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ORIGINAL RESEARCH

Platelet-Derived Growth Factor as Biomarker of Clinical Outcome for Autologous Platelet Concentrate Therapy in Grade I Knee Osteoarthritis

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Introduction: Autologous platelet concentrates (APC) are widely used in the infiltrative treatment of knee osteoarthritis (OA) to enhance tissue healing and relieve pain. Aim of this study was to identify predictive biomarkers for clinical outcomes in patients with grade I knee OA.

Methods: A panel of growth factors (GFs) and cytokines was determined in peripheral blood (PB) and APC. The Numeric Pain Rating Scale (NPRS) was used as a clinical readout before and after the APC infiltration.

Results: A lower white blood cell (WBC) count and higher Monocyte-chemoattractant Protein-1 levels in PB were associated with APC-induced pain relief. Platelet-derived Growth Factor (PDGF) levels in APC were significantly higher in OA patients displaying a larger NPRS reduction, independent of platelet count. Finally, the simultaneous determination of PDGF, Vascular Endothelial Growth Factor, and Macrophage Inflammatory Protein-1α in APC discriminated OA patients with very poor or no response.

Conclusion: Platelet-released GFs rather than platelet counts may predict clinical outcomes in grade 1 knee OA.

Keywords: growth factors, cytokines, regenerative medicine, inflammation, pain relief

Introduction

Platelets are a natural reservoir of soluble mediators, including cytokines and growth factors (GFs), located within α-granules.[1] Platelet activation may occur at the site of injury with the release of bioactive molecules, which synergistically promote tissue repair processes and modulate immune and inflammatory responses.[2]

GFs are mainly polypeptides that stimulate proliferation, chemotaxis, migration, and wound healing, through paracrine, autocrine, or endocrine mechanisms.[3,4] These molecules bind to specific cell receptors, triggering a cascade of molecular events that influence cell functions.[5,6] GFs are released after tissue damage; among those mostly involved in tissue regeneration, Fibroblast Growth Factors (bFGF), Vascular Endothelial Growth Factor (VEGF), and Platelet-Derived Growth Factor (PDGF) are known to play a major role.[5]

Articular cartilage is a specialized connective tissue with limited regenerative capacity.[7] The inflammatory process does not contribute to healing cartilage injuries or diseases, thus regenerative approaches are widely used.[8] Osteoarthritis (OA) is mainly characterized by an inflammatory state and abnormal "rubbing" exerted on the joint structures with progressive cartilage deterioration, wear and tear, and painful conditions.[9] The severity is identified by a five-degrees

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scale, ranging from cartilage softening without fissuring, to localized diffuse fissuring, to loss of cartilage substance with consequent exposure of the bone.[10]

The goal of regenerative medicine is tissue repair; therefore, the translational approaches currently being developed appear promising for clinical use. In patients with OA, several clinical trials have been carried out using biological products, including platelet concentrates and cell therapies, using infiltrative treatment.[11,12] These are valuable therapeutic agents with the main advantages of reduced immune reactions, ease of collection and preparation, and relatively low costs.[4,13,14]

Autologous Platelet Concentrate (APC) is generally obtained from the patient's peripheral blood. Several different procedures have been developed to obtain APC with higher concentrations of platelets compared to whole blood.[15] Due to their autologous nature, APC use is not expected to elicit severe adverse reactions. The rationale for APC therapy is the intra-articular administration of platelet concentrates at sites of injury with the release of soluble mediators, GFs, and other bioactive components.[16] These molecules have immunomodulatory, angiogenic, pain-relieving, and wound-healing functions.[17–19] Thus, APC delivers GFs and cytokines from platelet granules to the affected area, enhancing regenerative and innate repair processes and contributing to restoring cartilage functions and relieving pain.

Although APC therapy is considered an effective approach for managing OA, patients' response is still variable, and there is no evidence in literature of standard personalized therapeutic approaches. In this regard, this study is aimed to evaluate whether the determination of soluble factors in peripheral blood (PB) and APC could identify predictive biomarkers for clinical outcomes in patients with grade I OA.

Materials and Methods

Population Enrolment

51 patients with knee grade I OA, defined according to Kellgren and Lawrence grading, were recruited at Local Health Unit Napoli 2 Nord. The inclusion criteria for APC infiltration therapy were as follows: age > 18 years; presence of pain, spasm, or functional disability with failure of conservative treatment for at least 3 months but less than 5 years. The exclusion criteria were inflammatory or autoimmune diseases, pregnancy, and bleeding disorders.

APC infiltration into the articular cavity was performed at enrolment (T0) and after 30 days (T1). For each patient, biometrical, biochemical, and clinical data were collected at outpatient hospital admission before APC infiltration (T0), and follow-up biochemical and clinical data were collected at T1, before the second APC infiltration, and at 120 days (T2). Numeric Pain Rating Scale (NPRS) scores were also recorded at T0, T1, and T2. The NPRS is a one-dimensional, 11-point scale that assesses pain intensity in adults. It consists of a horizontal line, ranging from 0 to 10, corresponding to "no pain" and "worst pain imaginable.[20]

The investigations were carried out following the guidelines of the Declaration of Helsinki of 1975, revised in 2013. Informed consent was obtained from all patients before the procedure. The study protocol was approved by the ethics committee "Università Federico II - AORN A. Cardarelli" (Prot. N. 172/2023).

