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Characterization of platelet rich plasma in feline immunodeficiency virus-infected cats: Cell, and PDGF-BB and TGF-ß1 growth factor analysis

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ABSTRACT

Autologous platelet-rich plasma (PRP) contains growth factors (GFs) that modulate the expression of inflammatory cells; thus, these products could be considered a good strategy to favor tissue regeneration in feline immunodeficiency (FIV) positive cats. However, there is no scientific documentation on obtaining PRP in FIV-positive cats.

Authors hypothesized that PRP can be obtained in FIV cats following the PRGF®-Endoret® methodology. The objectives of this study were to compare the platelet, erythrocyte, and leukocyte concentration between whole blood (WB) and the PRP; and determine the concentration of platelet-derived growth factor BB (PDGF-BB) and transforming growth factor β1 (TGF-β1) in FIV-positive cats. Sixteen adults FIV-positive asymptomatic cats were included in the study. WB samples were drawn and the PRP was obtained by centrifugation at 265*g* for 10 min. Erythrocyte and leukocyte, platelets, and mean platelet volume (MPV) were determined both in WB and in PRP. PDGF-BB and TGF-β1 concentrations were additionally determined in PRP.

Platelet concentration increased 1.1 times in PRP fraction compared to WB, but no significant differences were reported. MPV was statistically higher in WB than in PRP ($p = 0.001$). Erythrocytes and leukocytes counts were decreased by 99% and 92%, respectively in the PRP fraction ($p < 0.001$). Regarding TGF-β1, a higher concentration was shown in the PRP ($p < 0.02$). Although the product obtained could not be classified as PRP according to the PRGF®-Endoret® methodology, based on the drastic reduction of RBC and WBC, the PLT concentrate is of high purity.

1. Introduction

Plasma rich in growth factors (PRGF) uses autologous proteins and growth factors (GFs) derived from plasma and platelets (PLT) as therapeutic formulations. PLT release a pool of GFs, they control growth, differentiation, and metabolism of cells, interfere on the wound healing process and repair cascade (Steed, 1997; Wu et al., 2016), promoting tissue repair, stimulating angiogenesis, and regulating inflammation (Werner and Grose, 2003; Grazul-Bilska et al., 2003; Anitua et al., 2004).

PRGF®-Endoret® system is an autologous biological approach based on platelet-enriched plasma. In veterinary medicine, PRGF®-Endoret® has been obtained in horses (Rushton et al., 2018), rabbits (Chicharro et al., 2018; Chicharro-Alcántara et al., 2018; Torres-Torrillas et al., 2021), dogs (Damià Giménez, 2012; Vilar et al., 2013; Cuervo et al., 2014), and more recently in healthy (Miguel-Pastor et al., 2022) and leukemia cats (Miguel-Pastor et al., 2023).

Feline immunodeficiency virus (FIV) is a lentivirus with worldwide distribution. Four subtypes of FIV have been isolated, and cats can be concurrently infected with more than one subtype (Hofmann-Lehmann et al., 1997). The primary mode of transmission is through bite wounds

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(Hosie et al., 2009). The worldwide prevalence rate of FIV ranges from 2 to 44% (Pedersen et al., 1987; Hartmann, 1998), registering 3 to 18% in the USA (Levy et al., 2006), 6 to 44% in Asia (Ishida et al., 1989; Sprißler et al., 2021), 13 to 20% in Australia (Westman et al., 2016; Carlton et al., 2022) and up to 30% in Europe (Carlton et al., 2022; Priolo et al., 2022). In Spain, it ranges from 1 to 20.9% (Ayllón et al., 2012; Miró et al., 2014; Montoya et al., 2018; Alcover et al., 2021; Villanueva-Saz et al., 2022; Candela et al., 2022).

There are three phases of FIV infection: a) acute phase, in which cats are viremic and symptomatic b) asymptomatic (or latent) phase and c) progressive or chronic phase (Torten et al., 1991; Hofmann-Lehmann et al., 1997). FIV has a primary tropism for lymphocytes, including B lymphocytes, CD4+ T lymphocytes, CD8+ T lymphocytes, and monocytes/macrophages (Hosie et al., 2009). The cytopathic effect of FIV causes a progressive loss of CD4+ lymphocytes, inversion of the CD4/CD8 ratio and loss of CD8+ lymphocytes (Joshi et al., 2004; Murphy et al., 2012). After a long period of clinically latency, the progressive loss of T lymphocytes results in an immunodeficiency syndrome characterized by chronic and recurrent infections (Hartmann, 2011; Carlton et al., 2022).

In addition to lymphopenia, other alterations such as neutropenia, eosinopenia, leukopenia (Hofmann-Lehmann et al., 1995; Callanan et al., 1996), anemia, thrombocytopenia and pancytopenia (Fujino et al., 2009; Gleich and Hartmann, 2009) have been reported. These cytopenia's occur in response to infection of myeloid progenitor cells and inhibition of hematopoiesis (Beebe et al., 1992; Fujino et al., 2009). Long-term FIV infection can also lead to the development of hematopoietic malignancies (Kaye et al., 2016).

