Individual Immune Cell and Cytokine Profiles Impact Platelet-Rich Plasma Composition

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Abstract

Objective: Platelet-rich plasma (PRP) is increasingly popular to treat musculoskeletal diseases, including tendinopathies and osteoarthritis (OA). To date, it remains unclear to which extent PRP compositions are determined by the immune profile of individuals or by the preparation method. To investigate this, we compared leukocyte and cytokine distributions of different PRP products to donor blood samples.

Design: For each of three PRP preparations (ACP®, Angel™, and nSTRIDE® APS), products were derived using blood samples from twelve healthy donors. The cellular composition of PRP products was analyzed by flow cytometry using DURAClone antibody panels (DURAClone IM Phenotyping Basic and DURAClone IM T Cell Subsets). The MESO QuickPlex SQ 120 system was used to assess cytokine profiles (V-PLEX Proinflammatory Panel 1 Human Kit, Meso Scale Discovery).

Results: All three PRP products showed elevated portions of leukocytes compared to baseline levels in donor blood (p < 0.0001). Further, the pro-inflammatory cytokines IFN-γ (p = 0.039) and TNF-α (p = 0.013) were significantly increased in nSTRIDE® APS samples compared to donor blood and other PRP products. The characteristics of all other cytokines and immune cells from the donor blood, including pro-inflammatory T cell subsets, were maintained in all PRP products.

Conclusions: Individuals with elevated levels of cells with pro-inflammatory properties maintain these in the final PRP products. The concentration of pro-inflammatory cytokines strongly varies between PRP products. These observations may help to unravel the previously described heterogeneous response to PRP in OA therapy. Both the individual's immune profile and the concentration method appear to impact the final PRP product.

Trial registration: This study was prospectively registered in the Deutsches Register Klinischer Studien (DRKS) on 4th November 2021 (registration number DRKS00026175).

Introduction

The Food and Drug Administration (FDA) defines platelet-rich plasma (PRP) as a centrifuged plasma product obtained by an uninterrupted venipuncture with at least 250,000 platelets per µl (1). Despite limited evidence for its efficacy, PRP is widely used to treat acute musculoskeletal injuries including rotator cuff tears and chronic degenerative joint disorders like osteoarthritis (OA) (2). A wide variety of PRP products is currently available. Products differ in terms of the manufacturing process, their cellular (e.g. platelets and leukocytes) and molecular (e.g. growth factors and anti-inflammatory cytokines) compositions as well as their exogenous activation (3). It is assumed that PRP products have anti-inflammatory and distinct immunomodulatory properties (4), which can be adjusted to individual needs of the patient and the treated disease.

It is currently unclear which patients respond well to PRP treatment, and to which extent their response to treatment is related to the cellular and molecular composition of the initial blood sample from which the
PRP product is derived. Previous studies have shown that the concentration of pro-angiogenic growth factors and catabolic proteases is positively correlated with leukocyte counts in the PRP product (5). Among leukocytes, granulocytes and lymphocytes are known to be important modulators of endogenous regeneration (6), but their concentration in peripheral blood is strongly dependent on donor age (7), sex (8), and physical activity (9). Locally elevated granulocyte counts appear to be crucial for facilitating fracture and wound healing (10–12) and elevated granulocyte concentrations in the peripheral blood are being discussed as predictive biomarkers for OA severity (13). Furthermore, elevated granulocyte counts have previously been correlated with an increased incidence of osteonecrosis of the femoral head (14).

Each individual has an unique adaptive immune profile that reflects the personal immune experience resulting from exposure to different antigens throughout life. Whether individual lymphocyte signatures or corresponding circulating cytokine levels are directly transferred into PRP products is unknown. PRP compositions differ between women and men (15) and are dependent on the PRP manufacturing system (4). Thus, it is of great importance to clarify whether cellular and cytokine profiles of individuals are transferred into the PRP product. Further, uncovering the impact of the manufacturing process on PRP compositions is necessary, when considering individualized treatment strategies.

We hypothesize that the individual profile of distinct leukocyte subsets in the donor blood determines the PRP composition. We assume that the degree of leukocyte and cytokine enrichment depends on the specific PRP manufacturing process. To address this, we systematically analyzed the cellular and molecular composition of PRP products from three commercially available systems (ACP®, Angel™ and nSTRIDE® APS) and compared them to the corresponding blood samples from healthy donors.

