Growth Factors in the Platelet-Rich Plasma

Maczy Gonzalez[1,*], Melvis Arteaga-Vizcaino[2], Ana Ruiz[1], Jesus Estevez[2], Jesus Quintero[2], Maribel Quintero[3], Olga Briceño[1], Ricardo Atencio[2] and Ivis Marcano[4]

[1]*Director Chair of Hematology, Faculty of Medicine, University of Zulia, Venezuela, USA*

[2]*Institute of Clinical Research, Faculty of Medicine, University of Zulia, Venezuela, USA*

[3]*Professional Practice of Hematology, Faculty of Medicine, University of Zulia, Venezuela, USA*

[4]*Obesology Specialist*

Abstract: PRP is an useful bioproduct to tisular regeneration. The aim of study was evaluate the concentration of growth factors: PDGFBB (platelet-derived growth factor), EGF(epidermal growth factor) and VEGF (vascular endothelial growth factor) present in the Platelet-rich plasma (PRP) in subjects treated with drugs which inhibit platelet aggregation as acetylsalicylic acid (ASA) and clopidogrel before and after administration. We determined by ELISA PDGFBB, EGF and VEGF levels in PRP, Platelet Poor Plasma (PPP), lysate and exudate from 32 healthy subjects before and 24 hours after ingesting acid Acetyl salicylic acid (ASA) and clopidogrel as a single dose. The PRP and PPP were obtained by the method of Anitua by single centrifugation method. To analyze the results of student test and Pearson correlation was applied, with statistical significance level of $p < 0.05$. PPP and exudate (Clopidogrel: $p < 0.001$), PRP (Clopidogrel: $p < 0.01$) statistically significant differences for PDGFBB in PPP ($p < 0.01$ AAS) were found, and for VEGF in lysate (ASA and Clopidogrel: $p < 0.05$). No significant difference was found for EGF. Only was no correlation between baseline values of EGF in the ASA group and the respective PRP platelet count ($r = 0.726$). The results show that the average basal values of the three growth factors measured were considered particularly high in the PRP and lysate, showing the significant decrease for PDGFBB after antiplatelet therapy, especially of Clopidogrel and a significant increase for the VEGF only for the lysate. Although the behavior of three different soluble mediators was different to antiplatelet agents, the observed changes support the conclusion that a single dose of these drugs not markedly affect the secretion and availability of the three growth factors measured in various platelet derived obtained.

Keywords: PRP, PPP, lisate, growth factors, platelet antiagregants.

1. INTRODUCTION

Platelets contain cytoplasmic organelles whose contents secrete to the extracellular medium once activated, among which are the dense bodies, granules α and lysosomes. Of these organelles, the most abundant are α-granules, which have an important protein content, such as β-thromboglobulin, platelet factor 4 (PF-4) neutralizing heparin and other non-protein products such as fibronectin, thrombospondin, Fibrinolysis, fibrinogen, von Willebrand factor, soluble mediators such as cytokines, growth factors (GFs), and among the latter are pro and anti-angiogenic proteins including vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), transforming growth factor beta (TGFβ) and insulin-like growth factor), Among others [1,2].

In this context, Platelet Rich Plasma (PRP) provides a high platelet content and this is prepared through numerous techniques from blood donations that are subjected to differential centrifugation, achieving a platelet concentrate (600,000 to 1500,000 X mm^3). It is a plasma fraction that has a platelet concentration of 2 to 5 times higher than the number of platelets in peripheral blood (6). Therefore, it is considered a platelet concentrate, generally obtained by centrifugation of the blood of the patient to whom it is applied (autologous) (5-8). Platelets, when activated with the thrombin and calcium mixture, generate a product of gelatinous consistency that can be applied topically to a wound, enhancing the mechanisms of regeneration, quickly and effectively [3,4].

PRP has been recognized as a powerful hemostatic and adhesive agent since the 1970s, as well as a potent source of GFs since 1990 (5). Among the most studied soluble mediators released from PRP are PDGFAB, VEGF and EGF and Interleukin 4 (IL-4) and 6 (IL-6) [6-9].