Since FIV-infected cats are susceptible to co-infections and the development of other pathologies, PRP therapy could be beneficial to alleviate the effects induced by the virus, as it has been proved using different PRP obtention protocols in wound healing (Angelou et al., 2022), corneal ulcers (Farghali et al., 2021), and spinal cord multiple sclerosis (Farid et al., 2022). The aim of the present study was to determine the concentrations of PLT, platelet-derived growth factor-BB (PDGF-BB) and transforming growth factor-ß1 (TGF-ß1), in platelet-poor plasma (PPP) fraction and PRP fraction in FIV-infected cats using the PRGF®-Endoret® protocol previously standardized for its application in cats by these same researchers (Miguel-Pastor et al., 2022). The authors hypothesize that PRP can be obtained in FIV cats following the PRGF®-Endoret® methodology as in healthy (Miguel-Pastor et al., 2022) or leukemia-infected (Miguel-Pastor et al., 2023) cats.

2. Materials and methods

2.1. Serum samples

2.1.1. Study population, clinical examination, and inclusion criteria

All animals were used by following ethical approval from the Animal Welfare Ethics Committee (CEEA) of the CEU Cardenal Herrera University in Valencia (Spain) in accordance with the Spanish Animal Protection Policy (RD53/2013), which complies with the European Union Directive European 2010/63/EU, with the following approval code: 2018/VSC/PEA/0196.

The study was designed as a prospective study and included cats presented to the CEU-Cardenal Herrera University Veterinary Clinical Hospital, between September 2022 and May 2023 as part of a medical clinical study. During this period, blood samples of sixty non-pedigree cats were tested for the presence of FIV, that were clinically healthy at the time of blood collection. All animal owners agreed to participate in the study after being informed and signed a consent form. Whole blood (WB) samples were tested for the presence of FIV antibody and FeLV antigen using a commercially available enzyme linked immunosorbent assay (ELISA; IDEXX SNAP® Combo FeLV Ag/FIV Antibody Test). Cats with FIV positive results were retested from 6 to 9 months after using the same assay and only considered true-positive if they also tested positive in the second test. Discordant test results were considered negative and there were excluded of the study. Clinically ill animals, or hematological alterations presentation as anemia, leukopenia, or real thrombocytopenia were excluded. Animals receiving any treatment for the previous or current 6 months of the study and those that developed diseases or tested positive FeLV, or double infected (FIV and FeLV) cats were not included in the study. Each cat was directly overseen by a veterinarian throughout the whole practice to ensure no harm incurred during study participation.

2.2. Blood processing

Under sedation (butorphanol 0.3 mg/kg, dexmedotomidine 12 µg/kg and alfaxalone 0.8 mg/kg intramuscularly), a small aliquot of blood (0.5 mL) was collected in a tube containing K3-EDTA (BD Vacutainer; Becton, Dickinson) to the cephalic vein using a 22 G catheter for WB analysis. Subsequently, if no hematology alterations were presented, 9 mL of blood were collected in sterile conditions from the jugular vein by means of a vacutainer sodium citrate 3.8% tube (Blood-Collecting Tubes®, BTI Biotechnology Institute, Alava, Spain) for PRGF®-Endoret® preparation. Finally, each cat received 9 mL of acetated Ringer's solution IV during the first 20 min to restore the vascular volume and to prevent hemodynamic complications.

2.3. Preparation of the PRGF®-Endoret®

After cat samples were collected in sodium citrate tubes, were immediately centrifugated at room temperature using a single-spin method as a protocol previously described (Miguel-Pastor et al., 2022) in the PRGF®-Endoret® System IV centrifuge (BTI Biotechnology Institute S.L.). Once the blood was centrifugated at 265 g for 10 min, the supernatant that consisted of plasma and buffy coat were obtained. According to BTI-Endoret manufactures, the 60% upper of the supernatant was considered PPP fraction, and the 40% lower (just above buffy coat) was considered PRP fraction (Fig. 1). Two plasma fractions (PPP and PRP) were analyzed and compare to WB samples to certificated if PRGF®-Endoret® were obtained. Both, PRP and PPP were activated by adding calcium gluconate 10% (PRGF® activator, BioTechnology Institute, Álava, Spain) (5% of the plasma volume) to achieve PLT degranulation and GFs release, hence obtaining PRGF. The obtained samples were aliquoted into eppendorf tubes and, immediately after activation, they were frozen at −80 °C before the PLT plug was formed for subsequent determination of TGF-β1 and PDGF-BB concentrations. In no case time exceeded 30 min between blood centrifugation, PRP obtention, activation, and freezing.

2.4. Hematological analysis

Cell counts were performed using an automated hematology analyzer validated for cat blood (Advia® 2120i Siemens Healthcare Diagnostics Inc.). Red blood cell (RBC; 10^6/µL), hemoglobin concentration (HB; g/dL), packed cell volume (PCV; %), mean corpuscular volume (MCV; fL), mean corpuscular hemoglobin (MCH; pg), mean corpuscular hemoglobin concentration (MCHC; g/dL), mean hemoglobin content (CH; pg), hemoglobin concentration distribution width (HDW; d/dL), red blood cell distribution width (RDW; %), reticulocytes absolute count (RET; 10^9/L), white blood cell (WBC; 10^3/µL), neutrophils (NFS; 10^3/µL), lymphocytes (LYMPH; 10^3/µL), monocytes (MON; 10^3/µL), eosinophils (EOS; 10^3/µL), basophils (BAS; 10^3/µL), platelet (PLT; 10^3/µL) counts, and mean platelet volume (MPV; fL) were analyzed in WB (Table 1). PLT, MPV, RBC, WBC, LYMPH, NFS and MON counts also were analyzed in PRP and PPP fractions (Table 1). The absolute numbers of PLT obtained by ADVIA were verified in blood smears.

