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Characterization of Growth Factors, Cytokines and Chemokines in Bone Marrow Concentrate and Platelet Rich Plasma: A Prospective Analysis

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Objectives: Autologous platelet-rich plasma (PRP) and bone marrow concentrate (BMC) are orthobiologic therapies with numerous growth factors and cytokines. Mesenchymal stem cells (MSCs) are also present in BMC; however, comprise a very limited component of the available monocytes. Other clinically relevant factors and cytokines, including interleukin-1 receptor antagonist (IL-1Ra), are implicated in the anti-inflammatory and regenerative processes. Prior to optimizing the clinical utility of PRP and BMC as a combined or monotherapy, an improved understanding of the components and respective concentrations is necessary. The purpose of this study was to prospectively measure and compare anabolic, catabolic, anti-inflammatory and pro-inflammatory factors, proteins and cytokines present in bone marrow aspirate (BMA), BMC, whole blood, leukocyte poor (LP)-PRP and leukocyte rich (LR)-PRP from samples collected and processed concurrently from patients presenting for elective knee surgery.

Methods: A total of 31 patients presenting for elective knee surgery were prospectively enrolled over a three-week period. Whole blood from peripheral venous draw and BMA from the posterior iliac crest were immediately processed using centrifugation and manual extraction methods to create LR- and LP-PRP and BMC, respectively. BMA, BMC, whole blood, LR-PRP and LP-PRP samples were immediately assayed and analyzed to measure factor and cytokine concentrations. We strictly adhered to the minimum reporting requirements for biological outcomes (MIBO). An a priori power and sample size calculation was performed. We conservatively assumed a Bonferroni correction among all 10 pairwise comparisons, two-tailed testing, and an overall alpha level of 0.05. Eighteen subjects was sufficient to detect this magnitude of effect size with 80% statistical power.

Results: BMC had a significantly higher IL-1Ra concentration than all other preparations (all $p < 0.0009$, Figure 1). LR-PRP had a significantly higher IL-1Ra concentration than LP-PRP ($p = 0.0006$). There were no significant differences in IL-1Ra concentration based on age, gender, body mass index or chronicity of injury among all preparations (Table 1). BMC had significantly higher concentrations of leukocytes and monocytes compared to the other biologic preparations including LR-PRP. LP-PRP had significantly higher concentrations of matrix metalloproteinase (MMP)-2, MMP-3 and MMP-12 than all other preparations (all $p < 0.007$), while BMC had a significantly lower concentration of MMP-2 than all other preparations. LR-PRP had significantly higher concentrations of MMP-1, serum soluble CD40 ligand (sCD40L), platelet derived growth factor (PDGF)-AA and PDGF-AB/BB than all other preparations (all $p < 0.004$).

Conclusion: BMC is a clinically relevant source of anti-inflammatory biologic therapy that may be more effective in treating osteoarthritis and for use as an intra-articular biologic for augmented healing in the post-surgical inflammatory and healing phases due to its significantly higher concentration of IL-1Ra compared to LR-PRP and LP-PRP. Additionally, LR-PRP had a significantly higher concentration of IL-1Ra than LP-PRP. In cases where increased vascularity and healing are desired for pathological or injured tissues including muscle and tendon, LR-PRP may be optimal due to its higher overall concentrations of PDGF, TGF-β, EGF, VEGF, and sCD40L.