**Materials And Methods**

This study was conducted following local ethic’s committee approval (Ethikkommission Charité – Universitätsmedizin Berlin, application and approval number 400886 [EA2/218/21]) and in accordance with the Declaration of Helsinki. All participants have given written informed consent to the study protocol. Sample size and effect sizes were estimated using nQuery (nQuery + nTerim, Version 4.0, GraphPad Software DBA Statistical Solutions, San Diego, CA, United States of America). Estimations were based on a confidence level of 95% and a power of 80% to detect effects.

**Platelet-rich Plasma Production**

Twelve healthy participants (five female) were recruited for study inclusion and blood samples of 115 ml were taken from each participant in order to produce the PRP products.

A maximum volume of 15 ml of donor blood was used for the ACP® system (Autologous Conditioned Plasma, ACP®, Arthrex GmbH, Naples, FL, United States of America). Without adding an anticoagulant, the blood was directly drawn into the double syringe provided by the manufacturer. Using a Hettich Centrifuge (Rotofix 32 A 220 V, Hettich GmbH & Co. oHG, Kirchhangel, NRW, Germany), each sample was
centrifuged at 1,500 revolutions per minute (rpm) for five minutes. Double syringes were handled according to the manufacturer’s instructions (16). The resulting PRP product was left in the inner syringe for immediate processing and analysis.

For the Angel™ system (Arthrex Angel™, Arthrex GmbH, Naples, FL, United States of America), 40 ml of blood were drawn into the syringe provided in the manufacturer’s kit. An acid-citrate-dextrose solution (ACD-A 30 ml, Zimmer Biomet Holdings, Warsaw, IN, United States of America) was added to the syringe in a 1:7 ratio to the blood volume. For further PRP preparation, we followed the manufacturer’s recommendations (17). The Angel™ centrifuge was set to reach a hematocrit of 2% in the final product. PRP was collected in a syringe for immediate processing and analysis.

The nSTRIDE® APS (nSTRIDE® Activated Protein Solution, Zimmer Biomet Holdings, Warsaw, IN, United States of America) was manufactured by mixing 55 ml of donor blood and 5 ml of acid-citrate-dextrose solution (ACD-A 30 ml, Zimmer Biomet Holdings, Warsaw, IN, United States of America) in the provided syringe, which was then placed in the cell separator containers and centrifuged at 3,200 rpm for 15 minutes. The supernatant was prepared according to the manufacturer’s protocol (18) and the resulting fraction was again centrifuged at 2,000 rpm for 2 minutes. The final product was transferred into a syringe which was then used for immediate processing and analysis.

The remaining 5 ml of the donor blood was used as the control for all subsequent laboratory analyses. Samples were collected in standard ethylenediaminetetraacetic acid (EDTA) tubes (Vacuette® EDTA tubes, Greiner Bio-One, Greiner Group AG, Kremsmünster, Austria) for immediate processing and analysis.

**Flow Cytometry Analysis of Immune Cell Composition**

DURACLone antibody panels were used for flow cytometry. For basic immune subset identification, CD16-FITC, CD56-PE, CD19-ECD, CD14-PC7, CD4-APC, CD8-A700, CD3-APC-A750, and CD45-KrO were assessed (DURACLone IM Phenotyping Basic, kit #B53309, Beckman Coulter, Washington, D. C., United States of America). For identification of T cell subsets CD45RA-FITC, CD197-PE, CD28-ECD, CD279-PC5.5, CD27-PC7, CD4-APC, CD8-A700, CD3-APC-A750, CD57-PB, and CD45-KrO were measured (DURACLone IM T Cell Subsets, kit #B53328, Beckman Coulter, Washington, D. C., United States of America).