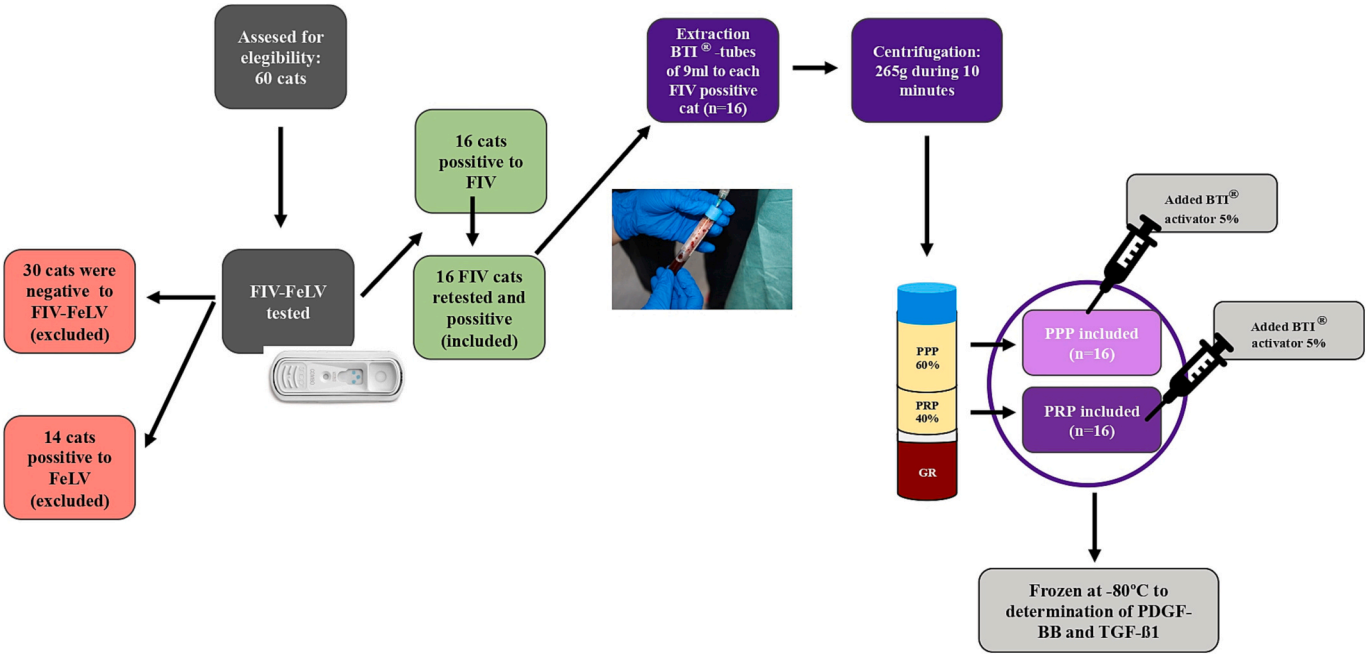

Fig. 1. Schematization of the study design: blood sample obtention, centrifugation following feline BTI protocol using the PRGF®-Endoret® methodology, PRP and PPP fraction obtention, subsequent activation of both fractions with BTI-activator and freezing at −80 °C for subsequent GFs analysis.

Table 1
Descriptive statistic of hematological parameters.

Parameter	Mean	SD	Minimum	Maximum	Reference value
WBC (10^3/μL)	11.36	5.19	5.06	24.4	5.5–19.5
NFS (10^3/μL)	6.86	2.78	2.32	11.34	2.5–12.5
LYMPH (10^3/μL)	3.8	2.65	1.46	10.61	1.5–7.0
MON (10^3/μL)	0.35	0.16	0.17	0.79	0–0.9
EOS (10^3/μL)	0.27	0.44	0	1.7	0–0.8
BAS (10^3/μL)	0.02	0.02	0	0.08	0–0.2
RBC (10^6/μL)	7.72	1.3	6.06	10.8	5.0–10.0
HB (g/dL)	10.19	1.43	7.7	12.9	8.0–15.0
PCV (%)	29.11	4.10	22.1	35.7	24.0–45.0
MCV (fL)	38.05	5	25.8	44.3	39.0–55.0
MCH (pg)	13.54	1.48	9.8	15.6	12.5–17.5
MCHC (g/dL)	34.99	1.07	33.5	37.3	30.0–36.0
CHCM (g/dL)	36.07	1.26	34.3	38.8	30.0–36.0
CH (pg)	13.93	1.56	9.9	16.3	12.0–16.0
RDW (%)	17.86	1.9	15.6	22.1	14.0–18.1
RET (10^9/L)	42.82	18.5	15.2	84.4	15.0–81.0
PLT (10^3/uL)	329.5	94.74	143	537	200.0–500.0
HDW (g/dL)	2.66	0.34	2.18	3.18	1.6–2.9
MPV (fL)	18.63	3.07	13.4	24.9	8.6–18.9

Mean ± SD or median and the 95% CI of all the hematological parameters considered in whole blood: leukocytes (WBC), neutrophils (NFS), lymphocytes (LYMPH), monocytes (MON), eosinophils (EOS), basophils (BAS), erythrocytes (RBC), hemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), cellular hemoglobin concentration mean (CHCM), cellular hemoglobin content for reticulocyte (CH), red blood cell distribution width (RDW), reticulocytes (RET), platelet (PLT), hemoglobin distribution width (HDW), and mean platelet volume (MPV) in whole blood samples in 16 FIV cats ($n = 16$).