Peripheral Blood Collection and Autologous Platelet Concentrate Sample Preparation

PB and APC samples were collected from each patient at T0 and T1. APC was obtained by centrifugation of venous blood samples using an automated and standardized cell separator (IMPACT – Plasmaconcept), according to the manufacturer's instructions. After obtaining the samples, 3 milliliters (mL) were infiltrated intra-articularly at the site of the injured cartilage and an aliquot of 500 microliters (μL) was collected for further analysis.

Determination of Cytokines, Chemokines, and Growth Factors

PB and APC were screened for the concentrations of interleukin (IL)-1ra, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, Eotaxin, basic fibroblast growth factor (bFGF), granulocyte-colony stimulating factor (G-CSF), granulocyte and macrophage-colony stimulating factor (GM-CSF), interferon-γ (IFN-γ), interferon-γ inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1) α, MIP-1β, platelet-derived growth factor (PDGF) bb, C-C motif chemokine ligand 5 (CCL5)/RANTES, tumor necrosis

factor (TNF) α, and vascular endothelial growth factor (VEGF) using the Bio-Plex Pro Human Cytokine GrpI Panel 27-Plex kit (cat. No. M500KCAF0Y) as previously described.[21] The magnetic bead-based assay was performed on a Bio-Plex 200 analyzer with Bio-Rad Bio-Plex Manager (Bio-Rad, Hercules, CA, United States).

Statistical Analysis

Statistical Analyses were performed using the R statistical platform (http://www.R-project.org/) in RStudio GUI software, version 4.1.2, and GraphPad Prism 8.4.2 software (GraphPad Software Inc., La Jolla, CA).

Continuous variables were reported as mean ± standard deviation or median (InterQuartile Range [IQR]). Shapiro–Wilk normality test was used to evaluate whether the continuous data were normally distributed and, according to the results, a Welch's two-tailed *t*-test for independent samples (for parametric data) or a Mann–Whitney *U*-test (for non-parametric data) was used for unpaired comparisons. Paired comparisons were performed using the paired *t*-test (for normally distributed data) or the Wilcoxon matched-pairs signed-rank test (for non-normally distributed data). Fold change tests were analyzed with either a one-sample *t*-test (for normally distributed data) or a Wilcoxon signed-rank test, with a hypothetical value set at 1. NPRS progression throughout the study was analyzed with a mixed effect analysis, Geisser-Greenhouse correction, and Tukey's post hoc multiple comparisons test. Categorical variables were reported as counts of occurrences (% - percentages) and were compared using Fisher's exact test. Correlations among continuous variables were assessed using non-parametric Spearman correlation. Receiver operating characteristic (ROC) curves were constructed using the 95% Confidence Interval (CI) De Long's method. The ROC curve for the combined effect of multiple continuous parameters was evaluated by generating a multivariate logistic regression model and combining each prediction value with their relative outcomes. Statistical significance was set at p-value (p) <0.05.

Results

Patients Phenotyping

51 consecutive patients with grade I OA eligible for APC infiltration therapy were recruited for this study. Forty patients were female (78.43%). The population had an overall mean age of 67.22 years and a body mass index (BMI) of 28.4. Nineteen patients (37.25%) were obese (BMI > 30 Kg/m^2), and nine (17.65%) had Type-2 Diabetes. Fifteen patients (29.41%) were smokers (Table 1). Blood cell and platelet counts, glycemia, and C-reactive protein, ferritin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels were within the expected ranges (Supplementary Table 1).

At enrolment (T0), the mean Numeric Pain Rating Scale (NPRS) score was 8.12 ± 1.6, indicating severe pain. Upon 30 days (T1) from APC infiltration, the NPRS score was 6.64 ± 1.93 (paired p <0.001); the reduction was more pronounced upon 120 days (T2), with a mean value of 5.08 ± 2.01 (paired p <0.001) (Supplementary Table 2 and Figure 1).

