The use of platelets in regenerative medicine and proposal for a new classification system

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DOI:
10.1111/jth.14223

Guidance on the use of platelets in regenerative medicine and proposal for a new classification system: a consensus of the working party from the platelet physiology subcommittee of SSC/ISTH

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Abstract. Autologous and single donor allogenic platelet preparations are increasingly being used in many areas of regenerative medicine. However, there are few properly controlled randomized clinical trials, and the preparation, content and characteristics of platelet preparations are generally poorly defined and controlled. The Platelet Physiology Subcommittee of the Scientific and Standardization Committee (SSC) of the ISTH formed a working party of experts with the aim of producing consensus recommendations for guidance on the use of platelets in regenerative medicine. Due to a lack of investigations that provide definitive evidence for the efficacy, definition and use of different platelet preparations in regenerative medicine, there were insufficient data to develop evidence-based guidelines. Therefore, the RAND method was used, which obtains a formal consensus among experts particularly when scientific evidence is absent, scarce and/or heterogeneous. Using this approach, each expert scored as "appropriate", "uncertain" or "inappropriate" a series of 45 statements about the practice of regenerative medicine with platelets, which included different sections on general aspects, platelet preparations, clinical trial design; and potential utility in different clinical scenarios. After presentation and public discussion at SSC meetings, the assessments were further refined to produce final consensus recommendations which is the subject of the present report.

Introduction

Platelet-Rich Plasma (PRP), an autologous or allogeneic derivative of whole blood that contains a supra-physiological concentration of platelets, has gained increasing attention in both the scientific literature and the wider media for its potential application as a regenerative adjunct therapy [1-5]. The platelet α-granules provide a vast array of growth factors and cytokines that are important in wound healing [1]. These factors can promote local angiogenesis, stem cell homing, local cell migration, proliferation and differentiation coupled with the deposition of matrix proteins, such as collagen, which all play a key role in enabling the restoration of normal tissue structure and function. The regenerative effect of PRP exerted by producing a local environment for tissue regeneration has been supported by *in vitro* and *in vivo* studies that suggest a positive influence on the migration and proliferation of a number of cell types. Although PRP therapy is increasingly popular and more and more widely employed, clinical use initially centred on its potential application in dental and maxillofacial surgery. The regenerative effects of PRP on bone, cartilage, skin, tendon and muscle have also attracted interest in orthopaedic and plastic surgery where restoration of poorly vascularised and damaged tissue is a critical determinant of successful clinical outcome [6-12]. Given the complexity of the material, the rudimentary knowledge of the mechanism of action of PRP and the limitations of current studies, any future clinical trial should be carefully designed to both accurately determine any clinical effects, and importantly should use disease-specific outcome tools. Imprecision in effect size estimates from underpowered studies with PRP has also undoubtedly led to unreliable conclusions [2]. The field is also plagued with poor standardization and variability in the methods used to generate PRP with variability in the terminology, purity, content and quality of products utilized [13, 14]. Depending upon its method of preparation, PRP can also be defined as platelet-enriched plasma(PeRP), platelet-rich concentrate(PRC), platelet concentrate, leukocyte-rich PRP(L-PRP), platelet-rich fibrin(PRF), platelet-rich in growth factors(PRGF), platelet-rich fibrin matrix(PRFM), autologous concentrated plasma(ACP), platelet gel, pure platelet-rich plasma(PurePRP) or platelet releasate etc. [15-19]. Platelet products therefore have varying concentrations of blood cells (platelets, leukocytes and erythrocytes) plasma or fibrinogen. Unsurprisingly, the content, purity and hence the biological properties of

those products therefore vary widely and impact upon their potential efficacy. Several clinical trials have therefore been conducted without clear definition or quantification of the PRP biological properties, leading to varying efficacy and outcomes.

