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## GROWTH FACTORS AND CYTOKINES IN ORTHOPAEDICS. COMPARISON OF TWO BLOOD PROCESSING TECHNIQUES: ORTHOKINE® AND ACP®.

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**Summary:** ORTHOKINE[1], PRP[2], ACP[3] are successful blood-processing based autologous biological treatment options in orthopaedics. Platelet-Rich Plasma contains platelets  $\geq 3x$  over baseline. ORTHOKINE and ACP do not concentrate platelets, but yield elevated Signalling Proteins (SP). ORTHOKINE outperforms ACP in SP content.

**Introduction:** Autologous blood preparations for orthopaedic SP therapy are popular. Platelet-rich plasma (PRP) has become trendy and starting to present RCT Level 1 proof of evidence. However, many different PRP techniques are used making comparison of results difficult. The SP contained in PRPs include cytokines and growth factors (GF) considered helpful once SP are released by coagulation in situ. Two non-PRP techniques were compared: ORTHOKINE-technique producing Autologous Conditioned Serum (ACS) and ACP plasma-separation technique producing autologous plasma (AP). ORTHOKINE bases on accumulation of SP from all blood cells in the supernatant serum through incubation at body temperature of coagulating venous whole blood in specially tailored syringes (conditioning of serum). Conditioned cell free serum is injected intra-articularly, peri- and intra-tendinous and radicular. ACP platelet-separation technique bases on physical separation of plasma from red and white blood cells (WBC) from anticoagulated venous blood. ACP-AP is injected in similar indications as ORTHOKINE-ACS.

**Patients and Methods:** Blood from 9 volunteers was collected with ORTHOKINE (10mL) and ACP (12mL) devices. Blood was cell counted and processed according to manufacturers' instructions. ORTHOKINE-ACS: blood was incubated 6 h, then fast centrifuged separating serum from coagulum. ACP-AP was prepared by slow centrifugation of anticoagulated whole blood in ACP device. Supernatant plasma was collected for measurements. Blood cell counts were performed externally on ACP-AP and whole blood. ORTHOKINE -ACS contains no cells. All samples were stored at  $\leq -20^{\circ}\text{C}$  until SP ELISA-measurement. ELISAs for IL-1Ra, IL-10, IL-6, EGF, HGF, IGF-1, PDGF-AB, TGF $\beta$ -1, VEGF were performed with R&D Systems Kits within 1 week after sampling.

**Results:** Mean yield for AP: 3.4mL; for ACS: 3.7mL (Table1). GF concentration in ORTHOKINE-ACS: all 1.3-3.9x over ACP-AP (Figure1). Cytokine concentrations in ORTHOKINE-ACS: all 2-31x over ACP-AP (Figure1). AP contained mean platelet count 1.9x over baseline. Mean yield of platelets in AP: 85% (Table1). Highest differences: ACS over AP: IL-1Ra: 5.5x; IL-6: 31x; EGF: 3.9x.

**Discussion/Conclusions:** ORTHOKINE-ACS and ACP-AP provide SP considered helpful to support injured [cartilage]tissue. Here ACS has higher SP content than AP. GF content is largely dependent on platelet degranulation, but WBC can contribute to GF and cytokine production. ACS gains from 100% of platelets and WBC. AP lacks  $\pm 15\%$  platelets and virtually all WBC. Lower GF concentrations detected in AP are possibly attributable to this. More GF may partly derive from white blood cells (e.g. EGF and VEGF) while IGF-1 is present in high baseline concentrations in the circulation ( $\pm 70\text{ng/mL}$ ). Apparently there are GF reservoirs in WBC being liberated after blood processing. Arguably, a higher SF dose may be predictive for better clinical effect. The high IL-1Ra content may explain the additional anti-inflammatory action of ORTHOKINE-ACS[1]. Comparison of both approaches in experimental and clinical settings should provide more insight.

[1] Wehling et al. *Biodrugs*2007; 21(5): 323-332

[2] Nguyen et al. *PM&R*2011; Vol.3, 226-250

[3] Schippinger et al. *JSSM*2011; 10, 439-444



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	ACP (10mL)	ORTHOKINE (10mL)	Typical PRP system
initial blood volume [mL]	12 mL (10,8 + 1,2 Citr)	10 mL	10-60mL
Final Volume [mL]	3,4 ±0,45	3,7 ±0,37	1-8 mL
Total Platelet Yield	1,6 x 10 <sup>9</sup> (85%)	1,8 x 10 <sup>9</sup> (100%)*	approx. 40-90%
Processing Time	15 min	20 min**	approx. 20-35 min
Storage possible?	n.d.	Yes, 7 months (≤20°C)	n.d.
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Table 1: Baseline Data for ORTHOKINE, ACP and typical PRP systems. \*ORTHOKINE uses whole blood containing all cellular and a-cellular components. \*\*Processing time plus 6 h incubation at body temperature.

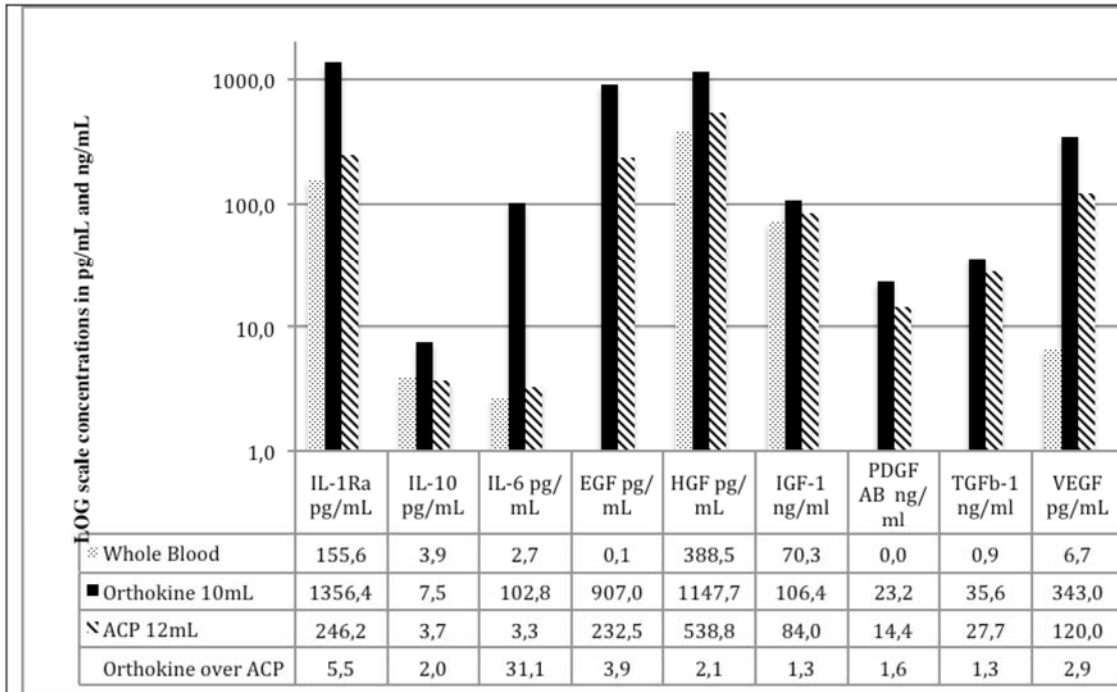


Figure 1: Comparison of effector protein content in ORTHOKINE-ACS and ACP-AP. Cytokines and Growth Factors. ORTHOKINE contains higher concentrations than ACP. Note LOG scale on the Y-Axis.