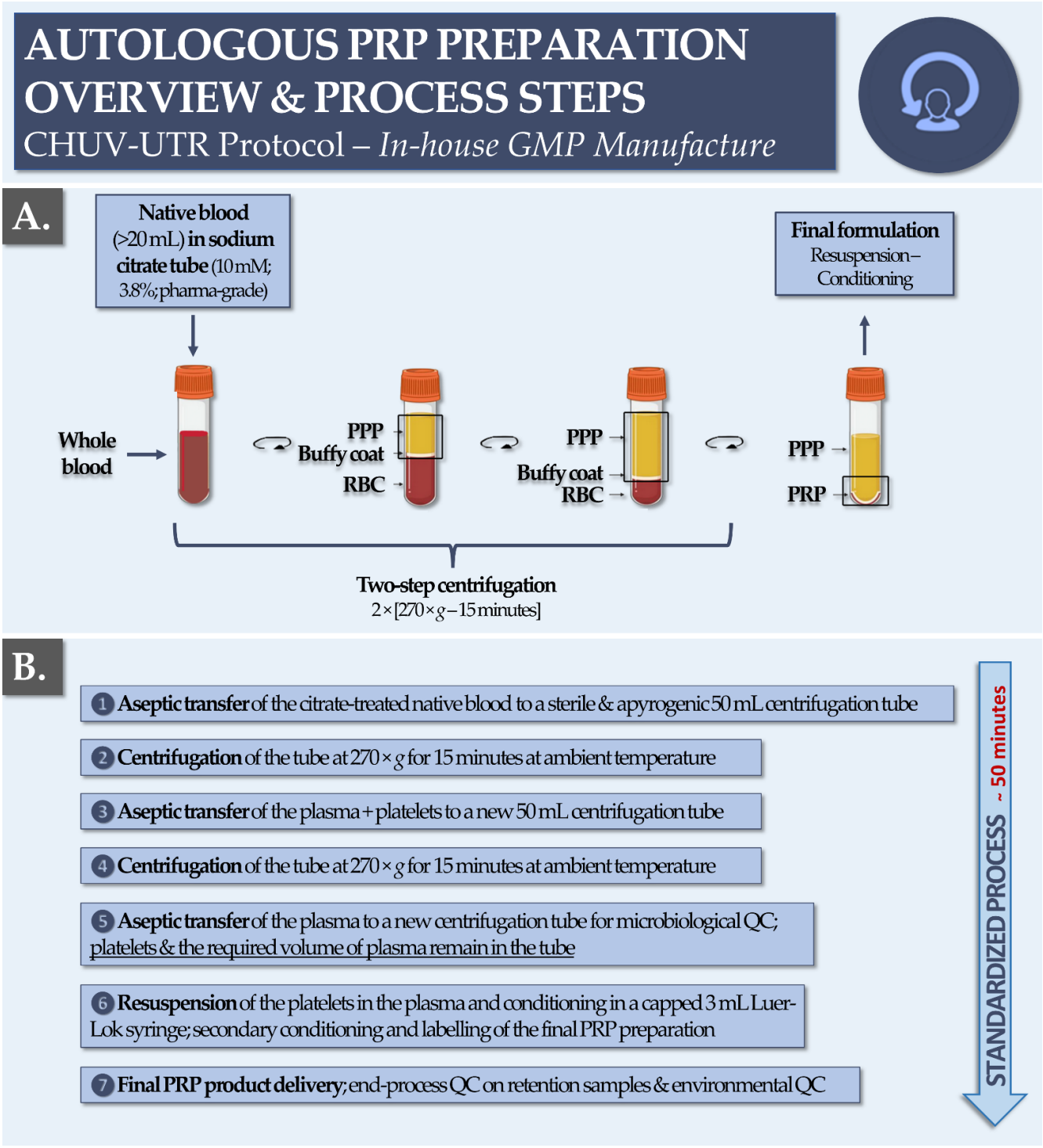

Figure 2. Illustrated and step-by-step process description for standardized autologous PRP manufacture under GMP at the Lausanne University Hospital. (**A**) Illustrated overview of autologous blood product processing, with two-step serial centrifugation for eventual platelet concentration. (**B**) Step-by-step description of the autologous blood product processing method for the obtention of the prescribed volume in the final autologous PRP product. Platelet counts were performed using the native blood sample and the final PRP product. CHUV, centre hospitalier universitaire vaudois; GMP, good manufacturing practices; PPP, platelet-poor plasma; PRP, platelet-rich plasma; QC, quality control; RBC, red blood cell; UTR, regenerative therapy unit.

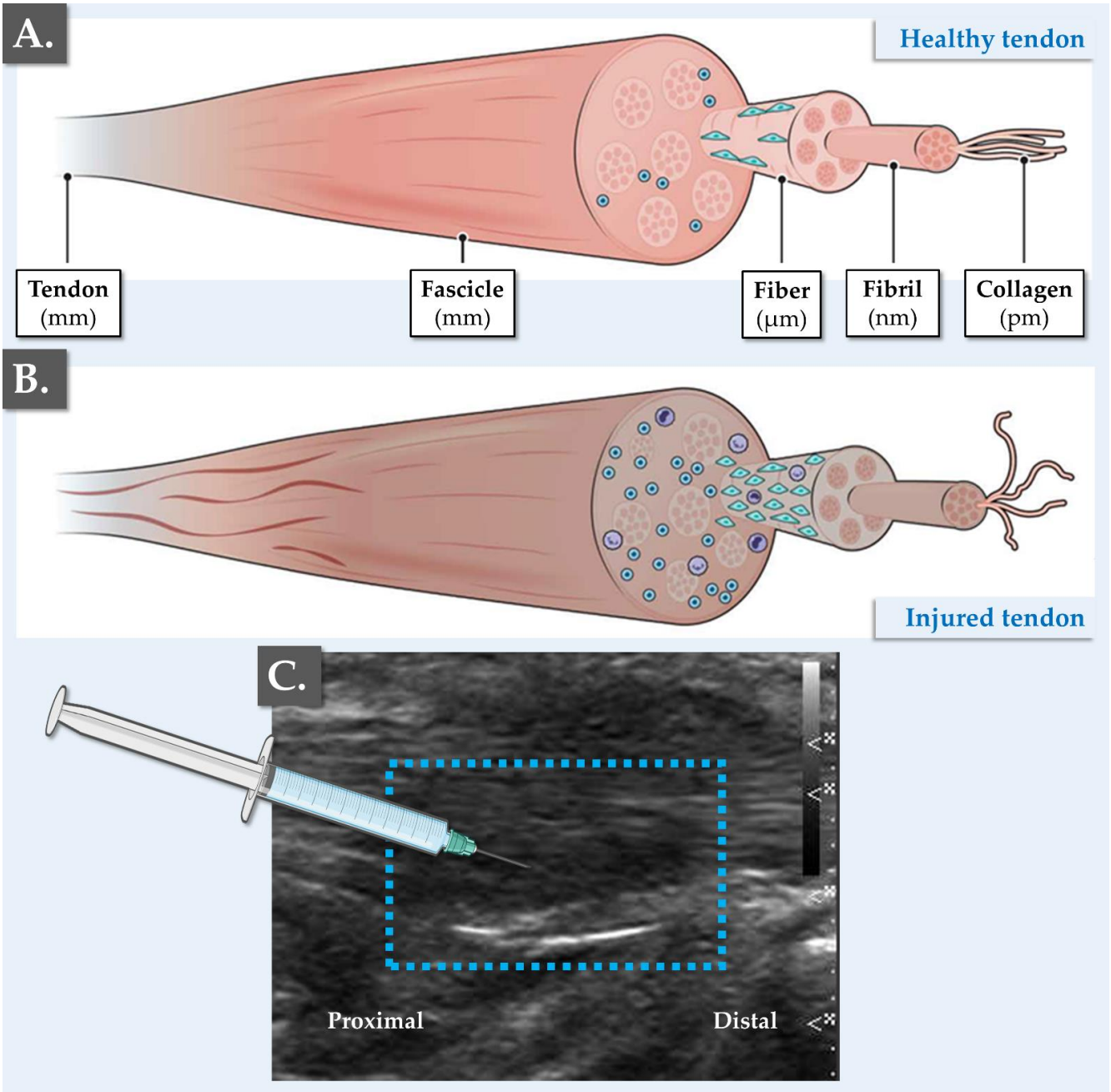

Figure 3. (**A**) Healthy tendon structure and components, including size scales. In normal conditions, the tendon ECM undergoes constant regeneration and re-modeling. (**B**) Unhealthy tendon structure and components. In case of vascular, inflammatory, degenerative pathology, or mechanical overload, tendon homeostasis is disrupted. This leads to a gradual accumulation of ECM damage and disorganization, with alteration of collagen architecture, glycosaminoglycan deposition, lipid accumulation, and heterotopic ossification. Compared to healthy tendons, diseased tendons have elevated collagen disorganization, smaller fibers, hypercellularity, increased cell rounding, elevated presence of immune cells, and increased denatured collagen. (**C**) Fissured Achilles tendon structure as observed by ultrasound, with illustration of the autologous PRP injection point. ECM, extracellular matrix; PRP, platelet-rich plasma.