2.5. Growth factors quantification: PDGF-BB and TGF-ß1

The concentration of both GFs in both plasma fractions (PRP and PPP) were determined by an ELISA of development with antibodies to human TGF-ß1 (Human TGF-beta 1 DuoSet, DY240E, R&D System, Minneapolis, MN, USA) and human PDGF-BB (Human PDGF-BB Duoset DY220E, R&D Systems, Minneapolis, MN, USA). ELISA was performed following the manufacturer's instructions (Table 1).

2.6. Statistical methods

The data were processed using SPSS 20.0 for Windows (SPSS® Inc., Chicago, USA). For each variable, a descriptive study of the mean, standard deviation and confidence intervals was performed. The Shapiro-Wilk test was used to assess the normality of data in every quantitative variable, and the Leven test was used to assess the variance homogeneity. The means of the variables were studied using a linear mixed model. These models included the treatment group as fixed effects and the cat as a random effect. If the interaction was found statistically significant, analyses using a one-way ANOVA and a Bonferroni test were used. Non-parametric Kruskal-Wallis tests were used to compare variables not adjusted to a normal distribution. A p-value <0.05 was considered significant.

3. Results

Blood was collected from a total of 16 asymptomatic FIV adult cats. There were 1 spayed female and 15 neutered males. The mean weight was 5.2 kg (range 3.4–5.4 kg) and the mean age was 5.3 years old (range 2–9 years).

Table 1 shows the hematological parameters in the WB samples individually for each animal included in the study. Table 2 shows the PLT, RBC, WBC, LYMPH, NFS and MON counts and the MPV both in WB and in the PRP and PPP fractions; and the concentrations of PDGF-BB and TGF-β1 in PRP and PPP.

3.1. Cellular analysis

3.1.1. Platelet concentration and mean platelet volume

A statistically significant difference was found between the PLT mean PRP (359.3 ± 90.6 10^3/μL) and PPP fraction (258.8 ± 79.6 10^3/μL) ($p = 0.007$), with no statistical differences between WB and PRP fraction or between WB and PPP fraction (Table 2, Fig. 2A). MPV was statistically higher in WB than in PRP or PPP fractions ($p = 0.001$ and $p < 0.001$, respectively) with no statistical differences between PRP and PPP fractions (Table 2).

Of the 48 total samples analyzed, PLT aggregates were obtained in 8

Table 2
Comparative values among WB, PRP and PPP fractions.

Variable	Mean ± SD			*P*-value		
	WB	PRP	PPP	WB-PRP	WB-PPP	PRP-PPP
PLT (10^3/μL)	329.5 ± 95.7	359.3 ± 90.6	258.7 ± 79.5	0.610	0.072	0.007
MPV (fL)	18.6 ± 3.1	14.8 ± 2.8	14.1 ± 2.4	0.001	< 0.001	0.734
RBC (10^6/μL)	7.7 ± 1.3	0.1 ± 0	0 ± 0	< 0.001	< 0.001	0.995
WBC (10^3/μL)	11.4 ± 5.2	0.9 ± 0.7	0.5 ± 0.5	< 0.001	< 0.001	0.935
LYMPH (10^3/μL)	3.7 ± 2.7	0.7 ± 0.7	0.5 ± 0.5	< 0.001	< 0.001	0.909
NFS (10^3/μL)	6.9 ± 2.7	0.1 ± 0.1	0 ± 0	< 0.001	< 0.001	0.995
MON (10^3/μL)	0.3 ± 0.1	0 ± 0	0 ± 0	< 0.001	< 0.001	0.848
PDGF-BB (pg/mL)		199.8 ± 133.9	159.7 ± 104			0.352
TGF-ß1 (pg/mL)		14,381.9 ± 7044.5	9403.7 ± 4031.9			<0.002

Mean ± SD of platelet (PLT) concentration, mean platelet volume (MPV), erythrocytes (RBC), leukocytes (WBC), lymphocytes (LYMPH), neutrophils (NFS), and monocytes (MON) concentrations in whole blood samples (WB) and in the PRP and PPP fractions; platelet-derived growth factor BB (PDGF-BB) and transforming growth factor β1 (TGF-β1) concentrations in the PRP and PPP fractions in 16 FIV cats (n = 16). Statistical differences $p < 0.05$.

WB samples and 4 PRP samples. According to ADVIA 2120i (Mitander, 2008) criteria, and the number of PLT aggregates in the smears, all samples, showed from 1 to 7 PLT aggregates with >10 PLT each in every PLT aggregate.

3.2. Erythrocyte concentration

The mean concentration of RBC in the PRP and PPP fractions (0.07 ± 0.02 10^6/μL and 0.04 ± 0.02 10^6/μL, respectively) decreased significantly by 99% compared to WB ($p < 0.001$), without significant differences between the PRP and PPP fractions (Table 2, Fig. 2B).

3.3. Total leukocytes and leukocyte populations

Compared to WB, the mean concentration of WBC in the PRP and PPP fractions (0.9 ± 0.7 10^3/μL and 0.5 ± 0.5 10^3/μL, respectively) decreased significantly by 92% ($p < 0.001$) in all the samples analyzed (Table 2, Fig. 2C).

