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### Abstract preview

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### Content English

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**Title:** Evaluation of the influence of the leukocyte concentration and composition on growth factor and protease concentrations in platelet-rich plasma (PRP)

**Abstract text:**

**Objectives**

Platelet-rich plasma (PRP) therapy has become an increasingly popular treatment for sports-related injuries, and various clinically available PRP preparation methods exist. However, the differences of PRP quality among numerous preparation methods remain unclear. Specifically, the benefit of including leukocytes in the PRP product thus remains controversial, and few studies have been conducted to evaluate the effects of the interaction between platelets and leukocytes on the growth factor concentrations. The aim of the present study was to compare the biological characteristics of PRPs focusing on the leukocyte concentration and composition.

**Methods**

Leucocyte rich (LR)-PRP, leucocyte poor (LP)-PRP, and pure-PRP were prepared from peripheral blood of 6 healthy male volunteers (mean age:31.3 years). The concentrations of platelet, leukocyte, erythrocyte, growth factors (transforming growth factor-beta 1: TGF-  $\beta$  1, fibroblast growth factor-basic: FGF-b, platelet-derived growth factor-BB: PDGF-BB, vascular endothelial growth factor: VEGF) and matrix metalloproteinase-9 (MMP-9) from each of the PRP samples were measured. Considering the interaction between platelets and leukocytes, correlations between platelet/leukocyte and growth factors/MMP-9 were analyzed using partial correlation coefficients. All p-values were two-sided and p-values of less than 0.05 were considered to be

statistically significant. Statistical analyses were performed using the SPSS Statistics version 20.0.

### **Results**

The platelet concentration did not differ among the three PRP preparation methods. Conversely, the leukocyte concentration was dramatically different; 14.9 ( $10^3 / \mu\text{l}$ ) in LR-PRP, 2.4 ( $10^3 / \mu\text{l}$ ) in LP-PRP, 0.2 ( $10^3 / \mu\text{l}$ ) in pure-PRP. Considering the interaction between platelets and leukocytes, we evaluated the partial correlation coefficients. The platelet concentration correlated positively with all growth factors (TGF- $\beta$  1:  $r=0.58$ ,  $p=0.004$ , FGF-b:  $r=0.90$ ,  $p<0.001$ , PDGF-BB:  $r=0.87$ ,  $p<0.001$ , VEGF:  $r=0.62$ ,  $p<0.001$ ). However, the leukocyte concentration positively correlated with PDGF-BB and VEGF concentration (PDGF-BB:  $r=0.56$ ,  $p=0.006$  VEGF:  $r=0.65$ ,  $p=0.001$ ), while it negatively correlated with FGF-b ( $r=-0.44$ ,  $p=0.036$ ) and TGF- $\beta$  1 ( $r=-0.20$ ,  $p=0.357$ ). Regarding catabolic factor, MMP-9 concentration strongly correlated with the leukocyte concentration ( $r=0.81$ ,  $p<0.001$ ), while there was no correlation in platelet and MMP-9 concentration ( $r=0.33$ ,  $p=0.125$ ).

### **Conclusions**

These findings demonstrated that leukocytes strongly influence the quality of PRPs. Therefore, modifying the PRP preparation method according to the pathology is indispensable to achieve better clinical results with PRP therapy.

### **Keywords:**

platelet-rich plasma (PRP), growth factor, preparation method, leukocyte, matrix metalloproteinase (MMP)