Observational Study

Plasma Rich in Growth Factors (PRGF) in the Treatment of Patients With Chronic Cervical and Lumbar Pain: A Prospective Observational Clinical Study

Eduardo Anitua, PhD[1,2], Isidro Milani, MD[3], Àlex Martínez[3], Freddy Cabello, MD[3], Roberto Prado, PhD[1,2], Sabino Padilla, PhD[1,2], and Luis Sanado, PhD[4]

From: [1]BTI-Biotechnology Institute ImasD, Vitoria, Spain; [2]University Institute for Regenerative Medicine and Oral Implantology - UIRMI (UPV/EHU-Fundación Eduardo Anitua), Vitoria, Spain; [3]Clínica Eduardo Anitua, Vitoria, Spain; 4Hospital Quirónsalud Vitoria, Vitoria, Spain

Address Correspondence:
Eduardo Anitua, PhD
Eduardo Anitua Foundation for Biomedical Research
Jacinto Quincoces, 39, 01007
Vitoria, Spain
E-mail: eduardo@fundacioneduardoanitua.org

Disclaimer: There was no external funding in the preparation of this manuscript

Conflict of interest: The authors declare that EA is the Scientific Director of, and RP and SP are scientists at BTI Biotechnology Institute, a biomedical company that investigates in the fields of regenerative medicine and PRGF-Endoret technology. The rest of the authors state that that they have no conflicts of interest that are relevant to the content of this article.

Manuscript received: 05-08-2023
Revised manuscript received: 05-18-2023
Accepted for publication: 07-17-2023

Free full manuscript: www.painphysicianjournal.com

Background: Chronic back pain is a long-lasting disorder, whose main source of pain is often the intervertebral disc that undergoes a degenerative process associated with low-grade inflammation, leading to a reduced quality of life.

Objectives: The aim of our study was to assess the efficacy of intradiscal and epidural injections of plasma rich in growth factors (PRGF) in patients with chronic clinical symptoms due to intervertebral disc (IVD) degeneration.

Study Design: Prospective observational study.

Setting: A single spine unit in a private clinic.

Methods: Thirty-two patients with cervical and lumbar chronic pain due to IVD degeneration were treated with 2 or 3 series of intradiscal and epidural PRGF infiltrations with 2 weeks between each procedure. The procedures were performed under fluoroscopic guidance and grade 3 sedation in an operating theater. Treatment efficacy was evaluated using the Spine Tango Core Outcome Measure Index questionnaire, Numeric Rating Scale for back pain, and the Oswestry Disability Index questionnaire. In addition, the number of patients who successfully achieved the minimal clinically important change was also determined. These assessments were evaluated at pretreatment (baseline) and at one, 3, and 6 months posttreatment.

Results: The Oswestry Disability Index, COMI Spine Tango Core Outcome Measure Index total score, and Numeric Rating Scale showed a statistically significant reduction from the baseline level to the posttreatment first month, third month, and sixth month ($P < 0.001$). Moreover, 78.1% of the patients reached a pain reduction superior to 30% one month posttreatment, and 87.5% at 6 months posttreatment, which is considered as a clinically significant improvement.

Limitations: This study was prospective and did not have a control group. Only patient-reported outcomes were evaluated.

Conclusions: This observational, prospective study of patients with chronic back pain showed that 2-3 intradiscal and epidural injections of PRGF significantly decreased pain and disability at one month posttreatment and this improvement was maintained, and in some patients even improved, at 3, and 6 months posttreatment.

Key words: Platelet-rich plasma, plasma rich in growth factors, degenerative disc disease, intervertebral disc degeneration, back pain, growth factors, regenerative medicine

Pain Physician 2023: 26:E725-E736

Cervical and lumbar intervertebral disc (IVD) degeneration are chronic disorders strongly correlated with nonspecific back pain and a reduced quality of life (1), representing the fourth leading cause of disability-adjusted life-years (2,3). Back pain is a complex and personal experience that likely develops as a consequence of the interaction between multidimensional physiologic, sensory, affective, cognitive, behavioral, and sociocultural phenomena with several risk factors (genetic, unhealthy lifestyle, type of work-job activity involving heavy loads and awkward postures, and aging) (1,4). The source of the pain related to the whole IVD joint may originate from its integrant tissues including periarticular muscles, IVD, vertebral subchondral bone, the annulus fibrosus, the vertebral endplate, and vertebral facet joints (5-8).

As a long-lasting disorder associated with low-grade inflammation (chronic pain persists for more than 3 months), cervical and low back pain brings about a reduced quality of life, psychological disorders, and loss of productivity. Its economic burden challenges the health care budgets of even the richest economies (1,9,10). Therefore, there are medical motivations and economic interests for reducing chronic pain in order to improve patients' quality of life and return them to their activities of daily living, including work.

In order to overcome some of the current available treatment limitations, new, innovative, biologically inspired, and minimally invasive therapeutic strategies that shorten recovery time compared with current surgical approaches are emerging to accurately deliver in situ different therapeutic biomolecules (5,11,12). Among them, autologous platelet-rich plasma (PRP) products are administered for treating chronic low back pain (13-16). These products deliver trophic and anti-inflammatory growth factors and cytokines to a degenerated disc through minimally invasive procedures (17-23) in order to reverse pain associated with low-grade inflammation and to restore homeostasis (5,24). More specifically, plasma rich in growth factors (PRGF) technology, a pioneering PRP (25,26) applied in multiple medical fields, has been shown to be an efficient treatment to attenuate pain associated with sterile, low-grade inflammation in a wide range of musculoskeletal conditions (24,27), including cervical and low back pain (13,14,28), thereby improving patients' clinical conditions.

The aim of our study was to assess the efficacy of intradiscal and epidural injections of PRGF in patients with chronic clinical symptoms due to IVD degeneration.

Methods

Study Design and Patient Population

This study was designed as a prospective observational study and carried out in a single private center in Victoria, Spain. The study protocol (code BTIIMD_02_EP/20/DISC) was approved on February 26, 2021 by the Ethics Committee CEIm-E and conducted in accordance with the international ethical standards from the revised World Medical Association Declaration of Helsinki amended in 2013 in Brazil. Patients provided written informed consent. This study is reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement (29). Patients were recruited from March 2021 through March 2022. The Inclusion and exclusion criteria for study participation are described in Table 1.

Preparation of PRGF

PRGF was prepared according to previously published methods (14,30). Briefly, peripheral venous blood was withdrawn (72 mL) and collected in 9-mL tubes containing sodium citrate (3.8% wt/vol) as an anticoagulant (EDK2_ENV kit, BTI Biotechnology Institute, S.L.). Next, the blood tubes were centrifuged for 8 minutes at 580 gravity (Endoret System V). The upper plasma volume (F1 fraction) was discarded, and the 2 mL plasma fraction located just above the buffy coat (F2 fraction) was collected without collecting leukocytes or erythrocytes. Activation of PRGF was conducted just prior to infiltration with the addition of a PRGF activator (10% calcium chloride) at a ratio of 20 µL of PRGF activator per mL of PRGF.