Compared to WB, the number of LYMPH was significantly decreased by 81% ($p < 0.001$) and 86.5% ($p < 0.001$) in the PRP and PPP fractions, respectively (Table 2, Fig. 2D). The mean NFS concentration was significantly decreased by 99% in both PRP and PPP fractions respect to WB ($p < 0.001$) (Table 2, Fig. 2E). Mean MON concentrations were significantly decreased by 90% in PRP and 97% in PPP fractions ($p < 0.001$) (Table 2, Fig. 2F).

3.4. Quantification of growth factors: PDGF-BB and TGF-β1 concentration

TGF-ß1 concentrations were significantly higher in the PRP than in the PPP fractions ($p = 0.02$), with no differences in PDGF-BB levels (Table 2, Fig. 3A and B, respectively).

Compared to the samples without aggregates, the concentrations of PDGF-BB and TGF-ß1 ($p = 0.021$ and $p < 0.001$, respectively) are higher in the samples with PLT aggregates.

4. Discussion

This research describes for the first time a simple centrifugation manual protocol (PRGF®-Endoret®) to obtain PRP from feline blood, thus concentrating TGF-ß1 in cats naturally infected with FIV. The degree of PLT enrichment in the PRP fraction achieved in this study was 1.1. Since the PRGF®-Endoret® technology considers a PRP product to be one in which the number of PLT must be 1.5 times higher than the baseline (Anitua et al., 2007), the PLT concentrate obtained in this study would not be characterized as a PRP according to PLT concentration, but meets the characteristics free of RBC and WBC.

Previous studies carried out in 30 healthy cats (Miguel-Pastor et al., 2022) and in 11 cats with leukemia (Miguel-Pastor et al., 2023) reported values of 1.5 and 1.4 times higher than in WB, respectively. Since that the same technology was used, differences in the degree of PLT enrichment, in principle, should not be related to methodology. However, the authors consider necessary to optimize the protocol for obtaining PRP to recover a greater number of PLT in the PRP fraction following the characteristics of PRGF®-Endoret®. Increasing the sample volume of WB, modifying the gravitational force or the centrifugation time, or even considering a second centrifugation could be an alternative.

However, some authors describe as a quality concentrate a minimum of >300.000 PLT/uL (Anitua et al., 2004), 1.5 to 2 times or 1.3–4 for WB (Anitua et al., 2009; Carmona et al., 2012; Anitua et al., 2013; Dhurat and Sukesh, 2014) although these PLT ranges have been described in other species, therefore, given the characteristics of PLT in cats, they could not be applied to this species in any case.

Other methodologies used in several studies have obtained higher PLT concentrations than those shown in this investigation, even though the animals used were physiologically normal. Some authors obtained a 183% and 173% PLT enrichment in the plasma concentrates (PC) compared with WB (Silva et al., 2012). More recently, other studies reported an increase of PLT up to 1.8-fold and 1.5-fold from baseline in overage, respectively (Ferrari and Schwartz, 2020; Chun et al., 2020). Finally, it has been reported a 2 to 8.2-fold increase of PLT in PRP concentration with respect to WB (Angelou et al., 2022).

Thrombocytopenia plays an important role in FIV-positive cats. In fact, thrombocytopenia (PLT < 145.000/μL) has been reported in 10% of animals seropositive to FIV (Hart and Nolte, 1994), establishing a prevalence of 6% (Shelton et al., 1989) or even 16% (Yamamoto et al., 1989). Of note, thrombocytopenia is more common in cats coinfected with FIV and FeLV, in which the risk of myeloproliferative disorders leading to thrombocytopenia due to myelopthisis is 77-fold (Shelton et al., 1990; Gleich and Hartmann, 2009). Given the normality of PLT counts in WB samples in this study, the PLT concentration reached in the PRP fraction could not be explained based on thrombocytopenia and/or pseudothrombocytopenia either. Pseudothrombocytopenia is a common finding in cats, reporting in 36% to 72% (Moritz and Hoffmann, 1997; Zelmanovic and Hetherington, 1998; Norman et al., 2001a; Norman et al., 2001b). In our study, clumping has described in 50% in WB samples and in 25% of PRP fraction. No PLT clumps have obtained in PPP fraction. Since pseudothrombocytopenia is common in cats, all data obtained regarding the presence or absence of aggregates were included in the same statistical analysis, which could have induced the variations in PLT numbers in WB in some of the animals. The large platelet size, the secretion of granules and the activation of PLT when exposed to high concentrations of serotonin or low concentrations of ADP are phenomena related to platelet aggregation in this species. However, the quality of the extraction of the blood samples also induces the aggregation of PLT in samples anticoagulated in EDTA (Weiser and Kociba, 1984; Moritz and Hoffmann, 1997; Russel, 2010). Indeed, the damage caused to the endothelium by venipuncture produces PLT adherence to the von Willebrand factor located in the subendothelial collagen with PLT receptors inducing additional PLT enrolment (Brooks and Catalfamo, 2010). However, other researchers disagree with these last observations