Patient activity-related parameters were compiled and used to classify the patient sample into various groups, as presented in Figure 4.

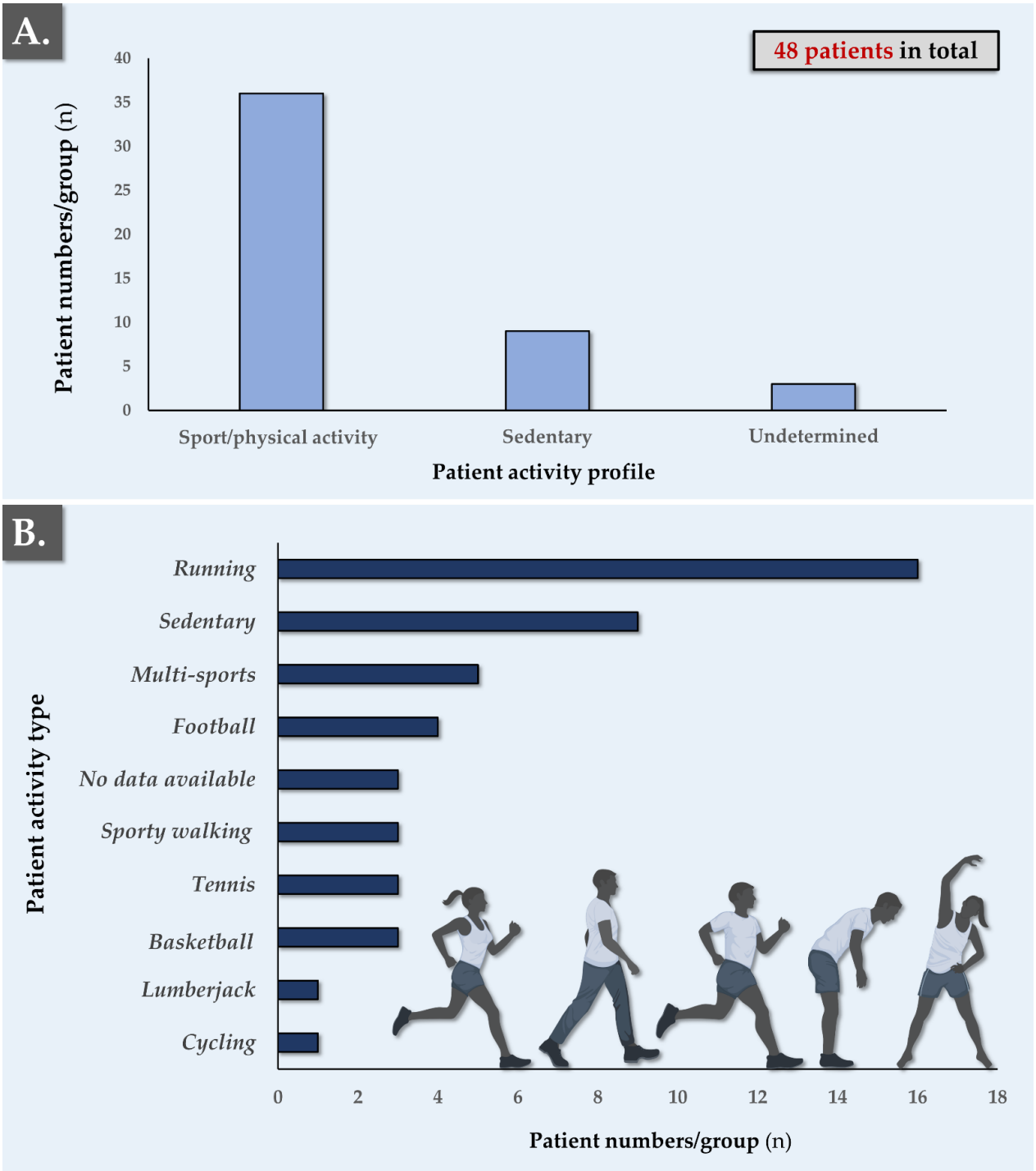

Figure 4. Activity-related parameters for the patients included in this retrospective study. (**A**) Patient physical activity status distribution at the time of autologous PRP treatment. (**B**) Type of sport or physical activity distribution for the included patients at the time of autologous PRP treatment. PRP, platelet-rich plasma.

An analysis of the clinical files revealed that a majority of the treated patients reported having a regular sport activity or an active physical professional activity (e.g., lumberjack), with 75% of the patients leading active lives (Figure 4). An examination of the background of the individual patients revealed that those who benefited from therapeutic autologous PRP injections were either highly involved athletes or patients who had previously remained refractory to several therapeutic approaches.

The diagnosis-related information gathered from the clinical files confirmed that all 48 patients presented severe tendon tissular alterations or a functional disorder. Regarding the follow-up of the patients after their PRP treatment and the evaluation of intervention efficacy, a positive evolution was defined as a complete recovery of physical condition, with complete disappearance of pain. An analysis of the patient follow-up data revealed that 61% of the patients who reported a positive evolution received a single autologous PRP injection (Figure 5A).

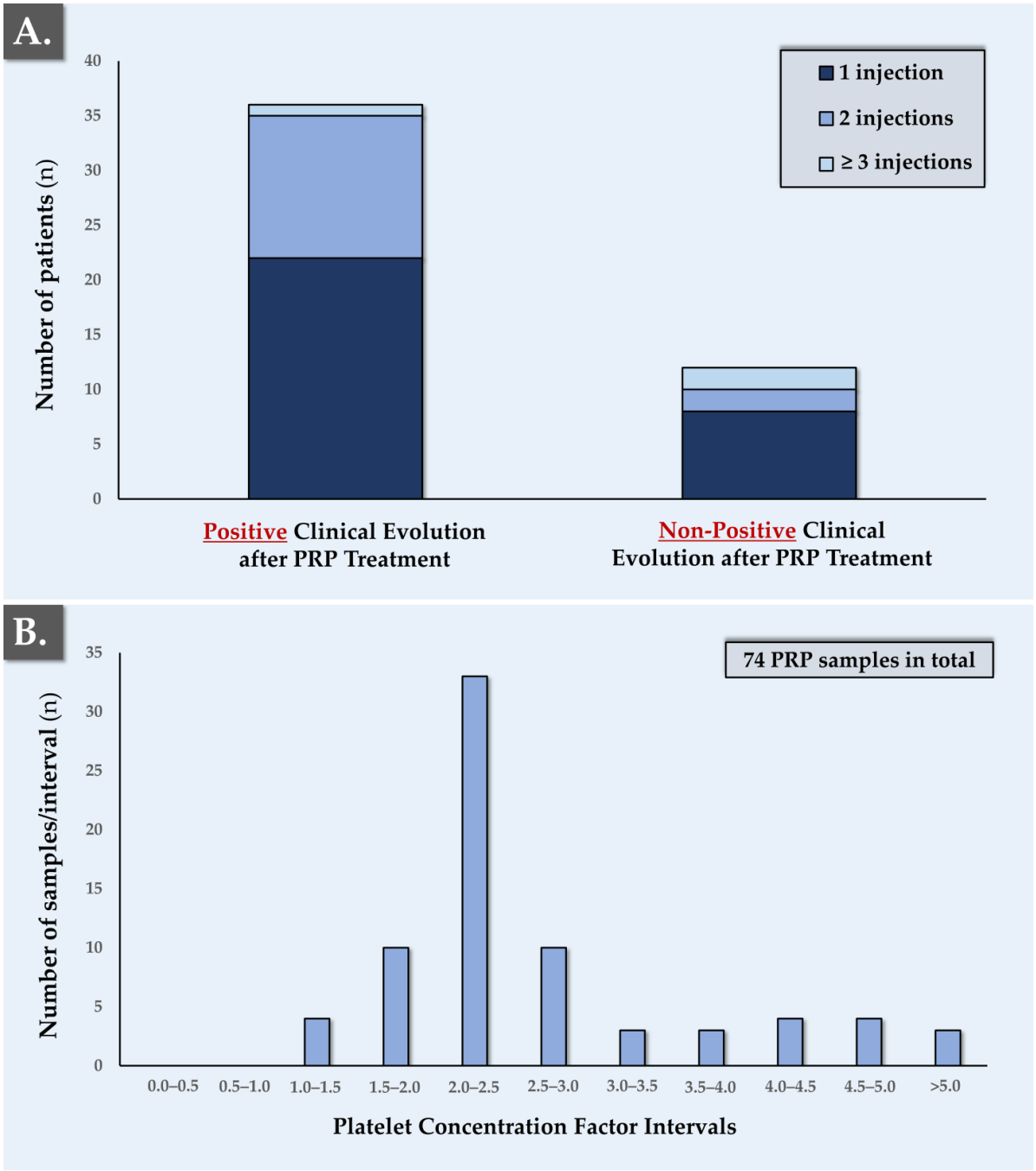

Figure 5. (**A**) Number of local autologous PRP injections received by the patients reporting positive or non-positive evolution of tendinopathy. It should be noted that, in both groups, some patients required more than three PRP injections. (**B**) Distribution of the platelet concentration factors in autologous PRP among the 48 patients, who received 74 PRP injections in total. PRP, platelet-rich plasma.