Characterization of PRGF

A characterization of the PRGF at each withdrawal and application was carried out. For this purpose, a complete blood count with 5-part differential (Pentra ES 60, Horiba ABX SAS) of both peripheral blood and nonactivated liquid PRGF (F1 and F2) was performed. In addition, leukocyte, erythrocyte, and platelet concentration factors were analyzed in relation to their levels in peripheral blood, as well as the platelet yield (%). A summary of the coding and classification of the PRGF-Endoret system using 9 different classification systems has recently been published by Anitua et al (31). The MIBO (Minimum Information for Studies Evaluating Biologics in Orthopaedics) guideline was followed for reporting methodological details (32).

Table 1. *Inclusion and exclusion criteria for study participation.*

Inclusion Criteria	Exclusion Criteria
1. Patients over 18 years of age. 2. Patients diagnosed with intervertebral disc degeneration by magnetic resonance imaging (MRI). 3. Patients with positive visible MRI signs, including rupture of the annulus fibrosus, annular fissure, with or without disc herniation in its protrusive form. 4. Symptoms of 3 months evolution that have not responded to conventional pharmacological treatment. 5. Numerical pain scale (NRS-11) between 6 and 10, average of the last month. 6. A complete blood test carried out in the last 2 months. 7. Informed consent form signature. 8. Agreement in the informed consent to be available for posttreatment follow-up for 6 months.	1. Presence of lumbar fracture, extruded disc herniations and herniations with signs of calcification. 2. Patients who have previously undergone spinal surgery or lumbar rhizolysis within the previous 8 months. 3. Patients with severe cardiovascular diseases, central nervous system diseases, epilepsy, coagulopathies, immunological diseases, infectious diseases (e.g. hepatitis, HIV, syphilis) and cancer. 4. Patients with a history of drug use (e.g., alcoholism or other) or mental illness or marked psychological conditions related to pain. 5. Morbidly obese patients. 6. Women who are pregnant or breastfeeding or women of childbearing age who are not taking contraceptive measures. 7. Pathologies that produce marked alterations in the efficacy of PRGF or coagulation, such as poorly controlled diabetes mellitus (glycosylated haemoglobin greater than 9%), hematological alterations (thrombopathy, thrombopenia, anemia with Hb < 9), being subjected to immunosuppressive and/or anticoagulant treatments, or any treatment with corticoids during the 6 months prior to inclusion in the study.

Intradiscal and Epidural PRGF Infiltration Protocol

After the blood collection and prior to the infiltrations, antibiotic prophylaxis and sedation of the patient were carried out. Cefazolin (2 g/vial, Normon Laboratories) was intravenously administered as an antibacterial prophylaxis. Mild intravenous sedation was carried out with a combination of 2.5 mg of midazolam hydrochloride (5 mg/5 mL, Normon Laboratories, Madrid, Spain) and 3.2 µg/kg/min of remifentanil hydrochloride (0.05 mg/mL, Ultiva, Aspen Pharmacare, GSK, Barcelona, Spain) and 0.025 µg/kg/min of propofol (BBraun Medical, Barcelona, Spain). Additionally, and depending on the duration of the procedure, a single dose, or repeated doses of 1-2 mg/kg of propofol (1%, BBraun Medical) were administered.

The intradiscal and epidural infiltration technique has been recently described (14). Briefly, IVD infiltrations with PRGF were performed under a fluoroscope with C-arm (Ziehm Solo, ZiehmImaging GMBH). Once the patient was prepared and placed prone for lumbar and supine for cervical, a small incision was made at the entry point for the 22G, 0.7 diameter x 178 mm long spinal needle (BD Spinal Needle Quincke, BD Spain).

The infiltration process was performed using an oblique angle of 25° to 35° at the lateral margin of the superior articular process of the lower vertebra, between the inferior endplate of the upper vertebra and the superior endplate of the lower vertebra, and always lateral to the neuroforamen for nerve root preservation. The spinal needle was manually bent at the tip (approximately 20°). Its position was confirmed with fluoroscopy in the 3 usual views, namely, oblique, for the approach; anteroposterior, to confirm the needle within the disc; and lateral, to measure the depth of the needle in the disc.

Once the tip of the spinal needle was placed in the degenerated disc (depth of the nucleus pulposus), and checked under fluoroscopy by infiltrating a small amount of PRGF, 3 mL of freshly activated PRGF were injected into the nucleus pulposus of the IVD in each injured disc. The infiltration procedure was performed without the use of any type of contrast agent. The procedure was performed on up to 3 levels. Afterwards, at the fluoroscopic lateral view, the posterior body wall reference was sought and, using the same procedure, peridural (epidural) infiltration was performed, injecting 2 mL of freshly activated PRGF.

Once the procedure was completed, the patient was transferred from the operating room to the recovery room and observed for 1-2 hours to monitor the patient's vital signs and any adverse reaction. An ice pack was kept on the treated area to avoid possible swelling while saline solution (100 mL) was administered by intravenous route. Acetaminophen (one g/8 h) and/or dexketoprofen (25 mg/12 h) was prescribed depending on the clinical evolution. After the procedure the patient was on relative rest for 7 days. During the posttreatment follow-up period (6 months) the patient could only be prescribed acetaminophen (one g/8 h) as a rescue analgesic. As an exception, no medication was allowed to be taken during the 48 hours prior to the follow-up visits.

Each patient was treated with a series of 2 14-day infiltrations. In each series the patient received IVD (between 1-3 levels) and epidural infiltrations. After the

one-month posttreatment follow-up visit, patients who did not obtain a decrease in pain of at least 2 points on the Numeric Rating Scale (NRS-11) received a new PRGF infiltration. Thus, patients received a series of 2-3 infiltrations depending on their clinical outcome.

Follow-up and Outcome Measures

Patients completed validated questionnaires in the pre- and posttreatment phases. Follow-up was performed one, 3, and 6 months posttreatment.

Treatment efficacy was evaluated using different patient-reported outcomes. The Spine Tango Core Outcome Measure Index (COMI) questionnaire for lumbar and cervical cases, NRS-11 for back pain, and the Oswestry Disability Index (ODI) questionnaire (for lumbar cases only). Furthermore, pain reduction over time was classified as excellent (an NRS-11 score of 0-3), moderate (an NRS-11 score of 3.1-6.5), and ineffective (an NRS-11 score of 6.6-10) (33). Finally, the number of patients who successfully achieved the minimal clinically important change (MCIC) for each of the scales evaluated was also determined (34-36).

Adverse Events

Complications and adverse events during all procedures and at each follow-up visit were also recorded.