There are also many types of commercial PRP preparation devices resulting in further variation in product content [20]. PRP regenerative properties are based on the production and release of bioactive proteins, including multiple growth and differentiation factors, upon platelet activation. However, the role of other cells that may be present in PRP, such as leukocytes and erythrocytes, is still unclear and cannot be excluded. The presence of leukocytes may have great impact on the biology of these preparations, not only because of their immune and antibacterial properties, but also because they are key players in tissue healing and local growth factor regulation [21]. PRP also implies the application of autologous plasma along with platelet-derived proteins. Therefore, it promotes the development of a fibrin scaffold at the desired location that can act as a temporary matrix to assist repair of the injured tissue. Defining the potential role(s) of all the bioactive factors present in any platelet preparation is therefore essential to understand the biological activities.

Autologous PRP can be prepared in a laboratory, operating theatre or a clinic room from anticoagulated blood collected at the time of the therapeutic application. Trisodium citrate is the most widely used anticoagulant with few negative effects on PRP preparation. ACD (acid citrate dextrose) and CPD (citrate phosphate dextrose), with and without adenine (ACD-A and CPD-A), are also effective anticoagulants. EDTA is not usually recommended for PRP preparation as this causes platelet swelling and activation. Traditionally, a relatively pure preparation and good yield of PRP can be easily obtained in a single step by centrifugation of anticoagulated blood at low force (170-200 g) for 10 minutes at room temperature [22]. For clinical applications there are essentially three main methods available that can rapidly provide a sterile PRP product: 1) Gravitational centrifugation techniques 2) Standard cell separators and 3) Autologous selective filtration technology (plateletpheresis). Standard cell separators and salvage devices generally operate on a full unit of blood. In general, they use continuous flow centrifuge bowl or continuous flow disk separation technology coupled with hard and soft centrifugation steps. High g-force centrifugation is normally used to isolate the buffy coat layer containing platelets and

leukocytes. Differences in g-force and centrifugation time used in each preparation technique will also result in significant differences in yields, concentration, purity, viability and activation status of the isolated platelets but also affect eventual clinical efficacy. PRP must also be activated for the platelets to release their α-granule contents, with the clot providing a scaffold to capture the secreted proteins and maintain their presence at the application site. This introduces another set of variables including method of activation (e.g. thrombin, re-calcification, etc), duration of activation and whether this should be performed prior to clinical application. Although popular and widely used, the use of platelets in regenerative medicine is clearly poorly standardized. Although some guidelines have been published, if this field is to progress then standardization of the methods for the generation of the various platelet-rich preparations and evidence based guidelines for future clinical trials are urgently required [23, 24]. In January 2016, the Platelet Physiology Subcommittee of the ISTH formed a working Party of 10 well known experts in the field of regenerative medicine with the aim of producing a series of consensus recommendations for standardizing the use of platelets in regenerative medicine. The working party then used a formal consensus method (RAND method) to develop its final recommendations.

Methods

The RAND method – developed by the RAND corporation in the 1980s – obtains a consensus among expert groups about the appropriateness of healthcare interventions when scientific evidence is absent, scarce or heterogeneous [25]. A series of statements about platelets in regenerative medicine were therefore formulated and each member of the working party then scored each one for appropriateness from 1 (completely inappropriate) to 9 (fully appropriate). Individual scores were blinded to other panel members and the two extreme scores (highest and lowest) then discarded. The median of the remaining 8 scores were then calculated and the statements classified as either inappropriate (scores 1-3), uncertain (4-6) or appropriate (7-9). The panel was also asked to give any further comments of relevance to this field. Recommendations were then presented at ISTH SSC meetings and it was agreed that these could form the basis of a consensus document.

The recommendations of the working party on the use of Platelets in regenerative medicine derive from 45 statements grouped into four main sections:

i) General aspects;
ii) Platelet preparation;
iii) Clinical trial design;
iv) Utility of platelets in different clinical scenarios.

Overall 26 statements were scored as appropriate (11 statements were with complete agreement with an additional 15 statements also in agreement after removal of the 2 extreme scores) and 19 statements were judged as uncertain. No statements were judged as inappropriate. The term PRP used in the statements below encompasses all platelet and platelet-related products used for regenerative medicine.

Results

General aspects

1. **It is uncertain** whether PRP preparations (autologous or allogeneic) are clinically useful in tissue regenerative techniques (median score 6, range 5-8).

