Accepted Manuscript

Authors: Ana María Ojea-Pérez, Andrea Acebes-Huerta, Tamara Arias-Fernández, Laura Gutiérrez, María Carmen Muñoz-Turrillas

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

Ana María Ojea-Pérez,[1,$] Andrea Acebes-Huerta,[2,$] Tamara Arias-Fernández,[1,$] Laura Gutiérrez[2,3,#] and María Carmen Muñoz-Turrillas[1,2,#]

1 Centro Comunitario de Sangre y Tejidos de Asturias, Oviedo, Spain.
2 Platelet Research Lab, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain
3 Dept. of Medicine, University of Oviedo, Spain

$,# Equal contribution

Corresponding author: Laura Gutiérrez: gutierrezglaura@uniovi.es, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA) Edificio FINBA Platelet Research Lab NO-F15 Avenida de Roma S/N 33011 Oviedo, Spain

Abstract

Platelet-rich plasma (PRP) is used with increasing demand as autologous adjuvant therapy in many areas of regenerative medicine, thanks to the platelet rich content of growth factors and bio-active molecules. However, to date there is a lack of consensus on PRP preparation methods, on processing and application forms, on clinical application guidelines and on knowledge-based composition at the cellular and molecular level, making difficult the assessment of clinical results from different groups in different clinical areas. Here we describe the implementation of PRP production on a closed-system using the infrastructure of a certified blood bank, detailing methodology, and validation and production results 1 year after its implementation. Our methodology provides a reproducible, safe, practical and yet affordable PRP bio-product that will allow further studies to better define PRP applications in regenerative medicine and personalized therapeutic regimes.

Keywords: Platelet Rich Plasma (PRP), closed-system, blood bank, safety, growth factors

1.   Introduction

Platelet-rich plasma (PRP) is a hemoderivative that contains a supraphysiological platelet concentration in plasma. It is used with increasing demand as adjuvant therapy in regenerative medicine (*i.e.* traumatology, dermatology and ophthalmology [1, 2]) in the form of infiltrations, implantations (gel) or serum eye-drops [3-5]. However, PRP refers to many types of platelet-based bio-products, without making any distinction as to the preparation method, cellular and molecular composition, processing and application form (platelet activation, freezing, fresh, liquid, jellified, drops). In some cases, the final product safety (sterility) is largely compromised [6-8]. Furthermore, in some special cases, once the PRP is prepared, it is frozen prior its application or, alternatively, infiltrations are done with fresh PRP containing live platelets/cells. These factors do not add uniformity to PRP-based products, which are *per se* subject to donor-dependent variation, and represent an obstacle to interpret clinical data among different groups [7, 8]. There is a general acceptance internationally, that, while the benefits of PRP-based therapies are not questioned, there are some aspects, including the preparation method, composition (at the cellular and molecular level) and the real efficacy of the bio-product and therapy application-wise, that need to be agreed upon and studied [1, 6-9]. Furthermore, not all hospitals or clinics have PRP preparation in their service portfolio and not all patients can afford PRP-therapies. Therefore, a highly standardized PRP preparation method that assures homogeneity and safety, storage longevity and is yet affordable for health institutions, remains as a high priority internationally (J. Seghatchian, personal communication, see comment in TRASCI 58.4 Editorial) [1, 6-9].

In order to circumvent these issues, at the local blood bank of the Principality of Asturias (Centro Comunitario de Sangre y Tejidos de Asturias -CCSTA-), we decided to validate and implement a closed-system PRP preparation method using the infrastructure of a certified blood bank. This initiative was started after several rmeetings of the Spanish Society of Blood Transfusion and Cell Therapy, inspired by the pioneer views of Dr. Bueno (Hospital Puerta de Hierro, Madrid) and Dr. Arroyo (Blood Bank of Cantabria).