PDGF is a thermostable cationic protein with a PM of 30-40 Kd, is a dimer composed of two identical or different peptide chains linked by disulfide bridges and its most known isoforms are PDGFAA, PDGFBB, PDGFAB, PDGFCC and PDGFDD. Its main source is platelets, where it is stored in the α-granules and released when the platelets are added and the

*Address correspondence to this author at the School of Bioanalysis, Faculty of Medicine, Clinical Research Institute, University of Zulia, Maracaibo, Venezuela, Final 20th Avenue next to the Maternity Castle Plaza, USA;
Tel: 58 261 4127 225, 04246692707; Fax: 58 261 4127 224;
E-mail: maczy.gonzalez@gmail.com, maczygonzalez@hotmail.com

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activation of the coagulation system is initiated [10,11]. The most important function of PDGF is to promote chemotaxis, in addition it can stimulate the recruitment, proliferation and survival of mesenchymal cells, smooth muscle, endothelial cells, fibroblasts, and other repair cells. Its angiogenic function is weaker than that developed by VEGF [1,12,13].

VEGF, also called Vascular Permeability Factor (VPF), is the most powerful of known vascular growth promoters, presents 5 different isoforms and the most abundant in platelets is VEGF-A. It belongs to the family that includes the Placental Growth Factor (PLGF). Among the cells that produce this factor in addition to platelets, there are macrophages, osteoblasts and smooth muscles mainly in the state of hypoxia. It acts on tyrosine kinase receptors in endothelial cells and is considered a potent angiogenic during the embryonic and postnatal stages [14].

EGF is a 53 amino acid polypeptide, synthesized and secreted by platelets, fibroblasts and endothelial cells, as well as renal cells and salivary glands. Only one isoform has been described. It possesses mitogenic, proapoptotic, migration and differentiation properties of fibroblasts, epithelial, renal and glial cells from mesenchymal cells. It is also attributed the ability to stimulate proliferation and differentiation of the epidermis, dermis, corneal epithelium, lungs and trachea during tissue repair [15,16].

The medical use of PRP today is recognized in several areas such as dentistry [1,17,18], cosmetic surgery [19,20,21], as well as in other branches of medicine in which these experiences were assumed for Justify its use in other pathologies, such as Hemophilia A and B, lower limb ulcers in patients with Diabetes Mellitus, osteoarthritis and other diseases [22].

The use of certain antiplatelet drugs on platelets such as acetylsalicylic acid (ASA) or aspirin and Clopidogrel / Ticlopidine (Tienopyridines) are widely known, which is universal in the treatment of patients with a tendency to thrombus Causes such as congenital or acquired heart disease, cerebrovascular disease, etc. These drugs act by inhibiting platelet aggregation, thereby reducing the risk of forming a platelet thrombus. However, they may interfere with the release of the FCs contained in the α [23-26] granules. Thus, in theory, PRP from subjects treated with antiplatelet agents would not be useful in the areas of medicine where it has been proven its great influence in the healing of various types of wounds.

This research hopes to contribute to a better understanding of the effective use of PRP in various medical procedures, from which it was intended to evaluate the concentration of growth factors (PDGF, EGF and VEGF) present in the PRP of subjects treated with different drugs which inhibit platelet aggregation such as AAS and Clopidogrel, before and after administration.

2. MATERIAL AND METHODS

2.1. Type and Design of Research

A descriptive, field, non-experimental and transversal investigation was performed [27].

2.1.1. Population and Sample

The study population consisted of adult subjects, male and female, apparently healthy, who came to the Institute of Clinical Research Dr. Américo Negrette of the Faculty of Medicine of the University of Zulia, Bolivarian Republic of Venezuela, during the period between February 2014 and February 2015. The sample was of a non-probabilistic type [28].

2.1.2. Inclusion Criteria

Subjects between 18 and 50 years of age, male and female, who wished to participate in this study, without basic clinical disease, were apparently healthy (previous general clinical evaluation) and with normal results in the study of platelet aggregation before treatment with the respective antiplatelet agent and strictly adhered to the indicated treatment. Those who had previously ingested platelet antiaggregants or alcoholic beverages 11 days prior to the study, or who were receiving any type of treatment, were excluded.

2.1.3. Process

Thirty-two subjects, 20 female and 12 male, were included and were divided into two groups as follows: 16 subjects treated with ASA at a single dose of 100 mg and 16 subjects receiving Clopidogrel at a dose of 75 mg in doses only.