Table 1 Biometrical and Clinical Phenotyping of the Recruited Population (N= 51) Continuous Data are Reported as Mean ± Standard Deviation (SD) or Median [Interquartile Range - IQR], While Categorical Variables are Reported as Count (Percentage - %)

Sex (Females)	40 (78.43%)
Age (Years)	67.22 ± 10.21
Weight (Kilograms - Kg)	77.12 ± 14.73
Height (meters - m)	1.65 ± 0.08
BMI (Kg/m^2)	28.4 ± 5.17
Obesity	19 (37.25%)
Diabetes	9 (17.65%)
Smoker	15 (29.41%)

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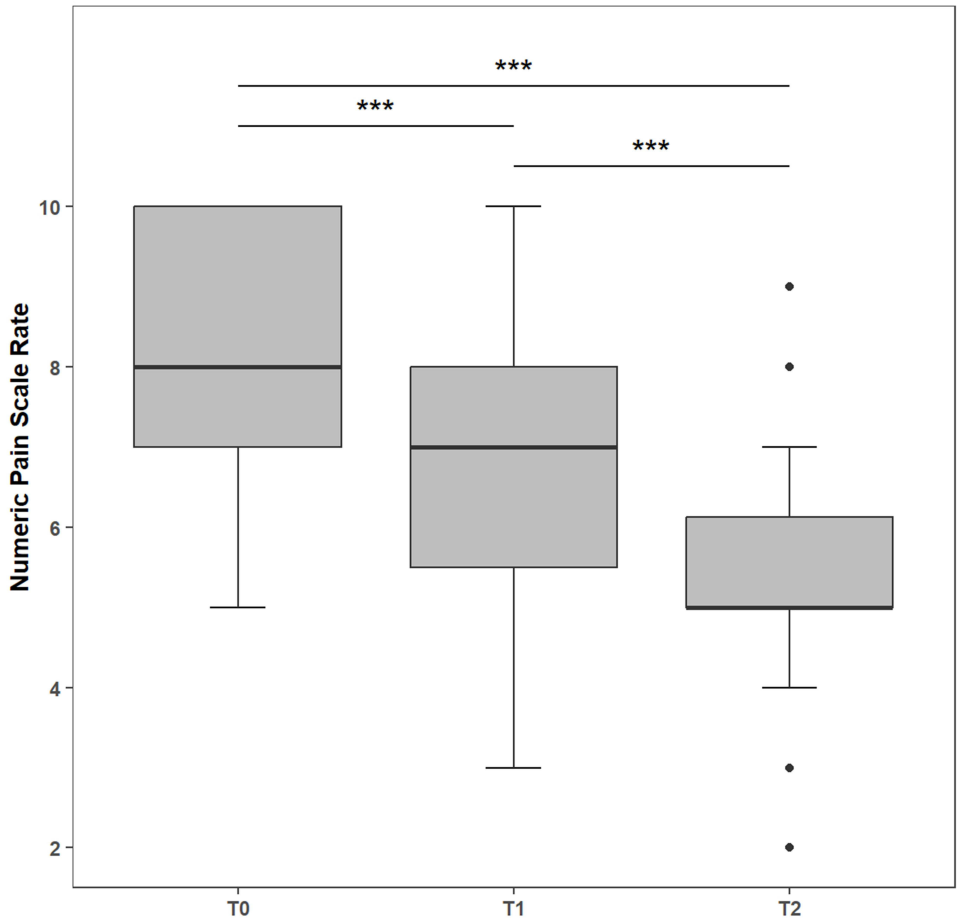

Figure 1 Numeric Pain Rate Scale distribution. NPRS was recorded at the enrolment (T0), after 30 days (T1) and 120 days (T2). Box plots denote the median and 25th to 75th percentiles (boxes), while whiskers represent the Tukey interval. Asterisks denote statistical significance (***p<0.001).

Cytokines and Growth Factors Screening in PB and APC

PB and APC samples were collected and the concentrations of cytokines and GFs were determined (Table 2). All tested factors, except interleukin (IL)-7 and IL-15, were detectable in both PB and APC. IL-4, IL-5, IL-10, IL-13, Platelet-Derived Growth Factor (PDGF), C-C motif Chemokine Ligand 5 (CCL5/RANTES), and Vascular Endothelial Growth Factor (VEGF) increased by more than 1.5-fold in APC compared with PB (Table 2). No significant difference was detected between cytokine and GF levels in APC at T0 compared to T1 (Supplementary Table 3).

In APC, a correlation matrix displayed a cluster of PDGF, RANTES/CCL5, and VEGF, which were mainly representative of platelet-released factors.[17] An additional cluster included IL-4, IL-5, IL-10, IL-17, basic Fibroblast Growth Factor (bFGF), Interferon-γ (IFN-γ), and Macrophage Inflammatory Protein-1 (MIP-1) α (Figure 2A). In PB, at T0, MIP-1α was negatively correlated with NPRS ($\rho = -0.306$, $p = 0.029$) and positively correlated with White Blood Cells (WBC) count ($\rho = 0.455$, $p = 0.001$). Moreover, a negative correlation between the WBC count and NPRS was detected ($\rho: -0.419$, $p = 0.003$) (Figure 2B). No other significant correlations were observed.

