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Procedures for production, storage and utilization of platelet-rich plasma and correlated products in Brazil: a cross-sectional study

Práticas de produção, armazenamento e utilização do plasma rico em plaquetas e produtos relacionados no Brasil: estudo transversal

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ABSTRACT

Introduction: Platelet-rich plasma (PRP) is a technology with potential application in several areas of Medicine and Dentistry. The high variability observed during the production process imposes a challenge for the regulation of its production and use. **Objective:** To evaluate current practices of production, storage and use of platelet-rich plasma and related products in Brazil, allowing the identification of operational variability throughout these processes. **Method:** Cross-sectional study with data collection carried out by using a self-applied electronic questionnaire, with protection of anonymity. Questions were defined based on a literature review and consultation with a specialist in the field. The study questionnaire was sent to researchers and professionals who are producing PRP identified by different strategies, such as previous participation in events and publications in the area. **Results:** 64 responses were obtained, with 36 complete responses (56.3%) and 28 partial responses (43.7%). The rate of respondents could not be calculated, considering the means for dispatching the questionnaire. For PRP, there was a predominance of use of protocols with double centrifugation and platelet activation by different methods. Most respondents reported using PRP in the areas of orthopedics, physiatry and sports medicine. For fibrin-rich plasma (PRF), there was a predominance of responses using a single centrifugation protocol, without the use of platelet activation methods. Most respondents indicate the use of PRF in areas of dentistry. Variability of procedures and usage profile were observed for all platelet concentrates. Analyses were carried out comparatively according to the three main types of platelet concentrates. **Conclusions:** Findings corroborate the need to develop regulatory norms aiming at reducing variability and at promoting safety along the production and use of platelet concentrates.

KEYWORDS: Platelet-Rich Plasma; Platelet Concentrates; Blood Preservation; Blood Safety

RESUMO

Introdução: O plasma rico em plaquetas (PRP) é uma tecnologia com potencial aplicação em diversas áreas da medicina e odontologia. A alta variabilidade no processo de produção desta tecnologia e de tecnologias relacionadas desafiam a regulamentação de sua produção, do controle de qualidade e do uso. **Objetivo:** Avaliar práticas de produção, armazenamento e utilização do PRP e produtos relacionados no Brasil, permitindo a identificação de variabilidade operacional ao longo destes processos. **Método:** Estudo transversal com coleta de dados realizadas por meio de um questionário eletrônico, autoaplicado e com preservação de anonimato. As perguntas foram definidas a partir de revisão de literatura e por consulta a especialista da área. O questionário foi encaminhado a pesquisadores e a profissionais de várias áreas atualmente envolvidas na produção do PRP. Esses profissionais foram identificados por diferentes estratégias, tais como participação prévia em eventos relacionados e publicações na área. **Resultados:** Foram obtidas 64 respostas, sendo 36 respostas completas (56,3%) e 28 respostas parciais (43,7%). A taxa de respondentes não pôde ser calculada, considerando-se as formas de divulgação do questionário. Para a produção de PRP, as respostas indicaram predomínio de protocolos com centrifugação dupla e utilização de métodos variados para ativação plaquetária. A maioria dos respondentes referiu utilizar o PRP nas áreas de ortopedia,

fisiatria e medicina esportiva. Para a produção do plasma rico em fibrina (PRF), houve predomínio de respostas indicando utilização de protocolo de centrifugação única, sem utilização de métodos de ativação plaquetária. A maioria dos respondentes indicou utilizar o PRF em áreas da odontologia. No geral, observou-se grande variabilidade nos procedimentos de obtenção e do perfil de utilização entre os principais tipos de concentrados de plaquetas. **Conclusões:** Os achados corroboram a necessidade do desenvolvimento de medidas regulatórias com foco nos pontos críticos identificados, visando promover a segurança durante a produção e utilização dos concentrados de plaqueta.

PALAVRAS-CHAVE: Plasma Rico em Plaquetas; Concentrado de Plaquetas; Preservação de Sangue; Segurança do Sangue

INTRODUCTION

Platelet-rich plasma (PRP) is a blood component characterized by the supraphysiological concentration of platelets, which, unlike transfusional platelet concentrates, does not aim to promote hemostasis or normalization of coagulation, being used for non-transfusional purposes[1].

Several types of growth factors are found inside platelets, such as platelet-derived growth factors (PDGF) and vascular endothelial growth factor (VEGF). These growth factors play an important role in biological functions related to tissue repair, through increased cell differentiation and proliferation, collagen production, and angiogenesis[2,3,4,5].

Based on this physiological rationale, platelet concentrates of autologous origins such as PRP, fibrin-rich plasma (FRP) and platelet-rich plasma without leukocyte reduction (PRP-L) have been used in a range of clinical situations in dentistry[6,7,8,9] and in medicine, in subareas such as orthopedics and sports medicine[10,11,12], wound care[13], dermatology[14], among others.