In all subjects, fasting and prior asepsis of the area, both before and 24 hours after the respective treatment, 20.5 mL of antecubital venous blood were extracted, with scalp No. 21 using the double syringe technique to avoid activation of platelets [29]. The first syringe with 2.5 mL of blood was dispensed into a glass tube containing EDTA for complete hematology and platelet count, which was processed using a Beckman Coulter AC-T automatic blood cell counter.

The second syringe with 18 ml venous blood was distributed in three plastic tubes containing 3.2% sodium citrate (9/1), as follows: in one poured 9 mL and in the other two 4.5 mL. In a tube of the latter, the platelet aggregation study was performed with a Chrono-log aggregometer (Corp. Haverton, PA, USA) according to the turbidimetric method of Born [30]. In each of these samples the platelet count was also performed.

All tubes with sodium citrate were centrifuged in a clinical centrifuge. The one containing 9 ml of blood was allowed to stand 20 minutes, then centrifuged at a speed of 1,400 rpm for 7 minutes, following the technique of Anitua [5]. From the total plasma volume obtained, 0.5 mL was obtained corresponding to Platelets-Poor Plasma (PPP), according to the protocol established by Anitua. The remainder (PRP) was taken through a very meticulous pipetting and with a different tip, to the area that was above the red fraction. One mL of the total PRP volume was aliquoted for platelet counting and GFs measurement. On the other hand, 1 mL of the PRP obtained was added 50 uL of 10% calcium chloride, mixed and allowed to stand for 15-30 minutes to obtain the gelled PRP and subsequently centrifuged at 2,500-3,000 rpm per 10 minutes, to obtain the exudate.

The remaining tube with 4.5 mL of blood was allowed to stand for 20 minutes and then subjected to 2 centrifugations, the first at 1400 rpm for 7 minutes (Anitua Single Spin Method) [5] and the second at a speed of 3,600 rpm for 10 minutes (Modified method of Ghandi *et al*.) [31].

The total volume obtained in the PRP after the second centrifugation (fraction of the PRP most concentrated in platelets), was discarded 0.5 mL of the PPP and the rest of the PRP was mixed with a different pipette tip in order to obtain a Plasma was further concentrated in platelets, then a portion was taken to perform a platelet count and finally separated and stored in Eppendorf tubes.

PPP, unactivated PRP, gelled PRP exudate and second centrifugation PRP were aliquoted and stored in Eppendorf plastic tubes at -70°C in a deep freezer (Form Scientific U95-18) until the analysis of GFs, with the technique of Enzyme Linked Immunoassay (ELISA) [32].

The PRP of the second centrifugation at the time of analysis of the GFs was subjected to a consecutive thawing and freezing process to promote the lysis of the platelets contained therein according to the following procedure: The first thawing of the aliquots (three for each subject) was performed at 37 °C for 25 minutes and then carefully mixed and organized into a single aliquot per individual. Each aliquot was re-frozen at -20°C for 1 hour, this procedure was repeated four times more consecutively by thawing each time at 37°C for 15 minutes and then freezing at -20°C (modified method applied by Perseghin *et al*.) [33]. At the end, each aliquot of the lysate was distributed in three aliquots 200 µL for the corresponding processing and analysis of each GF.

Levels of PDGFBB, EGF and VEGF growth factors were measured in samples and standards using the indirect ELISA method, whose assays for PDGFBB and EGF were supplied by ABCAM (ABCAM INC, CAMBRIDGE, USA: 1 Kendall Square, Ste B2304 Cambridge, MA 02139-1517 USA) with lot number: GR 85403-1, GR85404-1 and GR 56644-1 for the PDGF, GR81587-1, GR81588-1 and GR81589-1 for the EGF. The VEGF assays were supplied by Thermo Scientific (Pierce Biotechnology, 3747 N. Meridian Road, PO Box 117, Rockford IL61105, USA) with lot number: ME 156238, MH 161815 and NE 168666. All assays were performed according to the manufacturer's instructions.

It was considered as a reference value for peripheral blood platelets between 150,000 and 450,000 per mm^3 [34].