Relationship Between Early NPRS Variation and Biochemical Parameters

The population was then stratified according to the variation in NPRS score from T0 to T1 (indicative of an early response to therapy). Patients with an NPRS reduction above the mean (ΔT0-T1:1.48, Supplementary Table 2) were labeled as early responders (n= 28), while patients with an NPRS reduction lower than the mean were labeled as poor early responders (n= 23). Basal (T0) NPRS was significantly higher in early responders compared to their counterparts

Table 2 PB and APC Screening for Cytokines, Chemokines, and Growth Factors at T0. Results are Reported as Median [IQR] and Expressed as Picograms/Milliliters (pg/ml). Fold Change Is Reported as Estimate (Est) and 95% Confidence Interval (CI). Emboldened p-values are Statistically Significant (P<0.05)

Marker	T0 PB	T0 APC	Paired p	Fold Change	
				Est (95% CI)	p
IL-1β	0.71 [0.42; 1.30]	0.79 [0.57; 1.15]	0.713	1.31 (1.08–1.54)	**0.009**
IL-1ra	165 [139; 274]	206 [165; 293]	0.187	1.34 (1.07–1.6)	**0.013**
IL-2	2.55 [1.55; 4.01]	3.04 [2.05; 4.72]	0.531	2.25 (0.40–4.10)	0.158
IL-4	1.91 [1.29; 2.68]	2.43 [1.91; 3.2]	**<0.001**	1.79 (1.32–2.25)	**0.001**
IL-5	34.5 [20.4: 42.1]	43.6 [43.6; 54]	0.054	2.07 (1.11–3.03)	**0.03**
IL-6	0.6 [0.17; 1.48]	1.19 [0.46; 2.38]	0.147	9.90 (−2.48–21.3)	0.148
IL-8	5.88 [4.4; 8.87]	6.87 [5.88; 8.37]	0.138	1.19 (1.03–1.35)	**0.022**
IL-9	267 [209; 329]	329 [284; 379]	**<0.001**	1.31 (1.19–1.43)	**<0.001**
IL-10	3.01 [1.5; 4.98]	4.98 [3.51; 7.9]	**<0.001**	2.57 (1.95–3.19)	**<0.001**
IL-12	12.5 [7.63; 17.3]	26.8 [17.3; 41]	**<0.001**	8.65 (−2.30–19.6)	0.166
IL-13	1.91 [1.59; 3.64]	2.65 [2.16; 4.47]	**<0.001**	1.70 (1.41–1.99)	**<0.001**
IL-17	23.5 [16.4; 30]	29.1 [25.7; 32.5]	**<0.001**	1.34 (1.20–1.49)	**<0.001**
Eotaxin	55.9 [37.9; 77.9]	49 [33.1; 65.7]	0.11	0.95 (0.84–1.06)	0.388
bFGF	14.9 [12.8; 16.8]	20.6 [16.8; 25.1]	**<0.001**	1.43 (1.27–1.59)	**<0.001**
G-CSF	93.2 [65.8; 135]	109 [87.9; 151]	**0.004**	1.26 (1.13–1.38)	**<0.001**
GM-CSF	1.9 [1.14; 2.9]	3.51 [2.25; 5.18]	**<0.001**	4.03 (0.73–7.34)	0.071
IFN-γ	7.55 [5.48; 9.63]	9.63 [8.07; 12.5]	**<0.001**	1.41 (1.26–1.56)	**<0.001**
IP-10	255 [181; 440]	230 [155; 332]	**<0.001**	0.88 (0.83–0.94)	**<0.001**
MCP-1	16.5 [11; 26.6]	15.7 [12.6; 24.5]	0.058	1.15 (1.02–1.28)	**0.026**
MIP-1α	1.44 [1.11; 1.86]	1.73 [1.44; 2.12]	**<0.001**	1.27 (1.15–1.39)	**<0.001**
MIP-1β	105 [92.6; 133]	132 [108; 149]	**<0.001**	1.21 (1.12–1.30)	**<0.001**
PDGF	170.74 [92; 242.9]	423.22 [232; 688.56]	**<0.001**	3.96 (2.91–5.01)	**<0.001**
RANTES	1461 [1171; 2270]	2823 [2201; 4399]	**<0.001**	1.97 (1.68–2.25)	**<0.001**
TNF-α	107 [89; 136]	140 [119; 174]	**<0.001**	1.37 (1.25–1.48)	**<0.001**
VEGF	106 [77.7; 152]	148 [68.7; 202]	**0.026**	1.77 (1.33–2.21)	**0.001**

(8.71 ± 1.46 vs 7.39 ± 1.48, respectively; p = 0.003). The WBC count was lower in early responders than in poor early responders (6.3 [5.33; 7.88] vs 8.05 [6.57; 10.4]; p = 0.005) (Supplementary Table 4).

Moreover, early responders had significantly higher levels of Monocyte Chemoattractant Protein-1 (MCP-1) in PB at T0 than poor early responders (p = 0.012; Figure 3A). Interestingly, early responders displayed significantly higher values of PDGF (p = 0.009), RANTES/CCL5 (p = 0.023), and VEGF (p = 0.027) in APC than poor responders (Figure 3B–D and Supplementary Table 4).