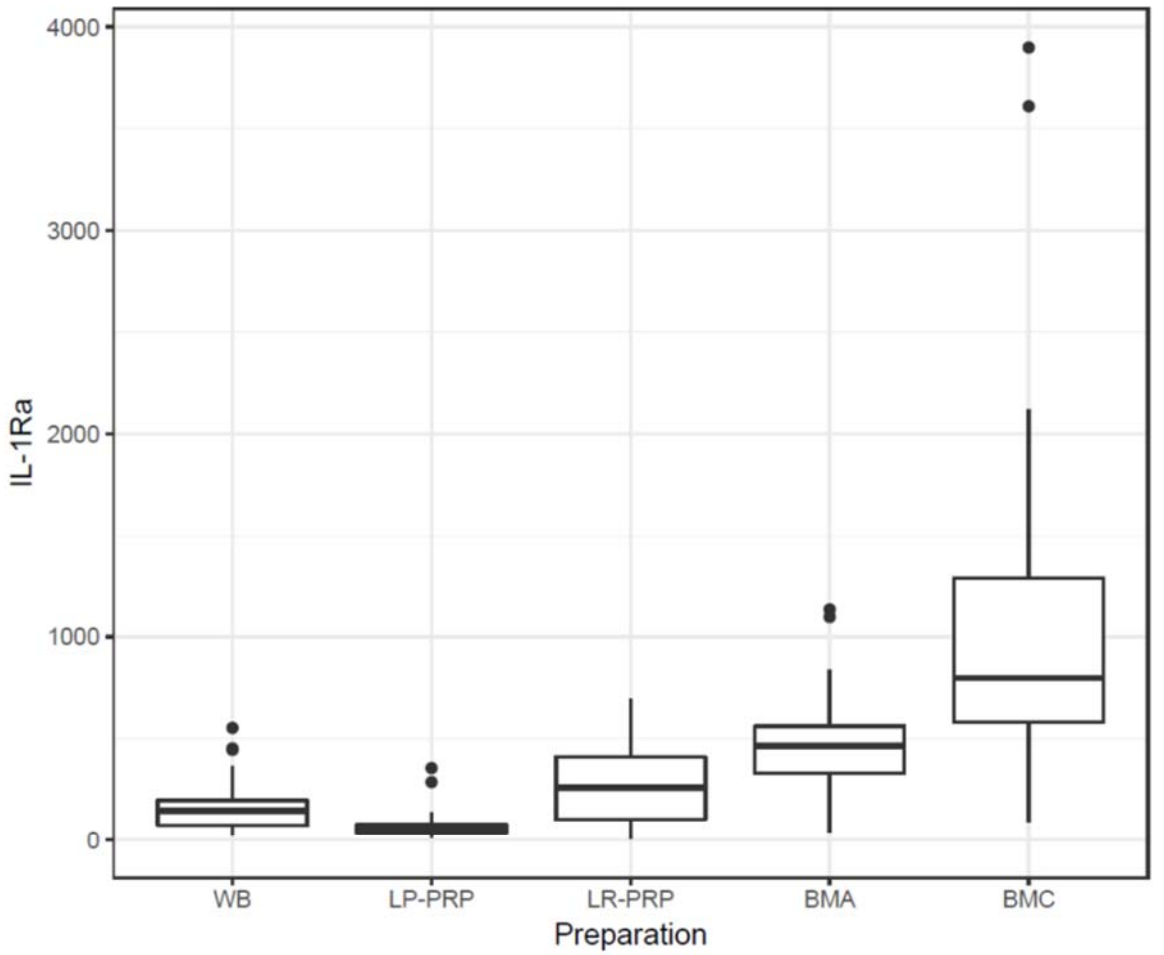

Figure 1. Interleukin-1 receptor antagonist (IL-1Ra) concentration (pg/μL) in whole blood (WB), leukocyte-rich platelet rich plasma (LR-PRP), leukocyte-poor platelet rich plasma (LP-PRP), bone marrow aspirate (BMA) and bone marrow concentrate (BMC). All pairwise comparisons were significant ($p < 0.003$) with BMC being significantly higher in IL-1Ra than all other preparations ($p < 0.0009$).

Table 1. Interleukin-1 receptor antagonist (IL-1Ra) concentration across biologic preparations including results based on age, gender and injury chronicity

	Preparation					
IL-1Ra	**WB**	**LR-PRP**	**LP-PRP**	**BMA**	**BMC**	***p value***
Overall	143	257.8	48.9	463	**796.6**	**<0.0009**
	[72.4, 195.4]	[100.7, 408.6]	[34.3, 76.8]	[328.7, 560.2]	[579.2, 1288]	*<0.003
Age (years)						>0.440
<35 (n=15)	124.9	257.8	55.1	492.3	842.1	
	[74.2, 163.3]	[92.2, 408.6]	[28.1, 77.1]	[362.1, 602.9]	[619.1, 1050]	
>35 (n=16)	172.4	239.2	41.5	419.3	679.6	
	[66.3, 255.6]	[113, 389]	[35.7, 71.7]	313.1, 541.4]	[463.5, 1353]	
Gender						>0.15
Male (n=18)	145.9	267.7	55.3	478.1	973.4	
	[73.4, 200.1]	[111.6, 426.9]	[36, 104.7]	[377, 626.8]	[683.2, 1375]	
Female (n=13)	142.4	176.5	35.8	352.6	616.3	
	[58.7, 176.3]	[85.2, 374.6]	[31.6, 67.9]	[173.4, 499.1]	[366, 1006]	
BMI (kg/m^2)						>0.20
<25 (n=11)	124.9	281.2	48.9	383	790.9	
	[56.4, 177]	[67.5, 396.8]	[35.8, 77.1]	[216.5, 511]	[295.6, 1050]	
>25 (n=20)	155.6	229.3	46.2	463.4	796.6	
	[78.3, 217.1]	[110.6, 407.8]	[34.1, 75]	[334.1, 730.1]	[605.2, 1462]	
Chronicity						>0.21
Acute (n=12)	152.7	188.7	47.5	494.3	1063	
	[87.1, 338.9]	[64, 349.9]	[34.4, 94.3]	[307.8, 605.9]	[506.9, 1505]	
Chronic (n=19)	124.9	281.2	48.9	383	784	
	[72.4, 177]	[107.1, 417.3]	[34.7, 70.8]	[328.7, 544.1]	[607, 1040]	

Results given as median [1st quartile, 3rd quartile]. Values given in bold were significantly higher than all other preparations. *All pairwise comparisons were significant ($p < 0.003$). There were no significant differences based upon age, gender, BMI or chronicity of injury with lowest p-value among the preparations listed in table. WB = whole blood; LR-PRP = leukocyte rich platelet rich plasma; LP-PRP = leukocyte poor platelet rich plasma; BMA, bone marrow aspirate; BMC = bone marrow concentrate. Acute = <6 weeks. Chronic = >6 weeks.

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