2.   Implementation of PRP preparation using a closed-system at the local blood bank in the Principality of Asturias

2.1. *Methodology (Figure 1A):*

i.- Extraction of 150mL blood (plus sample-tubes for Transfusion-Transmitted Infections (TTI), blood group/irregular antibodies screening and CBC analysis).

ii.- Transportation to the CCSTA in butanediol plates (constant temperature 20-24ºC) and sample registration and barcoding in e-Delphyn software, upon arrival.

iii.- First centrifugation (425g, 5 min, acc6, no brake, 22ºC), to separate plasma fraction containing platelets with a press Compomat G5 (Fresenius) into a new bag.

iv.- Second centrifugation (1328g, 12 min, acc9, break 4, 22ºC), to separate approximately 30mL of PRP into a new bag from the remaining platelet poor plasma.

v.- The PRP is allowed to rest for 4 hours and kept overnight agitating at room temperature. This allows settling of the platelets and time to obtain TTI screening results.

vi.- CBC/ADAM-rWBC measurement is performed the next day: $> 350 \times 10^3$ platelets/mm^3 and $< 1 \times 10^6$ residual leukocytes/unit (a PRP unit refers to the total 30mL) is required.

vii.- The PRP is then distributed into 3 pediatric transfusion bags (10 mL each).

viii.- Aliquots are barcoded/labeled displaying all quality control parameters of a transfusion product, plus platelet count and personal data (name, ID), as it is for autologous use.

ix.- Aliquots are frozen at -40ºC to allow platelet lysis and cargo release, and can be stored for 2 years. As scheduled, the PRP is distributed to the petitioner hospital on dry ice.

It should be noted that upon request, the sealing tubes could be used as compartmentalized containers of smaller volumes (*i.e.* when PRP has to be applied in drops). Additionally, jellification of the thawed PRP is possible using CaCl$_2$, and if that texture is required, instructions and reagents are provided. Furthermore, all fractionation is done using the equipment of a certified blood bank, including centrifuges, presses, sterile sealing devices and certified materials (CE), assuring that the whole process is done within a closed-system. The PRP is prepared within 24 hours of extraction and stored frozen and the cold chain is maintained until its use. Most Importantly, PRP

preparation is done for autologous use under clinical prescription and after informed consent is given by the patient. The exclusion criteria are serious heart diseases, hemostasis disorders, severe autoimmune diseases and a history of neoplasia at the site of PRP application. In case of infection suspicion, blood extraction and PRP preparation should be postponed until after its remission. In the event of positivity in any of the TTI markers, PRP cannot be delivered and must be destroyed, according to local regulations (unless special permission is granted by authorities), and such action must be registered in the e-Delphyn software. The patient is thereof informed accordingly.

2.2.*Validation and demographics 1 year after the implementation:* The validation process was performed using interrupted blood donations (Table 1). We were able to improve on the quality control parameters on monitored distributed PRP units produced after the validation process (Table 1).

The CCSTA obtained permission by the local authorities to produce PRP in February 2018, abiding by the law (RD 1088/2005 and Informe/VI/23052013). Since then and until May 2019, the CCSTA has prepared 553 PRP units (Figure 1B). Of these, 14 units had to be discarded due to positive serology test results (2.5%). There are nine public hospitals (total of 3132 beds) in the Principality of Asturias (1028 million inhabitants). To date, two major public hospitals in the Principality of Asturias prescribe PRP as treatment, one of them (436 beds) requesting 52.6% and the other one (463 beds) requesting 38.6% of the produced PRP units, while 6.3% requests were done by Foundation Hospitals or private clinics. Most of the PRP units (97.2%) were used in traumatology/rheumatology, and the rest (3%) in the areas of ophthalmology, maxillofacial surgery and plastic surgery.

We assessed, at the Platelet Research Lab (Instituto de Investigación Sanitaria del Principado de Asturias – ISPA), the concentration of several relevant growth factors by Multiplex Technology on a number of PRPs from the validation period (Figure 2 and Table 2). We observed a donor-dependent variation in concentration of these factors, however, and importantly, the concentration of factors amongst the three frozen aliquots derived from a single-donor remained constant (Table 2).