ROC curves for both MCP1 in PB (Area Under the Curve - AUC, 0.751; 95% CI, 0.563–0.867; p = 0.005; Figure 3A) and PDGF, RANTES/CCL5, and VEGF in APC (PDGF AUC: 0.726, p = 0.004, RANTES AUC: 0.686, p = 0.014; VEGF AUC: 0.679, p = 0.025; Figure 3B–D) displayed a significant accuracy for early response to APC treatment concerning NPRS score variation. No other biometric, biochemical, or clinical data showed significant differences between the two groups.

Relationship Between Long-Term NPRS Variation and Biochemical Parameters

Patients were then stratified according to the NPRS reduction from T0 to T2 (mean ΔT0-T2:3.04; 25th percentile, ≤ 2.0; 75th percentile, ≥ 5.0); thus, 12 patients were classified as overall low responders, 23 as mid responders, and 16 as high responders. Again, a larger decrease was observed in individuals with a higher NPRS (p <0.001) and a lower WBC count (p = 0.015) at T0 (Supplementary Table 5).

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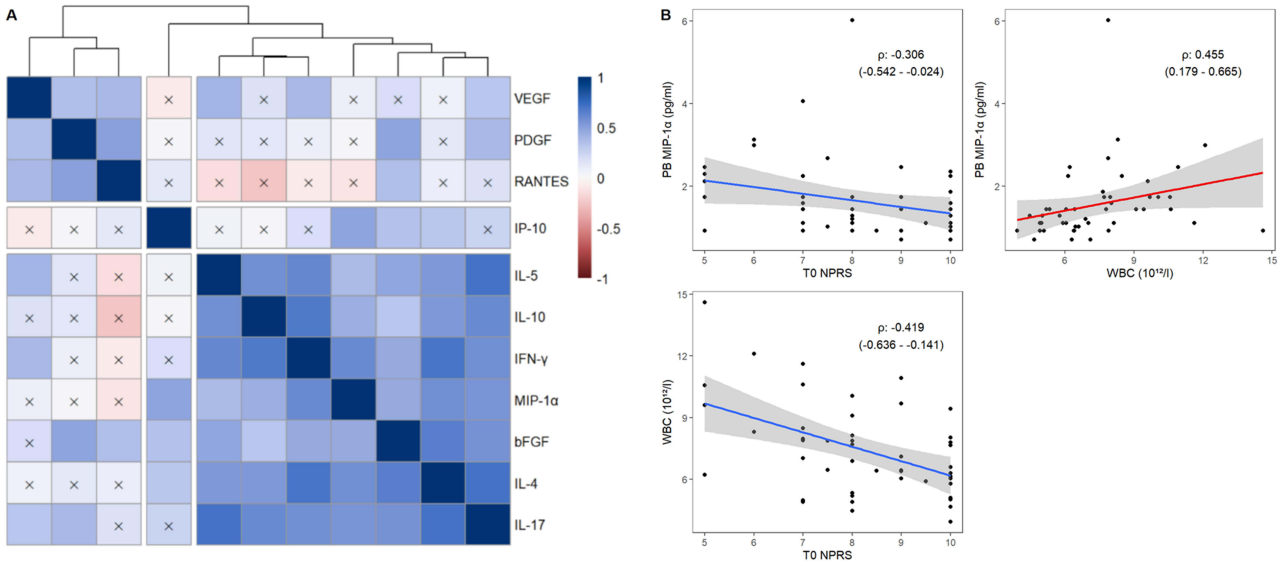

Figure 2 Correlation Analysis in PB and APC. (**A**) APC correlation matrix and hierarchical clustering. Spearman's rho (ρ) correlation coefficients for APC cytokine and growth factor scaled measurements at T0 are visualized by tile-color intensity (according to the legend on the right). ρ-values closer to 1 show a positive correlation; ρ-values closer to −1 show a negative correlation; ρ-values closer to 0 denote the absence of a correlation among the considered variables. All values not labeled with a black X are statistically significant (p<0.05). Dendrograms on top of the matrix show the hierarchical clustering. **(B)** Correlations among biochemical data in peripheral blood and NPRS. Scatter plots represent the patients' distributions: T0 NPRS and PB MIP-1α (top left), T0 NPRS and WBC (bottom left); T0 WBC and PB MIP-1α (top right). For each plot, a linear regression is reported, with a 95% confidence interval (CI) marked in grey, as well as the ρ Spearman correlation value with the relative 95% CI. Every correlation reported is statistically significant (p<0.05).