Despite its wide potential for use and investigation through randomized clinical trials, the evidence regarding the efficacy of non-transfusion platelet concentrates is still not considered consistent, due to methodological limitations of the studies[15] and the expressive variability of the methods of obtaining platelet concentrates and the composition of the product obtained[16,17,18].

In addition to having implications for the effectiveness of platelet concentrates, the high variability in production, storage, and use methods can pose issues related to product quality and patient safety. The present study was developed to evaluate current practices in the production, storage and use of PRP and related products in Brazil, allowing the identification of variability according to the different types of platelet concentrates (PRP, FRP, PRP- L), from information collected by applying a questionnaire, to support the development of regulatory standards by the Brazilian National Health Surveillance Agency (Anvisa).

METHOD

Study design and location

Cross-sectional study, with data collection performed using an electronic self-applied questionnaire conducted at the Health Technology Assessment Center of Hospital Sírio-Libanês.

Construction of the questionnaire

The questions in the questionnaire were identified based on the findings of a literature review, carried out previously, seeking to reflect the stages of production of the PRP and related products that are more subject to variability and, therefore, can be considered as critical. In addition, a specialist hematologist was consulted in order to identify additional questions and to construct alternative answers to the questions, in order to ensure the scope and adequacy of the questionnaire (Chart 1).

The questionnaire was designed to be self-administered in electronic format, through the SurveyMonkey® platform, and was available for access between July 30th and September 30th, 2019. The final version of the questionnaire was piloted by one of the authors.

Application and dissemination of the questionnaire

The questionnaire was disseminated through different means, aiming to increase the number of respondents and the representation of different areas of knowledge. The following dissemination strategies were adopted:

- Sending an e-mail to researchers and health professionals participating in the International Seminar on the Use and Regulation of PRP held in August 2018 in Brasília.
- Sending an e-mail to a list of events related to the areas of hematology, orthopedics, and biomedicine, previously held at the Teaching and Research Institute of Hospital Sírio-Libanês.
- Contact with professional societies and sending the *link* with the questionnaire for dissemination among registered members (Chart 2).
- Sending an email to Brazilian researchers who have authored studies investigating the effects of PRP and related products registered on the ClinicalTrials.gov clinical trial registry platform.

Analysis and presentation of results

The data obtained from SurveyMonkey® were extracted using a Microsoft Excel® spreadsheet for evaluation and interpretation. Considering the small number of expected responses and the preliminary exploratory objective of this study, detailed statistical analyzes were not planned for the treatment and presentation of data.

Chart 1. Topics covered in the questionnaire.

Topics covered in the questionnaire.
• Production site
• Type of system used in production (open or closed system)*
• Type of product produced
• Anticoagulant used
• Number of centrifugations
• Centrifugal force (single centrifugation or first and second steps of double centrifugations)
• Centrifugation duration (single centrifugation or first and second steps of double centrifugation)
• Maximum time between blood collection and the end of product processing
• Temperature conditions during the period between blood collection until the end of processing
• Identification of samples at the time of collection and during processing
• Frequency of evaluation of cell concentrations of the final product
• Method used to assess the cell concentrations of the product obtained
• Platelet concentration of the product obtained, in absolute and relative values
• Leukocyte concentration of the product obtained, in absolute and relative values
• Frequency of evaluation of the final product growth factor concentrations
• Method used to assess the concentration of growth factors
• Frequency of microbiological assessment
• Methods for microbiological assessment
• Frequency of the patient's serological and/or molecular assessment
• Serologies and/or molecular tests performed
• Maximum storage time (from obtaining the final product until its use)
• Storage temperature (from obtaining the final product until its use)
• Identification of samples during the storage period
• Activation method used before its use
• PRP usage areas
• Product form of application
• Administration of PRP (or its related products) concurrently with other substance(s)
• How to use PRP or related products
• Checking the identification of samples before using the product
• Adverse events related to the use of PRP and related products

Source: Elaborated by the authors, 2020.
PRP: platelet-rich plasma.
*Open system defined by blood collection using a needle and syringe, where it is necessary to disconnect the needle to transfer the blood to the collection tube, and a closed system characterized by a vacuum collection system.

Ethical aspects

The answers were evaluated after the automatic generation of an identifying number, fully preserving the anonymity of the answers. There was no identification of respondents through names or other attributes. Analyzes were performed based on the respondents' agreement regarding the terms of the project.

Chart 2. List of contacted professional societies and associations.

- Brazilian Association of Hematology, Hemotherapy, and Cellular Therapy
- Brazilian Association of Aesthetic Medicine
- Brazilian Dental Association
- Federal Council of Biomedicine
- Federal Council of Medicine
- Federal Council of Dentistry
- Brazilian Society of Dermatology
- Brazilian Society of Ophthalmology
- Brazilian Society of Orthopedics and Traumatology
- Brazilian Society of Rheumatology
- Brazilian Society of Urology

Source: Elaborated by the authors, 2020.