4

This added quality factor assures that a patient requiring a regime therapy with serial applications is going to receive a PRP product with the same characteristics.

3.   Conclusions

The regulation of collection, processing, analysis, traceability, safety, quality control and distribution of platelet derived bio-products is most efficient when the infrastructure is standardized and validated procedures used in blood component processing are applied. Targeting the implementation of the local blood banks and logistics, the PRP becomes affordable and available to all patients that could benefit. Multiplex analysis of relevant growth factors showed donor-dependent variation, however, and importantly, the same analysis proved that aliquots prepared with this method maintain the same characteristics, allowing a better follow-up of patients requiring serial applications. Moreover, PRP production using a closed-system (which improves the safety criteria of a hemoderivative) and a reproducible standardized production method will allow the design of prospective studies to evaluate the efficacy and benefit of PRP based-therapies in multiple clinical settings as well as its usefulness in regenerative medicine, required for the development of personalized therapy regimes.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

[1] Marques LF, Stessuk T, Camargo IC, Sabeh Junior N, dos Santos L, Ribeiro-Paes JT. Platelet-rich plasma (PRP): methodological aspects and clinical applications. Platelets. 2015;26:101-13.

[2] Samadi P, Sheykhhasan M, Khoshinani HM. The Use of Platelet-Rich Plasma in Aesthetic and Regenerative Medicine: A Comprehensive Review. Aesthetic Plast Surg. 2019;43:803-14.

[3] Drew VJ, Tseng CL, Seghatchian J, Burnouf T. Reflections on Dry Eye Syndrome Treatment: Therapeutic Role of Blood Products. Front Med (Lausanne). 2018;5:33.

[4] Ghanaati S, Herrera-Vizcaino C, Al-Maawi S, Lorenz J, Miron RJ, Nelson K, et al. Fifteen Years of Platelet Rich Fibrin in Dentistry and Oromaxillofacial Surgery: How High is the Level of Scientific Evidence? J Oral Implantol. 2018;44:471-92.

[5] Piccin A, Di Pierro AM, Canzian L, Primerano M, Corvetta D, Negri G, et al. Platelet gel: a new therapeutic tool with great potential. Blood Transfus. 2017;15:333-40.

[6] Kikuchi N, Yoshioka T, Taniguchi Y, Sugaya H, Arai N, Kanamori A, et al. Optimization of leukocyte-poor platelet-rich plasma preparation: a validation study of leukocyte-poor platelet-rich plasma obtained using different preparer, storage, and activation methods. J Exp Orthop. 2019;6:24.

[7] Magalon J, Bausset O, Serratrice N, Giraudo L, Aboudou H, Veran J, et al. Characterization and comparison of 5 platelet-rich plasma preparations in a single-donor model. Arthroscopy. 2014;30:629-38.

[8] Magalon J, Chateau AL, Bertrand B, Louis ML, Silvestre A, Giraudo L, et al. DEPA classification: a proposal for standardising PRP use and a retrospective application of available devices. BMJ Open Sport Exerc Med. 2016;2:e000060.