Statistical Analysis

A priori power analysis was performed for determining the sample size based on data from Tuakli-Wosornu et al (18). With a power of 80% (β error of 0.2) and a one-sided α error of 0.05, it was estimated that a minimum sample size of 28 patients would be needed. Under the assumption of a drop-out rate of 20% throughout this prospective study, the number of patients we determined needed to be recruited was 35.

Descriptive data were presented as frequencies and percentages. The results of the patient-reported outcomes are reported as median (interquartile range [IQR]). No imputation method was used for missing data. All data values were tested for normality using the Shapiro-Wilk test. Changes in outcome measures between pre- and posttreatment were assessed using the Friedman test with Dunn's multiple comparisons test. Comparison of the success rate of pain reduction (classification), and in the MCIC over time (discrete variable data) was carried out using a χ^2 test. The relationship between demographics, clinical outcomes, and the characterization of whole blood and PRGF was explored using Spearman correlation coefficient. Box and whisker plots were drawn following the Tukey style (37), i.e., boxes show the median and IQR, while whiskers indicate the 25th percentile -1.5 × the IQR and the 75th percentile -1.5 × the IQR. Dots indicate outliers outside the whisker interval. Differences were considered statistically significant at a P value of < 0.05. GraphPad Prism version 9 (GraphPad Software) was used to analyze and graph the data.

Results

Patients' Demographics

Our study had 35 patients, but 3 were lost during follow-up and were not included in the analysis (drop-out rate of 8.6%). A total of 32 patients completed the follow-up. Their outcomes were measured at baseline pretreatment and at one, 3, and 6 months posttreatment (Table 2).

Of these, 40.6 % were women, with a mean age at baseline of 54.9 ± 10.1 and a mean body mass index (BMI [kg/m^2]) of 24.8 ± 3.1. The median chronic pain period was 4 months.

A total of 59 IVDs were infiltrated in the 32 patients, most of them in the lumbar region (90.6%). As revealed by magnetic resonance imaging, Pfirrmann grade III was the most prevalent radiological finding by far (40.7%), followed by grade IV (20.3%). Most of the patients (59.4%) received 2 series of infiltrations. Regarding the number of infiltrated levels, 53.1% of the patients received treatment on 2 discs, 31.3% on a single disc, and only 15.6% were infiltrated on 3 levels. If we consider specific levels, most of the infiltrations (79.7%) were in the lower lumbar back at the L4-L5 (40.7%) and L5-S1 (39.0%) levels (Table 2).

PRGF Characterization

The characterization of whole blood and PRGF fractions of patients included in the analysis is shown in Table 3. Fraction 2 (F2) was infiltrated in the patient after activation with calcium chloride, but the data shown are prior to activation. The biological variability of these data is illustrated in Fig. 1. The F2 was almost free of leukocytes and erythrocytes with a platelet concentration of 2.2 ± 0.4 in comparison with the peripheral blood level. The components of both whole blood and PRGF for each of the 2-3 series of infiltrations (intrapatient) showed no statistically significant difference ($P > 0.05$).

Table 2. *Patient baseline and demographic characteristics.*

Patients (n)	32
Gender	
Women (n, %)	13 (40.6 %)
Men (n, %)	19 (59.4 %)
Age (years, mean ± SD)	54.9 ± 10.1
Height (cm, mean ± SD)	171.7 ± 8.1
Weight (kg, mean ± SD)	73.4 ± 12.0
Body mass index (kg/m^2, mean ± SD)	24.8 ± 3.1
Pain evolution period (months, median [IQR])	4 [3-6]
Intervertebral discs (n)	59
Spine Region	
Lumbar (patients, n, %)	29 (90.6 %)
Cervical (patients, n, %)	3 (9.4 %)
MRI Pfirrmann Grade	
II (discs, n, %)	9 (15.3 %)
III (discs, n, %)	24 (40.7 %)
IV (discs, n, %)	12 (20.3 %)
V (discs, n, %)	14 (23.7 %)
Series of infiltration	
2 series (n, %)	19 (59.4 %)
3 series (n, %)	13 (40.6 %)
Multiple levels injected	
One level (n, %)	10 (31.3 %)
2 levels (n, %)	17 (53.1 %)
3 levels (n, %)	5 (15.6 %)
Levels infiltrated	
C5-C6 (n, %)	3 (5.1 %)
C6-C7 (n, %)	1 (1.7 %)
L2-L3 (n, %)	2 (3.4%)
L3-L4 (n, %)	6 (10.2 %)
L4-L5 (n, %)	24 (40.7 %)
L5-S1 (n, %)	23 (39.0 %)

Analysis of Clinical Outcomes

The PRGF infiltrations, assessed by the scores of ODI and COMI questionnaires, exerted a statistically significant clinical and functional improvement since the first month after the procedure as shown in the Fig. 2 and Table 4. The ODI showed a statistically significant reduction from 36 (IQR, 28-50) at baseline to 12 (IQR, 3-23) at posttreatment month one, to 6 (IQR, 0-16) at posttreatment month 3, and to 8 (IQR, 2-16) at posttreatment month 6 ($P < 0.001$, Table 4, Fig. 2). Similarly, the COMI total score presented a statistically significant reduction from 6.4 (IQR, 4.9-7.3) at baseline to 2.1 (IQR, 0.7-3.1) at posttreatment month one,

Table 3. *Characterization of whole blood and PRGF fractions (F1 and F2) of patients included in the analysis. Fraction 2 (F2) is infiltrated in the patient. A complete blood count with 5-part differential was conducted. Leukocyte, erythrocyte, and platelet concentration factor relative to the level of peripheral blood and platelet yield (%) are also indicated. Data are expressed as mean ± SD, n = 32, n.d., not detected.*

	Whole blood	PRGF F1	PRGF F2
Leukocytes (x 10^3/µL)	6.41 ± 2.05	0.08 ± 0.08	0.26 ± 0.19
Lymphocytes (%)	33.1 ± 8.4	n.d.	n.d.
Monocytes (%)	4.5 ± 2.3	n.d.	n.d.
Neutrophils (%)	59.5 ± 9.5	n.d.	n.d.
Eosinophils (%)	3.0 ± 1.4	n.d.	n.d.
Basophils (%)	0.6 ± 0.2	n.d.	n.d.
Erythrocytes (x 10^6/µL)	4.43 ± 0.42	n.d.	0.01 ± 0.01
Platelets (x 10^3/µL)	214 ± 47	300 ± 69	465 ± 137
Mean platelet volume (fL)	7.4 ± 0.6	6.7 ± 0.5	7.1 ± 0.7
Leukocyte concentration factor	1	0.01 ± 0.01	0.04 ± 0.03
Erythrocyte concentration factor	1	0	0
Platelet concentration factor	1	1.4 ± 0.2	2.2 ± 0.4
Platelet yield (%)	100	23.1 ± 8.9	46.4 ± 8.3

