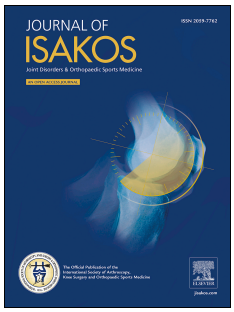

Review of dohan eherenfest et al. (2009) on “classification of platelet concentrates: from pure platelet-rich plasma (p-prp) to leucocyte- and platelet-rich fibrin (l-prf)”

Theodorakys Marín Fermín, MD, Jacob G. Calcei, MD, Franco Della Vedova, MD, Juan Pablo Martinez Cano, MD, Claudia Arias Calderon, MD, Mohamed A. Imam, MD, MSc, D. SportMed, PhD, FRCS (Tr and Orth), Miguel Khoury, MD, Markus W. Laupheimer, MD, MBA, MSc, MFSEM, Pieter D’hooghe, MD, MSc, MBA

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REVIEW OF DOHAN EHERENFEST ET AL. (2009) ON "CLASSIFICATION OF PLATELET CONCENTRATES: FROM PURE PLATELET-RICH PLASMA (P-PRP) TO LEUCOCYTE- AND PLATELET-RICH FIBRIN (L-PRF)"

Theodorakys MARÍN FERMÍN, MD[1]#

Jacob G. CALCEI, MD[2]#

Franco DELLA VEDOVA, MD[3]#

Juan Pablo MARTINEZ CANO, MD[4]#

Claudia ARIAS CALDERON, MD[5]#

Mohamed A. IMAM, MD, MSc, D. SportMed, PhD, FRCS (Tr and Orth)[6,7]#

Miguel KHOURY, MD[1]

Markus W. LAUPHEIMER, MD, MBA, MSc, MFSEM[1]

Pieter D'HOOGHE, MD, MSc, MBA[1]#

Affiliations:

[1]Aspetar Orthopaedic and Sports Medicine Hospital, Sports City Street, Inside Aspire Zone, Al Buwairda St, 29222, Doha, Qatar.

[2]University Hospitals Drusinsky Sports Medicine Institute, Case Western Reserve University, 11100 Euclid Ave, Cleveland, OH, 44106, USA

[3]Instituto "Dr. Jaime Slullitel", Rosario, Santa Fe, Argentina.

[4]Departamento de Ortopedia, Fundación Valle del Lili, Cra 98 No. 18-49, 760032, Cali, Colombia.

[5]Department of Trauma and Orthopedic Surgery, Hospital Nacional Edgardo Rebagliati Martins, Jesús María, Peru.

[6]Smart Health Centre, University of East London, London, UK.

[7]Rowley Bristow Orthopaedic Unit, Ashford and St Peter's Hospital, Ashford, UK.

ISAKOS Young Professionals Task Force.

*Corresponding author: Theodorakys Marín Fermín, MD. Aspetar Orthopaedic and Sports Medicine Hospital, Sports City Street, Inside Aspire Zone, Al Buwairda St, 29222, Doha, Qatar.

E-mail: theodorakysmarin@yahoo.com.

Telephone number: +58 4143727046.

Contributions:

TMF: conceptualization, investigation, writing original draft, visualization, project administration; JGC: investigation, writing - original draft; FDV: resources, writing - review & editing; JPMC: resources, writing - review & editing; CAC: resources, writing - review & editing; MI: writing - review & editing, supervision; MK: validation, data curation, writing - review & editing, supervision; MWL: validation, writing - review & editing, supervision; PDH: validation, writing - review & editing, supervision.