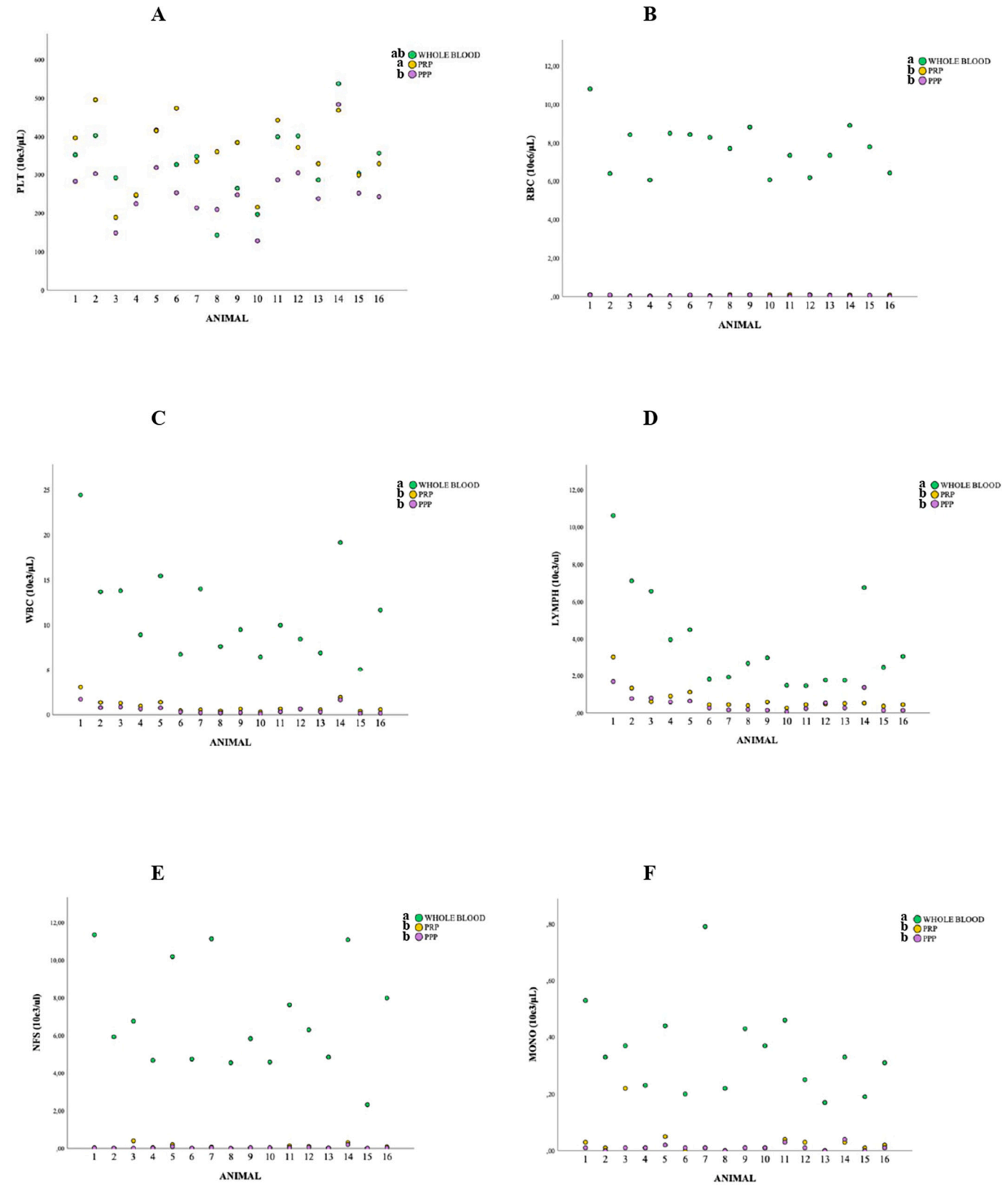

Fig. 2. Comparison of the platelet [PLT; (A)]; red blood cell [RBC; (B)]; white blood cell [WBC; (C)]; lymphocytes [LYMPH; (D)]; neutrophils [NFS; (E)]; monocyte [MON; (F)] concentrations (mean ± SD) in FIV cats (n = 16) between whole blood (WB) and PRP and PPP fractions. Different letters (a, b) indicate differences between groups. $P < 0.05$ statistically different. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