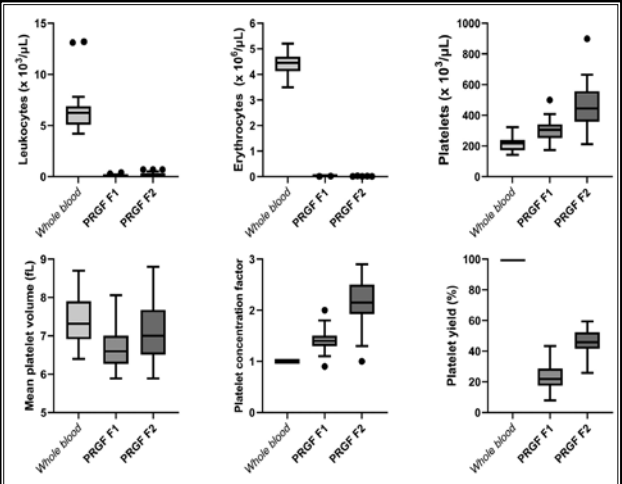

Fig. 1. *Characterization of whole blood and PRGF F1 and F2 fractions. Leukocyte, erythrocyte and platelet count are shown. Specifically for platelets, the mean platelet volume is represented, as well as the platelet concentration factor (the increase of platelets with respect to the basal value of peripheral blood), and the platelet yield, or percentage of platelets recovered in each PRGF fraction with respect to peripheral blood.*

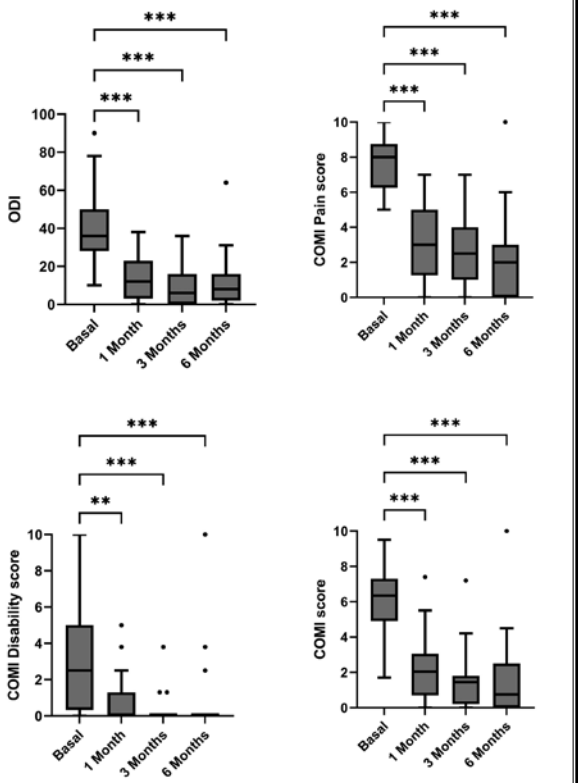

Fig. 2. *Clinical outcomes for all patients (lumbar and cervical) with complete follow-up. Oswestry Disability Index (only for lumbar cases), NRS-11, COMI Disability score, and COMI total score are displayed. ** indicates P < 0.01 and *** indicates P < 0.001.*

Table 4. *Outcome assessment at baseline, one, 3 and 6 months posttreatment for all patients (lumbar and cervical cases) with complete follow-up (n = 32). Results are reported as median [interquartile ranges]. Friedman test with Dunn's multiple comparisons test. Statistically significant differences (P < 0.05) are in boldface. Oswestry Disability Index only for lumbar cases (n = 29).*

Outcome variable	Median [interquartile ranges]	*P* Value vs Baseline	*P* Value vs One month	*P* Value vs 3 months
NRS-11				
Baseline	8.0 [6.3 - 8.8]			
One month	3.0 [1.3 - 5.0]	< **0.001**		
3 months	2.5 [1.0 - 4.0]	< **0.001**	> 0.99	
6 months	2.0 [0.0 - 3.0]	< **0.001**	0.8	> 0.99
COMI Disability score				
Baseline	2.5 [0.3 - 5.0]			
One month	0.0 [0.0 - 1.3]	**0.006**		
3 months	0.0 [0.0 - 0.0]	< **0.001**	> 0.99	
6 months	0.0 [0.0 - 0.0]	< **0.001**	> 0.99	> 0.99
COMI total score				
Baseline	6.4 [4.9 - 7.3]			
One month	2.1 [0.7 - 3.1]	< **0.001**		
3 months	1.5 [0.2 - 1.8]	< **0.001**	0.88	
6 months	0.8 [0.0 - 2.5]	< **0.001**	0.20	> 0.99
Oswestry Disability Index				
Baseline	36 [28 - 50]			
One month	12 [3 - 23]	< **0.001**		
3 months	6 [0 - 16]	< **0.001**	> 0.99	
6 months	8 [2 - 16]	< **0.001**	> 0.99	> 0.99

to 1.5 (IQR, 0.2-1.8) at posttreatment month 3, and to 0.8 (IQR, 0.0-2.5) at posttreatment month 6 ($P < 0.001$, Table 4, Fig. 2).

The pain NRS-11 was significantly reduced at posttreatment month one (3.0 [IQR,1.3 - 5.0]), as well as at posttreatment month 3 (2.5 [IQR, 1.0 - 4.0]), and posttreatment month 6 (2.0 [IQR, 0.0 - 3.0]) compared with the baseline value (8.0 [IQR, 6.3 - 8.8]) ($P < 0.001$, Table 4, Fig. 2).

On the other hand, 20 patients (62.5%) reached an NRS-11 score < 3 at posttreatment month one, which is considered as "excellent" pain reduction (33); at the last follow-up, 26 patients (81.3 %) were included in that category. By contrast, only one patient (3.1%) continued to have a pain score considered to be minimal or of no benefit (between 6.6-10) (Fig. 3). Moreover, a pain reduction greater than 30%, which is considered as MCIC (23,34), was reached by 78.1% of the patients after one and 3 months of treatment, and 87.5 % of patients at 6 months of follow-up.

The data are similar if we analyze the MCIC in the global score of the COMI scale (≥ 2.2 points) (35), with 87.5% of responders at posttreatment month one and posttreatment month 6 and 84.4% at posttreatment month 3. The highest percentage of responders (≥ 10 points) was obtained in the ODI scale (36) at posttreatment month 6 (89.7%), while at posttreatment months one and 3 the percentages were 82.8% and 86.2%, respectively. Taking all the scales together, the ratio of nonresponders at posttreatment month 6 is estimated to be between 10.3% and 12.5%.