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REVIEW OF DOHAN EHERENFEST ET AL. (2009) ON "CLASSIFICATION OF PLATELET CONCENTRATES: FROM PURE PLATELET-RICH PLASMA (P-PRP) TO LEUCOCYTE- AND PLATELET-RICH FIBRIN (L-PRF)"

ABSTRACT

This classic discusses the original publication of Dohan Eherenfest et al. on "Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF)", in which the authors propose four categories of platelet concentrates depending on their leukocyte and fibrin content (P-PRP, leucocyte- and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF), and L-PRF) to group a "jungle" of products in which the term platelet-rich plasma (PRP) was used indistinctly. They were able to identify common factors such as: (1) the use of anticoagulant and immediate centrifugation of the blood after its collection, (2) most preparation techniques allowed platelet concentrate preparation within an hour, (3) the centrifugation aimed to separate the blood in layers that would allow the extraction of specific fractions, and (4) the product was activated with thrombin or calcium chloride. The reviewed manuscript has been listed among the most cited PRP articles in regenerative medicine, with more than 800 citations, driving the current scientific research and clinical practice by categorizing L-PRP and P-PRP (now, leukocyte-poor PRP). The classification has also opened the door to understanding intrinsic biological mechanisms between the platelets, leukocytes, fibrin, and growth factors, later considered for studying the proliferation and differentiation of cells in different tissues affected by PRP. Since the initial classification of platelet concentrates, several other classification systems have been proposed and published in the current literature, such as the PAW, Mishra, PLRA, DEPA, MARSPILL, etc. These classifications have identified important aspects of PRP that affect the biological composition and, ultimately, the indications and outcomes. To date, there is still a lack of standardization in sample

preparation, cohort heterogeneity, and incomplete reporting of sample preparation utilized, leading to a lack of clarity and challenging researchers and clinicians.

Keywords: platelet-rich plasma, classification, platelet concentrates, orthobiologics, growth factors, leukocytes.

ABBREVIATIONS

DEPA	Dose of platelet, efficiency, purity, and activation
L-PRF	Leucocyte- and platelet-rich fibrin
L-PRP	Leucocyte- and platelet-rich plasma
MARSPILL	Method, activation, red blood cells, spin, platelets, image guidance, leukocytes, and light activation
MIBO	Minimum information for studies evaluating biologics in orthopaedics
PAW	Platelet, activation, white blood cell
PLRA	Platelet, leukocyte, red blood cells, and activation
P-PRF	Pure platelet-rich fibrin
P-PRP	Pure platelet-rich plasma (now, leukocyte-poor platelet-rich plasma)
PRP	Platelet-rich plasma

INTRODUCTION

Platelet-rich plasma (PRP) is a worldwide implemented regenerative medicine therapy. The clinical applications and use of platelet-rich therapy in medicine and surgery have thrived over the past two decades. It was described in 1970 as a plasma portion from autologous blood with increased platelet concentration obtained by a centrifugation process [1].

PRP harnesses the signaling molecules and growth factors of platelets such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), epidermal growth factor, insulin-like growth factors (e.g., IGF-1, IFF-2), and others that enhance the natural healing potential of tissues, pain and inflammation modulation, and functional improvement [1].

PRP is currently used to treat multiple musculoskeletal conditions, including knee osteoarthritis, cartilage injuries, patellar tendonitis, and tennis elbow [2, 3]. The media, celebrity athletes, the desire for novel treatments, and its autologous nature have boosted its use in multiple sports-related injuries during the last decades [4-7]. As a result, its high demand has led to an industry-driven development of various platelet concentrate systems and products exceeding the pace of evidence-based practice [1, 3, 7, 8].

In 2009, Dohan Eherenfest et al. proposed the first attempt at PRP classification to categorize the platelet concentrates concerning their fibrin and leukocyte content and the degree of standardization of the procedure, providing an overview of the available systems [9]. This classification later inspired authors to investigate the role of the different PRP components and the development of new classifications and reporting guidelines, highlighting leukocyte properties in orthobiologics products. However, the goal of standardization still seems far. A systematic review by Magalon et al. [8] revealed a great heterogeneity among fifty platelet concentrate products from

forty companies, which may explain the inconsistent outcomes in the literature. Thus, as a scientific orthopedic community, we should question ourselves: how far have we gotten from the first attempt at orthobiologics classification? or has the panorama changed since then?