RESULTS

A total of 64 responses were obtained from the questionnaire, 29 responses were obtained from a *link* previously made available and 35 responses were obtained from communication via personal e-mail.

Among the responses obtained, 36 were complete responses (56.3%) and 28 were partial responses (43.7%). The rate of respondents in the form of response via communication via personal email was 6.0% (64 responses from 1,065 emails sent). The mean time to complete the questionnaire, as shown by SurveyMonkey®, was 12 min. The definition of the total percentage of respondents was not possible due to the disclosure of the *link* by professional societies.

Type of product

Regarding the type of platelet concentrate produced, there was a predominance of PRP production (54.7% of responses), followed by FRP (17.2%), PRP-L (14.0%), PRP gel (6.3%), lyophilized PRP (3.1%), or other types of platelet concentrates (4.7%) (Figure 1).

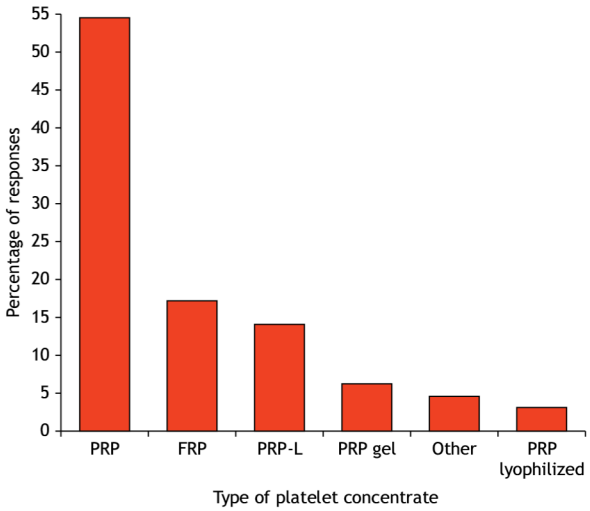
Analyzes were performed according to the three main types of platelet concentrates (PRP, PRP-L, FRP), to identify technical specificities and usage profiles.

Production site

It was observed that PRP processing is carried out predominantly in medical offices or clinics (62.9% of responses) and/or hospitals (60.0% of responses), while FRP processing is performed mainly in offices or dental clinics (63.4% of responses) or in medical offices or clinics (54.6% of responses) (Figure 2).

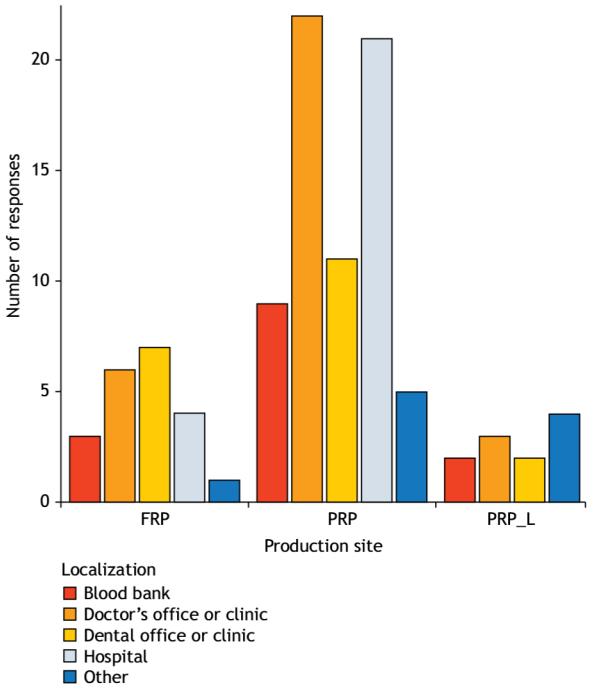
Type of system

The processing of the FRP is performed, in most cases, using closed systems (90.9% of responses), while the processing of the PRP and PRP-L is performed using both types of systems (Figure 3).



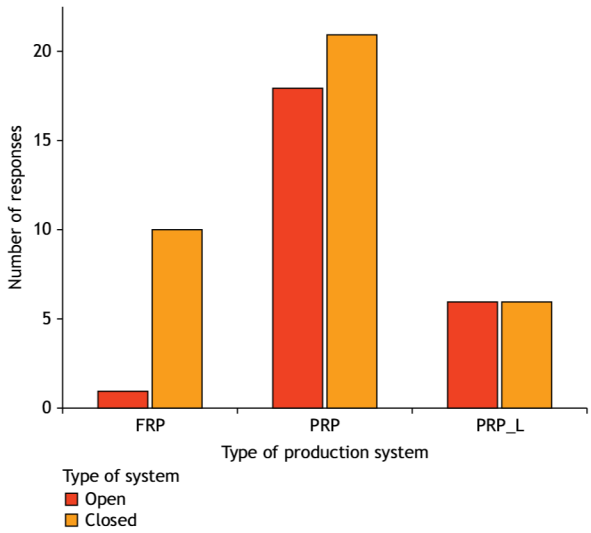
Source: Elaborated by the authors, 2020.
PRP: platelet-rich plasma; FRP: fibrin-rich plasma; PRP-L: platelet-rich plasma without leukocyte reduction.