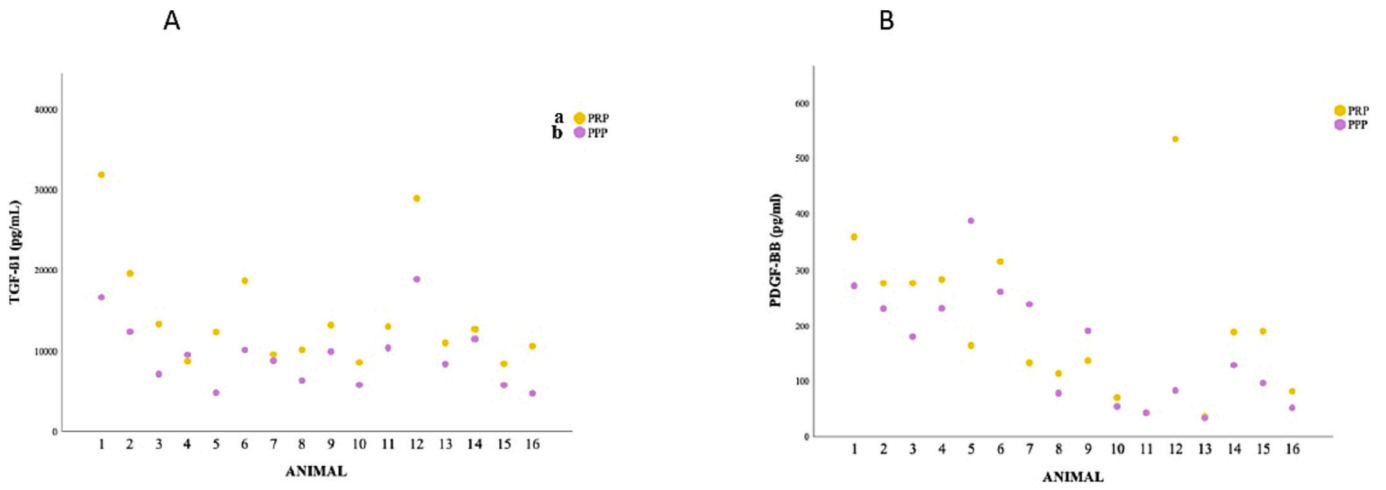

Fig. 3. Comparison of transforming growth factor ß1 [TGF-ß1; (A)] and Platelet-Derived Growth Factor-BB [PDGF-BB; (B)] concentrations (mean ± SD) in FIV cats (n = 16) between PRP and PPP fractions. Different letters (a, b) indicate differences between groups. $P < 0.05$ statistically different.

(Riond et al., 2015).

According to previous investigations (Miguel-Pastor et al., 2022; Miguel-Pastor et al., 2023), in the present study MPV was higher in WB compared to PRP and PPP fractions, although higher values in PLT concentrates have also been reported (Silva et al., 2012). MPV represents the average size of PLT and increase in PLT activation (Vagdatli et al., 2010). It is possible that the increase in large PLT would have conditioned an increase in MPV in the WB samples. Indeed, three of the animals, the MPV presented values >18.9 fL, probably due to activation or platelet aggregation since it was confirmed by the blood smear. However, several factors such as the type of anticoagulant employed, the temperature and storage could be involved in these changes in MPV (Handagama et al., 1986; Jackson and Carter, 1993; Mylonakis et al., 2008).

WB hematological parameters were consistent with previously described reference ranges for the feline species (Moritz et al., 2004). However, a physiologic leukocytosis with lymphocytosis accompanied by mild polycythemia in response to catecholamine-mediated fear or arousal occurred in one of the animals. Likewise, in five samples MCV values <39.0 fL were obtained, Although, the blood smear study revealed that these erythrocytes were crenated or echinocytes, so in no case there were recognized as true microcytes. Nonetheless, various studies have evaluated potential hematologic differences between naturally FIV-infected cats and FIV-uninfected cats, with variable and often inconsistent findings (Carlton et al., 2022). In fact, cytopenic hematologic abnormalities including neutropenia, lymphopenia, anemia, and pancytopenia have been reported in cats naturally infected with FIV (Shelton et al., 1995; Fujino et al., 2009; Gleich and Hartmann, 2009). A study carried out in 3784 cats of owners compared the hematological parameters in cats infected with FIV, infected with FeLV and uninfected (Gleich and Hartmann, 2009) showing that although anemia and thrombocytopenia did not vary between infected and uninfected animals, neutropenia affected 25% of the FIV positive cats.

Development mechanisms of these cytopenia in FIV-infected cats have been related to the infection and, therefore, to the presence of viral RNA in megakaryocytes and other marrow precursor cells (Pedersen and Barlough, 1991), although others refute it (Shelton et al., 1991). Likewise, other serum inhibitors (possibly antibodies) (Shelton et al., 1991) can also trigger immune-mediated mechanisms against stem cells and circulating cells (Pedersen and Barlough, 1991). Based on this evidence, the absence of hematological abnormalities in this study could be associated with the subclinical stage of infection of the animals at the time of sampling, reinforcing the absence of bone marrow involvement.

The BTI system employed in this study allowed to drastically reduce the concentrations of RBC and WBC including LYMPH, NFS and MON in both fractions, PRP and PPP compared to WB, so that the product obtained showed high purity. The WBC percentage in feline PRP differs depending on the method used: there was registered a decrease of 36% (Chun et al., 2020), 65% (Silva et al., 2012), 80% (Ferrari and Schwartz, 2020) or 95% (Miguel-Pastor et al., 2022; Miguel-Pastor et al., 2023) compared with WB in different protocols feline PRP obtention. The role of WBC in PRP therapy is a controversial topic. PRGF®-Endoret® technology recommended exclusion of WBC based on neutrophils release pro-inflammatory substances (Anitua et al., 2008). However, increase WBC in PRP fraction increase the concentration of GFs (Zimmermann et al., 2001), and can promote an anti-infectious effect (Moojen et al., 2008; Cieslik-Bielecka et al., 2009). This fact manifest that more studies are necessary to conclude WBC should be included or excluded from PRP according tissue or injury (McLellan and Plevin, 2011). According to some authors, PRP is a useful regenerative therapy, but this product is more than just PLT, as it contains many bioactive factors that act on anabolic, catabolic, pro-inflammatory, and anti-inflammatory pathways. The precise combination and concentration of PLT, WBC, and other plasma components that are best for different lesions remain unknown. It is likely that there is a maximum effective concentration beyond which the concentration of PLT does not provide additional clinical benefit. Although the effects of many of the PRP proteins on various tissues are still unknown, it is likely that they contribute to the biological healing process (Boswell et al., 2012). Based on this evidence, it is logical to think that basing the design of the clinical study solely on the variation of the PLT count may fail the use of PRP in some patients.