CONSIDERATION

Historical perspective

In 2009, Dohan Eherenfest et al. [9] faced a scenario where commercial interests were obscuring real clinical benefits, a "jungle" in their own words, developing a plethora of preparation methods, systems, and centrifuges, and multiple platelet-derived products were covered by the umbrella term PRP which did not allow a distinction between them. However, the authors were able to identify common factors among the available products, such as: (1) the use of anticoagulant and immediate centrifugation of the blood after its collection, (2) most preparation techniques allowed platelet concentrate preparation within an hour, (3) the centrifugation aimed to separate the blood in layers that would allow the extraction of specific fractions, and (4) the product was activated with thrombin or calcium chloride. The situation led his team to propose a classification to provide an objective approach to the growth and advance of PRP therapy [9].

Back then, Dohan Ehrenfest and colleagues were implementing Choukroun's leukocyte- and platelet-rich fibrin (L-PRF) protocol in oral and maxillofacial surgery (Box 1 and 2) [10]. The technique's benefits included a high efficiency in retrieving and concentrating platelets and leukocytes and its semisolid and three-dimensional fibrin matrix structure mimicking a natural blood clot. However, it was technically demanding because its success depended on rapid blood collection and centrifugation. After all, the lack of anticoagulant deemed almost instant coagulation of the blood once in contact with the walls of the dry-glass tubes. Otherwise, the fibrin would polymerize diffusely in the tube, failing to concentrate most of the available platelets.

Understanding the rise of the idea – The clinical implication

The proposed platelet concentrates classification of Dohan Eherenfest and colleagues included three main parameters (Table 1), allowing the characterization of platelet concentrates in pure platelet-rich plasma (P-PRP), leukocyte- and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF), and L-PRF [9].

This classification brought new insights into the relevance of other components of platelet concentrates, the leukocytes, and fibrin. By then, anti-microbial and immunomodulatory properties were already attributed to the leukocytes in platelet concentrates and their role in angiogenesis with vascular endothelial growth factor synthesis [11-14]. The role of L-PRP in tendon healing was starting its rise [15]. Similarly, they supported the theory that the fibrin matrix and its composition (including cytokines) were crucial for the platelet concentrates clinical efficacy. In fact, the clotting pattern they were inducing in Choukron's L-PRF technique enhanced platelet growth factor release. The author confirmed his claims with the publication of another article in 2012 [16], where his team proved not only an increased release of growth factors but also a more extended release period (seven days) due to the naturally formed dense fibrin network in contrast to the light fibrin network present in P-PRP, observed when artificial activation was triggered with bovine thrombin, calcium chloride or other clotting agents [17-19].

Scientific and societal impact

In a recent publication by the European Society for Sports Traumatology, Knee Surgery, and Arthroscopy Orthobiologic Initiative, the paper by Dohan Ehrenfest et al. [9] was listed fourth among the most cited PRP articles in regenerative medicine with more than 800 citations and including two additional papers from the author in the top 100 [6]. The current scientific research and clinical practice, driven by the main categorization between L-PRP and P-PRP (now, leukocyte-poor PRP),

confirms the impact of this article. Indeed, recent studies have proposed specific roles of different leukocytes in PRP clinical efficiency depending on the healing stage and the type of injury [20]. Lymphocytes, for example, have an anti-inflammatory role by steering monocyte differentiation from M1 to the M2 subtype. On the other hand, neutrophils lead the so-called "regenerative inflammation" by secreting chemokines to recruit macrophages and promoting an inflammatory process desired to trigger the healing process [20, 21].

Before the classification system proposed by Dohan Ehrenfest et al. [9], the characterization of platelet concentrates was confusing and contradictory, and created a methodological bias in many publications. The authors addressed this challenging problem by simply proposing a classification framework. Additionally, the classification opened the door to understanding intrinsic biological mechanisms between the platelets, leukocytes, fibrin, and growth factors later considered for studying the proliferation and differentiation of cells in different tissues affected by PRP. In other words, the authors proposed evaluating these products as living tissues instead of pharmaceutical preparations with a simple and precise composition.

Current evidence

After highlighting the importance of classifying the platelet concentrates according to the presence of leukocytes, several clinical and laboratory studies conducted during the last decade have demonstrated clinical benefit, especially in tendinopathies. In a controlled laboratory study, Lin et al. [22] revealed a higher induction of platelet growth factors and tenocyte proliferation with L-PRP preparations than P-PRP. Furthermore, a network meta-analysis of 18 randomized controlled trials by Fitzpatrick et al. [3] showed that the most significant positive outcomes were obtained from a single ultrasound-guided L-PRP injection in tendinopathies such as rotator cuff, tennis elbow, patellar tendon, and Achilles tendon.