Figure 1. Type of platelet concentrate produced in Brazil, 2019.



Source: Elaborated by the authors, 2020.
PRP: platelet-rich plasma; FRP: fibrin-rich plasma; PRP-L: platelet-rich plasma without leukocyte reduction.

Figure 2. Place of production of non-transfusion platelet concentrates, in relation to the type of health establishment.



Source: Elaborated by the authors, 2020.
PRP: platelet-rich plasma; FRP: fibrin-rich plasma; PRP-L: platelet-rich plasma without leukocyte reduction.

Figure 3. Type of system used in the production of non-transfusion platelet concentrates.

Type of anticoagulant

The use and choice of the type of anticoagulant were more homogeneous for the processing of PRP and PRP-L, with a predominance of sodium citrate or citrate dextrose A (ACD-A) (78.8% and 77.8% of responses, respectively), to the detriment of ethylenediaminetetraacetic acid (EDTA) or absence of anticoagulant use (21.2% and 22.2%, respectively), while for the processing the PRF, the use of sodium citrate or ACD-A was identified in 50.0% of the responses, the absence of anticoagulants in 45.5% of the responses and the use of EDTA in 4.5% of the responses.

Number of centrifugations

Regarding the number of centrifugations, the protocol for processing PRP or PRP-L, in most cases, involved double centrifugation (62.9% and 88.9% of responses, respectively), followed by single centrifugation (31.4% and 11.1%, respectively) or by unspecified protocols (5.7% of responses related to PRP). Processing of FRPinvolved single centrifugation (72.7% of responses).

Centrifugation Force - single centrifuge protocol

The centrifugation force of the most frequently used FRP single centrifugation protocols was 100 to 500 *g* (57.1% of responses), followed by greater than 500 to 1,000 *g* (14.3%). The centrifugation force at *g* was not reported in 28.6% of responses. The centrifugation force of the single centrifuge protocols for PRP processing was more heterogeneous, ranging from 100 to 1,000 *g* in all valid responses.

Centrifugation duration - single centrifugation protocol

The duration of centrifugation in the most frequently used single centrifugation protocols was 7 to 10 min for all platelet concentrates (60.0%, 100.0%, and 57.1% of responses, for PRP, PRP-L, and FRP, respectively). Other responses to the PRP included time greater than 10 min (20.0%) or lack of knowledge about the information (20.0%). For the PRF, the use of a centrifugation time greater than 10 min occurred in 42.9% of the responses.

Force of the first centrifugation - double centrifugation protocol

The force of the first centrifugation in the PRP double centrifugation protocols was 500 to 1,000 *g*, followed by 100 to 500 *g* (35.0% and 30.0% of responses, respectively). Other responses included strength greater than 1,000 *g* (5.0%), or no valid responses (30.0%). For the PRP-L, most answers indicated the use of force from 100 to 500 *g* (57.1% of the answers), followed by a force greater than 1,000 *g* (14.3%). The other responses obtained did not present the *g* force values.

Duration of first centrifugation - double centrifugation protocol

The duration of the first centrifugation in the double centrifugation protocols for PRP processing was 7 to 10 min (45.0% of responses), followed by 4 to 6 min (30.0% of responses), and for a time longer than 10 min (10.0% of responses). The other responses obtained did not identify the duration of the centrifugation time. For the production of the PRP-L, there was a predominance of responses of 4 to 6 min (42.8% of responses), followed by 7 to 10 min (28.6% of responses), and for a time of 1 to 3 min (14.3%). The other responses obtained did not identify the duration of the centrifugation time. For FRP, only one response was obtained, referring to a duration of 4 to 6 min.

Force of the second centrifugation - double centrifugation protocol

The force of the second centrifugation of the most frequently used PRP double centrifugation protocols was 500 to 1,000 *g* (35.0% of responses), however, values below and above this range were also reported, showing great variability of methods. The same was observed for PRP-L. No responses were obtained for the FRP.

Duration of second centrifugation - double centrifugation protocol

The duration of the second centrifugation in the most frequently used double centrifugation protocols for processing the PRP was 7 to 10 min (35.0% of responses). However, values below and above this range were also reported, showing great variability of methods. For PRP-L processing, the duration most frequently reported was greater than 10 min (57.1% of responses), followed by responses reporting centrifugation duration of 7 to 10 min (28.6%), and for 4 to 6 min (14.3%). A single response was obtained regarding the processing of the FRP, referring to a duration of 4 to 6 min.

Maximum time between collection and completion of processing

The maximum time between collection and the end of processing was up to 4 h in most responses related to PRP and PRP-L (89.5% and 100.0% of responses, respectively). In the other responses obtained for the PRP, the time was longer, between 8 and 12 h (5.2%) and between 12 and 24 h (5.25%). For FRP, two responses were obtained, indicating processing time up to 4 h and between 4 and 8 h.