[9] Harrison P, Subcommittee on Platelet P. The use of platelets in regenerative medicine and proposal for a new classification system: guidance from the SSC of the ISTH. J Thromb Haemost. 2018;16:1895-900.
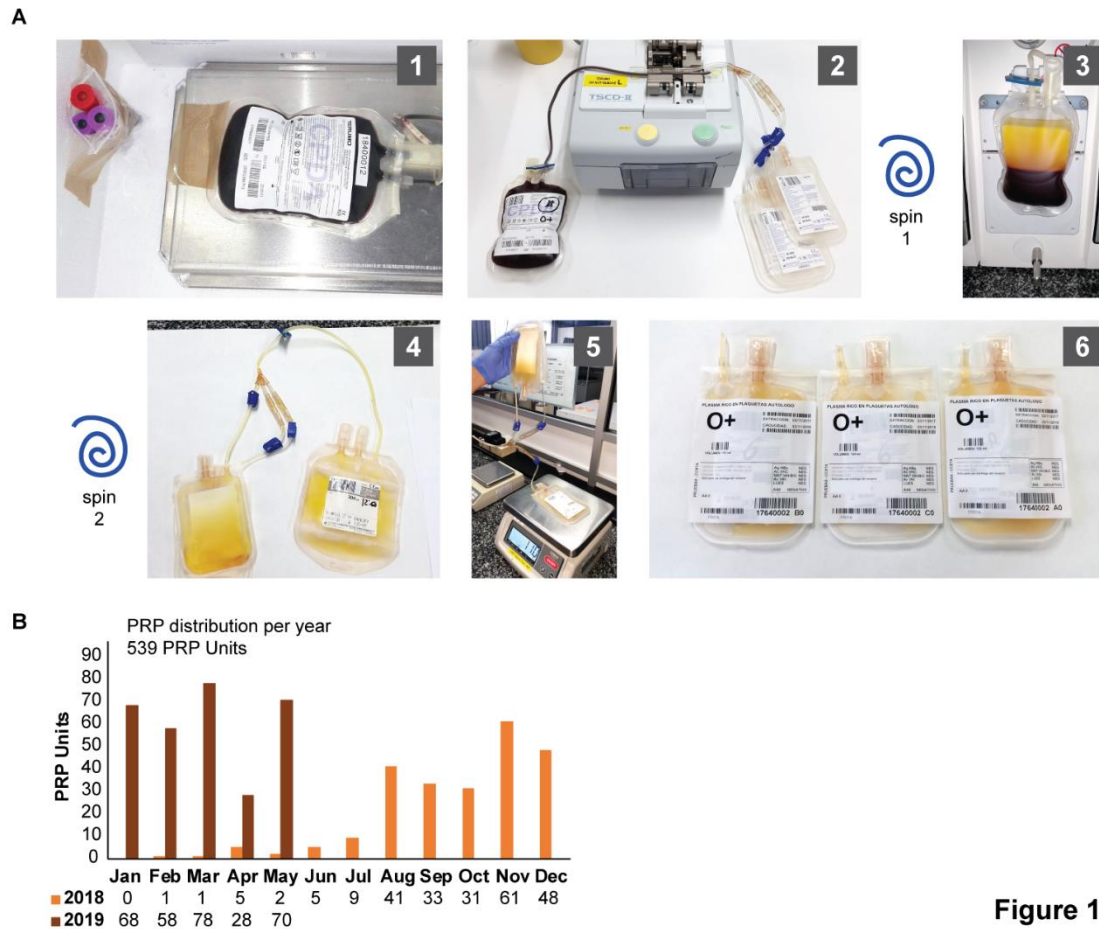
6