For the tabulation and analysis of the results that were obtained, the respective statistic was used. The data are shown in tables, in absolute values and percentages, as well as mean ± standard deviation. For the comparison of the variables in the study, paired Student's t-test, Tukey-Kramer multiple comparison test and post test was used, while the Pearson test was used for the correlation study and considered a $p < 0,05$ as the least statistical probability [35].

All subjects were required to have their written informed consent before being included in the study [36] and proceeded according to the principles of the Helsinki Declaration of 1975, updated in 2013, at the 64th General World Medical Assembly of Fortaleza, Brazil and the recommendations made by the Council of International Organizations of Medical Sciences (CIOMS) in 2002 [37].

3. RESULTS

In Table **1**, it is observed that the average blood platelet count of healthy subjects incorporated into the present investigation, is within the reference range.

Table 1: Number of Platelets in Healthy Subjects before Treatment with AAS or Clopidogrel

Sample n=32	# Platelets $10^3/mm^3$
Periferic Blood BP	272.281±69.66[a] (150-379)
PPP $10^3/mm^3$ Basal	375.14±112.6[b] (247-626)
PRP $10^3/mm^3$ Basal	505.40±140.83 (316-740)

Results are expressed as mean. standard deviation and range.
n: Number of subjects studied.
PPP: Platelet Poor plasma.
PRP: Platelet Rich Plasma.
[a]Platelets in blood vs platelets in basal PRP: p = 0.0001.
[b]Platelets in PPP basal vs platelets in basal PRP: NS.

While the value of the baseline PRP or pre-treatment PRP (505.40 ± 140.83 x 103 / mm^3) for all subjects studied (n = 32) presented statistically significant differences (p <0.0001) When compared to the peripheral blood value (272.281 ± 69.66 x 103 / mm^3), this was not statistically significant when compared to that obtained in the baseline PPP (375.14 ± 112.6 x 103 / mm^3).

The platelet values in the PRP obtained from the AAS-treated group were higher than those treated with Clopidogrel, both before and after receiving the drug (p < 0.002, p < 0.001), respectively. When comparing the number of platelets before and after receiving treatment in each group, there were no significant differences, as shown in Table **2**.

The comparison of mean concentrations of PDGFBB in PPP, PRP and its by-products of healthy subjects before and after treatment with ASA or Clopidogrel is shown in Table **3**. The lowest concentration of this factor was observed in PPP after receiving ASA with 8.96 ± 1.4 ng / mL, a value that was statistically different from that obtained before administration (p<0.01). In addition, a decrease of this GF in post-treatment PRP was observed, although it was not statistically significant. Comparing the mean values of PDGFBB before and after treatment with Clopidogrel, a statistically significant decrease was

Table 2: Number of Platelets in the PRP of Healthy Subjects before and after the Treatment with AAS or Clopidogrel

	Número de plaquetas $10^3/mm^3$		
Antiplatelet Drug	**Before**	**After**	**p**
AAS n=16	578.25±125.33 (404-803)	566.86 ± 109.16 (405-784)	NS
Clopidogrel n=16	432.56±118.18 (251-604)	435.22 ± 108.48 (333-620)	NS
p	0.002	0.001	

The results are expressed in mean. Standard deviation and range.
n: Number of subjects studied.
AAS: Acetyl salicylic acid.
NS: Not significant.

Table 3: Concentration of PDGFBB (ng/mL) in PPP, PRP and its Substances of Healthy Subjects before and after Treatment with AAS or Clopidogrel

Sample	AAS n=16		Clopidogrel n=16	
	Before	**After**	**Before**	**After**
PPP	10.68±1.9	8.96±1.4 a	10.6±1.8	8.53±0.59 b
PRP	12.12±2.51	11.36± 2.48	12.0±2.4	9.65±1.17 a
Exudated	10.84±1.68	11.11±1.14	10.7±1.55	8.51±0.75 b
Lisate	10.56±2.36	10.85±3.01	10.45±2.1	10.15±1.74

n: Number of subjects studied.
AAS: Acetyl salicylic acid.
PDGFBB: Growth Factor Derived from Platelets BB Isoform.
PPP: Platelet Poor Plasma.
PRP: Platelet Rich Plasma.
[a]With respect to its control (before) p<0.01.
[b]With respect to its control (before) p<0.0001.