On the other hand, the presence of leukocytes in PRP has been found to be chondrotoxic, while in the absence of leukocytes, PRP promotes chondrogenesis [23, 24]. However, clinical studies on knee osteoarthritis have shown conflicting results when comparing both PRP preparations [24-26]. Although L-PRP has offered comparable results to leukocyte-poor preparations, the latter is preferred due to a higher risk of swelling from the increased inflammatory response [27].

Lessons learned

As with many biologic therapies in medicine, particularly musculoskeletal medicine, additional research has often led to more questions than answers. Quite possibly the most important lesson learned with biologic treatments, and PRP specifically, is that there is a vast range of variability not only in the PRP preparation (instruments, devices, spin rate and time, activators, among others) but also in the quality of the product due to inter- and intra-human variability. Since PRP is an autologous product, the quality of the sample, growth factors concentration, and activity of the components within the specimen are likely affected by the health status of the individual, medications, diet, and cortisol stress levels, among others. Furthermore, the heterogeneity of clinical outcomes among the studies has also moved the spotlight to the key elements that allow patients to benefit from PRP therapy. Researchers have now classified patients into responders and non-responders and started phenotyping the ideal patient [28-31].

New developments – New classifications and research originated from the original study

Since the initial classification of platelet concentrates, several other classification systems have been proposed and published in the current literature. Rossi et al. [32] recently reviewed the available classification systems for PRP, examining the advantages and limitations of each (Figure 1). Rossi and colleagues acknowledge the Dohan Ehrenfest classification system and its basic component breakdown.

DeLong et al. [33] presented their classification system in 2012, known as the platelet, activation, white blood cell (PAW) classification. The PAW classification system was based on the absolute number of platelets (P1 to P4, depending on the number of platelets), the method of platelet activation, and the presence (α) or absence (β) of white blood cells. Mishra et al. [34] presented a similar classification system. However, they classified the variables differently, which resulted in four separate categories of PRP: elevated platelets and leukocytes without an external activator, elevated platelets and leukocytes with an external activator, elevated platelets without leukocytes and no external activator, and elevated platelets without leukocytes but with an external activator.

The classification systems continued to evolve as Mautner et al. [35] noted the importance of red blood cells and their potential detrimental effects in PRP; thus, they added red blood cell analysis to the classification known as platelet, leukocyte, red blood cells, and activation (PLRA). Magalon et al. [36] then proposed the dose of platelet, efficiency, purity, and activation (DEPA) classification. In this classification system, the proportion of the platelets recovered from PRP and the purity of the PRP sample was included as essential qualities of the sample preparation. Lana et al. [37] proposed a classification in 2017 evaluating method, activation, red blood cells, spin, platelets, image guidance, leukocytes, and light activation (MARSPILL). Finally, The Platelet Physiology Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis has recently proposed a classification system that includes the presence of leukocytes, red blood cells, activation product, platelet concentration, and preparation category [38]. Individually, these classification systems have identified important aspects of PRP that affect the biological composition and, ultimately, the indications and outcomes. In any situation, simple and elegant classification systems are often preferred due to their ease of use; however, the bulkier classification systems are typically more comprehensive. Based on the current indications of PRP, the presence or absence of leukocytes seems to be the main dividing point. Still, the complexity

of the makeup of PRP makes a simple and elegant classification system quite challenging to develop. While there is no perfect classification system to rely entirely on when evaluating biologics, including PRP, the field strives to characterize this biological product better. Until we determine exactly what elements of PRP are most important in affecting outcomes, we may be stuck relying on bulky systems or even combining multiple classification systems.