For each sample, 100 µl of donor blood or respective PRP were added into the DURACLone tubes. Following staining in the dark at room temperature, 2 ml of VersaLyse erylysis buffer was added and tubes were centrifuged in an Eppendorf centrifuge (Centrifuge 5810 R, Eppendorf SE, Hamburg, Germany) at 200 g for 5 minutes. While the supernatant was separately stored for cytokine profiling, 3 ml phosphate-buffered saline (PBS) were added to the tubes. The samples were centrifuged at 200 g for 5 minutes and respective supernatants were discarded. Each cell pellet was resuspended in 200 µl buffer (containing 1X PBS with 5% v/v of fetal bovine serum, 2 mM EDTA, and 2 mM sodium azide).
Samples were analyzed using a Navios EX cytometer (Navios EX, Beckman Coulter, Washington, D. C., United States of America). Acquired immune cells were described as follows: Leukocytes (CD45+), granulocytes, identified using forward and sideward scatters, and depicted as NGr+ (CD45+FSC/SSC), while lymphocytes and monocytes were depicted as NGr− (CD45+FSC/SSC), monocytes (CD45+CD14+), classical monocytes (CD45+CD14<sub>high</sub>CD16<sup>−</sup>), non-classical monocytes (CD45+CD14<sub>dim</sub>CD16<sup>+</sup>), intermediate monocytes (CD45+CD14<sub>high</sub>CD16<sup>+</sup>), B cells (CD45+CD14<sup>−</sup>CD3<sup>−</sup>CD19<sup>+</sup>), natural killer (NK) cells (CD45+CD14<sup>−</sup>CD19<sup>−</sup>CD3<sup>−</sup>CD56<sup>+</sup>), T cells (CD45+CD14<sup>−</sup>CD19<sup>−</sup>CD3<sup>+</sup>), T helper cells (CD45+CD14<sup>−</sup>CD19<sup>−</sup>CD3<sup>+</sup>CD4<sup>−</sup>CD8<sup>−</sup>CD4<sup>+</sup>), and cytotoxic T cells (CD45+CD14<sup>−</sup>CD19<sup>−</sup>CD3<sup>−</sup>CD4<sup>−</sup>CD8<sup>+</sup>). CD4<sup>+</sup> and CD8<sup>+</sup> T cells were further separated into central memory (CD45RA<sup>−</sup>CD197<sup>+</sup>), naive (CD45RA<sup>+</sup>CD197<sup>+</sup>), effector memory (CD45RA<sup>−</sup>CD197<sup>−</sup>), and terminally differentiated effector memory (TEMRA) T cells (CD45RA<sup>+</sup>CD197<sup>−</sup>). See supplementary Figure S1 for the detailed gating strategy.

**Determination of Cytokine Levels with Meso Scale Multiplexing Immunoassays**

Supernatants of PRP products and donor blood samples were stored at -80°C until needed for cytokine measurements. The concept of Meso Scale multiplexing immunoassays is based on the sandwich enzyme-linked immunosorbent assay (ELISA) principle. Commercially available capture antibodies are precoated on conductive plates to which the samples are applied. The data output was analyzed using the Meso QuickPlex SQ 120 system proinflammatory cytokine panel (V-PLEX Proinflammatory Panel 1 Human Kit, Meso Scale Discovery (MSD), Meso Scale Technologies, LLC, Rockville, MD, United States of America) which quantifies the concentrations of interferon gamma (IFN-γ), interleukin 1β (IL-1β), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and tumor necrosis factor alpha (TNF-α) (19).

**Analysis and Statistics**

Flow cytometry data files were analyzed using Kaluza Analysis (Kaluza Analysis, Version 2.1, Beckman Coulter, Washington, D. C., United States of America). MSD data, including controls and standards, were analyzed using MSD Workbench (MSD Workbench, Version 4.0, Meso Scale Technologies, LLC, Rockville, MD, United States of America).

Statistical analysis was performed using GraphPad Prism (GraphPad Prism for macOS, Version 9.3.0, GraphPad Holdings, LLC, San Diego, CA, United States of America). Donor blood and PRP product samples were defined as dependent samples. Accordingly, the Friedman test was used and a post hoc analysis was performed with Dunn's correction for multiple testing. We used the Pearson correlation to assess correlations between samples. Unless stated otherwise, frequencies are represented as numbers (portion of the whole [%]) and not normally distributed values are represented as median (IQR [25th percentile, 75th percentile]). All p-values are two-tailed and p-values ≤ 0.05 were considered statistically significant.

**Results**
The median age of this population was 31 years (range 26–51). Five participants (41.7%) were females, none of the participants showed signs of systemic or local infections at the time of PRP preparation, and none had any chronic medical condition.