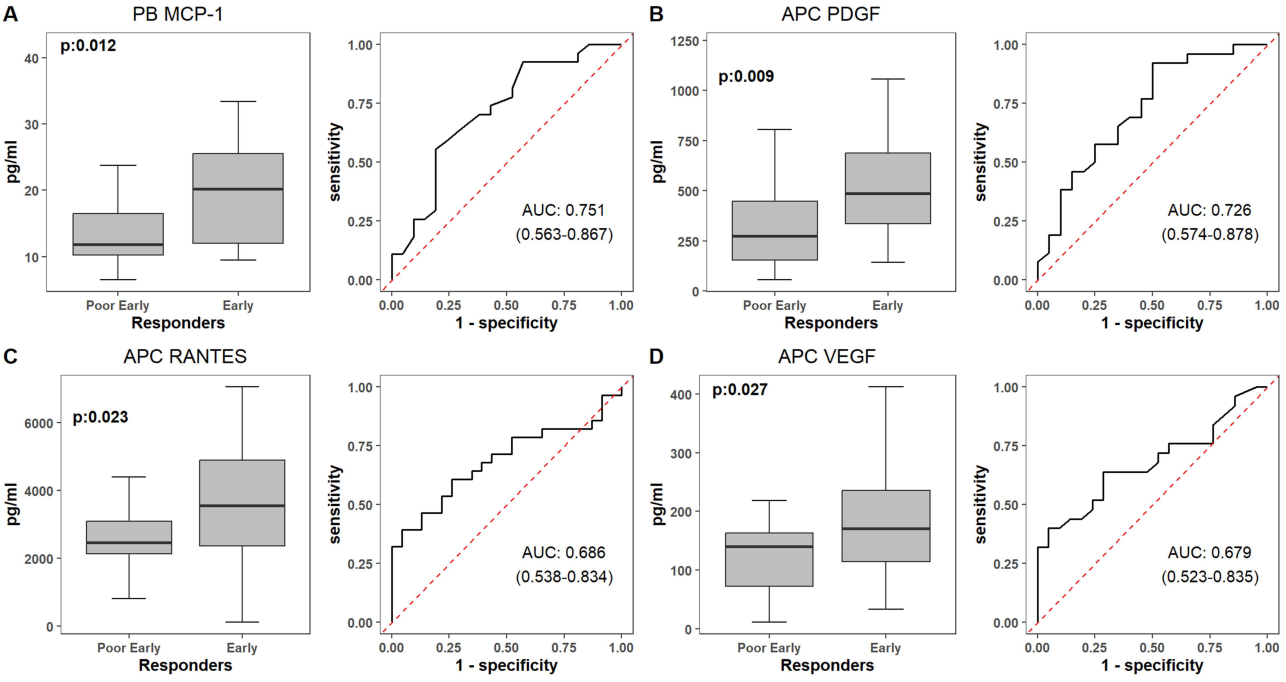

Figure 3 Soluble factors in PB and APC of early responders vs poor early responders. Boxplots depict concentrations at T0 of MCP-1 in PB **(A)** and of PDGF **(B)**, RANTES/CCL5 **(C)**, and VEGF **(D)** in APC for early responders and poor early responders as described in the text. Concentrations are expressed as pg/mL. For each molecule, the relative ROC Curve for discriminating among the two groups is shown. The figure reports only those factors displaying statistical significance (p<0.05).

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The concentration of PDGF in APC was higher in high- and mid-responders than in low-responders ($p = 0.004$; Supplementary Figure 1). No other significant differences were observed between the PB and APC of the groups.

Comparison Between Optimal Responders and Poor Responders

Of the 51 total patients, 11 (21.57%) showed NPRS reduction at both T1 and T2 (optimal responders), while 8 (15.69%) displayed slight to no NPRS reduction (never responders). These two groups did not display significant differences in biometric and clinical data other than the basal NPRS, which was higher in optimal responders ($p = 0.004$). The WBC count was lower in optimal responders than in never responders ($p = 0.008$) (Supplementary Table 6). No other significantly different biochemical features were found in the PB of the two groups (Supplementary Table 6).

Intriguingly, in APC, optimal responders displayed slightly lower levels of MIP-1α ($p = 0.033$) and 2.5- and 2.4-fold higher levels of PDGF ($p = 0.027$) and VEGF ($p = 0.032$), respectively, than never responders. These markers also displayed a significant predictive value in discriminating optimal responders from never responders (MIP-1α AUC, 0.801; $p = 0.006$; PDGF AUC, 0.793; $p = 0.011$; VEGF AUC, 0.781; $p = 0.013$) (Figure 4A and Supplementary Table 6).

Moreover, the combined effect of MIP-1α, PDGF, and VEGF resulted in higher predictive power in discriminating optimal responders from never responders (AUC: 0.931, $p < 0.001$) (Figure 4B).

Discussion

The use of platelet concentrates to enhance tissue regeneration and wound healing has been largely exploited in the last two decades for the repair of articular cartilage injuries.[4,16,22,23] The rationale for the use of platelets is their ability to exert specific effects on inflammation, proliferation, and differentiation of chondrocytes by releasing a large number of cytokines, chemokines, and GFs.[24,25] Their infiltration into articular cavities may allow the enrichment of soluble mediators in tissues with limited blood supply and slow cell turnover.[26] Effective pain relief and improvement of quality of life have also been documented following intra-articular injection of platelet concentrates.[27,28]

We observed a progressive decrease in pain after APC treatment in patients with grade I OA. Pain relief was detected one month after the first APC administration and was further enhanced following the second injection, as measured after four months. Nevertheless, the degree of response to APC varied among the patients, and they were classified based on NPRS variation. Currently, no validated thresholds are known for NPRS variation; hence, as a reasonable starting point aiming to standardize the approach, we classified our population according to its mean value and quartiles.