2. **It is uncertain** whether platelet gels or clots are clinically useful in regenerative medicine (median score 6, range 5-7).

3. **It is uncertain** whether platelet-rich fibrin is clinically useful in regenerative medicine (median score 6, range 5-7).

4. **Appropriate** The term PRP is a confusing, too general and incomplete terminology (median score 8.5, range 7-9).

5. **Appropriate** Autologous/Allogeneic sterile PRP preparations are clinically safe (median score 7.5, range 5-8).

6. **Appropriate** Clinical Preparations of PRP are poorly standardized (median score 8.5, range 7-9 with complete agreement by entire panel).

7. **Appropriate** The content, purity and biological properties of PRP all vary widely and will impact upon their clinical efficacy (median score 8.5, range 6-9).

8. **Appropriate** Many trials or studies fail to fully define the content, purity and biological properties of platelet preparations (median score 9, range 8-9 with complete agreement by entire panel).

Platelet preparation

1. **It is uncertain whether** PRP should be prepared from citrate anticoagulated whole blood (median score 5.5, range 3 -7).

2. **It is uncertain whether** PRP should be prepared from ACD anticoagulated whole blood (median score 6.5, range 4-8).

3. **It is uncertain whether** PRP should be prepared from CPD anticoagulated whole blood (median score 4.5, range 2-7).

4. **Appropriate** EDTA anticoagulant should not be used for PRP preparation (median score 8, range 6-9).

5. **It is uncertain** whether PRP should ideally be produced by low g centrifugation for short periods (e.g. 170 g for 10 minutes) to maximise platelet yield and minimise cellular contamination (median score 5, range 5-8).

6. **Appropriate** Platelet concentration, yield and recovery are dependent upon centrifugation protocol and collection methods utilised (median score 8, range 8-9 with complete agreement by entire panel).

7. **It is uncertain** whether PRP once prepared is stable for clinical use up to 6 hours post preparation (median score 6, range 4-8).

8. **It is uncertain whether** PRP must be activated for platelets to release their granule contents before application (median score 5, range 4-8).

9. **Appropriate** In some applications collagen-rich tissues may also activate PRP and eliminate the need for a pre-application activation step (median score 7, range 5-8).

Clinical trial design

Clinical trials on the use of platelets in regenerative medicine should be adequately powered and controlled with some essential design requirements including:-

1) **Appropriate** Randomized placebo controlled design (median score 8, range 7-9).

2) **Appropriate** Sample size calculation based on clear predefined endpoints (median score 8.5, range 7-9 with complete agreement by entire panel).

3) **Appropriate** Clear inclusion/exclusion criteria (median score 9, range 7-9 with complete agreement by entire panel).

4) **Appropriate** A homogeneous study population (median score 8, range 7-9).

5) **Appropriate** Standardised clinical assessments (median score 9, range 6-9 with complete agreement by entire panel).

6) **Appropriate** Clinical endpoints objectively measured (median score 9, range 7-9).

7) **Appropriate** Validated PRP production and delivery methods (median score 9, range 6-9).

8) **Appropriate** Details on the applied treatment including number of platelets, number of applications, duration of treatment etc (median score 9, range 7-9 with complete agreement by entire panel).

9) **Appropriate** Robust clear clinical outcomes (median score 9, range 7-9).

10) **Appropriate** Standardized post treatment follow up protocol (median score 9, range 7-9 with complete agreement by entire panel).

11) **Appropriate** Full description of PRP preparation methodology (median score 9, range 8-9 with complete agreement by entire panel).

12) **Appropriate** Details of the amount of autologous blood collected (median score 9, range 7-9).

13) **Appropriate** Baseline number, volume and concentration of platelets utilised (median score 8, range 7-9 with complete agreement by entire panel)

14) **It is uncertain whether** the overall yield of platelets obtained during PRP preparation should be reported (median score 6.5, range 6-9).

15) **Appropriate** The purity of the final PRP preparation should be reported, i.e. how many RBC and WBC are contaminating the PRP and whether the preparation contains plasma (median score 8, range 7-9 with complete agreement by entire panel).