Sub-group Analysis

Patients with either lumbar or cervical back pain were included in our study. However, as shown in Table 3, most had lumbar pain (90.6 %), thus, the lumbar

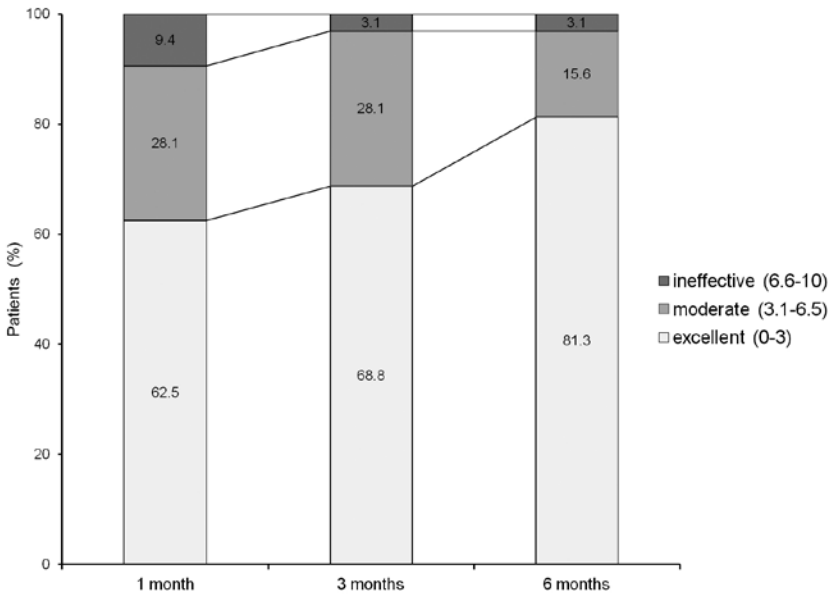

Fig. 3. *Representation of treatment response in terms of pain reduction during follow-up. This reduction was classified as excellent (score 0-3 on the NRS-11 pain scale), moderate (NRS-11 3.1-6.5) and ineffective (NRS-11 6.6-10). According to the inclusion criteria, patients started (pretreatment, baseline) with an NRS-11 for pain ≥ 6. The percentage of patients in each of the 3 categories is shown.*

level is a major contributor to our data. Both subgroups were analyzed separately; the statistical results obtained for low back pain (n = 29, $P < 0.001$ vs baseline, Table 5, Fig. 4) were similar to those for the total number of patients (n = 32, $P < 0.001$ vs baseline, Table 4, Fig. 2). In the case of patients with cervical pain, it was not possible to perform a statistical analysis due to the low number of individuals in this subgroup (n = 3), although all improved as is noted in Fig. 5, which shows the dispersion of the data.

Correlation Analysis

There was no clear correlation in any of the patients that might shed light on parameters that could predict the efficacy of the treatment. No correlation was observed between hematological and demographic parameters vs clinical outcomes ($P > 0.05$).

Adverse Events

Two patients in the study, one with cervical pain and one with lumbar pain, experienced slight sensory alteration, such as numbness, which resolved after 4-6 weeks.

Discussion

This observational, prospective study shows that intradiscal and epidural injections of PRGF significantly attenuated pain and disabilities at one, 3, and 6 months of follow-up in patients with chronic back pain due to vertebral disc degeneration. Significantly, 20 patients

Table 5. *Outcome measures at baseline, one, 3, and 6 months for lumbar cases with complete follow-up (n = 29). Results are reported as median [interquartile ranges]. Friedman test with Dunn's multiple comparisons test. Statistically significant differences (P <0 .05) are in boldface.*

Outcome variable	Median [interquartile ranges]	*P* Value vs Baseline	*P* Value vs 1 month	*P* Value vs 3 months
NRS-11				
Baseline	8.0 [6.0 - 9.0]			
One month	3.0 [0.5 - 4.5]	< **0.001**		
3 months	2.0 [1.0 - 4.0]	< **0.001**	> 0.99	
6 months	2.0 [0.5 - 3.0]	< **0.001**	> 0.99	> 0.99
COMI Disability score				
Baseline	2.5 [0.7 - 5.0]			
One month	0.0 [0.0 - 1.3]	**0.01**		
3 months	0.0 [0.0 - 0.0]	< **0.001**	> 0.99	
6 months	0.0 [0.0 - 0.0]	< **0.001**	> 0.99	> 0.99
COMI total score				
Baseline	6.4 [4.8 - 7.3]			
One month	1.9 [0.6 - 3.0]	< **0.001**		
3 months	1.5 [0.2 - 1.8]	< **0.001**	> 0.99	
6 months	0.9 [0.1 - 2.7]	< **0.001**	0.56	> 0.99
Oswestry Disability Index				
Baseline	36 [28 - 50]			
One month	12 [3 - 23]	< **0.001**		
3 months	6 [0 - 16]	< **0.001**	> 0.99	
6 months	8 [2 - 16]	< **0.001**	> 0.99	> 0.99

(62.5%) reached a pain NRS-11 score < 3, which is considered as "excellent" in the literature (33). At the end of follow-up, 26 patients (81.3 %) had a pain NRS-11 score < 3. Only one patient's (3.1%) pain score was in the 6-10 range which is considered to be minimal or no benefit (6-10). Moreover, 78.1% of the patients reached a pain reduction superior to 30% after one month of treatment and 87.5% at 6 months of follow-up, which is considered to be a clinically significant improvement (23,34).

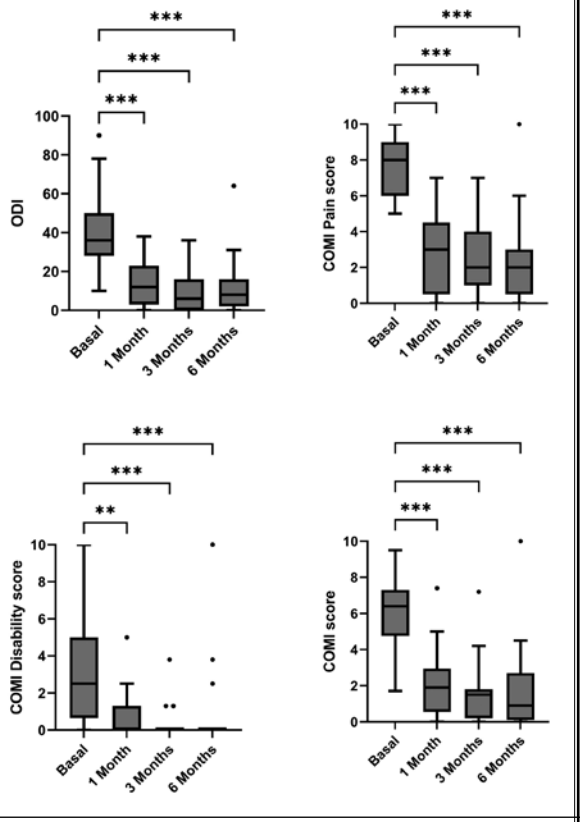

Fig. 4. *Clinical outcomes for patients with lumbar pathology with complete follow-up. Oswestry Disability Index, NRS-11, COMI Disability score, and COMI total score are showed. ** indicates P < 0.01 and *** indicates P < 0.001.*

The short-term pain reduction after one month after intradiscal injection of PRP observed in our patients is consistent with previous studies and equates with the acute analgesic and anti-inflammatory effects of glucocorticoids as first-line and the most common treatment for inflammatory pathologies of musculoskeletal tissues (19,38-40). Kirchner et al (13) reported that the largest pain reduction, 53% over a total of 93%, was attained one month after a PRGF procedure. In the same vein, Akeda, et al (38) found that pain reduction after 2 months of treatment represented 48% of the total pain reduction reached 60 weeks after an intradiscal injection of autologous PRP, which is consistent with the results reported by Tuakli-Wosurnu et al (18).