**Individual Leukocyte Profile Is Maintained In PRP Products**

To determine the cellular composition of the different PRP products and the corresponding donor blood, we first performed a basic characterization of granulocytes, the major lymphocyte subsets, and monocyte subpopulations. This analysis revealed a significant enrichment of leukocytes in all PRP products (ACP®: 55.9% [IQR 29.2, 62.4], p = 0.003; Angel™: 57.0% [IQR 45.7, 68.6], p < 0.0001; nSTRIDE® APS: 40.2% [IQR 20.4, 56.0], p = 0.027) compared to donor blood (1.1% [IQR 0.7, 9.7]) (Fig. 1a). In all but one product, the increased leukocyte levels were accompanied by a decrease in the relative amount of granulocytes compared to donor blood (NGr) (Donor blood: 62.3% [IQR 49.3, 69.5]; ACP®: 8.2% [IQR 5.3, 11.3], p < 0.0001; Angel™: 8.5% [IQR 5.2, 15.4], p < 0.0001; nSTRIDE® APS: 39.6% [IQR 29.1, 56.0], p = 0.347) (Fig. 1b), while monocytes and lymphocytes (NGr) were concentrated in all PRP products compared to the corresponding values in the blood (Donor blood: 37. % [IQR 30.5, 50.7]; ACP®: 91. % [IQR 88.7, 94.7], p < 0.0001; Angel™: 91. % [IQR 84.6, 94.8], p < 0.0001; nSTRIDE® APS: 60. % [IQR 44.0, 70.9], p = 0.347) (Fig. 1c).

Although the relative amount of NGrCD14+ monocytes was slightly reduced in the different PRP products compared to donor blood (Donor blood: 15.3% [IQR 12.8, 16.6]; ACP®: 10.0% [IQR 7.7, 14.2], p = 0.009; Angel™: 13.2% [IQR 12.0, 14.7], p = 0.683; nSTRIDE® APS: 14.8% [IQR 11.5, 20.0], p > 0.999) (Fig. 1d), the changes of the donor specific ratio between the monocyte subpopulations was more pronounced. The non-classical subpopulation was significantly reduced in nSTRIDE® APS (2.4% [IQR 1.7, 3.3]) compared to donor blood and the other products (Donor blood: 6.7% [IQR 4.8, 7.8], p = 0.005; ACP®: 5.8% [IQR 4.3, 9.9], p = 0.009; Angel™: 7.6% [IQR 4.2, 11.2], p = 0.0001) (Supplementary Figure S2a). The classical subpopulation was significantly reduced in ACP® (71.8% [IQR 51.0, 74.8]) compared to donor blood (81.8% [IQR 77.1, 87.4], p = 0.0009) and nSTRIDE® APS (79.8% [IQR 74.2, 86.4], p = 0.027), as well as in Angel™ (68.1% [IQR 62.0, 75.2], p = 0.003) compared to donor blood (Supplementary Figure S2b). Lastly, the intermediate subpopulation was significantly concentrated in nSTRIDE® APS (8.9% [IQR 5.9, 11.7]) compared to donor blood and the other PRP products (Donor blood: 3.1% [IQR 2.0, 4.6], p = 0.043; ACP®: 2.6% [IQR 1.8, 4.8], p = 0.043; Angel™: 2.0% [IQR 1.0, 3.0], p < 0.0001) (Supplementary Figure S2c).

The levels of CD19+ B cells were significantly lower in nSTRIDE® APS (11.1% [IQR 8.1, 15.7]) than in the other two PRP products (ACP®: 14.0% [IQR 10.8, 20.3], p = 0.002; Angel™: 15.2% [IQR 9.9, 18.1], p = 0.043), but no statistical difference was observed between the PRP products and donor blood (13.4% [IQR 9.2, 17.2]) (Fig. 1e). The correlation analysis revealed that the relative amount of CD19+ B cells within the different PRP products was consistent with baseline donor blood levels (Supplementary Figure S3a). No significant difference in relative proportions of CD3+ T cells (Donor blood: 81.7% [IQR 75.3, 86.2]; ACP®:
82.7% [IQR 77.7, 86.2]; Angel™: 81.0% [IQR 78.9, 86.2]; nSTRIDE® APS: 83.6% [IQR 82.0, 87.0]) (Fig. 1f) or CD3 CD56+ NK cells (Donor blood: 13.2% [IQR 8.4, 18.7]; ACP®: 13.5% [IQR 9.6, 19.0]; Angel™: 12.4% [IQR 8.9, 16.4]; nSTRIDE® APS: 13.1% [IQR 8.5, 14.2]) (Fig. 1g) could be found between the PRP products and donor blood. Correlation analysis showed that relative proportions of CD3+ T cells and CD3 CD56+ NK cells were consistent with baseline levels in donor blood for ACP® and Angel™, but not for nSTRIDE® APS (Supplementary Figure S3b-c).