Table 4: Concentration of EGF (pg/ml) in PPP, PRP and its Subproducts in Healthy Subjects before and after the Treatment with AAS and Clopidogrel

Sample	AAS (n=16)		Clopidogrel (n=16)	
	Before	After	Before	After
PPP	96±141.1	172.75±167.4	11.9 ± 6.8	8.25±2.75
PRP	296.1±203.6	353.95±204.5	51.75±14.35	58.75±29.15
Exudated	150.25±120.8	159.7±71.1	29.75±12.95	36.8±11.95
Lisate	280±70	275±35	37±18.9	48±13

n: Number of subjects studied.
AAS: Acetyl salicylic acid.
EGF: Epidermal Growth Factor.
PPP: Platelet Poor Plasma
PRP: Platelet Rich Plasma.

observed in both PPP and exudate (p <0.0001), as well as in PRP (p <0.01), posttreatment.

Table **4** shows the concentrations of EGF in PPP, PRP and their respective by-products in the studied population. The highest concentrations were obtained from PRP in both groups. No statistically significant differences were found when comparing GF values in the different platelet derivatives before and after treatment.

Plasma concentrations of VEGF in PPP, PRP and its byproducts before and after administration of ASA or Clopidogrel in healthy subjects are shown in Table **5**. The highest value found for this factor was in the lysate of the group that Received Clopidogrel with 1,076.8 ± 534 pg / mL and the PRP of the group receiving AAS with 913.6 ± 380.84 pg / mL. When pretreatment and posttreatment values were compared, there were only statistically significant differences for the lysate (p <0.04) in both groups.

There was only significant correlation (r: 0.71, p <0.001) between baseline PRP platelet counts and EGF pretreatment concentrations in the ASA group (data not shown in table).

4. DISCUSSION

In the present investigation, the platelet numbers of the subjects studied were within the reference range before treatment with the antiplatelet agents used [34]. Likewise, the mean baseline platelet count in the PRP of the 32 subjects studied in the present study showed a significant increase (p <0.0001) when compared to the blood count. This increase was 1.2 to 2.72 times, was within the range established for the protocol of a centrifugation described by Anitua [5,38] and adjusted to the definition of PRP enunciated in the literature [5]. These values are comparable to those described by several researchers including Castillo *et al*. (39), who obtained PRP through commercial cell separation systems and found a platelet count of 566.2 ± 292.6 x 103 / mm3 by the GFS method and 443.8 ± 24.7 x 103 / mm^3 per the Magellan method.

Table 5: Concentration of VEGF (pg/ml) in PPP, PRP and its Substances of Healthy Subjects. Before and after the Treatment with AAS and Clopidogrel

Sample	AAS (n=16)		Clopidogrel (n=16)	
	Before	After	Before	After
PPP	658.8±262.68	708.8±353.88	504±288.52	542±272.12
PRP	833.6±446.4	913.6±380.84	663.2±419.2	680.4±383.6
Exudated	756.4±375.56	870±387.76	568±366.24	568.4±309.32
Lisate	632.8±212	801.6±151.72[a]	728.8±387.88	1076.8±534[a]

n: Number of subjects studied.
AAS: Acetyl salicylic acid.
VEGF: Vascular Endothelial Growth Factor.
PPP: Platelet. Poor Plasma.
PRP: Platelet Rich Plasma.
[a]with respect to its control (before): p<0.04.

An important finding of the present investigation was that there was no statistically significant difference in the platelet count obtained in baseline PPP when compared with baseline PRP. This suggests that the PPP obtained through the single-centrifugation protocol of Anitua [5], does not produce a poor concentration of platelets as it is cataloged by Anitua´s research, so its incorporation into PRP must be supposed to increase its cellular content and GFs.