In addition, in our study PRGF injections showed a sustained analgesic effect at 6 months posttreatment, an observation already reported by other studies (13,18,19,38). The pain reduction at 6 months follow-up in patients of our study holds an important meaning considering that the range of IQR baseline pain value of the NRS-11 was between 6.3-8.8. This pain level has a great effect on quality of life and is associated with severe disability. However, at 6 months posttreatment with intradiscal injections of PRGF, our patients improved their quality of life, including their activities of daily living and their physical activity, with only mild

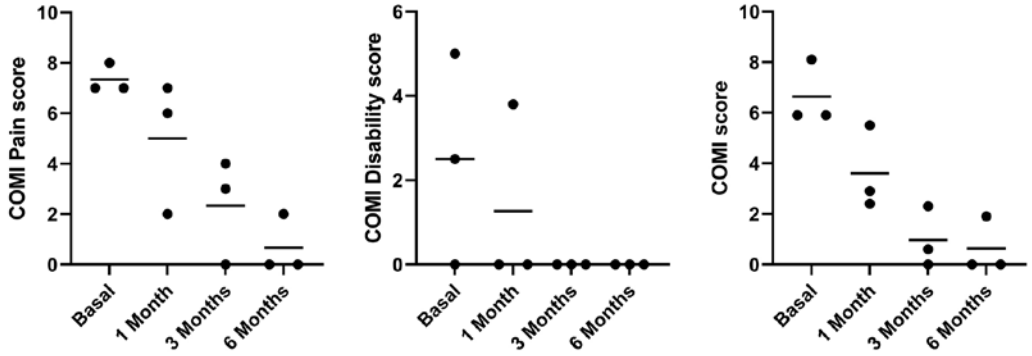

Fig. 5. *Clinical outcome for the 3 patients with cervical pain. NRS-11, COMI Disability score, and COMI total score are represented. No statistics were performed due to low number of patients.*

disability and pain. The residual-persistent pain at the end of follow-up did not interfere with patients' participation in work, social, and self-care activities, meaning that the remaining mild pain did not impinge on daily functioning, nor did it entail the prescription of painkillers or anti-inflammatory drugs, a fact conceptualized or known as the impact of chronic pain (the impact of pain on daily functioning) (1,41).

There were, however, 4 patients (12.5%) who did not reach the MCIC for pain (nonresponders) at the end of the study. Several factors could be related to this outcome, such as the main origin of the pain, the biological composition of PRGF, or even patient lifestyles (1,5). Due to the low number of nonresponders, no correlations between PRGF characteristics and clinical outcomes could be performed.

Pain is a hardwired signal generated within the brain where biological messages from the peripheral tissues are integrated with other sensory and emotional stressor experiences already recorded as centralized pain (42-44). Consistent with this concept of pain, it is worth considering that the residual mild pain showed by the patients of this study at 6 months posttreatment of PRGF intradiscal infiltrations might be considered as neuroplastic pain, whose major feature may be the absence of a noxious peripheral stimulus, and whose therapeutic approach should contemplate other therapeutic interventions, such as pain reprocessing therapy (45). In this respect, a recent randomized controlled clinical trial (46) reported that a mindfulness intervention in adult patients with low back pain reduced their stress level as measured by lowered circulating cortisol and IL-1β levels. These lowered levels are associated with a significant decrease in pain and other disability domains, and improved an improved quality of life compared with the control group (46).

The molecular mechanism by which PRP exerts a short-term and persistent analgesic effect on musculoskeletal pathologies is poorly understood. Several studies point to the dual immediate and sustained release of several growth factors with neuro-immunomodulatory effects including TGF-β , HGF, and IGF-1, provided that fibrin is the autologous biomimetic scaffold that conveys the plasma and platelet pool of growth factors and other biomolecules to the dysregulated pathological tissue (5,13,24,47). Other potential mechanisms include the NF-kB signalling pathway inhibition that mediate in the inflammatory response of stressed cells (48,49), the antiapoptotic, ECM-protective, and IL-Ra mediated anti-inflammatory and pain reduction. Interestingly, the antalgic effects is mediated by a peripheral endocannabinoid-related mechanismd (50-53).

When considering the potential therapeutic properties of PRP, it is crucial to emphasize the significance of accurately characterizing the administered PRP (30) as well as the precise dosage applied. Moreover, it is essential to consider the effect of previous injections or procedures both in the intervertebral disc and in the rest of the spinal tissues. In our study, a hematological count of both blood and PRGF was performed in all patients at each withdrawal and application. No statistically significant differences were found in the intrapatient values for each of the 2-3 series of infiltrations.

The PRGF infiltrated presented an average 2.2-fold increase in the peripheral platelet concentration without leukocytes. In this respect, there is in vitro evidence indicating that leukocytes enhance inflammation and catabolic degenerative changes in the IVD after endplate fracture without infiltrating the disc (54) and leukocytes in PRP stimulate inflammatory and catabolic effects on nucleus pulposus-derived stem cells from an early degenerated IVD (55). Supporting this in vitro evidence, clinical studies show that one intradiscal injection of PRP with leukocytes (56) do not show improvements in pain reduction compared with a saline injection and the best and the worst pain even increased after one month of leukocyte-rich PRP injection (56). Furthermore, it has recently been shown that the inclusion of leukocytes in PRP contributes significantly to scaffold instability and to a reduced ability to induce cell proliferation (57).

Finally, the disparate clinical outcomes when it comes to intradiscal PRP infiltration on chronic back pain is associated with the different biological composition, the PRP dose, and the prior injections of other products into the disc to be treated with PRP, such as contrast medium, anesthetics and antibiotics, and compounds that could be detrimental in the healing process expected to be induced by PRP (58-60).