The highest degree of response was observed in the patients with the highest initial NPRS scores. Interestingly, however, an early response was also associated with a low WBC count and low MIP-1a levels in PB, suggesting that systemic inflammation could be a detrimental factor for the APC response. Indeed, MIP-1α plays a major role in the development of systemic inflammation and may interfere with the organ repair process.[29] Surprisingly, we also found that MCP-1 levels were higher in the peripheral blood of patients displaying better early response (as recorded 1 month after injection) to APC treatment. MCP-1 is one of the key chemokines that regulate the migration and infiltration of monocytes/macrophages.[30] High circulating MCP-1 levels may contribute to monocyte recruitment into healing tissues. However, the MCP-1 role in cartilage repair requires further investigation.

To assess whether predictive factors for APC response could be identified, we measured the concentrations of GFs and cytokines in APC. The concentrations of PDGF, VEGF, and RANTES/CCL5 were positively associated with early response to APC. Notably, a hierarchical cluster of PDGF, VEGF, and RANTES/CCL5 was detected in platelet concentrates, which was consistent with the degranulation pattern and release of healing mediators.[17,31,32]

When the overall response was evaluated four months after intra-articular injections of APC, better outcomes were achieved in the presence of higher concentrations of PDGF and VEGF, and lower concentrations of MIP-1α. Interestingly, no association was found with platelet count, suggesting that growth factor release, rather than platelet number, is relevant to clinical outcomes.

There is still controversy regarding the most effective platelet concentration for in vivo applications.[33] In vitro studies have shown conflicting results in terms of cell proliferation or other regenerative effects.[34] Some reports have shown that high concentrations of platelets are most beneficial,[35] while others have provided evidence that very high concentrations of platelet-rich plasma (PRP) could be counterproductive, with a potential risk of cell death.[36–38] We have previously

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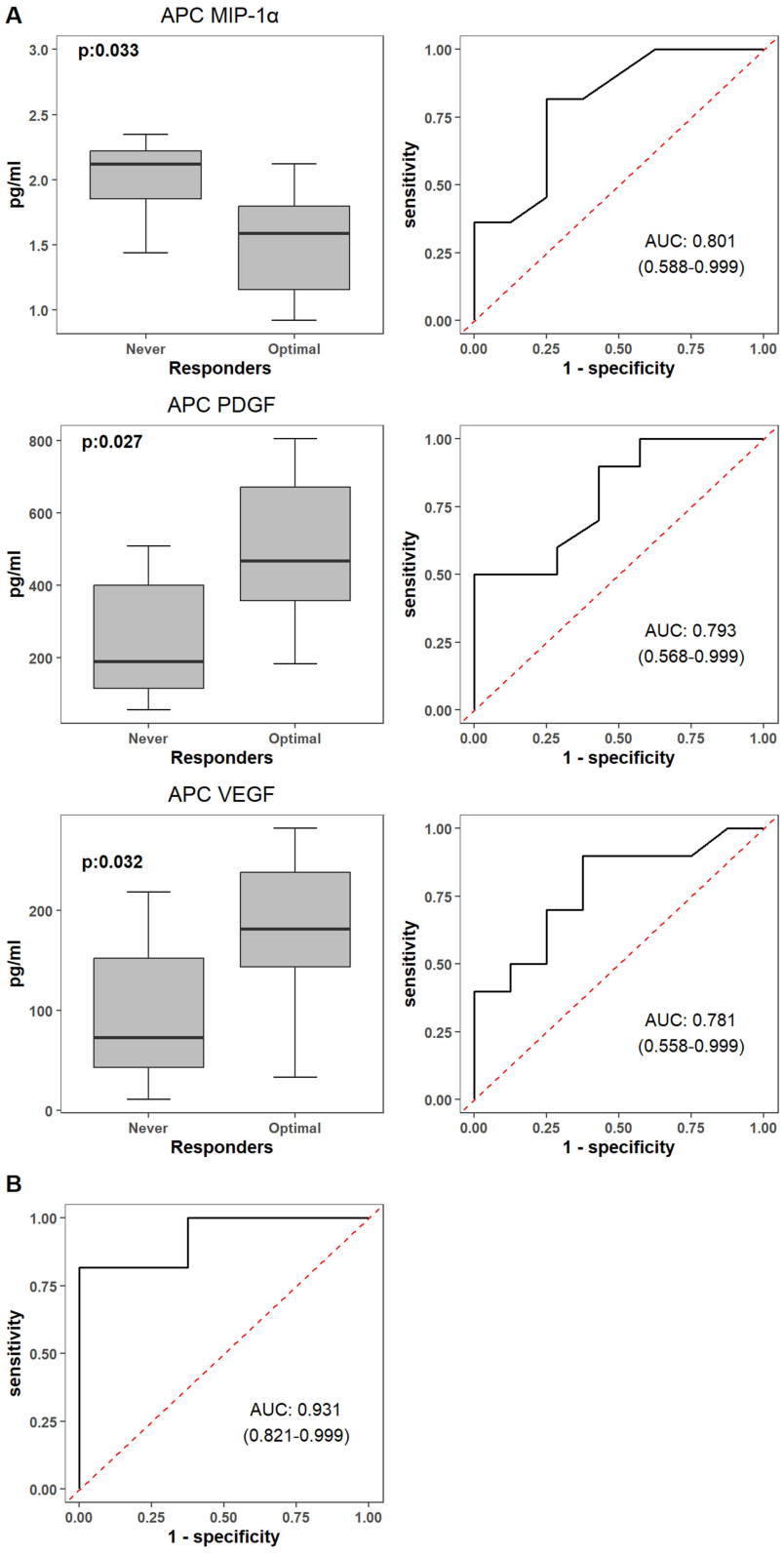