16) **Appropriate** A measurement of PRP quality is required – i.e. platelet activation status prior to clinical use (to clarify if platelets have already lost their granular content) (median score 7, range 5-9).

17) **It is uncertain whether** the measurement of Growth Factor content of the PRP preparation used should be reported (median score 6.5, range 4-9).

18) **Appropriate** The activation procedure, if used, should be reported (median score 9, range 7-9).

19) **Appropriate** A new classification system for PRP is required taking into account all key variables (median score 9, range 7-9).

Utility in different clinical scenarios.

1) **It is uncertain whether** PRP can be utilised to treat burn injuries (median score 6.5, range 5-7).

2) **Appropriate** PRP can be utilised for general wound healing (median score 7, range 5-7).

3) **It is uncertain whether** PRP can be utilised for treating tendon Injuries (median score 5.5, range 5-8).

4) **It is uncertain whether** PRP can be used for acute muscle injuries (median score 5, range 5-6).

5) **It is uncertain whether** PRP can be utilised for bone healing (median score 6, range 4-7).

6) **It is uncertain whether** PRP can be utilised in Maxillofacial injuries (median score 5.5, range 4-8).

7) **It is uncertain whether** PRP can be utilised for treating Sports injuries (median score 5.5, range 5-7).

8) **It is uncertain whether** PRP can be utilised to treat osteoarthritis (median score 6.5, range 5-8)

9) **It is uncertain whether** PRP can be utilised to prevent skin ageing as a beauty therapy (median score 4, range 2-5)

All of these statements are also summarized in supplementary table 1.

Proposed New Classification System for PRP and related products

The authors of this guideline suggest a new classification system, which incorporates previous classification systems, and includes the preparation method technique[16, 18].

Activation is divided into three subcategories:

- I for PRP application without activator
- II for the use of PRP with activation
- III for use of frozen-thawed preparations

Platelet concentrates are subdivided into three categories (A, B and C) based on the platelet count range in the samples. The three categories are:

A Platelet count < 900 $x10^3$ /µl

B Platelet count 900 – 1700 $x10^3$ /µl

C Platelet count > 1700 $x10^3$ /µl

The preparation methods are classified into three categories:

1 The Gravitational centrifugation techniques

2 Standard cell separators

3 Autologous selective filtration technology (plateletpheresis)

The modified classification is summarized in table 1. This includes the activation method if used, the total volume used, the frequency of dosing and subcategories of activation, platelet concentration and preparation technique and would include the overall average counts and range(low–high) of platelets, red cells and differential leukocyte counts(neutrophils, lymphocytes and monocytes).

Conclusions

Autologous or allogeneic platelet administration remains an attractive and popular strategy in many clinical scenarios given the cost-effective, minimally invasive and safe nature of this therapy [1]. The statements presented here should contribute to improve the standardization and design of future clinical trials using platelets in regenerative medicine and agree with some parts of recent recommendations [23, 24]. Future trials should not only be appropriately controlled and adequately powered, but also take into account the content and quality control of the platelet preparations to ensure that clear correlations between the products and outcomes are established [23, 24, 26-28]. Further studies into the mechanism of platelet tissue regeneration and optimal platelet preparation may help elucidate the best combination of bioactive factors to achieve maximal regenerative activity [29]. It will be important that guidelines such as this one should also be reviewed and updated in the future as the field continually changes and evolves.

Acknowledgements

The authors would like to thank Lacey Schmeidler and Cary Clark from the ISTH for their help with the distribution and grading of the RAND method used in this manuscript. The authors would also like to thank the ISTH platelet physiology SSC for support in organizing this study.

Disclosure of Conflicts of Interests

The authors state that they have no conflict of interests.