Figure 1

Figure 1: A. Workflow of PRP preparation using a closed-system. *The photographs depict the protocol of action: (1) Blood sample collection to perform the PRP extraction and viral screening. (2) Bypass to collecting pediatric bags. (3-4) PRP and PPP fraction obtained by the system after two cycles of centrifugation. (5-6) Distribution into equal aliquots and labelling of the product for autologous use.* *B. PRP production stats.* *Bar-graph depicting the PRP production distribution through the year from its implementation (February 2018) to date.*
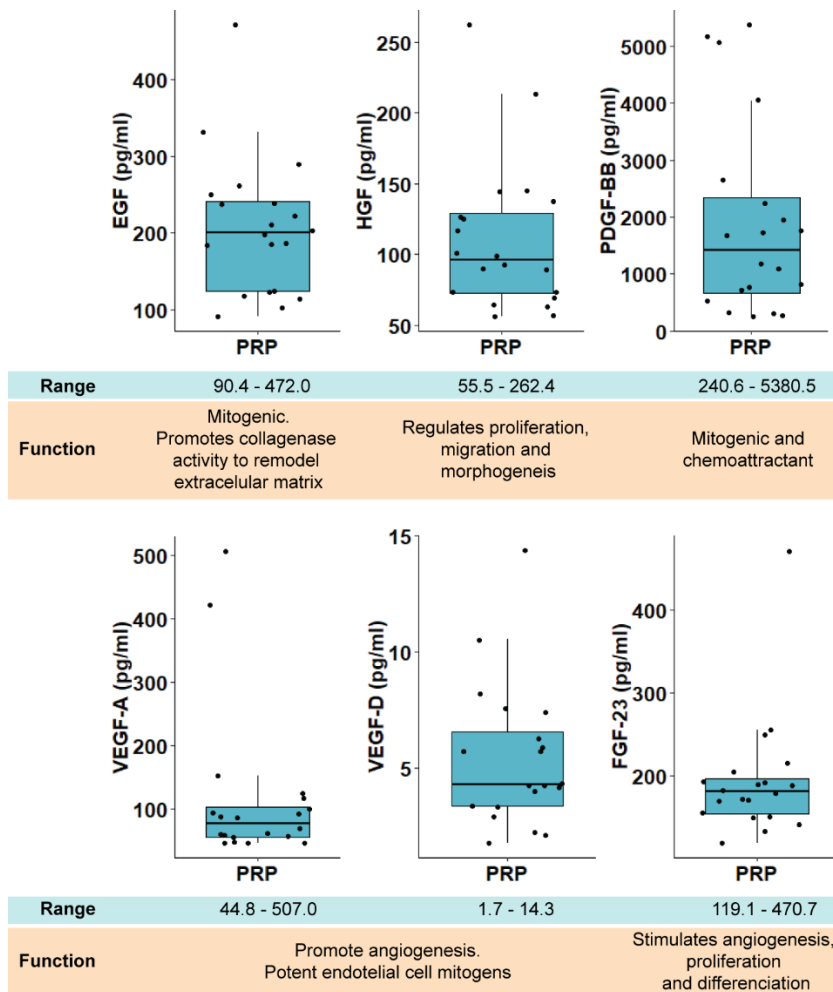
Figure 2: Multiplex quantification of growth factor concentration of PRP processed during the validation period. *The figure shows the concentration (pg/ml) of EGF, HGF, PDGF-BB, VEGF-A, VEGF-D and FGF-23 in PRP samples. Horizontal bars, boxes and whiskers represent median, 25%/75% quartiles and range, respectively. The analyte concentration range (pg/ml) and the function of each growth factor in wound healing is indicated.*

8

Table 1: Parameters during the validation process and of a representative number of distributed PRP units. Average and Standard Deviation are given. Vol: volume; PLT: platelets; WBC: white blood cells (counted with ADAM-rWBC).

	Whole Blood (WB)		Platelet Rich Plasma (PRP) Unit (30mL)		
	Vol (mL)	PLT ($x10^3/mm^3$)	Vol (mL)	PLT ($x10^3/mm^3$)	WBC per unit
VALIDATION N = 14	App. 150mL	145.07 ± 38.66	30	431.93 ± 145.97	$0.97x10^6 ± 0.22x10^6$
PRODUCTION N = 5	App. 150mL	208.60 ± 28.40	30	598.40 ± 95.26	$0.66x10^6 ± 0.19x10^6$

Table 2. Quantification of growth factors in the three PRP aliquots prepared from a single blood extraction from two different donors (D1 and D2). Concentrations are given in pg/mL. EGF. Epidermal growth factor; HGF. Hepatocyte Growth Factor; PDGF. Platelet Derived Growth Factor; VEGF. Vascular Endothelial Growth Factor; FGF-23 Fibroblast Growth Factor.

Donor	Aliquot	*EGF*	*HGF*	*PDGF-BB*	*VEGFA*	*VEGFD*	*FGF-23*
D1	1	113.6	100.7	1760.4	115.8	4.2	204.4
	2	102.0	89.7	1715.2	86.6	3.2	189.6
	3	90.4	88.8	1668.6	85.6	4.1	191.2
D2	1	221.9	145.0	770.3	55.7	3.3	133.1
	2	185.6	137.0	716.7	45.2	2.8	119.1
	3	197.7	144.2	812.3	54.4	3.9	141.0