Temperature conditions between collection and completion of processing

For all types of platelet concentrates, there was a report of controlled room temperature, between 20 and 24°C (52.6% to 57.1% of responses), but a greater variability of responses was observed for PRP, with 28.6% of the answers referring to uncontrolled ambient temperature and 19.0% referring to refrigeration conditions between 2 and 6ºC.

Sample identification methods between collection and processing

The methods reported for identifying samples between PRP collection and processing involved a single identifier (47.4% of responses), followed by the use of two identifiers (42.1% of responses), by the absence of identification (5.0%), or by using another identification method (5.0%). Most of the responses obtained for the PRP-L indicated the use of two identifiers (57.14% of the responses), followed by a single identifier (28.6%), or by the absence of the use of identification (14.3%). For the FRP, two responses were obtained pointing to the use of a unique identifier.

Frequency of evaluation of cell concentrations in the final product

The responses obtained for PRP showed variability in terms of the frequency of evaluation of cell concentrations in the final product, with the evaluation of all samples for 34.6% of the responses and with the evaluation of cell concentrations in at least some of the samples in 30.8% of the responses. Other responses included the absence of assessment of cell concentrations in the product (19.2%) or another type of assessment routine (15.4%). For PRP-L, the evaluation of cell concentrations in the final product in all samples was indicated in 50.0% of the responses, followed by another evaluation routine (25.0%), evaluation in some of the samples (12.5%), or lack of evaluation (12.5%). For the PRF, most answers pointed to the absence of an evaluation of the cell concentrations of the product obtained (62.5% of the answers), followed by evaluation in all samples, in some of the samples or other evaluation routines (12.5% for each answer).

The method used to assess cell concentrations

The responses obtained for the PRP showed a predominance of using automated methods for counting (94.4% of the responses), followed by manual counting (5.6%). For the PRP-L, a predominance of automated methods was also reported, corresponding to 57.1% of the responses, followed by manual methods (28.6%), and other methods (14.3%). For the FRP, there was a greater diversity of responses, indicating the use of automated and manual methods, similarly.

Platelet concentration of the obtained product

The number of platelets in the product ranged from 500,000 to 750,000 platelets/mm^3 in 44.4% of responses, however, values lower and higher than this range were also reported, showing great variability of methods. For the PRP-L, the values reported in 50.0% of the responses were between 750,000 and 1,000,000 platelets/mm^3 and for the FRP two responses were obtained, one being 750,000 and 1,000. 000 fibrins/mm^3 and the other above 1,000,000 fibrins/mm^3.

Considering the relative values, there was great variability in the responses on the platelet concentration of the product obtained, in relative values for the PRP, with responses between 1.5 and 2.5 times the baseline values (38.9%) or more than 4.5 times the baseline values (33.3%). Other responses obtained were 1.5 to 2.5 times baseline (16.7%) or 3.5 to 4.5 times baseline (11.1%). The same variability was observed for PRP-L. For the PRF, two responses were obtained, indicating that the platelet concentration is 3.5 to 4.5 times higher than the baseline values.

Leukocyte concentration of the obtained product

Valid responses for PRP indicated that the number of leukocytes in the final product ranged from 1,000 leukocytes/mm^3 (46.7% of responses) to values between 1,000 and 5,000 leukocytes/mm^3 (46.7% of responses). Other responses obtained were between 5,000 to 10,000 leukocytes/mm^3 (6.7%). For the PRP-L, there was greater variety of responses, with values observed ranging from levels below 1,000 leukocytes/mm^3 to the range between 10,000 and 15,000 leukocytes/mm^3. For the FRP, responses were obtained between 5,000 and 15,000 leukocytes/mm^3.

In relative values, there was great variability of responses on the leukocyte concentration of the product obtained, in relative values, for the PRP, with responses referring to a leukocyte concentration higher, equal to, or lower than the baseline values. The same variability was observed for PRP-L. For the FRP, two responses were obtained, indicating that the leukocyte concentration is higher than the baseline values.

Frequency and methods for evaluating the concentration of growth factors

The responses obtained for all platelet concentrates showed a lack of routine assessment of the concentration of growth factors in most cases. In cases where the concentration of growth factors is evaluated, the only method reported was flow cytometry. The use of flow cytometry has been reported in five PRP-related responses, two PRP-L-related responses, and one FRP-related response.

Microbiological evaluation

Microbiological assessment is not performed in most cases of FRP processing (85.7% of responses). For the PRP, variable responses were identified, with 45.5% of the responses referring to the absence of microbiological assessment routines, and 31.8% of the responses indicating the performance of microbiological assessment for some samples. Other responses obtained were microbiological assessment in all samples (18.2%) or another type of assessment routine (4.5%).

The method used for microbiological assessment involved culture media in most cases of PRP processing (63.6% of responses), followed by Gram stain (18.2%), or another method (18.2%). The use of culture media was reported in all cases of PRP-L processing where this type of assessment was performed. For the FRP, two responses were identified, referring to the use of cultures or Gram stain.