Future directions

Much of the challenge in practicing evidence-based medicine in the case of biological treatments is the lack of standardization in sample preparation, the heterogeneity in cohorts, and the incomplete reporting of sample preparation utilized. Thus, there has been a call for minimum reporting standards for studies involving biologics in musculoskeletal care [39-42]. Specifically, Murray et al. [41] reported the 23-item checklist compiled by the PRP working group to report minimum information for studies evaluating biologics in orthopaedics (MIBO). In an assessment of the 50 most cited articles related to PRP in musculoskeletal medicine, Bugarin et al. [43] reported a high level of evidence in approximately 50% of the studies. Still, most of the studies were of only fair methodological quality. Systematic reviews by Chahla et al. [44], DeClercq et al. [45], and Marín Fermín et al. [5] have revealed that less than 10% of the studies provided a clear description of the implemented PRP preparation protocol, which significantly limits the study's reproducibility. A paucity of accurate reporting of a highly variable product has led to a lack of clarity and continues challenging researchers and clinicians. In this sense, journal editors can play an essential role in evidence quality improvement by requesting mandatory adherence to acceptable orthobiologics reporting guidelines in submission and review [46]. The goal of the present decade is to build a new body of evidence with high-quality reporting and reproducibility that will serve as the foundation of its so-longed-for standardization.

ADDITIONAL EXPERT OPINION

Importantly, when approaching a novel treatment option, we must do our best to practice evidence-based medicine. This first requires research-driven processes to identify the crucial components of the product so that it can be appropriately characterized and reliably recreated. In the case of biologics such as PRP, where countless variables may ultimately impact the preparation and final product, it becomes vital to identify the key elements and differences in the preparation method. Classification systems can play a key role in driving this standardization process. As mentioned above, the available classification systems have identified essential aspects of PRP that affect the biological composition and, ultimately, the indications and outcomes. The simple and elegant system proposed by Dohan Ehrenfest et al. [9] identified what seems to be the main dividing point based on the current indications for PRP in musculoskeletal medicine: the presence or absence of leukocytes. While there is no perfect classification system to rely entirely on when evaluating PRP, there is no question that the Dohan Ehrenfest classification system began the conversation, leading to many more comprehensive classification systems.

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390 **TABLES**

391 Table 1. Dohan Eherenfest et al. (2009) platelet concentrate classification.

PARAMETERS		**SUBPARAMETER**
A	Preparation kits and centrifuges	1. Size of the centrifuge 2. Duration of the procedure 3. Cost of the device and kits 4. Ergonomics of the kit and the complexity of the procedure
B	Content of the concentrate	1. Final volume of usable concentrate 2. Efficiency in collecting platelets 3. Leucocytes 4. Preservation of the components
C	Fibrin network	1. Concentration and density 2. Polymerization process

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393 **BOXES**

BOX 1. Choukroun's L-PRF protocol [10]

This novel method was characterized by its simplicity, reproducibility, and low cost. In this protocol, developed in Nice (France), venous blood was collected in 10 ml tubes and instantly centrifuged without anticoagulant. The lack of anticoagulant allowed the natural formation of a clot (platelet activation) that would facilitate the manipulation of L-PRF, avoiding the implementation of any additive. Thus, after centrifugation at 3000 rpm (400g) for 10 minutes, three distinct layers are visualized: the red blood cell layer in the bottom of the tube, a top acellular plasma layer, and a L-PRF clot in between, containing most of the platelets.

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BOX 2. Short interview with Pr. Lars Rasmusson – Co-author of the classic paper on "Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF)"

Head of Department, Department of Maxillofacial Surgery, Sahlgrenska Academy, University of Gothenburg

Q1: What motivated the development of the classification system?

A-LR: The motivation to develop a classification system was the somewhat conflicting PRP and PRF handling methodology at the time.

Q2: Who were the researchers involved in the development of the classification and their contribution to it?

A-LR: It was Pr. Tomas Albrektsson, Head of the Department of Biomaterials, University of Gothenburg, and I at the time together with our postdoc, David Dohan Ehrenfest.

Q3: What were the clinical uses of PRP in your Institution?

A-LR: PRP and PRF were used both clinically in maxillofacial reconstruction and in experimental work at our lab. In clinical practice here at our unit, L-PRF has replaced PRP since it is easier to use and possible to manufacture in different consistency/preparations, for example, injectable and as membranes.

Q4: What are the current challenges of PRP therapy?