Class	Leukocytes + = > 1%	Red Cells + = > 10%	Activation	Platelet Concentration	Preparation category
PRP	-	**-**	**I**	**A**	**1**
			II	**B**	**2**
Red-PRP		**+**	**III**	**C**	**3**
L-PRP	**+**	**-**	**I**	**A**	**1**
			II	**B**	**2**
Red-L-PRP		**+**	**III**	**C**	**3**
PRF	**-**	**-**	**I**	**A**	**1**
			II	**B**	**2**
Red-PRF		**+**	**III**	**C**	**3**
L-PRF	**+**	**-**	**I**	**A**	**1**
			II	**B**	**2**
Red-L-PRF		**+**	**III**	**C**	**3**

Table 1. A proposed new PRP classification system. This includes the activation method if used, the total volume used, the frequency of dosing) and subcategories of activation, platelet concentration and the preparation techniques - 1) Gravitational centrifugation techniques 2) Standard cell separators and 3) Autologous selective filtration technology (plateletpheresis). (e.g recalcified 5 ml (x1) – Red-L-PRP IB1 has been used in the PATH-2 trial [26] and would include the overall average counts and range (low – high) of platelets, red cells and differential leukocyte counts (neutrophils, lymphocytes and monocytes). (Abbreviations: PRP= platelet rich plasma; Red-PRP= Red Cell Rich platelet Rich Plasma; L-PRP= Leukocyte rich platelet rich plasma; Red-L-PRP Red Cell and Leukocyte rich platelet rich plasma. PRF= Platelet Rich Fibrin; Red-PRF= Red cell rich Platelet Rich Fibrin; L-PRF= Leukocyte rich platelet rich fibrin; Red-L-PRF= Red cell and Leukocyte rich platelet rich fibrin.

Supporting Information

General Comments from the panel :-

1) Clearly the application procedure for these products is as important as the product "per se". For example, volume, injection techniques, sonography assisted interventions or debridement in wound care.

2) We do not know if platelet dosage is more important than platelet enrichment, or if the balance between both plasma and platelet derived molecules determine the efficacy of the product.

3) It may be also important to consider the status of host tissue being treated including chronicity, inflammatory or degenerative status.

4) We also need more research before deciding which anticoagulants are optimal, what growth factors or chemokines or cytokines are important; thus quality control and how to optimally implement this is still challenging.

5) Recent research in ulcer care has also focussed on the lipidomic content of PRP, which is largely ignored in the majority of studies.

6) In addition, the age of patients and metabolic associated co-morbidities, or systemic inflammatory conditions (e.g. in burns patients) may significantly influence the quality of autologous PRP [30].

7) In complex pathologies such as tendinopathies or osteoarthritis the effort to advance in PRP therapies should be accompanied by identifying different phenotypes.

8) In oral and maxillofacial surgery, the various forms of PRF (mostly the L-PRF family) are developing very strongly as an inexpensive, open-access and efficient surgical adjuvant.

9) The effects in regenerative medicine of PRF are more dependent on the fibrin architecture (which may be dependent on the platelets and defines the bio-mechanical properties of the material) and the cell content (particularly the proportions of various leukocytes and circulating cells). All of these will participate in the definition of the "biological signature for growth factors release" of each kind of product and its final clinical potential.

10) There remains so much to investigate about appropriate number of (activated) platelets, quality of platelets, addition (or not) of leukocytes, multiple to single applications and this for all different indications.

11) The role of white and red blood cells may be very important in PRP and need to be properly investigated?

12) We will need profound basic studies and randomized clinical trials to really verify the use of PRP or any other platelet preparations in any clinical application. RCT should include deep characterization of injected products with GF quantification in link with the disease treated.

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Supplementary Material

Supplementary Table 1. A summary of the median scores and ranges of the working party for each of the 45 statements in the RAND survey about platelets in regenerative medicine. Each statement is classified as either inappropriate (scores 1-3), uncertain (4-6) or appropriate (7-9). The term PRP used in the statements encompasses all platelet and platelet-related products used for regenerative medicine.