Serological and/or molecular assessment of the patient

Serological and/or molecular assessment of the patient was not performed in most cases of PRP-L processing (85.7% of responses). For PRP and FRP, responses with great variability were identified, with a predominance of responses referring to the absence of serological and/or molecular assessment routines. The most used tests were anti-HIV, anti-HBC, HBS-Ag, Anti-HCV and serology for syphilis.

Maximum storage time

For the three products, most responses indicated immediate use and therefore no storage (68.2% for PRP, 100.0% for PRP-L, and 71.4% for PRF). A minority of responses related to PRP pointed to the need to store the product for up to 6 h (9.5% of responses), from 6 to 12 h (4.8% of responses), from 12 to 24 h (4, 8% of responses), or more than 24 h (4.8% of responses). All responses related to PRP-L indicated immediate use of the product. For the FRP, storage periods of up to 12 h were reported.

Storage temperature

For the PRP, 38.1% of the responses indicated the storage of the PRP between 20 to 24ºC and 38.1% of the responses indicated storage under uncontrolled room temperature. Other responses included refrigerated storage (9.5%), other storage conditions (9.5%), and freezing (4.8%). For PRP-L or FRP, the responses showed greater variability.

Identification of samples during storage

For the PRP, the responses indicated that the samples are most often identified through a unique identifier (52.4% of responses), followed by responses pointing to the use of two identifiers (28.6% of responses), another type of identification system (14.3%), or lack of identification (4.7%). One response indicated the absence of product identification during the storage period. For PRP-L or FRP, the responses showed greater variability, with some responses indicating the lack of identification of the stored samples.

Activation method used

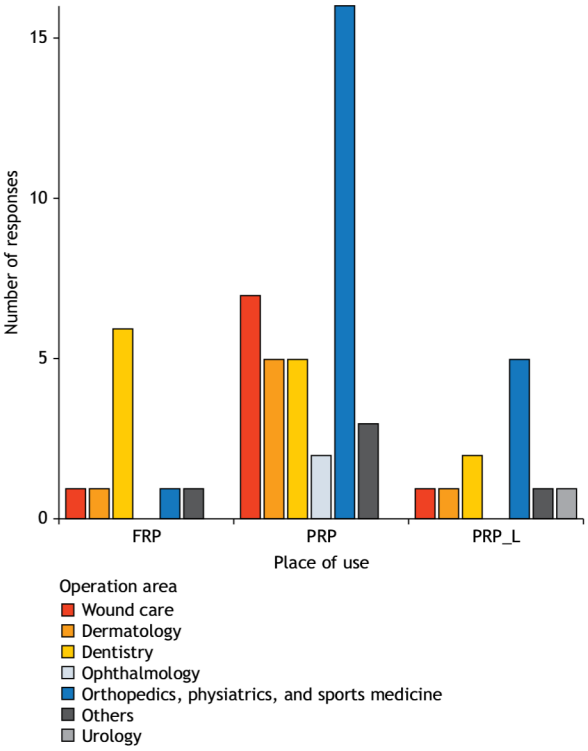
Most responses related to the PRF pointed to the absence of use of activation methods (85.7% of responses), followed by activation with thrombin (7.1%), or another method of use (7.1%). The responses for PRP and PRP-L indicated greater variability in relation to the activation methods used.

Areas of expertise in health in which the products were used

The answers related to PRP and PRP-L indicated the use of these concentrates in the areas of: orthopedics, physiatrics, and sports medicine (50.0% of responses), wound care (18.4%), dermatology (13.15%), dentistry (13.15%), ophthalmology (5.3%) and others (7.9%). The answers related to the FRP indicated a predominance of use in the field of dentistry (60.0% of the answers), followed by wound care (10.0%), dermatology (10.0%), orthopedics (10.0%), and others (10.0%) (Figure 4).

Form of application

There was a predominance of use of PRP by infiltration (75.0% of respondents) or intraoperative period (60.0% of respondents). The use of PRP-L was mainly due to infiltration (80.0% of respondents). The responses related to the FRP indicated predominantly intraoperative use in dental procedures (71.4% of respondents).



Source: Elaborated by the authors, 2020.
PRP: platelet-rich plasma; FRP: fibrin-rich plasma; PRP-L: platelet-rich plasma without leukocyte reduction.

Figure 4. Areas of use of non-transfusion platelet concentrates, according to medical or dental specialty.

Concurrent application to other substances

Variability was observed in relation to the concomitant application of PRP with other substances for all platelet concentrates, including the use associated with topical anesthetics (30.0% of responses) and hyaluronic acid (45.0% of responses).

Modalities of use

Most responses related to PRP identified use as part of a research protocol (80.0% of responses), followed by clinical practice (60.0% of responses). For the PRP-L and FRP, the predominance of responses referred to use as clinical practice (80.0% and 100.0% of responses, respectively).