A total of 36% of the patients who reported a positive evolution required a second autologous PRP injection to maintain this positive evolution, and 3% of the patients who reported a positive evolution required three or more PRP injections to fully recover (Figure 5A). Overall, 36 patients reported a positive evolution following local PRP treatment, and 12 patients reported a non-positive evolution (Figure 5A).

3.3. PRP GMP Manufacturing Data Analysis and Clinical Efficacy Evaluation

The analysis of PRP manufacturing records for the patients included in this retrospective study revealed that the average native blood samples drawn from the patients for autologous PRP preparation were characterized as having a volume of 20 mL. The platelet concentration factors of the final autologous PRP products for clinical use were 2.79 on average, whereas the most frequent platelet concentration factor interval was of 2.0–2.5 in value (Figure 5B, Table 2).

Table 2. Manufacturing data for the autologous PRP products. For the patients in the non-positive evolution group, a non-significant trend of lower (i.e., comparison of mean quantitative values) platelet concentration factor values is evident when compared to the concentration factor values of the positive evolution group. The platelet concentration factor data were found to be normally distributed. PRP, platelet-rich plasma; SD, standard deviation.

Patient Follow-Up	Number of Patients	Patient Age (Mean ± SD)	Platelet Concentration Factors (Mean ± SD)
All patients	48	43.3 ± 16.6	2.79 ± 1.34
Positive evolution	36	41.7 ± 22.9	2.92 ± 1.46
Non-positive evolution	12	47.5 ± 28.5	2.28 ± 0.28

Overall, no significant relationship was found between the final PRP product attributes (e.g., platelet concentration factor values) and efficacy-related outcomes in the analyzed dataset. On average, the patients who reported a positive evolution were younger than those who reported a non-positive evolution (Table 2). However, due to the absence of a statistically significant (i.e., with $p < 0.05$) difference in mean patient age values between the two groups, it was concluded that patient age was not a predictive criterion for autologous PRP treatment efficacy in the studied patient population.

An analysis of patient gender in relation to autologous PRP treatment efficacy revealed that males reported a positive evolution more often than females (i.e., 69.4%, Table 3).

Table 3. Patient gender distribution in relation to local autologous PRP treatment efficacy evaluation for the 48 patients included in this retrospective study. PRP, platelet-rich plasma.

Patient Follow-Up	Male Patients (n) Percentage of Subgroup (%) Average Number of PRP Injections (n)	Female Patients (n) Percentage of Subgroup (%) Average Number of PRP Injections (n)
Positive evolution	25 69.4% 1.6	11 30.6% 1.6
Non-positive evolution	6 50.0% 1.0	6 50.0% 2.0

However, no gender-related patient distribution differences were found in the group of patients who reported a non-positive evolution (Table 3). Interestingly, the data revealed that the six males who did not benefit from a positive evolution received only one PRP injection compared to an average of 1.6 PRP injections in the group that reported a positive evolution (Table 3). In comparison, the females who did not report a positive evolution

received 2 PRP injections on average compared to the average of 1.6 PRP injections in the positive evolution group (Table 3). It was not possible to determine whether the group of male patients who did not improve became discouraged more quickly or whether their attending physician discontinued the treatment too soon. For the female patients, it was found that patient care was more sustained (Table 3).

Further investigation of the clinical patient files was performed for the patient group who reported a non-positive evolution following local autologous PRP treatment of tendinopathy. Within this group, there were three elderly patients (i.e., age > 70 years) and two overweight patients (i.e., BMI value > 25). There were also two sports teachers, a sedentary 41-year-old patient, two athletes aged 21 and 32, a patient who was applying for disability insurance benefits, and a patient for whom no further information was available. It should be noted that five patients within the non-positive evolution group presented a significant discrepancy between anamnestic complaints and ultrasound findings.

3.4. Standardized PRP GMP Manufacturing Process Statistical Evaluation

When considering the distribution of platelet concentration factors for the 48 included patients, relatively low values were most frequent (i.e., 33 patients in the 2.0–2.5 concentration factor group, Figure 5B). Despite the presence of extreme values (e.g., platelet concentration factors > 5.0), the variability in the platelet concentration factor value distribution was assessed as being low overall. Therein, the recorded values of platelet concentration factor in the autologous PRP were found to be tightly normally distributed around a mean value of 2.79 $\pm$ 1.34 (Table 2). Specifically, it was determined that 79.7% of the individual concentration factor values fell within one standard deviation of the mean value. For reference, a theoretical Gaussian distribution would comprise 68.27% of the individual values within one standard deviation of the mean value (Table S1). This reduced variability in platelet concentration factors when compared to a Gaussian distribution may be considered positively from a manufacturing process standardization point-of-view, especially for inherently variable biological starting materials, such as native blood and its derivatives.

Furthermore, intra-patient variability concerning platelet concentration factors was also studied to assess the robustness of the PRP manufacturing process. Therefore, an analysis of platelet concentration factor variability for each patient having received $\geq$2 autologous PRP injections was performed (i.e., 19 patients included in the analysis, representing 45 PRP preparations). In detail, the mean platelet concentration factor was determined for every patient, where applicable. Then, a gap analysis was performed for each patient to study the difference between the individual values of platelet concentration factor and the mean value of platelet concentration factor for the specified patient (Table S2). The results of the gap analysis are presented in Table 4.

Table 4. Results of the intra-patient gap analysis performed between individual and mean platelet concentration factor values. A total of 45 autologous PRP preparations were included in the analysis, corresponding to 19 patients. The data were found to be normally distributed. PRP, platelet-rich plasma.

Parameters	Experimental Results
Average gap value	14.0% $\pm$ 6.3%
Average gap value in male patients	18.7%
Average gap value in female patients	9.8%

The relatively low average results of the gap analysis presented in Table 4 can also be considered positively from a manufacturing process standardization point-of-view. Such analyses demonstrate that within a standardized GMP process for PRP manufacture, high reproducibility and consistency in autologous final product attributes (e.g., platelet concentration) could be attained. This aspect is especially important for the assurance of consistency in the quality and critical attributes of the final therapeutic product administered to a specific patient.

4. Discussion

4.1. Applicable Legal Bases and Advantages of GMP Autologous PRP Manufacture

The clinical use of autologous PRP has expanded rapidly in recent years despite the absence of a clear consensus on product preparation and characterization methodologies [14,33,44,51]. In particular, the high diversity observed in the domain of autologous PRP preparation and use may be partly attributed to the heterogeneity of clinical users (e.g., hospitals, private clinics and medical offices, and esthetic institutes) and manufacturing materials (i.e., commercial PRP kits) [27,44]. This in turn may potentially explain the highly contrasted clinical reports on orthobiologic intervention efficacy assessment, as clinical results are highly dependent on the therapeutic indication and on the PRP preparation/administration method [24–27,30,40,44]. In addition to the fragmented and inhomogeneous technical aspects of current autologous PRP preparation and use, the application of regulatory requirements is specific to member states in the European Union (EU), and several gray zones exist with regard to responsibility attribution [29,44].