RAND survey statements	Median Score and Range	Classification
General Aspects		
PRP preparations are clinically useful in tissue regenerative techniques	6 (5-8)	Uncertain
Platelet gels or clots are useful in regenerative medicine	6 (5-7)	Uncertain
Platelet-rich fibrin is clinically useful in regenerative medicine	6 (5-7)	Uncertain
Autologous/Allogeneic sterile PRP preparations are clinically safe	7.5 (5-8)	Appropriate
The term PRP is a confusing, too general and incomplete terminology	8.5 (7-9)	Appropriate
Clinical preparations of PRP are poorly standardized	8.5 (7-9) Complete agreement	Appropriate
The content, purity and biological properties of PRP all vary widely and will impact upon their clinical efficacy	8.5 (6-9)	Appropriate
Many trials or studies fail to define the content, purity and biological properties of platelet preparations	9 (8-9) Complete agreement	Appropriate
Platelet Preparation		
PRP should be prepared from citrate anticoagulated blood	5.5 (3 -7)	Uncertain
PRP should be prepared from ACD anticoagulated blood	6.5 (4-8)	Uncertain
PRP should be prepared from CPD anticoagulated blood	4.5 (2-7)	Uncertain
EDTA anticoagulant should not be used for PRP preparation	8 (6-9)	Appropriate

PRP should be ideally produced by low g centrifugation for short periods (e.g. 170 g for 10 minutes to maximise platelet yield and minimise cellular contamination	5 (5-8)	Uncertain
Platelet concentration, yield and recovery are dependent upon centrifugation protocol and collection methods utilised	8 (8-9) Complete agreement	Appropriate
PRP once prepared is stable for clinical use up to 6 hours post preparation	6 (4-8)	Uncertain
PRP must be activated for platelets to release their granule contents before application	5 (4-8)	Uncertain
In some applications collagen-rich tissues may also activate PRP and eliminate the need for a pre-application activation step	7 (5-8)	Appropriate
Clinical Trial Design		
Clinical trials on the use of platelets in regenerative medicine should be adequately powered and controlled with some essential design requirements including :-		
Randomized placebo controlled design	8 (7-9)	Appropriate
Sample size calculation based on clear predefined endpoints	9 (7-9) Complete agreement	Appropriate
Clear inclusion/exclusion criteria	9 (7-9) Complete agreement	Appropriate
A homogeneous study population	8 (7-9)	Appropriate
Standardised clinical assessments	9 (7-9) Complete agreement	Appropriate
Clinical endpoints objectively measured	9 (7-9)	Appropriate
Validated PRP production and delivery methods	9 (6-9)	Appropriate
Details on the applied treatment including number of platelets, number of applications, duration of treatment etc	9 (7-9) Complete agreement	Appropriate
Robust clear clinical outcomes	9 (7-9)	Appropriate
Standardized post treatment follow up protocol	9 (7-9) Complete agreement	Appropriate
Details of the amount of autologous blood collected	9 (7-9)	Appropriate
Baseline number, volume and concentration of platelets utilised	8 (7-9)	Appropriate
The overall yield of platelets obtained during PRP preparation should be reported	6.5 (6-9)	Uncertain
The purity of the final PRP preparation should be reported, i.e. how many RBC	8 (7-9) Complete agreement	Appropriate

and WBC are contaminating the PRP and whether the preparation contains plasma		
A measurement of PRP quality is required – i.e. platelet activation status prior to clinical use (to clarify if platelets have already lost their granular content)	7 (5-9)	Appropriate
The measurement of Growth Factor content of the PRP preparation used should be reported	6.5 (4-9)	Uncertain
The activation procedure if used should be reported	9 (7-9)	Appropriate
A new classification system for PRP is required taking into account all key variables	9 (7-9)	Appropriate
Utility in different clinical scenarios		
PRP can be utilised to treat burn injuries	6.5 (5-7)	Uncertain
PRP can be utilised for general wound healing	7 (5-7)	Appropriate
PRP can be utilised for tendon injuries	5.5 (5-8)	Uncertain
PRP can be utilised for acute muscle injuries	5 (5-6)	Uncertain
PRP can be utilised for bone healing	6 (4-7)	Uncertain
PRP can be utilised for maxillofacial injuries	5.5 (4-8)	Uncertain
PRP can be used for treating sports injuries	5.5 (5-7)	Uncertain
PRP can be used to treat osteoarthritis	6.5 (5-8)	Uncertain
PRP can be used to prevent skin ageing as a beauty therapy	4 (2-5)	Uncertain