Although PDGF-BB and TGF-β1 concentrations were lower than those obtained in healthy cats (Miguel-Pastor et al., 2022) and FELV-positive cats (Miguel-Pastor et al., 2023), lower TGF-β1 values in both PRP and PPP fractions have also been found (Silva et al., 2012). These differences between results were expected since the concentration of factors depends on the PLT concentrate in the PRP fraction. Since the PLT concentration in this study was 1.1, it is logical that the concentration of both GFs was lower than those of previous studies in which PLT concentrates were 1.5 (Miguel-Pastor et al., 2022) and 1.4 (Miguel-Pastor et al., 2023), respectively. However, in samples with PLT aggregates these GFs increased compared to those in which the aggregates were not present. It is important to highlight that these samples were analyzed in serum, so in this fluid, fibrinogen becomes a fibrin clot in which the PLT are trapped, aggregate, activate (Jennings, 2009), and release the GFs contained in the α-granules (Silva et al., 2012). Therefore, pseudothrombocytopenia affects the concentration of GFs in FIV positive contrary to what happens in healthy cats. Based on the elevation of GFs in samples with aggregates, it could be suggested that the

presence of PLT aggregates could provide better biological actions for the deposition of extracellular matrix, angiogenesis, and cell migration than those devoid of aggregates (Wasterline et al., 2012).

Some recent studies in some areas in feline medicine have shown promising results, although the methodology for obtaining PRP has not been specific for the feline species. Thus, in a model of multiple sclerosis in cats, concluded neuroprotective and neurotrophic effects of PRP. In this case, the PRP was obtained according to the method described previously in another study in horses (Giraldo et al., 2015), not verifying the concentration of PLT nor of GFs in this fraction (Farid et al., 2022). Another study on conjunctival ulcers in cats showed that the subconjunctival application of PRP provided curative effects (Farghali et al., 2021), after the preparation of PRP based on a protocol described for humans (Kececi et al., 2014), likewise, without confirmation either of the PLT or GFs concentration prior to its application. Since the composition of the PRP varies between protocols, kit used, centrifuge and species, further research must be carried out in this area to achieve a species-specific PRP with adequate clinical potential.

The FIV infection progresses through several stages, similar to human immunodeficiency virus infection, including an acute phase, a clinically asymptomatic phase of variable duration, and a terminal phase sometimes named "feline acquired immunodeficiency syndrome" (Goto et al., 2000). All the cats that participated in our study were in the subclinical phase of the disease, being asymptomatic cats. However, the FIV leads to immunodeficiency in infected cats, making them more susceptible to secondary infections by opportunistic agents of viral, bacterial, fungal, or protozoal origin. Also, immunodeficiency and immunostimulation in FIV cats can produced inflammatory diseases as chronic gingivostomatitis, chronic rhinitis, lymph adenopathy or immune-mediated glomerulonephritis that impairs the patient's quality of life (Tenorio et al., 1991; del Fierro et al., 1995; Hughes et al., 1999). PRGF®-Endoret® is a versatile technology with proven effects and great clinical potential in various medical fields as traumatology and orthopedic diseases or wound healing among others in regenerative medicine in other animal species as in rabbits, dogs, and horses (Damià Giménez, 2012; Vilar et al., 2013; Cuervo et al., 2014; Chicharro et al., 2018; Chicharro-Alcántara et al., 2018; Torres-Torrillas et al., 2021), but it has not been tested on cats. Then, the authors consider necessary to increase PRP studies in cats susceptible to developing diseases such as cats infected with FIV, especially chronic inflammatory pathologies therapies with minimal side effects and low cost as PRGF®-Endoret® technology, even as it isn't the minimum of PLT concentrate but it is an RBC and WBC-free plasma concentrate, since the plasma contains other substances such as cytokines that may exert beneficial effects in inflammatory processes.

On the other hand, although hemostatic disorders in FIV-positive cats are rare (Hart and Nolte, 1994), it is possible that PLT have their own characteristics, are defective or more fragile and do not withstand centrifugation protocols, or even that soluble factors exist that prevent PLT from concentrating, since PLT counts do not differ from those reported in previous studies in healthy (Miguel-Pastor et al., 2022) animals. For this reason, the authors believe that it is necessary to expand the studies on the standardization of the protocol for obtaining PRGF in cats, especially in cats positive for FIV using this PRGF®-Endoret® methodology to allow reproducibility.

One of the limitations of the study was the small sample size, in which only FIV-positive but asymptomatic or subclinical cats were included, so that the results obtained cannot be extrapolated to the entire FIV-positive feline population. Future studies with a larger number of animals in the same phase would be needed to verify this.

5. Conclusions

To the best of our knowledge, this is the first experimental study that has made possible to obtain a PLT concentrate in FIV-positive cats using PRGF®-Endoret® technology. Although the product obtained did not meet the minimum requirements regarding PLT concentration according to the BTI-PRGF®-Endoret® technology, the absolute reduction of RBC and WBC have allowed it to be identified as a high purity PLT concentrate. New studies are required to guarantee the recovery of a greater number of PLT in these patients, so that it can finally be used to mitigate the possible collateral effects induced by the virus in these patients.

CRediT authorship contribution statement

Laura Miguel-Pastor: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Katy Satué:** Writing – original draft, Writing – review & editing. **Deborah Chicharro:** Investigation, Writing – original draft. **Elena Damiá:** Investigation. **Belén Cuervo:** Investigation. **Marta Torres-Torrillas:** Investigation, Writing – review & editing. **Emma Martins:** Investigation. **María Gemma Velasco-Martínez:** Investigation. **José M. Carrillo:** Conceptualization, Data curation, Funding acquisition, Supervision. **Joaquín J. Sopena:** Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing. **José J. Cerón:** Investigation. **Mónica Rubio:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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