Product identification prior to use

Responses related to PRP indicated the use of a unique identifier before applying the platelet concentrate (65.0% of responses), followed by the use of two identifiers (35.0% of responses). There was great variability of responses for PRP-L and FRP.

Adverse events

Most responses related to PRP identified the presence of at least one adverse event (60.0% of responses). The most common adverse event reported was pain at the application site (40.0% of responses). The same finding occurred in relation to the PRP-L, with 60.0% of the responses reporting the occurrence of pain at the application site. For the FRP, most respondents reported no adverse events (85.7% of responses).

In summary, great variability of responses was observed, in several stages of processing, as well as in relation to quality control and the use of platelet concentrates. The main findings are listed in Chart 3.

DISCUSSION

The variability of production methods for PRP and other platelet concentrates has been reported in previous studies[16,19]. These studies highlight the role of using different commercial systems[17,20] and different centrifugation protocols as sources of variability[19], including aspects of strength and centrifugation duration[21]. Other relevant issues include the use of platelet activation methods[1,22] and the type of anticoagulant used at the time of collection[23].

In the present study, the sources of production variability were identified for the three main platelet concentrates, PRP, PRP-L and PRP-F. The methods used for the production and the profile of use of these platelet concentrates were evaluated individually, for each one of them. Therefore, the interpretation of the results considered the specificities in terms of the procedures for production and use.

For the PRP, some guiding characteristics were identified from the set of responses. Product processing has predominantly taken place in medical offices and clinics and/or hospitals, with a double centrifuge protocol. The first centrifugation step has been carried out for 7 to 10 min with a centrifugal force of 100 to 1,000 g. The second centrifugation seems to be more susceptible to variability in terms of duration and gravitational force. The concentration of platelets in the final product obtained varies between 1.5 and 4.5 times higher than the basal value. The leukocytes' concentration seems to be related to greater variability and the growth factors' concentration is not routinely performed in most cases. PRP is predominantly used by professionals in the field of orthopedics, physiatrics and sports medicine. The product is applied by infiltration or intraoperatively in medical procedures.

The responses related to PRP-L exhibited variability in relation to the place of production. The product is obtained, in most cases, using double centrifugation, using, in the first centrifugation, a gravitational force of 100 to 500 *g* for 4 to 10 min. The second centrifugation most often employs a force of 500 to 1,000 *g* and lasts longer than 10 min. The platelet concentration of the final product ranges between values 1.5 to 4.5 times higher than the basal value. WBC concentration is highly variable, with responses indicating a decrease or increase in WBC concentration from baseline. The concentration of growth factors is not routinely performed. The product is predominantly applied by infiltration, in the areas of orthopedics, physiatrics, and sports medicine.

PRF exhibits a different profile of production and use methods compared to PRP and PRP-L, being produced predominantly by a single centrifugation protocol, employing a gravitational force between 100 and 500 *g*, by a period equal to or greater than 7 min. The concentration of platelets in the final product was 3.5 to 4.5 times higher than the baseline value. Leukocyte concentration above baseline was reported, without monitoring the concentration of growth factors. The product is predominantly used through intraoperative application in dental procedures.

The assessment of the adequacy of the methods described in the responses obtained is hampered by the scarcity of well-defined recommendations in the scientific literature. In a recently published consensus on the use of PRP in knee osteoarthritis, some recommendations regarding the standardization of the use of platelet concentrates are presented, such as the number of applications needed, the indication criteria, and recommendations on the use in conjunction with other products. This consensus, however, does not bring recommendations regarding technical production procedures or minimum safety requirements[24].

Several critical points were identified, subject to variability and risks throughout the production process. A primeira questão refere-se à qualidade do produto obtido, em termos das concentrações celulares e da concentração de fatores de crescimento. Considering that the low concentration of cellular components and growth factors may compromise the effectiveness of platelet concentrates, minimum standards for platelet, leukocyte, or growth factor concentrations should be stipulated as requirements for evaluating the quality of the concentrate. Additionally, the monitoring routine and methods for documenting the results must be defined.

Chart 3. Production characteristics of platelet concentrates.