Regarding the number of platelets obtained in the PRP after 24 hours of treatment with ASA or Clopidogrel, no statistically significant differences were found when compared to the mean values in the baseline PRP before treatment. The finding that both antiplatelet drugs in a single dose did not significantly affect the number of platelets in the studied subjects could be explained, firstly because their effect on platelet function and, second, for the fact that platelet replacement under normal conditions after administration of these drugs is approximately 7 days [40, 41]. These results agree with those obtained by Hayasaka *et al*. [42], who performed a comparative study of the involvement of hematologic parameters in 2 groups of patients treated for 2 months with aspirin plus Clopidogrel and aspirin alone, and found no statistically significant difference in platelet count (baseline and posttreatment) but there are statistically significant difference for the red cells, white cells, hemoglobin an hematocrit counts.

However, when comparing PRP platelet counts before and after treatment between the two groups, despite being within the expected limit for PRP, there were statistically significant differences ($p < 0.002$ and $p < 0.001$, respectively). However, these variations in the number of platelets in healthy subjects clearly respond to physiological fluctuations described in the scientific literature [5, 34,38].

When the mean PDGFBB concentrations determined in PPP, PRP and its byproducts were analyzed before and after treatment with the platelet drugs used, a marked decrease in the levels of this factor was observed in two of the four platelet products (PPP and PRP) after AAS treatment (although it was only significant for PPP, $p < 0.01$). However, the highest baseline mean PDGFBB values were noted in the PRP and not in the exudate or lysate as expected. These results are similar to those obtained by Passaretti *et al*. [43], who compared the contribution of GFs in PRP and Platelet Rich Fibrin (PRF) and described twice as PDGF levels in PRP (equivalent to the exudate of this research) as in PRP. These results could be due to the fact that PRP is the product derived from platelets in which there is the highest concentration of them.

It is possible that this occurred in the lysate more markedly, as this is a product obtained from repeated freezing and thawing, and hence the measured GF levels are similar to those of the PRP, confirming that this process does not appear to compromise the availability of GFs as described by Perseghin *et al*. (2006), who compared the levels of GFs in fresh blood *vs* lysate and did not find significant differences [33].

It is also important to emphasize, that mean values of PDGFBB in PRP and its byproducts at baseline and after administration of Clopidogrel showed significant differences for PRP, PPP and exudate, with levels lower than those found in the group of ASA. These results could confirm a greater level of alteration in the capacity of secretion of the PDGFBB by the platelet derivatives in the subjects treated with Clopidogrel.

It is important to note that the mean concentrations of PDGFBB before treatment were similar to those reported by other investigators, whose values ranged from 2.3 to 37 ng / mL [44-48]. Among these studies, the study by Christgau *et al*.

When comparing the mean concentration of PDGFBB in the various platelet derivatives of the subjects studied after AAS / Clopidogrel treatment, it can be seen that GF levels are higher in the different platelet byproducts of the ASA group, in contrast to Clopidogrel. This appears to reveal a lower degree of alteration (decrease) in PDGFBB levels in samples from subjects treated with ASA compared to clopidogrel. It is inferred that these differences may be due in part to the mechanism of specific pharmacological action that each drug possesses; Being the selective and irreversible inhibition of cyclooxygenase by the acetylation of its active site, and its effect usually lasts for 7 days [54].

In PRP, AAS can completely block arachidonic acid-induced platelet aggregation and partly reduce platelet activation induced by collagen, ADP, epinephrine and platelet activating factor (PAF). On the other hand, thienopyridines, such as clopidogrel, exert their main function by blocking the ADP receptor known as P2Y12, inhibit the secretion of α-granules that are rich in GFs and interfere with the platelet fibrinogen receptor (gIIb-IIIa) impeding the pathway of greater amplification of platelet aggregation [54-57], which may have mainly affected the secretion of PDGF in the Clopidogrel group.

Considering EGF levels before and after treatment with both ASA and Clopidogrel, there was an increase in mean concentrations (compared to baseline) after treatment in both study groups, however, no Statistically significant differences were found in none of the platelet derivatives. The baseline values of this GF were similar to those reported by other authors [48-50], such as Eppley B *et al*. (2004), who found EGF in the PRP of 470 ± 320 pg / ml [48], these levels being approximate to baseline and posttreatment for the AAS group, but not for the Clopidogrel group. For the latter group, the concentrations were lower than those of ASA.

In this sense, the differences in the EGF concentrations found, both at baseline and post-treatment with ASA and Clopidogrel, could be explained by the individual variability described in the cellular production, storage and secretion of GFs by Platelets and other cells, which may be present at the time of collecting the sample or released with the procedure to which the whole blood is subjected to obtain different platelet byproducts, such as PRP, exudate or lysate [44,48].