To investigate the extent to which the adaptive immune profile of the donor is maintained in PRP products, the composition of distinct T cell subsets was analyzed as a central component of individual immunity. No statistical difference was found between the proportion of total CD3+CD4+ T helper cells in donor blood (63.5% [IQR 58.5, 65.5]) and the different PRP products (ACP®: 66.9% [IQR 60.5, 70.4]; Angel™: 65.0% [IQR 61.1, 68.0]; nSTRIDE® APS: 65.1% [IQR 62.2, 68.8]) (Fig. 1h). Accordingly, all PRP products also contained comparable levels of CD3+CD8+ cytotoxic T cells when compared to corresponding donor blood samples (Donor blood: 32.1% [IQR 28.6, 33.1]; ACP®: 28.9% [IQR 25.3, 33.8]; Angel™: 29.5% [IQR 28.7, 35.4]; nSTRIDE® APS: 30.0% [IQR 24.2, 33.1]) (Fig. 1i). The correlation analysis showed that relative amounts of CD3+CD4+ and CD3+CD8+ T cells within PRP products were donor-dependent and determined by the individual levels in the donor blood (Supplementary Figure S4a-b). Correlation analyses of the CD4+ and CD8+ T cell subpopulations further showed that levels of naive, central memory, effector memory, and TEMRA T cells were also significantly correlated with the corresponding donor blood profile (Supplementary Figures S5 and S6).

**PRP Composition Shows Pro-Inflammatory Properties**

To assess the cytokine profiles of the different PRP products in comparison to donor blood samples, levels of ten cytokines were analysed as surrogate markers for the inflammatory composition of PRP products. In several samples of the PRP products and donor blood, the concentrations of IL-1β (Donor blood: n = 4; ACP®: n = 6; Angel™: n = 8; nSTRIDE® APS: n = 6), IL-12p70 (Donor blood: n = 1; ACP®: n = 1; nSTRIDE® APS: n = 2), and IL-13 (nSTRIDE® APS: n = 2) were below the limit of quantitation. Concentrations of IFN-γ, TNF-α, IL-2, IL-6, IL-8, IL4, and IL-10 were above the limit of detection in all samples (Table 1).
Table 1
Cytokine profile of donor blood and corresponding PRP samples.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Donor blood</th>
<th>ACP®</th>
<th>Angel™</th>
<th>nSTRIDE® APS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ [pg/ml]</td>
<td>1.765 (IQR 0.922, 3.999)</td>
<td>4.472 (IQR 3.174, 5.495)</td>
<td>3.832 (IQR 2.667, 4.706)</td>
<td>5.095 (IQR 3.357, 7.099)</td>
</tr>
<tr>
<td>TFN-α [pg/ml]</td>
<td>0.57 (IQR 0.316, 1.137)</td>
<td>0.988 (IQR 0.655, 1.33)</td>
<td>0.909 (IQR 0.652, 1.199)</td>
<td>1.264 (IQR 0.98, 1.523)</td>
</tr>
<tr>
<td>IL-1β [pg/ml]</td>
<td>0.417 (IQR 0.02, 0.708)</td>
<td>0.02 (IQR 0.02, 0.196)</td>
<td>0.02 (IQR 0.02, 0.041)</td>
<td>0.033 (IQR 0.02, 0.619)</td>
</tr>
<tr>
<td>IL-2 [pg/ml]</td>
<td>0.617 (IQR 0.234, 0.767)</td>
<td>0.557 (IQR 0.355, 0.959)</td>
<td>0.558 (IQR 0.424, 0.718)</td>
<td>0.315 (IQR 0.149, 0.581)</td>
</tr>
<tr>
<td>IL-6 [pg/ml]</td>
<td>0.355 (IQR 0.189, 0.52)</td>
<td>0.58 (IQR 0.293, 0.812)</td>
<td>0.478 (IQR 0.322, 0.636)</td>
<td>0.369 (IQR 0.25, 0.601)</td>
</tr>
<tr>
<td>IL-8 [pg/ml]</td>
<td>11.845 (IQR 2.504, 18.021)</td>
<td>7.139 (IQR 2.566, 8.48)</td>
<td>4.254 (IQR 2.507, 5.274)</td>
<td>7.089 (IQR 2.566, 26.494)</td>
</tr>
<tr>
<td>IL-4 [pg/ml]</td>
<td>0.057 (IQR 0.032, 0.134)</td>
<td>0.069 (IQR 0.008, 0.12)</td>
<td>0.041 (IQR 0.001, 0.08)</td>
<td>0.08 (IQR 0.006, 0.133)</td>
</tr>
<tr>
<td>IL-10 [pg/ml]</td>
<td>0.185 (IQR 0.128, 0.437)</td>
<td>0.367 (IQR 0.23, 0.507)</td>
<td>0.361 (IQR 0.234, 0.413)</td>
<td>0.312 (IQR 0.193, 0.473)</td>
</tr>
<tr>
<td>IL-12p70 [pg/ml]</td>
<td>0.201 (IQR 0.072, 0.359)</td>
<td>0.248 (IQR 0.038, 0.39)</td>
<td>0.143 (IQR 0.071, 0.311)</td>
<td>0.118 (IQR 0.038, 0.179)</td>
</tr>
<tr>
<td>IL-13 [pg/ml]</td>
<td>1.648 (IQR 0.934, 3.091)</td>
<td>1.985 (IQR 1.321, 2.235)</td>
<td>1.903 (IQR 1.151, 2.626)</td>
<td>1.079 (IQR 0.396, 1.847)</td>
</tr>
</tbody>
</table>