In the case of Switzerland, the applicable legal bases for PRP preparation and use are highly similar to European frameworks. Therein, PRP is considered as a "non-standard medicinal product", meaning that good manufacturing practice (GMP) standards must be applied [58]. Specifically, Directive 2005/62/EC clearly states that Good Practice Guidelines (GPG) based on the principles of GMP shall be implemented and defines the technical standards, which are very similar to GMP [48–50,53]. Such concepts and approaches are confirmed by the Swiss legislator in the Therapeutic Products Act (TPA, SR812.21) [58]. Authorizations from the national therapeutic products authority, Swissmedic, are therefore required for the GMP manufacturing of PRP preparations for clinical use [31]. Several exceptions and derogations to the aforementioned authorization regimen are specified, but Swiss private practices, clinics, or hospitals that do not rely on GMP autologous PRP manufacture must implement an appropriate ad hoc quality system [48–50]. Importantly, they must operate under defined procedures for the following activities:

(i) Training and qualification of personnel.
(ii) Validation of premises, equipment, testing procedures, and computerized systems.
(iii) Traceability assurance (i.e., proper labeling of samples and materials).
(iv) Storage and distribution of final products.
(v) Performance of self-inspections/audits for any complaints, recalls, and notifications to hemovigilance, along with implementation of appropriate corrective and preventive actions.

The purpose of the above-mentioned legal and regulatory procedures is to protect patients and to ensure that appropriate responsibility is assumed by clinicians for the assurance of the safety, quality, and efficacy of the administered autologous PRP treatments [48–50]. Overall and despite the elevated fixed costs of GMP manufacturing of autologous PRP products, optimal demonstration of compliance with regulatory standards is possible (i.e., use of a risk-based and process-oriented quality system) [31]. Therein, the autologous PRP manufacturing protocol developed by the Lausanne University Hospital (i.e., standardized CHUV-UTR protocol) has been validated from a regulatory standpoint within the scope of the Swissmedic authorization for the in-house GMP platform [28,31]. Importantly, ad hoc workflows for autologous PRP processing and trained personnel are available for the execution of medical prescriptions.

The validation of the GMP production platform and the qualification of the equipment and personnel contribute to guarantee the reproducibility of the quality of autologous PRP products, contrasting with extemporaneous preparation in a physician's office using a kit. Importantly, the use of sub-optimal preparation and administration protocols can potentially jeopardize the clinical efficacy of PRP intervention and incur elevated morbidity and healthcare costs. Based on the sensitive nature of blood products and on the invasiveness of the PRP administration route (i.e., local injection), maximal safety and quality of the final product are ensured by the use of an appropriate GMP production platform [31].

4.2. PRP Manufacturing Process Standardization for Enhanced Therapeutic Quality

The demographic data of the included patient sample in the present retrospective study show high diversity in patients affected by tendinopathies (Figure 4, Table 1). Therefore, the use of a standardized manufacturing process for autologous PRP preparation is considered as a prerequisite for sound evaluation of intervention clinical efficacy. Indeed, for diverse types of cytotherapies and orthobiologics, the manufacturing process itself is the product and the object of standardization [31,59,60]. Importantly, it was reported that for PRP, no clear relationship existed between increased platelet concentrations in the finished product and enhanced clinical outcomes [23–26,30]. Such reports were confirmed by the results of the present retrospective study, in which non-significant trends of higher concentration factors were noted in the patient group with a recorded positive evolution (Table 2). Importantly, the recorded platelet concentration factors were found to be in line with previously published reports on the use of autologous PRP (i.e., 2–3 concentration factors, Table 2) [27,44].

It is important to mention that general or specific patient-related factors (e.g., use of aspirin, anti-inflammatory drugs, estrogens, or hormone replacement therapy) can potentially impact platelet activity and negatively influence autologous PRP treatment efficacy. Furthermore, the technical specifications of the PRP manufacturing process (e.g., centrifugation speed and time) are known to affect platelet reactivity and platelet yields in the finished PRP preparation, which can also potentially affect the therapeutic efficacy of the intervention [23–25]. While patient-related variability and variable factors are difficult or impossible to modify, manufacturing process-related specifications may be optimized and validated in order to minimize the detrimental impacts on platelet yields and activity in the PRP therapeutic preparation. Specifically, development efforts should be directed toward the use of processing and formulation methods that enable the obtention of PRP products characterized by high platelet yields and purity, along with high levels of therapeutic activity [23,27,44].

From a manufacturing and control point-of-view, further autologous PRP product standardization could be performed, notably by normalizing the platelet concentration in the final PRP product; however, several factors argue against such steps. Firstly, the low dispersion of recorded platelet concentration factor values and the documented high process robustness in this retrospective study indicated that limited technical gains could be procured by such an endpoint normalization (Figure 5, Table 4). Secondly, the additional processing steps incurred by such a normalization would result in higher direct manufacturing costs, as the process would require adaptation to individual samples (e.g., use of larger volume syringes and conditioning materials). Thirdly, the risk level (i.e., microbial or particulate contamination) would be increased with a non-standardized final step of PRP manufacturing (e.g., sample-specific dilution in plasma for platelet concentration adjustment). Finally, as the relationship between platelet concentration in PRP and clinical efficacy is tendential at best, no significant clinical gains in terms of product efficacy may be considered or would justify a further effort to standardize the final product itself. For all of the aforementioned reasons and due to the specific nature of clinical treatment with autologous PRP, it may be concluded that optimal quality and efficiency may be attained with the appropriate use of an ad hoc and standardized GMP manufacturing workflow (Figure 2).

An alternative technical approach to PRP final product standardization would be individual growth factor or cytokine content-based standardization, which presents both advantages and disadvantages [36,44]. On the one hand, selection of a single quality control marker (i.e., or a small panel of markers) to be used for PRP product standardization is technically feasible, with the use of specific and sensitive analytical tools, and may be considered advantageous over the current controls performed using iterative platelet enumeration from an analytical viewpoint. However, inclusion of such analytical steps would incur additional manufacturing costs and may eventually not be accurate or robust due to inter-patient (i.e., natural variability) and intra-patient (i.e., depending on the

timepoint of blood collection) variability. Therefore, despite analytical precision advantages, high starting material variability and the need for the demonstration of enhanced clinical efficacy have not favored the widespread use of growth factor/cytokine-based autologous PRP product standardization [44].

Overall, the presented results confirmed the interest of using a standardized PRP manufacturing workflow, especially for patient cohorts presenting high demographic variability. Parallelly, it may be concluded that detailed investigation into patient demographic and diagnostic parameters is of prime importance for the appropriate demonstration of PRP intervention efficacy. By extension, the performance of large-scale retrospective studies on the therapeutic uses of PRP, taking into account all relevant demographic factors, could potentially enable the identification of predictive factors of standardized PRP intervention efficacy. This would, in turn, enable researchers to better define the applicable or optimal clinical pathway and treatment regimen using autologous PRP, thereby facilitating enhanced clinical success and rationalization of overall healthcare resources.

4.3. High Interest in Orthobiologics for Tendinopathy Management in Aging Populations

The overall prevalence of osteoarthritis, tendinopathy, and ligament injuries generally increases with age. Notably, rotator cuff tendinitis, rhizarthrosis, and carpal tunnel increase in women during perimenopause, without any direct link to physical activity or trauma [1–3,5,61]. In younger patients, physical activity or trauma-related injuries (e.g., cruciate ligaments, knee internal or external lateral ligaments, and Achilles tendon) are much more common [1–3,5]. Natural musculoskeletal tissue lesions are accentuated/aggravated following menopause or andropause [3,61]. Several points may be considered to tentatively explain these pathophysiological variations in connection with the endocrine status of patients. Specifically, estrogens and androgens have been shown to (i.e., directly or indirectly) influence cartilage tissue health [62]. Indirectly, as neurohormones, androgens affect behavior, mood, mental status, or cognition, and they impact on the willingness to undertake or exert physical effort [61,62]. Decreases in physical activity directly impact the release of growth hormone (GH), which can also be triggered by food intake and physical, psychic, or caloric stress during a fast [63]. Therefore, a decrease in metabolism associated with reduced physical activity favors an increase in patients' BMI, further reducing GH secretion and production of IGF-1, which regulates somatic growth and plays a central role in skeletal growth [61,63].

With regard to age-related variations in tendon tissue healing, the decline of the vascular bed can also contribute to the progression toward less efficient tissular repair, as well as to the emergence of clinically perceivable pain for minor lesions. Inflammation and vasculature in tendinopathy have been poorly studied as neo-vessels seem to be very different from the normal vasculature formed during tissue growth and are not fully functional [5,64]. Overall, there are three major mechanisms of tendon degeneration:

(i) Overuse of tendons implicating ECM degradation (e.g., improper training, muscle imbalance, and overload by repetition or acute excessive exercise) [2,3].
(ii) Formation of neo-vessels related to exogenous stresses and stimuli (e.g., pharmacological treatments, smoking and alcohol consumption, environmental factors, and diet) [5,64].
(iii) Tissue aging related to endogenous factors (e.g., gender, body weight, hormonal factors, genetics, prior injury, and co-morbidities) [5,61,63].