Limitations

This study has several limitations that need to be considered. The first is the lack of a longer follow-up time. The second is related to the absence of an image-based magnetic resonance imaging or radiographic study to assess structural changes in the disc. The third is that our therapeutic approach was exclusively biological. The treatment of chronic back pain should be a multidisciplinary approach, which should include several levels of intervention from biological to psychological and rehabilitation programs. The fourth

limitation is the low number of cervical cases, which makes robust statistical analysis of this subgroup difficult. Nevertheless, the cervical cases data were reported in this paper since the original protocol approved by the ethics committee included both cervical and lumbar cases. The fifth limitation is that we only targeted the IVD and the epidural space with PRGF. It is widely accepted that apart from the IVD, other tissues of the intervertebral disc joint contribute to the origin of back pain, such as the vertebral subchondral bone, the annulus fibrosus, the vertebral endplate, and vertebral facet joints (5).

Conclusions

Overall, this prospective study demonstrates that treating chronic back pain with PRGF is effective for reducing pain and improving the quality of life of patients at 6 months posttreatment. At the same time, our study lays the foundation for building stronger clinical evidence, such as conducting randomized clinical trials with long-term follow-up and including magnetic resonance imaging in those studies.

References

1. Vlaeyen JWS, Maher CG, Wiech K, et al. Low back pain. *Nat Rev Dis Primers* 2018; 4:52.
2. Vos T, Abajobir AA, Abate KH, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 2017; 390:1211-1259.
3. Hay SI, Abajobir AA, Abate KH, et al. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 2017; 390:1260-1344.
4. Mannion AF, Balague F, Pellise F, Cedraschi C. Pain measurement in patients with low back pain. *Nat Clin Pract Rheumatol* 2007; 3:610-618.
5. Anitua E, Padilla S. Biologic therapies to enhance intervertebral disc repair. *Regen Med* 2018; 13:55-72.
6. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: Pain and disc content. *Nat Rev Rheumatol* 2014; 10:44-56.
7. Lotz JC, Fields AJ, Liebenberg EC. The role of the vertebral end plate in low back pain. *Global Spine J* 2013; 3:153-164.
8. Shapiro IM, Risbud MV. *The Intervertebral Disc. Molecular and Structural Studies of the Disc in Health and Disease.* Springer, Vienna, 2014.
9. Dieleman JL, Baral R, Birger M, et al. US Spending on Personal Health Care and Public Health, 1996-2013. *JAMA* 2016; 316:2627-2646.
10. Knezevic NN, Candido KD, Vlaeyen JWS, Van Zundert J, Cohen SP. Low back pain. *Lancet* 2021; 398:78-92.
11. Moriguchi Y, Alimi M, Khair T, et al. Biological treatment approaches for degenerative disk disease: A literature review of in vivo animal and clinical data. *Global Spine J* 2016; 6:497-518.
12. Wang SZ, Rui YF, Tan Q, Wang C. Enhancing intervertebral disc repair and regeneration through biology: Platelet-rich plasma as an alternative strategy. *Arthritis Res Ther* 2013; 15:220.
13. Kirchner F, Anitua E. Intradiscal and intra-articular facet infiltrations with plasma rich in growth factors reduce pain in patients with chronic low back pain. *J Craniovertebr Junction Spine* 2016; 7:250-256.
14. Kirchner F, Milani I, A, et al. Plasma rich in growth factors (PRGF) in the treatment of cervical and lumbar back pain: A retrospective observational clinical study. *Pain Physician* 2021; 24:E649-E660.
15. Navani A, Manchikanti L, Albers SL, et al. Responsible, safe, and effective use of biologics in the management of low back pain: American Society of Interventional Pain Physicians (ASIPP) Guidelines. *Pain Physician* 2019; 22:S1-S74.
16. Barbieri M, Colombini A, Stogicza A, de Girolamo L. Effectiveness of plasma rich in growth factors in the management of chronic spinal pain: A case series of 32 patients. Regen Med 2022; 17:175-184.
17. Aufiero D, Vincent H, Sampson S, Bodor M. Regenerative Injection Treatment in the Spine: Review and Case Series with Platelet Rich Plasma. *J Stem Cells Res, Rev & Rep* 2015:2: 1019.
18. Tuakli-Wosornu YA, Terry A, Boachie-Adjei K, et al. Lumbar intradiskal platelet-rich plasma (PRP) injections: A prospective, double-blind, randomized controlled study. *PM R* 2016; 8:1-10.
19. Akeda K, Ohishi K, Masuda K, et al. Intradiscal injection of autologous platelet-rich plasma releasate to treat discogenic low back pain: A preliminary clinical trial. *Asian Spine J* 2017; 11:380-389.
20. Lutz GE. Increased nuclear T2 signal intensity and improved function and pain in a patient one year after an intradiscal platelet-rich plasma injection. *Pain Med* 2017; 18:1197-1199.
21. Broadhead DY, Douglas HE, Bezjian Wallace LM, et al. Use of ultrasound-guided platelet-rich plasma injection of the sacroiliac joint as a treatment for chronic low back pain. *Mil Med* 2020; 185:e1312-e1317.
22. Cheng J, Santiago KA, Nguyen JT, Solomon JL, Lutz GE. Treatment of symptomatic degenerative intervertebral discs with autologous platelet-rich plasma: Follow-up at 5-9 years. *Regen Med* 2019; 14:831-840.
23. Zhang J, Liu D, Gong Q, Chen J, Wan L. Intradiscal autologous platelet-rich plasma injection for discogenic low back pain: A clinical trial. *Biomed Res Int* 2022; 2022:9563693.
24. Anitua E, Nurden P, Prado R, Nurden AT, Padilla S. Autologous fibrin scaffolds: When platelet- and plasma-derived biomolecules meet fibrin. *Biomaterials* 2019; 192:440-460.