Abbreviations: IFN-γ: interferon γ, TNF-α: tumor necrosis factor α, IL-1β: interleukin 1β, IL-2: interleukin 2, IL-4: interleukin 4, IL-6: interleukin 6, IL-8: interleukin 8, IL-10: interleukin 10, IL-12p70: interleukin 12p70, IL-13: interleukin 13.

The median IFN-γ concentration in the PRP products was approximately two to three times higher than in the donor blood samples, but reached statistical significance only between donor blood and nSTRIDE® APS samples (p = 0.034) (Fig. 2a). Significantly elevated TFN-α concentrations were also found in nSTRIDE® APS compared to donor blood (p = 0.027) and Angel™ samples (p = 0.027) (Fig. 2b). The median concentration of IL-2 was comparable between donor blood and the different PRP products, but significantly lower in nSTRIDE® APS compared to ACP® and (p = 0.043) (Fig. 2d). The median concentrations of IL-1β, IL-6, IL-8, IL-4, IL-10, IL-12p70, and IL-13 were comparable between donor blood levels and the different PRP products without significant differences (Fig. 2c and 2e-i).

**Discussion**

Despite the constantly rising number of patients suffering from OA (20), there is, thus far, no causal treatment for OA and symptomatic treatment cannot halt disease progression (21). The local low-grade
inflammation in OA has been linked to cartilage degeneration and subsequent joint destruction (22). Complementary to surgical treatment strategies (23), various potentially disease modifying drugs, that may selectively disrupt inflammatory pathways present in OA, are currently being investigated (24). Of these, PRP is one of the most popular products already in clinical use. PRP was observed to be superior to other therapeutics, including hyaluronic acid, corticosteroids, and placebo, with respect to clinical outcomes and disease progression in OA (25, 26). However, response to therapy is highly heterogenous and PRP fails in a relevant proportion of affected patients. To date, reasons for absent responses to treatment remain elusive.

To the best of our knowledge, this is the first study assessing cellular and cytokine compositions of various commercially available PRP systems used in daily clinical practice and comparing these to corresponding donor blood samples.

All PRP systems resulted in a significant proportional enhancement of leukocytes. This observation is consistent with previous findings (27–34). Commercially available PRP systems result in products with varying leukocyte concentrations, that can be categorized in two groups: leukocyte-rich PRP (LR-PRP) and leukocyte-poor PRP (LP-PRP). While LR-PRP systems tend to aggregate leukocytes, LP-PRP systems reduce leukocyte concentrations compared to corresponding blood concentrations (27–34). Slow spin speeds of around 1,500 rpm are associated with an up to threefold concentration of platelets, an almost complete elimination of red blood cells (RBC), and a reduction of leukocyte concentrations (LP-PRP), whereas higher spin speeds of around 3,200 rpm are associated with an up to ninefold concentration of platelets, some loss of RBCs, and an increase in leukocyte concentrations (LR-PRP) (27). Previous studies ranked the ACP® and the Angel™ systems among the LP-PRP systems (2, 33, 35).