Many biological parameters and lifestyle choices are involved in chronic tendinopathies, leading to multifactorial etiologies and alterations. Among the possible alterations to normal tissular functions are perturbations of nerve function and vascularization, cell density and phenotypes changes, cell–cell interactions, cell–matrix interactions, cytokine balance, and overall tendon matrix alterations (Figure 3) [5,64]. Based on the high complexity of tendon-specific pathologies and healing process, an autologous biological-based therapeutic approach (e.g., PRP injections) appears to be an optimal management option [65,66]. PRP is easily available in standard clinical settings and contains a variety of bioactive

factors, such as PDGF, TGF, VEGF, IGF, or EGF, which are known to be actively involved in tissue healing [23,67,68]. Therefore, the use of autologous PRP may provide numerous beneficial outcomes for regenerative therapies in diverse clinical affections, especially if the manufacturing and administration processes are standardized [14,24,29].

Within autologous PRP treatments, high patient-related innate variability exists for the quantity and quality of platelets. A variety of reasons related to overall patient health, diet, genetics, and environmental factors may play a role in the efficacy obtained following the administration of autologous PRP [69–71]. Published reports have indicated that appropriate final platelet concentration in therapeutic PRP products is indication-specific [44,70]. Namely, a platelet concentrating factor of 7–12 is necessary to demonstrate efficacy in capillary transplantation, whereas musculoskeletal affections seem to respond with lower platelet concentrations and frequent PRP administrations [23–26,70,72]. Such reports were notably confirmed by the data gathered in the present retrospective study, where platelet concentration factors were most frequently found in the 2.0–2.5 interval (Figure 5). Therefore, indication-specific studies using standardized autologous PRP preparations appear to be a good starting point for devising optimal PRP dosing and administration regimens, with the endpoint objective being to enhance clinical efficacy and efficiency.

Taking all the abovementioned patient-related factors into account, it would seem logical to obtain poorer results in elderly and overweight patients with little or no physical activity and low androgen levels [61,62]. Such trends were partly confirmed by the detailed demographic analysis of the patients who reported a non-positive evolution following the PRP treatment (Figure 5). In this context, it could be advisable to consider different types of clinical regimens for autologous PRP enriched in platelets according to the age or the endocrinological status of patients, or to consider a chronic treatment pathway. This type of approach is considered to bear more potential for long-term success, reduced costs, and reduced morbidity when compared to more invasive techniques (e.g., autologous plasmapheresis) or alternative biological-based therapies, which require substantial manipulation.

4.4. Study Significance and Limitations

The present retrospective study sets forth the use of a fully GMP-compliant and accredited manufacturing platform for the preparation of finished autologous PRP products for clinical use. Although such practices should be applied in most cases according to the applicable legal bases in Europe and in Switzerland, very few clinical centers currently apply this level of quality and traceability for the preparation of autologous PRP. This is due to the well-known high variability in the content and application of the legal bases for PRP, as best shown within the European patchwork of laws, rules, and directives [29,51,52].

The first main study limitation consisted in the small size of the included patient sample (i.e., 48 patients included for retrospective analysis). This was mainly due to the limited number of patients treated for tendinopathies by PRP in Lausanne when compared to larger centers (e.g., in France or in the USA). However, this aspect is considered to be counterbalanced by the availability of high-quality original data pertaining to patient treatment and follow-up, as well as GMP manufacturing records for all the administered autologous PRP preparations. Notably, it is difficult to directly compare the present study to other published reports mainly due to the autologous PRP manufacturing method (i.e., GMP manufacturing of the final product versus the use of commercial PRP kits). However, similar trends were observed in the analyzed original data, such as the use of relatively low platelet concentration factors (e.g., 2–3 in value) for tendinopathy management and the absence of significant differences in efficacy related to final platelet concentrations.

Secondly, an accuracy issue was identified within the described GMP manufacturing process of autologous PRP with regard to the determination of platelet counts in whole blood and in the finished PRP preparations. Specifically, the mean platelet counts determined in whole blood were 73 $\pm$ 29 G/L for the samples included in the study, and 192 $\pm$ 96 G/L for the corresponding autologous PRP preparations. These values were found to be low with regard to normal whole blood platelet counts in the general popula-

tion (i.e., 150–450 G/L) [47,73–76]. It was concluded that the enumeration values recorded in the GMP manufacturing batch records did not accurately reflect the platelet counts in whole patient blood as the included patients were not clinically qualified as being thrombocytopenic. The ad hoc investigations into the origins of this systematic accuracy issue enabled us to exclude platelet counting mistakes as all sample analyses were performed on a Sysmex XN-9000 automated hematology instrument. Therefore, it is most probably the sample processing itself (i.e., use of multiple small blood harvest tubes, use of sodium citrate instead of EDTA, and prolonged time periods between blood collection and analysis) that systematically resulted in the obtention of low platelet counts in the samples. Despite the identified accuracy issues for platelet enumeration, the precision of the retained method was confirmed, notably in the context of the intra-patient variability investigation, as presented and discussed in this article.

Importantly, as the present study focused on the attributes of the final autologous PRP products and specifically on the platelet concentration factors characterizing the obtained PRP, the aforementioned systematic accuracy issues within platelet enumeration bear virtually no consequences (i.e., use of a relative value or platelet ratio, and not absolute values) and have not resulted in any kind of systematic clinical failures. However, this important element constitutes a prime real-world and experience-based example of the current need for additional standardization of manufacturing and control activities for PRP products [44,77–80]. Specifically, this could technically be performed by optimizing sample preparation workflows (i.e., primary containers, anticoagulant type, and analytical timelines) or by using and validating platelet enumeration methods, which are not only precise but also accurate in a variety of clinical settings.

5. Conclusions and Perspectives

This retrospective study considered the standardized GMP manufacturing and sports medicine-related clinical use of autologous PRP for tendinopathy management in 48 patients. Importantly, the results of this study have demonstrated the effectiveness of standardized autologous PRP preparations for tendinopathies in sports medicine. As shown in the presented results and related data interpretations, manufacturing process standardization and holistic integration of patient-related factors are necessary for an appropriate evaluation of autologous PRP administration efficacy. Notably, the available GMP manufacturing records have shown that relatively low platelet concentration factors (i.e., 2.0–2.5) are sufficient to obtain a positive evolution in most of the treated clinical cases. The statistical discussions of the available data enable us to outline the manufacturing process repeatability and robustness, which are critical factors to ensure the continuous provision of high-quality orthobiologic care to each patient. Furthermore, a detailed analysis of the demographic factors of the patients who reported a non-positive evolution following PRP treatment provides some insights into how to further perform clinical pathway optimization in complex cases. Overall, the presented results confirm the central importance of process and protocol standardization for autologous PRP manufacturing/control and clinical administration. These elements are considered as prerequisites for the reduction of inter-individual variability and for the sound assessment of therapeutic intervention efficacy. Finally, the analyses of process-based and patient-related elements in the present study may be useful in the establishment of enhanced clinical workflows for autologous PRP therapies, aiming to reduce tendinopathy-related morbidity and costs and ensure long-term patient remission. To further enhance the orthobiologic treatment's clinical efficiency for tendinopathies in sports medicine, relevant insights may be gained from best practices in transfusion medicine to ensure safe and appropriate PRP clinical use.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/bioengineering10040409/s1. Table S1: Theoretical model for statistical discussion of experimental data; Table S2: Detail of the gap analysis performed for the assessment of autologous PRP GMP manufacturing process robustness.

Author Contributions: Conceptualization, P.S., N.H.-B., C.S., P.A.-S., V.G., A.L., L.A.A. and G.G.; methodology, P.S., N.H.-B., W.R., A.L., L.A.A. and G.G.; validation, P.S., N.H.-B., C.S., P.A.-S., W.R., V.G., A.L., L.A.A. and G.G.; formal analysis, P.S., N.H.-B., C.S., W.R., V.G., A.L., L.A.A. and G.G.; investigation, P.S., V.G. and G.G.; resources, P.S., W.R., V.G. and L.A.A.; data curation, P.S., N.H.-B., C.S., A.L., L.A.A. and G.G.; writing—original draft preparation, P.S., A.L. and L.A.A.; writing—review and editing, P.S., N.H.-B., C.S., P.A.-S., W.R., V.G., A.L., L.A.A. and G.G.; visualization, N.H.-B., A.L. and L.A.A.; supervision, W.R., V.G., L.A.A. and G.G.; project administration, N.H.-B., W.R. and L.A.A.; funding acquisition, W.R. and L.A.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and was duly approved by the Vaud State Ethics Committee (N°CER-VD-ID#2022-00305, 2022) at the Lausanne University Hospital–CHUV, Lausanne, Switzerland [57].