	PRP	PRP-L	FRP
Processing location	Medical offices and clinics and/or hospitals	Dental offices/clinics and medical offices/clinics	Dental offices/clinics and medical offices/clinics
Type of system	Open or closed	Open or closed	Closed
Type of anticoagulant	Sodium citrate/ACD-A	Sodium citrate/ACD-A	None or sodium citrate/ACD-A
Number of centrifugations	Predominance of double centrifugation	Double centrifugation	Predominance of single centrifugation
Centrifugation force (single centrifugation)	100 to 1,000 g	No response obtained	100 to 500 g
Centrifugation duration (single centrifugation)	7 to 10 min	7 to 10 min	> 7 min
Force of the first centrifugation (double centrifugation)	100 to 1,000 g	100 to 500 g	No response obtained
Duration of the first centrifugation (double centrifugation)	4 to 10 min	4 to 10 min	4 to 6 min
Force of the second centrifugation (double centrifugation)	500 to 1,000 g	500 to 1,000 g	No response obtained
Duration of second centrifugation (double centrifugation)	7 to 10 min	Greater than 10 min	4 to 6 min
Maximum time between blood collection and end of processing	Up to 4 h	Up to 4 h	Up to 8 h
Sample identification methods between collection and end of processing	One or two identifiers	Two identifiers	One identifier
Frequency of evaluation of cell concentrations in the final product	All or some of the samples	All samples	Not evaluated
Method used to assess cell concentrations	Automated	Automated or manual	Automated or manual
Platelet concentration in the obtained product	1.5-4.5 times higher than baseline	1.5-4.5 times higher than baseline	3.5-4.5 times higher than baseline
Leukocyte concentration	Variable	Variable	> Baseline values
Growth factor concentration assessment	Not performed	Not performed	Not performed
Frequency of microbiological assessment	Not performed or performed on some samples	Variable	Not performed
Methods for microbiological assessment	Culture	Culture	Culture or Gram
Frequency of the patient's serological and/or molecular assessment	Variable	Not performed	Variable
Tests for serological and/or molecular assessment of the patient	Anti-HIV, anti-HBC, HBS-Ag, Anti-HCV<t5/>, and serology for syphilis.	Variable	Variable
Storage temperature	20 to 24°C or room temperature	Variable	Variable
Identification of samples during storage	One or two identifiers	Variable	Variable
Activation method	Variable	Variable	No activation
Usage areas	Orthopedics, physiatrics, and sports medicine	Orthopedics, physiatrics, and sports medicine	Dentistry
Forms of application	Infiltration or intraoperative application in medical procedures	Infiltration	Intraoperative application in dental procedures
Concomitant use with other substances	Topical anesthetics Hyaluronic acid Corticosteroids	Topical anesthetics Hyaluronic acid Radioisotopes	Topical anesthetics Hyaluronic acid
Modality of use	Research protocol	Clinical practice	Clinical practice
Identification of samples before application	Unique identifier or double identifier	Variable	Variable
Adverse events	Pain at the application site	Pain at the application site	None

Source: Elaborated by the authors, 2020.
PRP: platelet-rich plasma; PRP-L: platelet-rich plasma without leukocyte reduction; FRP: fibrin-rich plasma; ACD-A: sodium citrate or dextrose A citrate; min: minute; h: hour

In order to reduce the risks related to treatment with platelet concentrates in multiple scenarios, the development of regulatory standards should consider the practical aspects related to the identification of samples, throughout all stages of the process, from collection to application of the product, minimizing the risk of exchange of samples and contamination. Temperature conditions and maximum storage time must also be defined, as well as the standardization of previous serological assessment and microbiological assessment routines. The analysis of cell concentrations and growth factors

in the product and the minimum number of samples to be tested in quality control must also be established by standards so that the quality of the product, essential for its effectiveness, is guaranteed.

So far, there are no regulations to guide the technical aspects of the production of platelet concentrates without transfusion purposes. The Consolidated Ordinance of the Ministry of Health No. 5, of September 28, 2017[25], provides for the storage term and storage temperature of PRP, as an intermediate product in the production of transfusion platelet concentrates. The ordinance provides that the production of PRP occurs within 24 h after collection if the whole blood has been kept in conditions validated to maintain the temperature at 22 ± 2°C. It is noteworthy that these regulations refer to the production of PRP for transfusion purposes and, therefore, are not directed to the production of platelet concentrates without transfusion purposes.

Some of the responses obtained suggest that part of the production of platelet concentrates in Brazil occurs in non-recommended standards, which reinforces the importance of establishing regulations based on scientific evidence and international regulatory experience, which can address the critical issues associated with the process of production and use of PRP and related products, as well as the adoption of effective inspection measures.

The main advantage of the present study was the comprehensiveness of strategies for collecting responses. The study questionnaire was disseminated through e-mail lists and *websites* of professional societies, in order to capture the greatest number of responses. The questions in the questionnaire were defined in order to enable the analysis of all stages involved in the production, storage, and use of platelet concentrates. On the other hand, the main limitation of the study was related to the impossibility of evaluating the rate of respondents to verify the representativeness of the responses. This assessment was not possible, considering the dissemination of the questionnaire on open *sites*.

CONCLUSIONS

The process of production, storage and use of platelet concentrates without transfusional purposes involves considerable variability in several stages, with potential compromised effectiveness and increased risks related to the treatment. Ideally, the regulatory process should address these critical points to guide the production and use of these products effectively and safely.

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Author's Contributions

Pachito DV, Bagattini AM, Riera R - Conception, planning (study design), acquisition, analysis, data interpretation, and writing of the work. Mendrone Júnior A - Conception and planning (study design). All authors approved the final version of the work.

Conflict of Interests

The authors inform that there is no potential conflict of interest with peers and institutions, politicians, or financial in this study.