Figure 4 Soluble factors in APC in optimal vs never responders. **(A)** Boxplots depict concentrations of MIP-1α, PDGF, and VEGF in APC for optimal responders and never responders as described in the text. Concentrations are expressed as pg/mL. For each molecule, the relative ROC Curve for discriminating among the two groups is shown. The figure reports only those factors displaying statistical significance ($p<0.05$). **(B)** ROC Curve for the multivariable model including APC MIP-1α, APC PDGF, and APC VEGF ($p<0.05$).

shown that distinct effects on individual cell types may be due to the release of different amounts of specific GFs, including PDGF and VEGF.[31]

The functional relevance of PDGF has been largely addressed and is widely accepted. Indeed, PDGF plays a pivotal role in cell proliferation and tissue repair.[39,40] Moreover, PDGF has recently been shown to attenuate OA development by inhibiting inflammation and enhancing cell proliferation via the Janus Kinases (JAK) 2 / Signal Transducer and Activator of Transcription proteins (STAT) 3, Phosphoinositide 3-kinases (PI3K) / Protein kinase B (AKT), and p38 signaling pathways.[41,42] Consistently, PDGF has been used in functionalized scaffolds to promote the recruitment of synovial mesenchymal stem cells for osteochondral repair.[43]

Cartilage lacks blood and lymphatic supply. Normal articular cartilage resists vascular invasion due to its matrix composition and the production of diffusible angiogenesis inhibitors.[44,45] Hence, the action of VEGF on cartilage repair is still not well defined. Undoubtedly, VEGF is a relevant factor for tissue repair and regeneration.[46,47] However, previous studies have shown that angiogenesis blockade promotes cartilage repair in animal models.[48,49] VEGF-attenuated PRP, through VEGF-binding microspheres, did not affect PRP-induced chondrogenic differentiation of stem cells in vitro and improved therapeutic effect on cartilage repair in rats.[49] On the other hand, VEGF may exert non-angiogenic actions.[50] It has also been described that VEGF may be involved in macrophage recruitment and M2 polarization,[51] which may contribute to relieving intra-articular inflammatory burden. Nevertheless, VEGF release by APC might represent a surrogate marker of platelet degranulation and, in addition to being a relevant biomarker of clinical outcome, might have a neutral or even inhibitory effect on chondropathic pain.

Finally, our data revealed that the presence of MIP-1α in both PB and APC might be detrimental to clinical outcomes. Low MIP-1α levels in APC of optimal responders may be due to low systemic inflammation in patients. Therefore, it is conceivable that favorable clinical outcomes are facilitated by the transfer of low levels of inflammatory cytokines into the articular cavity.

Notably, it is known that OA and pain can be influenced by various confounding factors, such as old age, smoking, obesity, and diabetes.[52,53] In our population, NPRS variation was not affected by these factors. However, our study only included a homogeneous population of grade I knee OA patients, thus further investigations are needed to assess how confounding factors may impact on larger sampling sizes, including higher grades of OA.

Conclusion

In conclusion, our study confirms the effectiveness of APC treatment on pain reduction in Grade I Knee OA. The search of biomarkers for clinical outcome has revealed that determination of growth factors and cytokines in peripheral blood has limited predictive value. Nevertheless, we have shown for the first time that PDGF in platelet concentrates is a highly reproducible predictive biomarker of APC-induced pain reduction in individuals with grade I Knee OA. High levels of PDGF, but not platelet counts, are associated with better clinical outcomes. The simultaneous determination of PDGF, VEGF, and MIP-1α in platelet concentrates may confer higher accuracy in identifying patients who would benefit from APC therapy.

Data Sharing Statement

The datasets analyzed in this study are available from the corresponding author upon reasonable request.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

This study received no funding. Author FA is an employee of NGmed SRL. All other authors declare no competing financial or non-financial interests.

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