Informed Consent Statement: Informed consent (i.e., formalized in a general informed consent agreement) was obtained from all patients or from their legal representatives at the time of treatment, for unrestricted use of the gathered and anonymized patient data.

Data Availability Statement: The data presented in this study are available upon reasonable request made in writing to the corresponding author.

Acknowledgments: The authors wish to thank the S.A.N.T.E and the Sandoz Family foundations, as well as the Lausanne Orthopedics Research Foundation (LORF), for their continuous support of the Regenerative Therapy Unit (CPR-UTR) at the CHUV, especially for the procurement of materials and consumables. The authors are grateful for the assistance of Murielle Michetti in the preparation of high-quality original graphical elements and professional illustrations. The artwork templates for the figures of the present study were partly created with www.biorender.com, accessed on 1 February 2023.

Conflicts of Interest: A.L. was employed by LAM Biotechnologies SA during the redaction of this work. The company LAM Biotechnologies SA had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. The remaining authors declare no conflicts of interest.

Abbreviations

BMI	body mass index
CHUV	centre hospitalier universitaire vaudois
EC	European Commission
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
EU	European Union
GAG	glycosaminoglycan
GH	growth hormone
GMP	Good Manufacturing Practices
GPG	Good Practice Guidelines
HA	hyaluronic acid
IGF	insulin-like growth factor
MD	medical device
NSAID	non-steroidal anti-inflammatory drugs
PDGF	platelet-derived growth factor
PPP	platelet-poor plasma
PRP	platelet-rich plasma
QC	quality control
RBC	red blood cells
SD	standard deviation
TGF	transforming growth factor
TPA	Therapeutic Products Act

USA	United States of America
UTR	regenerative therapy unit
VEGF	vascular endothelial growth factor

References

1. Scott, A.; Ashe, M.C. Common tendinopathies in the upper and lower extremities. *Curr. Sport Med. Rep.* **2006**, *5*, 233–241. [CrossRef]
2. Seinmann, S.; Pfeifer, C.G.; Brochhausen, C.; Docheva, D. Spectrum of tendon pathologies: Triggers, trails and end-state. *Int. J. Mol. Sci.* **2020**, *21*, 844. [CrossRef] [PubMed]
3. Clayton, R.A.; Court-Brown, C.M. The epidemiology of musculoskeletal tendinous and ligamentous injuries. *Injury* **2008**, *39*, 1338–1344. [CrossRef]
4. Hopkins, C.; Fu, S.C.; Chua, E.; Hu, X.; Rolf, C.; Mattila, V.M.; Qin, L.; Yung, P.S.; Chan, K.M. Critical review on the socio-economic impact of tendinopathy. *Asia Pac. J. Sport. Med. Arthrosc. Rehabil. Technol.* **2016**, *4*, 9–20. [CrossRef] [PubMed]
5. Sharma, P.; Maffulli, N. Tendon injury and tendinopathy: Healing and repair. *J. Bone Jt. Surg. Am.* **2005**, *87*, 187–202. [CrossRef]
6. Abate, M.; Schiavone, C.; Salini, V. The use of hyaluronic acid after tendon surgery and in tendinopathies. *BioMed Res. Int.* **2014**, *2014*, 783632. [CrossRef]
7. Ho, J.O.; Sawadkar, P.; Mudera, V. A review on the use of cell therapy in the treatment of tendon disease and injuries. *J. Tissue Eng.* **2014**, *5*, 2041731414549678. [CrossRef] [PubMed]
8. Lamplot, J.D.; Rodeo, S.A.; Brophy, R.H. A practical guide for the current use of biologic therapies in sports medicine. *Am. J. Sport. Med.* **2019**, *48*, 488–503. [CrossRef]
9. Costa-Almeida, R.; Calejo, I.; Gomes, M.E. Mesenchymal stem cells empowering tendon regenerative therapies. *Int. J. Mol. Sci.* **2019**, *20*, 3002. [CrossRef]
10. Romero, A.; Barrachina, L.; Ranera, B.; Remacha, A.R.; Moreno, B.; de Blas, I.; Sanz, A.; Vasquez, F.J.; Vitoria, A.; Junquera, C.; et al. Comparison of autologous bone marrow and adipose tissue derived mesenchymal stem cells, and platelet-rich plasma, for treating surgically induced lesions of the equine superficial digital flexor tendon. *Vet. J.* **2017**, *224*, 76–84. [CrossRef]
11. Van den Boom, N.A.C.; Winters, M.; Haisma, H.J.; Moen, M.H. Efficacy of stem cell therapy for tendon disorders: A systematic review. *Orthop. J. Sport. Med.* **2020**, *8*, 2325967120915857. [CrossRef]
12. Van der Vlist, A.C.; Winters, M.; Weir, A.; Ardern, C.L.; Welton, N.J.; Caldwell, D.M.; Verhaar, J.A.; de Vos, R.J. Which treatment is most effective for patients with Achilles tendinopathy? A living systematic review with network meta-analysis of 29 randomised controlled trials. *Br. J. Sport. Med.* **2021**, *55*, 249–256. [CrossRef]
13. Laurent, A.; Abdel-Sayed, P.; Grognuz, A.; Scaletta, C.; Hirt-Burri, N.; Michetti, M.; de Buys Roessingh, A.S.; Raffoul, W.; Kronen, P.; Nuss, K.; et al. Industrial development of standardized fetal progenitor cell therapy for tendon regenerative medicine: Preliminary safety in xenogeneic transplantation. *Biomedicines* **2021**, *9*, 380. [CrossRef] [PubMed]
14. Cash, C.; Scott, L.; Lane Walden, R.; Kuhn, A.; Bowman, E. Bibliometric analysis of the top 50 highly cited articles on platelet-rich plasma in osteoarthritis and tendinopathy. *Regen. Med.* **2022**, *17*, 491–506. [CrossRef]
15. Zhang, M.; Liu, H.; Cui, Q.; Han, P.; Yang, S.; Shi, M.; Zhang, T.; Zhang, Z.; Li, Z. Tendon stem cell-derived exosomes regulate inflammation and promote the high-quality healing of injured tendon. *Stem Cell Res. Ther.* **2020**, *11*, 402. [CrossRef] [PubMed]
16. Zhang, Z.; Li, Y.; Zhang, T.; Shi, M.; Song, X.; Yang, S.; Liu, H.; Zhang, M.; Cui, Q.; Li, Z. Hepatocyte growth factor-induced tendon stem cell conditioned medium promotes healing of injured Achilles tendon. *Front. Cell Dev. Biol.* **2021**, *9*, 654084. [CrossRef] [PubMed]
17. Laurent, A.; Porcello, A.; Fernandez, P.G.; Jeannerat, A.; Peneveyre, C.; Abdel-Sayed, P.; Scaletta, C.; Hirt-Burri, N.; Michetti, M.; de Buys Roessingh, A.; et al. Combination of hyaluronan and lyophilized progenitor cell derivatives: Stabilization of functional hydrogel products for therapeutic management of tendinous tissue disorders. *Pharmaceutics* **2021**, *13*, 2196. [CrossRef]
18. Lu, V.; Tennyson, M.; Zhang, J.; Khan, W. Mesenchymal stem cell-derived extracellular vesicles in tendon and ligament repair-A systematic review of in vivo studies. *Cells* **2021**, *10*, 2553. [CrossRef]
19. Yao, Z.; Li, J.; Xiong, H.; Cui, H.; Ning, J.; Wang, S.; Ouyang, X.; Qian, Y.; Fan, C. MicroRNA engineered umbilical cord stem cell-derived exosomes direct tendon regeneration by mTOR signaling. *J. Nanobiotech.* **2021**, *19*, 169. [CrossRef]
20. Song, K.; Jiang, T.; Pan, P.; Yao, Y.; Jiang, Q. Exosomes from tendon derived stem cells promote tendon repair through miR-144-3p-regulated tenocyte proliferation and migration. *Stem Cell Res. Ther.* **2022**, *13*, 80. [CrossRef]
21. Xue, Z.; Chen, Z.; Wu, T.; Li, R.; Chen, C.; Liu, J.; Hou, H.; Zheng, X.; Wang, H. VEGFA-enriched exosomes from tendon-derived stem cells facilitate tenocyte differentiation, migration, and transition to a fibroblastic phenotype. *BioMed Res. Int.* **2022**, *2022*, 8537959. [CrossRef]
22. Laurent, A.; Porcello, A.; Jeannerat, A.; Peneveyre, C.; Coeur, A.; Abdel-Sayed, P.; Scaletta, C.; Michetti, M.; de Buys Roessingh, A.; Jordan, O.; et al. Lyophilized progenitor tenocyte extracts: Sterilizable cytotherapeutic derivatives with antioxidant properties and hyaluronan hydrogel functionalization effects. *Antioxidants* **2023**, *12*, 163. [CrossRef]
23. Alsousou, J.; Thompson, M.; Hulley, P.; Noble, A.; Willett, K. The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery. *J. Bone Joint Surg.* **2009**, *91*, 987–996. [CrossRef]
24. Hsu, W.K.; Mishra, A.; Rodeo, S.R.; Fu, F.; Terry, M.A.; Randelli, P.; Canale, S.T.; Kelly, F.B. Platelet-rich plasma in orthopaedic applications: Evidence-based recommendations for treatment. *J. Am. Acad. Orthop. Surg.* **2013**, *21*, 739–748. [CrossRef]