The concentration of leukocytes was accompanied by an overall reduction in granulocytes and an proportional increase of lymphocytes and monocytes. Donor blood samples contained 1.1% of leukocytes, of which over 60% were granulocytes, which was reduced to at least 40% in final PRP products. The lymphocytes and monocytes proportion was increased from around 40% in donor blood to around 60% and above in the PRP samples. As previously observed (27), the nSTRIDE® APS system, categorized as an LR-PRP system, showed higher proportions of granulocytes when compared to the LP-PRP systems, ACP® and Angel™. Wakayama et al. compared the nSTRIDE® APS and the MyCells® (LP-)PRP system. Both systems concentrated lymphocytes, but only the nSTRIDE® system concentrated neutrophils in both healthy volunteers and OA patients (30). Fitzpatrick et al. compared the GPS III, SmartPrep® II, and the ACP®. They detected that neutrophils and lymphocytes were the most concentrated fractions within the leukocyte populations (33). These results were supported by a canine feasibility study that solely analyzed the nSTRIDE® APS system and observed a global concentration of leukocytes, neutrophils, lymphocytes, and monocytes (31). The aforementioned findings are not completely in accordance with our data, but only Wakayama et al. reported data on human subjects (30). As the proportion of neutrophils and monocytes directly influences the composition of pro- and anti-inflammatory cytokines in the final product (27), this may impact clinical outcomes in treated patients.
We observed considerably varying cytokine compositions between products. The concentrations of the pro-inflammatory cytokines IFN-γ and TNF-α were significantly increased in the nSTRIDE® APS when compared to donor blood samples. This was not the case for the other two systems. As PRP is mainly used for its anti-inflammatory properties, this finding appears counterintuitive. Previous studies have observed a concentration of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, especially in LR-PRP products (29, 30, 34–36). Increased levels of IL-1β and TNF-α directly contribute to cartilage degradation and enhanced IL-6 production, which then enhances inflammatory responses that cause bone resorption in OA (37). Accordingly, most authors do not recommend the use of LR-PRP for OA therapy, as they expect these products to increase or, at least, maintain the low-grade inflammation present in affected joints (34, 38, 39). In laboratory studies, however, LR-PRP prevented chondrogenic matrix degradation, increased chondrocyte cellularity, and inhibited the production of matrix metalloproteinase 13 (40–42). Interestingly, both Mariani et al. and Cole et al. observed no local changes in cytokine concentrations in synovial fluid after PRP injections (35, 43). These observations are confirmed by clinical studies, which showed overall effectiveness of products with various cytokine compositions, including LR-PRP, for OA treatment (44). While the relevance of cytokine compositions in PRP products on clinical outcomes in OA may be questioned, it is important to note that regardless of individual donor blood cytokine compositions, some PRP products will yield a high pro-inflammatory cytokine composition, which may affect immune cells already present in the affected joint.

When comparing donor blood and corresponding PRP samples, we observed a maintenance of each participant’s adaptive immune profile. This is highly relevant, since high systemic levels of distinct T cell subsets have been associated with impaired tissue healing (45, 46). These TEMRA T cells accumulate in large numbers also at the injury site and are the major local producers of pro-inflammatory cytokines (46), which are, again, linked to the age-related phenotype (47) and the development of OA (48). Further, a local downregulation of these cells led to a decreased concentration of pro-inflammatory cytokines and an improved bone regeneration in a preclinical fracture model (49). Our observations could help to understand the heterogeneous clinical effectiveness of PRP in OA therapy, as we observed that the individual adaptive immune profile is directly transferred into the PRP products. Further clinical studies are needed to examine this potential influence of patients’ individual adaptive immune system and its receptivity to regenerative therapies, such as PRP.