25. Anitua E. Plasma rich in growth factors: Preliminary results of use in the preparation of future sites for implants. *Int J Oral Maxillofac Implants* 1999; 14:529-535.
26. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost* 2004; 91:4-15.
27. Padilla S, Sanchez M, Orive G, Anitua E. Human-based biological and biomimetic autologous therapies for musculoskeletal tissue regeneration. *Trends Biotechnol* 2017; 35:192-202.
28. Kirchner F, Pinar A, Milani I, Prado R, Padilla S, Anitua E. Vertebral intraosseous plasma rich in growth factor (PRGF-Endoret) infiltrations as a novel strategy for the treatment of degenerative lesions of endplate in lumbar pathology: Description of technique and case presentation. *J Orthop Surg Res* 2020; 15:72.
29. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Lancet* 2007; 370:1453-1457.
30. Anitua E, Prado R, Nurden AT, Nurden P. Characterization of Plasma Rich in Growth Factors (PRGF): components and formulations, in Anitua E, Cugat R, Sánchez M (eds). *Platelet Rich Plasma in Orthopaedics and Sports Medicine*. Springer International Publishing, Cham, Switzerland, 2018, pp 29-45.
31. Anitua E, Padilla S, Prado R, Alkhraisat MH. Platelet-rich plasma: Are the obtaining methods, classification and clinical outcome always connected? *Regen Med* 2022; 17:887-890.
32. Murray IR, Geeslin AG, Goudie EB, Petrigliano FA, LaPrade RF. Minimum information for studies evaluating biologics in orthopaedics (MIBO): Platelet-rich plasma and mesenchymal stem cells. *J Bone Joint Surg Am* 2017; 99:809-819.
33. Oder B, Loewe M, Reisegger M, Lang W, Ilias W, Thurnher SA. CT-guided ozone/steroid therapy for the treatment of degenerative spinal disease--effect of age, gender, disc pathology and multi-segmental changes. *Neuroradiology* 2008; 50:777-785.
34. Ostelo RW, Deyo RA, Stratford P, et al. Interpreting change scores for pain and functional status in low back pain: Towards international consensus regarding minimal important change. *Spine (Phila Pa 1976)* 2008; 33:90-94.
35. Mannion AF, Porchet F, Kleinstuck FS, et al. The quality of spine surgery from the patient's perspective: Part 2. Minimal clinically important difference for improvement and deterioration as measured with the Core Outcome Measures Index. *Eur Spine J* 2009; 18 Suppl 3:374-379.
36. Ostelo RW, de Vet HC. Clinically important outcomes in low back pain. *Best Pract Res Clin Rheumatol* 2005; 19:593-607.
37. Krzywinski M, Altman N. Visualizing samples with box plots. *Nat Methods* 2014; 11:119-120.
38. Akeda K, Ohishi K, Takegami N, et al. Platelet-rich plasma releasate versus corticosteroid for the treatment of discogenic low back pain: A double-blind randomized controlled trial. *J Clin Med* 2022; 11:304.
39. Vaquerizo V, Garcia-Lopez M, Mena-Roson A, Prado R, Padilla S, Anitua E. Plasma rich in growth factors (PRGF) versus corticosteroid injections for management of chronic rotator cuff tendinopathy: A prospective double-blind randomized controlled trial with 1-year of follow-up. *J Shoulder Elbow Surg* 2023; 32:555-564.
40. Scott A, Khan KM. Corticosteroids: Short-term gain for long-term pain? *Lancet* 2010; 376:1714-1715.
41. Von Korff M, Scher AI, Helmick C, et al. United States National Pain Strategy for Population Research: Concepts, definitions, and pilot data. *J Pain* 2016; 17:1068-1080.
42. Clauw DJ. Diagnosing and treating chronic musculoskeletal pain based on the underlying mechanism(s). *Best Pract Res Clin Rheumatol* 2015; 29:6-19.
43. Tracey I. Getting the pain you expect: Mechanisms of placebo, nocebo and reappraisal effects in humans. *Nat Med* 2010; 16:1277-1283.
44. Vlaeyen JWS, Crombez G. Behavioral conceptualization and treatment of chronic pain. *Annu Rev Clin Psychol* 2020; 16:187-212.
45. Ashar YK, Gordon A, Schubiner H, et al. Effect of pain reprocessing therapy vs placebo and usual care for patients with chronic back pain: A randomized clinical trial. *JAMA Psychiatry* 2022; 79:13-23.
46. Diez GG, Anitua E, Castellanos N, Vazquez C, Galindo-Villardon P, Alkhraisat MH. The effect of mindfulness on the inflammatory, psychological and biomechanical domains of adult patients with low back pain: A randomized controlled clinical trial. *PLoS One* 2022; 17:e0276734.
47. Buchheit T, Huh Y, Maixner W, Cheng J, Ji RR. Neuroimmune modulation of pain and regenerative pain medicine. *J Clin Invest* 2020; 130:2164-2176.
48. Bonizzi G, Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 2004; 25:280-288.
49. Montaseri A, Busch F, Mobasheri A, et al. IGF-1 and PDGF-bb suppress IL-1β-induced cartilage degradation through down-regulation of NF-κB signaling: Involvement of Src/PI-3K/AKT pathway. *PLoS One* 2011; 6:e28663.
50. Descalzi F, Ulivi V, Cancedda R, et al. Platelet-rich plasma exerts antinociceptive activity by a peripheral endocannabinoid-related mechanism. *Tissue Eng Part A* 2013; 19:2120-2129.
51. Khatab S, van Buul GM, Kops N, et al. Intra-articular Injections of platelet-rich plasma releasate reduce pain and synovial inflammation in a mouse model of osteoarthritis. *Am J Sports Med* 2018; 46:977-986.
52. Lee HR, Park KM, Joung YK, Park KD, Do SH. Platelet-rich plasma loaded hydrogel scaffold enhances chondrogenic differentiation and maturation with up-regulation of CB1 and CB2. *J Control Release* 2012; 159:332-337.
53. van Buul GM, Koevoet WL, Kops N, et al. Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes. *Am J Sports Med* 2011; 39:2362-2370.
54. Dudli S, Boffa DB, Ferguson SJ, Haschtmann D. Leukocytes enhance inflammatory and catabolic degenerative changes in the intervertebral disc after endplate fracture in vitro without infiltrating the disc. *Spine (Phila Pa 1976)* 2015; 40:1799-1806.
55. Wang SZ, Fan WM, Jia J, Ma LY, Yu JB, Wang C. Is exclusion of leukocytes from platelet-rich plasma (PRP) a better choice for early intervertebral disc regeneration? *Stem Cell Res Ther* 2018; 9:199.
56. Schepers MO, Groot D, Kleinjan EM, Pol MM, Mylenbusch H, Klopper-Kes AHJ. Effectiveness of intradiscal platelet rich plasma for discogenic low back pain without Modic changes: A randomized controlled trial. *Interv Pain Med* 2022; 1:100011.
57. Anitua E, Zalduendo M, Troya M,

Tierno R, Alkhraisat MH. The inclusion of leukocytes into platelet rich plasma reduces scaffold stability and hinders extracellular matrix remodelling. *Ann Anat* 2022; 240:151853.

58. Hoelscher GL, Gruber HE, Coldham G, Grigsby JH, Hanley EN Jr. Effects of very high antibiotic concentrations on human intervertebral disc cell proliferation, viability, and metabolism in vitro. *Spine (Phila Pa 1976)* 2000; 25:1871-1877.
59. Chee AV, Ren J, Lenart BA, Chen EY, Zhang Y, An HS. Cytotoxicity of local anesthetics and nonionic contrast agents on bovine intervertebral disc cells cultured in a three-dimensional culture system. *Spine J* 2014; 14:491-498.
60. Iwasaki K, Sudo H, Yamada K, Ito M, Iwasaki N. Cytotoxic effects of the radiocontrast agent iotrolan and anesthetic agents bupivacaine and lidocaine in three-dimensional cultures of human intervertebral disc nucleus pulposus cells: Identification of the apoptotic pathways. *PLoS One* 2014; 9:e92442.