25. Samadi, P.; Sheykhhasan, M.; Khoshinani, H.M. The use of platelet-rich plasma in aesthetic and regenerative medicine: A comprehensive review. *Aesth. Plast. Surg.* **2019**, *43*, 803–814. [CrossRef] [PubMed]
26. Dong, C.; Sun, Y.; Qi, Y.; Zhu, Y.; Wei, H.; Wu, D.; Li, C. Effect of platelet-rich plasma injection on mild or moderate carpal tunnel syndrome: An updated systematic review and meta-analysis of randomized controlled trials. *BioMed Res. Int.* **2020**, *2020*, 5089378. [CrossRef]
27. Coulange Zavarro, A.; De Girolamo, L.; Laver, L.; Sánchez, M.; Tischer, T.; Filardo, G.; Sabatier, F.; Magalon, J. The top 100 most cited articles on platelet-rich plasma use in regenerative medicine–A bibliometric analysis–from the ESSKA orthobiologic initiative. *Bioengineering* **2022**, *9*, 580. [CrossRef]
28. Akhundov, K.; Pietramaggiori, G.; Waselle, L.; Darwiche, S.; Guerid, S.; Scaletta, C.; Hirt-Burri, N.; Applegate, L.A.; Raffoul, W. Development of a cost-effective method for platelet-rich plasma (PRP) preparation for topical wound healing. *Ann. Burns Fire Dis.* **2012**, *4*, 207–213.
29. Fiorentino, S.; Roffi, A.A.L.A.; Filardo, B.G.; Marcacci, M.; Kon, E. European definitions, current use, and EMA stance of platelet-rich plasma in sports medicine. *J. Knee Surg.* **2015**, *1*, 51–54. [CrossRef]
30. Yaman, R.; Kinard, T.N. Platelet rich plasma: Hope or hype? *Ann. Blood* **2022**, *7*, 1–7. [CrossRef]
31. Chemali, M.; Laurent, A.; Scaletta, C.; Waselle, L.; Simon, J.-P.; Michetti, M.; Brunet, J.-F.; Flahaut, M.; Hirt-Burri, N.; Raffoul, W.; et al. Burn center organization and cellular therapy integration: Managing risks and costs. *J. Burn Care Res.* **2021**, *42*, 911–924. [CrossRef]
32. Martinez, C.E.; Smith, P.C.; Palma Alvarado, V.A. The influence of platelet-derived products on angiogenesis and tissue repair: A concise update. *Front. Physiol.* **2015**, *6*, 290. [CrossRef] [PubMed]
33. Andriolo, L.; Altamura, S.A.; Reale, D.; Candrian, C.; Zaffagnini, S.; Filardo, G. Nonsurgical treatments of patellar tendinopathy: Multiple injections of platelet-rich plasma are a suitable option: A systematic review and meta-analysis. *Am. J. Sport. Med.* **2019**, *47*, 1001–1018. [CrossRef] [PubMed]
34. Senna, M.K.; Shaat, R.M.; Alaa Ali Awad, A. Platelet-rich plasma in treatment of patients with idiopathic carpal tunnel syndrome. *Clin. Rheumatol.* **2019**, *38*, 3643–3654. [CrossRef] [PubMed]
35. Hong, P.; Zheng, Y.; Rai, S.; Din, Y.; Zhou, Y.; Liu, R.; Li, J. Efficacy and safety of platelet-rich plasma in the treatment of carpal tunnel syndrome: A network meta-analysis of different injection treatments. *Front. Pharmacol.* **2022**, *13*, 906075. [CrossRef]
36. Galán, V.; Iñigo-Dendariarena, I.; Galán, I.; Prado, R.; Padilla, S.; Anitua, E. The effectiveness of plasma rich in growth factors (PRGF) in the treatment of nerve compression syndromes of the upper extremity: A retrospective observational clinical study. *J. Clin. Med.* **2022**, *11*, 4789. [CrossRef] [PubMed]
37. Gupta, A.; Ortiz-Babilonia, C.; Xu, A.L.; Rogers, D.; Vulcano, E.; Aiyer, A.A. The statistical fragility of platelet-rich plasma as treatment for plantar fasciitis: A systematic review and simulated fragility analysis. *Foot Ankle Orthop.* **2022**, *7*, 24730114221144049. [CrossRef]
38. Gupta, A.K.; Cole, J.; Deutsch, D.P.; Everts, P.A.; Niedbalski, R.P.; Panchaprateep, R.; Rinaldi, F.; Rose, P.T.; Sinclair, R.; Vogel, J.E.; et al. Platelet-rich plasma as a treatment for androgenetic alopecia. *Dermatol. Surg.* **2019**, *45*, 1262–1273. [CrossRef]
39. Gentile, P.; Garcovich, S. Systematic review: Adipose-derived mesenchymal stem cells, platelet-rich plasma and biomaterials as new regenerative strategies in chronic skin wounds and soft tissue defects. *Int. J. Mol. Sci.* **2021**, *22*, 1538. [CrossRef]
40. Bigby, M.; Grimalt, R. Platelet-rich plasma lacks evidence of clinically significant improvement in androgenetic alopecia. *J. Am. Acad. Dermatol.* **2021**, *84*, 1183–1185. [CrossRef]
41. Chan, G.K.L.; Guo, M.S.; Dai, D.K.; Lai, Q.W.S.; Fung, K.W.C.F.; Zheng, B.Z.; Wu, K.Q.; Man, B.K.K.; Dong, T.T.; Tsim, K.W.K. An optimized extract, named self-growth colony, from platelet-rich plasma shows robust skin rejuvenation and anti-ageing properties: A novel technology in development of cosmetics. *Skin Pharmacol. Physiol.* **2021**, *34*, 74–85. [CrossRef]
42. Chamata, E.S.; Bartlett, E.L.; Weir, D.; Rohrich, R.J. Platelet-rich plasma: Evolving role in plastic surgery. *Plast. Reconstr. Surg.* **2021**, *147*, 219–230. [CrossRef] [PubMed]
43. Gentile, P.; Alves, R.; Cole, J.; Andjelkov, K. 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. *Exp. Opin. Biol. Ther.* **2021**, *21*, 1443–1449. [CrossRef]
44. Fadadu, P.P.; Mazzola, A.J.; Hunter, C.W.; Davis, T.T. Review of concentration yields in commercially available platelet-rich plasma (PRP) systems: A call for PRP standardization. *Reg. Anesth. Pain Med.* **2019**, *44*, 652–659. [CrossRef] [PubMed]
45. Freedman, B.R.; Mooney, D.J.; Weber, E. Advances toward transformative therapies for tendon diseases. *Sci. Transl. Med.* **2022**, *14*, eabl8814. [CrossRef]
46. Dhurat, R.; Sukesh, M.S. Principles and methods of preparation of platelet-rich plasma: A review and author's perspective. *J. Cutan. Aesthet. Surg.* **2014**, *7*, 189–197. [CrossRef] [PubMed]
47. Klatte-Schulz, F.; Schmidt, T.; Uckert, M.; Scheffler, S.; Kalus, U.; Rojewski, M.; Schrezenmeier, H.; Pruss, A.; Wildemann, B. Comparative analysis of different platelet lysates and platelet rich preparations to stimulate tendon cell biology: An in vitro study. *Int. J. Mol. Sci.* **2018**, *19*, 212. [CrossRef]
48. European Parliament and Council. Directive 2002/98/EC setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending Directive 2001/83/EC. *Off. J. Eur. Union* **2003**, *33*, 30–40.