The above has also been reported by other authors who indicate that each individual would need different concentrations of their cells, especially of the platelets, to achieve a secretion of GFs with comparable biological effect [44,48]; And that the interindividual variations found may not be correlated with the platelet count, thus, an individual with high levels of CF can exhibit the lowest concentrations of another GF [44,49].

When the mean EGF concentrations are observed in the AAS and Clopidogrel post-treatment groups, it can be seen that the values of this GF are higher in the PRP than in the rest of the platelet derivatives, in the PPP they are the lowest and there is similarity between The concentrations shed for PRP and lysate, with no significant differences for any platelet derivative. This finding does not allow to establish a relevant difference between these platelet byproducts, but if we confirm what Woo LJ *et al*. (2013) who state that there is no significant influence of thrombin or calcium chloride used to achieve PRP activation and secretion of the GFs, nor the amount of platelets concentrated in the PRP, therefore, the addition of These substances are unnecessary to achieve their effective preparation [58].

It is important to note that there are no officially established reference values for the GFs measured in this research, since the commercial systems for measuring them are not standardized, hence different concentrations are reported for similar populations in research conducted by different authors. This makes it difficult to compare scientific work [59].

Observing the mean concentrations of VEGF in PRP and its platelet byproducts before and after administration of AAS / Clopidogrel, it can be seen that, with respect to baseline values, they coincide with those reported by other investigators [45,48,60,61]. In this regard, Agren MS *et al*. (2013) determined, among other HRs, VEGF in PRF in 10 volunteer donors, PRF was obtained through a commercial system called Vivostat®. VEGF levels were at 406 ± 141 pg / ml, as well as some variability in the concentration of measured GFs, indicating that this is due to the individual differences that may occur between donors (64). Also, Barsotti MC *et al*. (2013) measured the effect of platelet lysate on the different phases of the wound healing process, obtaining mean VEGF values of 740 ± 110 pg / ml, values similar to those of this study [62].

When the average levels of VEGF are seen in the study groups according to the drug used, it is noted that in the AAS group the highest average values are found in PRP (before and after), whereas in the Clopidogrel group are in the lysate with statistically significant difference for both the AAS group and Clopidogrel. This confirms the assurance of other authors such as Weibrich G *et al*. (2002), Perseghin P *et al*. (2005) and Barsotti MC *et al*. (2013), who point out that freezing at very low temperatures (-70ºC) and rapid thawing (37ºC), is a common and effective method to achieve the release of GFs, without affecting their concentrations, or biologically [33,52,62], thanks largely to the massive lysis of the platelets present in the platelet derivative. This aspect is of clinical utility when considering the use of frozen PRP concentrates from patients receiving antiplatelet agents such as those used in the present study.

Together, it can be seen that in both study groups (AAS / Clopidogrel) an increase in mean values of VEGF was observed after the respective treatment, which is more evident in the AAS group. This would seem to indicate alteration of the GFs that seems to be specific for each drug.

It is significant to note that the scientific literature did not find scientific papers that had carried out the determinations of PDGFBB, EGF and VEGF after the treatments with the antiplatelet agents used here, so no comparison could be established with another similar experience.

In summary, there are apparently 2 types of behavior in terms of the concentration of the three GFs after treatment with antiplatelet agents. The first one is the one exhibited by the PDGFBB whose mean values were kept below the basal levels in almost all platelet byproducts, being this more evident and significant decrease in the Clopidogrel group, which would allow to deduce that possibly this drug seems to propitiate a higher level of involvement of the secretory pattern of this GF in comparison to the AAS, although this decrease is not marked, it could not be said that a single dose of this drug could be responsible for the alteration of the bioavailability of this GF. The second type of behavior is the one developed by the EGF and VEGF, whose concentrations showed a predominance of the increase above the basal values after the respective treatment, which would seem to indicate that both drugs could favor or not affect negatively, the secretion of both GFs, with no apparent detriment to their biologically active levels.

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CONFLICT OF INTEREST STATEMENT

It is stated that the authors of this manuscript do not have any conflict of interest.

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