This study has some limitations. First, we provided data from a rather small cohort of healthy participants. Second, correlations of our results with clinical outcome data are not available. In future, prospective observational or randomized interventional studies are needed in order to analyse our findings for clinical relevance. On the other hand, this is the first study to merge the cellular and cytokine compositions of different PRP products and match these to individual donor immune profiles. This is highly relevant, as authors were previously able to highlight the influence of the individual adaptive immune capacities on tissue regeneration (46, 49). The systems used for PRP production in our study are commercially available and broadly used in clinical practice, allowing for a high comparability with other researchers’ results.
Conclusion

All PRP systems used examined significantly enhanced leukocytes, which was accompanied by an overall reduction in granulocytes and a proportional increase of lymphocytes and monocytes. Further, each participant’s adaptive immune profile was maintained in the final PRP products and the cytokine compositions highly varied between product. These observations may help to answer why patients differently benefit from PRP treatment in OA.

Abbreviations

PRP: Platelet-rich plasma; OA: Osteoarthritis; FDA: Food and Drug Administration; RPM: Revolutions per minute; ACD: Acid-citrate-dextrose; EDTA: Ethylenediaminetetraacetic acid; PBS: Phosphate-buffered saline; NK cells: Natural killer cells; TEMRA: Terminally differentiated effector memory; ELISA: Enzyme-linked immunosorbent assay; MSD: Meso Scale Discovery; IFN-γ: interferon gamma; IL-1β: Interleukin 1β; IL-2: Interleukin 2; IL-4: Interleukin 4; IL-6: Interleukin 6; IL-8: Interleukin 8; IL-10: Interleukin 10; IL-12p70: Interleukin 12p70; IL-13: Interleukin 13; TNF-α: Tumor necrosis factor alpha; IQR: Interquartile range;

Declarations

Ethics approval and consent to participate

This study was conducted following institutional review committee approval (Ethikkommission Charité – Universitätsmedizin Berlin, application and approval number 400886 [EA2/218/21]) and in accordance with the Declaration of Helsinki. All participants have given written informed consent to the study protocol.

Consent for publication

Not applicable.

Availability of data and materials

The data sets used and/or analyzed in the present study are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

MN takes full responsibility for the integrity of the presented work as a whole, from inception of the study to the finished article.

Conception and design: All authors.

Analysis and interpretation of the data: MN, MO, and TM.

Drafting of the article: MN, MO, and TM.

Critical revision of the article for important intellectual content: MS, AW, GG, FNF, FG, AB, SR, US, CP, GND, SG, and TW

Final approval of the article: All authors.

Provision of study materials or patients: SG and TW.

Statistical expertise: MN, MO, and TM.

Obtaining of funding: GND, MO, CP, SG, TW

Administrative, technical, or logistic support: MS, AW, GG, FNF, FG, AB, SR, US, CP, GND, SG, and TW.

Collection and assembly of data: MN and MO.

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References


Figures
Figure 1

Cellular composition of different PRP products and corresponding donor blood samples. a) Proportion of leukocytes (CD45+), b) granulocytes (CD45+FSC/SSC NGr+), c) lymphocytes and monocytes (CD45+FSC/SSC NGr-), d) monocytes (CD45*CD14+), e) B cells (CD45*CD14*CD19*CD3-), f) T cells (CD45*CD14*LyCD19*CD3+), g) NK cells (CD45*CD14*CD19*CD3*CD56+), h) CD4+ T helper cells
(CD45^+CD14^-CD19^-CD3^-CD4^-CD8^+), and i) CD8^+ cytotoxic T cells (CD45^+CD14^-CD19^-CD3^-CD4^-CD8^+) in donor blood compared to PRP samples. Abbreviations: NK cells: Natural killer cells, PRP: Platelet-rich plasma.

Figure 2
Cytokine profile of different PRP products and corresponding donor blood samples. a) Concentrations of IFN-γ, b) TNF-α, c) IL-1β, d) IL-2, e) IL-6, f) IL-8, g) IL-10, h) IL-12p70, and i) IL-13 in donor blood compared to PRP samples. Abbreviations: IFN-γ: interferon γ, TNF-α: tumor necrosis factor α, IL-1β: interleukin 1β, IL-2: interleukin 2, IL-4: interleukin 4, IL-6: interleukin 6, IL-8: interleukin 8, IL-10: interleukin 10, IL-12p70: interleukin 12p70, IL-13: interleukin 13, PRP: Platelet-rich plasma.

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