49. European Parliament and Council. Directive 2004/33/EC implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components. *Off. J. Eur. Union* **2004**, *91*, 25–39.
50. European Parliament and Council. Directive 2005/62/EC implementing Directive 2002/98/EC of the European Parliament and of the Council as regards Community standards and specifications relating to a quality system for blood establishments. *Off. J. Eur. Union.* **2005**, *256*, 41–48.
51. Anitua, E.; Prado, R.; Orive, G. Closing regulatory gaps: New ground rules for platelet-rich plasma. *Trends Biotech.* **2015**, *33*, 492–495. [CrossRef]
52. Anitur, E.; Prado, R.; Orive, G. A new regulatory framework for platelet-rich plasma in Spain. *J. Knee Surg.* **2015**, *28*, 355–356. [CrossRef]
53. European Parliament and Council. Directive 2016/1214 amending Directive 2005/62/EC as regards quality system standards and specifications for blood establishments. *Off. J. Eur. Union* **2016**, *199*, 14–15.
54. European Parliament and Council. Regulation (EU) 2017/745 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC. *Off. J. Eur. Union* **2017**, *117*, 1–175.
55. European Parliament and Council. Regulation (EU) 2017/746 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU. *Off. J. Eur. Union* **2017**, *117*, 176–332.
56. Sussman, W.I.; Mautner, K.; Malanga, G. The role of rehabilitation after regenerative and orthobiologic procedures for the treatment of tendinopathy: A systematic review. *Regen. Med.* **2018**, *13*, 249–263. [CrossRef] [PubMed]
57. World Medical Association. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA* **2013**, *310*, 2191–2194. [CrossRef]
58. Federal Assembly of the Swiss Confederation. *Federal Law on Medication and Medical Devices (Law on Therapeutic Products) SR 812.21*; Switzerland's Federal Council: Bern, Switzerland, 2000. Available online: https://fedlex.data.admin.ch/eli/cc/2001/422 (accessed on 1 February 2023).
59. Laurent, A.; Hirt-Burri, N.; Scaletta, C.; Michetti, M.; de Buys Roessingh, A.S.; Raffoul, W.; Applegate, L.A. Holistic approach of Swiss fetal progenitor cell banking: Optimizing safe and sustainable substrates for regenerative medicine and biotechnology. *Front. Bioeng. Biotechnol.* **2020**, *8*, 557758. [CrossRef] [PubMed]
60. Philippe, V.; Laurent, A.; Hirt-Burri, N.; Abdel-Sayed, P.; Scaletta, C.; Schneebeli, V.; Michetti, M.; Brunet, J.-F.; Applegate, L.A.; Martin, R. Retrospective analysis of autologous chondrocyte-based cytotherapy production for clinical use: GMP process-based manufacturing optimization in a Swiss university hospital. *Cells* **2022**, *11*, 1016. [CrossRef]
61. Fink, G.; Summer, B.E.H.; McQueen, J.K.; Wilson, H.; Rosie, R. Sex hormones, mood, mental state and memory. *Clin. Exp. Pharm. Physiol.* **1998**, *25*, 764–775. [CrossRef]
62. Sniekers, Y.H.; Weinans, H.; van Osch, G.J.V.M.; van Leeuwen, J.P.T.M. Estrogen is important for maintenance of cartilage and subchondral bone in a murine model of knee osteoarthritis. *Arthritis Res. Ther.* **2010**, *12*, R182. [CrossRef]
63. Dixit, M.; Bahadur Poudel, S.; Yakar, S. Effects of GH/IGF axis on bone and cartilage. *Mol. Cell. Endocrinol.* **2020**, *519*, 111052. [CrossRef] [PubMed]
64. Järvinen, T.A.H. Neovascularization in tendinopathy: From eradication to stabilization. *Br. J. Sport. Med.* **2020**, *54*, 1–2. [CrossRef] [PubMed]
65. Petrou, I.G.; Grognuz, A.; Hirt-Burri, N.; Raffoul, W.; Applegate, L.A. Cell therapies for tendons: Old cell choice for modern innovation. *Swiss Med. Wkly* **2014**, *144*, w13989. [CrossRef]
66. Grognuz, A.; Aeberhard, P.-A.; Michetti, M.; Hirt-Burri, N.; Scaletta, C.; de Buys Roessingh, A.; Raffoul, W.; Laurent-Applegate, L.A. Cell therapies for tendon: Treatments and regenerative medicine. In *Regenerative Medicine and Plastic Surgery*; Duscher, D., Shiffman, M.A., Eds.; Springer: Cham, Switzerland, 2019. [CrossRef]
67. Pavlovic, V.; Ciric, M.; Jovanovic, V.; Stojanovic, P. Platelet rich plasma: A short overview of certain bioactive components. *Open Med.* **2016**, *11*, 242–247. [CrossRef]
68. Boswell, S.G.; Cole, B.J.; Sundman, E.A.; Karas, V.; Fortier, L.A. Platelet-rich plasma: A milieu of bioactive factors. *Arthroscopy* **2012**, *28*, 429–439. [CrossRef]
69. Gupta, A.K.; Renaud, H.J.; Bamimore, M. Platelet-rich plasma for androgenetic alopecia: Efficacy differences between men and women. *Dermat. Ther.* **2020**, *33*, e14143. [CrossRef]
70. Bansal, H.; Leon, J.; Pont, J.L.; Wilson, D.A.; Bansal, A.; Agarwal, D.; Preoteasa, I. Platelet-rich plasma (PRP) in osteoarthritis (OA) knee: Correct dose critical for long term clinical efficacy. *Sci. Rep.* **2021**, *11*, 3971. [CrossRef]
71. Popescu, M.N.; Iliescu, M.G.; Beiu, C.; Popa, L.G.; Mihai, M.M.; Berteanu, M.; Ionescu, A.M. Autologous platelet-rich plasma efficacy in the field of regenerative medicine: Product and quality control. *BioMed Res. Int.* **2021**, *2021*, 4672959. [CrossRef]
72. Wasterlain, A.S.; Braun, H.J.; Harris, A.H.S.; Kim, H.J.; Dragoo, J.L. The systemic effects of platelet-rich plasma injection. *Am. J. Sport. Med.* **2013**, *41*, 186–193. [CrossRef] [PubMed]
73. Bernardi, M.; Agostini, F.; Chieregato, K.; Amati, E.; Durante, C.; Rassu, M.; Ruggeri, M.; Sella, S.; Lombardi, E.; Mazzucato, M.; et al. The production method affects the efficacy of platelet derivatives to expand mesenchymal stromal cells in vitro. *J. Transl. Med.* **2017**, *15*, 90. [CrossRef] [PubMed]
74. Harrison, T.E.; Bowler, J.; Levins, T.N.; Reeves, K.D.; Cheng, A.L. Platelet-rich plasma centrifugation changes leukocyte ratios. *Cureus* **2021**, *13*, e14470. [CrossRef]

75. Machado, E.S.; Leite, R.; Dos Santos, C.C.; Artuso, G.L.; Gluszczak, F.; de Jesus, L.G.; Caldas, J.M.P.; Bredemeier, M. Turn down—Turn up: A simple and low-cost protocol for preparing platelet-rich plasma. *Clinics* **2019**, *74*, e1132. [CrossRef] [PubMed]
76. Beitzel, K.; Allen, D.; Apostolakos, J.; Russell, R.P.; McCarthy, M.B.; Gallo, G.J.; Cote, M.P.; Mazzocca, A.D. US definitions, current use, and FDA stance on use of platelet-rich plasma in sports medicine. *J. Knee Surg.* **2015**, *28*, 29–34. [CrossRef]
77. Graiet, H.; Lokchine, A.; Francois, P.; Velier, M.; Grimaud, F.; Loyens, M.; Berda-Haddad, Y.; Veran, J.; Dignat-George, F.; Sabatier, F.; et al. Use of platelet-rich plasma in regenerative medicine: Technical tools for correct quality control. *BMJ Open Sport Exerc. Med.* **2018**, *4*, e000442. [CrossRef] [PubMed]
78. Tey, R.V.; Haldankar, P.; Joshi, V.R.; Raj, R.; Maradi, R. Variability in platelet-rich plasma preparations used in regenerative medicine: A comparative analysis. *Stem Cells Int.* **2022**, *2022*, 3852898. [CrossRef] [PubMed]
79. Magalon, J.; Brandin, T.; Francois, P.; Degioanni, C.; De Maria, L.; Grimaud, F.; Veran, J.; Dignat-George, F.; Sabatier, F. Technical and biological review of authorized medical devices for platelets-rich plasma preparation in the field of regenerative medicine. *Platelets* **2021**, *32*, 200–208. [CrossRef] [PubMed]
80. Sebbagh, P.; Cannone, A.; Gremion, G.; Gremeaux, V.; Raffoul, W.; Hirt-Burri, N.; Michetti, M.; Abdel-Sayed, P.; Laurent, A.; Wardé, N.; et al. Current status of PRP manufacturing requirements & European regulatory frameworks: Practical tools for the appropriate implementation of PRP therapies in musculoskeletal regenerative medicine. *Bioengineering* **2023**, *10*, 292. [CrossRef]