A-LR: The challenge has been (and still is) to prove long-term efficacy and superiority in bone healing.

Q5: What is the future of PRP therapy?

A-LR: I strongly believe that more indications for use will be discovered and evaluated in cartilage repair via injectable platelets.

BOX 3. Summary of The Classic

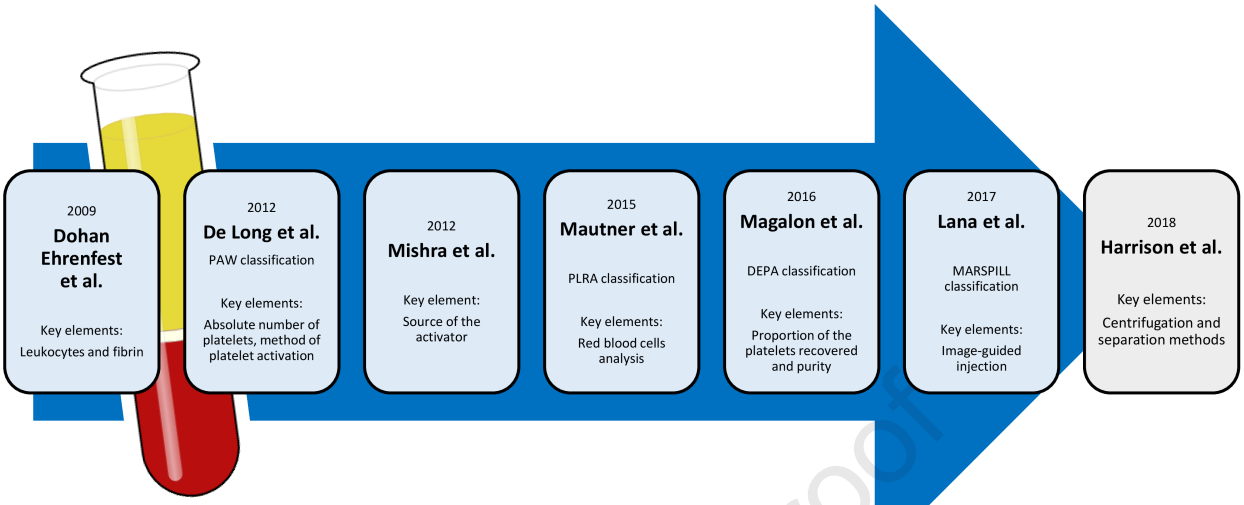
PRP is currently used to treat multiple musculoskeletal conditions, including knee osteoarthritis, cartilage injuries, patellar tendonitis, and tennis elbow. In recent decades, the media, celebrity athletes, the desire for novel treatments, and its autologous nature have boosted its use in multiple sports-related injuries. As a result, its high demand has led to an industry-driven development of various platelet concentrate systems and products exceeding the pace of evidence-based practice.

In 2009, Dohan Eherenfest et al. proposed the first attempt at PRP classification to categorize the platelet concentrates concerning their fibrin and leukocyte content and the degree of standardization of the procedure, providing an overview of the available systems. The authors proposed four categories of platelet concentrates (P-PRP, L-PRP, P-PRF, and L-PRF) to group a "jungle" of products in which the term PRP was used indistinctly.

The classification opened the door to understanding intrinsic biological mechanisms between the PRP components. Since the initial classification, several other classification systems have been proposed and published in the current literature, identifying important aspects of PRP. To date, there is still a lack of standardization in sample preparation, cohort heterogeneity, and incomplete reporting of sample preparation utilized, leading to a lack of clarity and challenging researchers and clinicians. A paucity of accurate reporting of a highly variable product has led to a lack of clarity and continues challenging researchers and clinicians. In this sense, journal editors can play an essential role in evidence quality improvement by requesting mandatory adherence to acceptable orthobiologics reporting guidelines in submission and review. The goal of the present decade is to build a new body of evidence with high-quality reporting and reproducibility that will serve as the foundation of its so-longed-for standardization.

397 **FIGURE LEGENDS**

398 Figure 1. Historical landmarks of platelet-rich plasma classifications.



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: