

# Could platelet rich plasma have effects on systemic circulating growth factors and cytokine release in orthopaedic applications?

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The use of platelet rich plasma (PRP) has been proposed for tendon and ligament healing. The rationale is the release of large amounts of growth factors and cytokines with regenerative, anabolic, and anti-inflammatory effects on damaged tissues.<sup>1-3</sup>

We applied PRP in tendinopathies of professional athletes. PRP was obtained using the gravitational platelet separation system GPS II (Biomet, Bridgend, Wales, UK) from 30 ml peripheral anticoagulated whole blood, and introduced by a syringe to multiple sites of injured tendon.

After obtaining informed consent, we measured a series of cytokines and growth factors in serum obtained from peripheral blood of five male subjects (age 20–35 years; four cases of patella tendinopathy and one elbow tendinosis) to evaluate the eventual, systemic effects of such local treatment. Blood was withdrawn before treatment and 30 minutes, three hours, and 24 hours after the treatment. Serum was separated by centrifugation at 2000 g for 10 minutes at 4°C and stored at -196°C in liquid nitrogen until analysis.

The analytes of interest were quantified by Randox Ltd (Crumlin, Northern Ireland, UK) with a biochip array analyser (the Evidence analyser). The biochip used consists of a 9 × 9 mm substrate on which discrete test regions have been constructed.

The binding ligands (antibodies) are attached to predefined sites on the chemically modified surface of the

biochip. After a simple enzyme linked immunosorbent assay (ELISA) procedure, each spot is imaged to capture chemiluminescent signals generated at each spot on the array. The light signal is captured by a charge coupled device camera as part of an imaging station and converted by image processing software to provide results compared with calibration curves for each location on the biochip.

We observed no modification of the following variables: interleukin 4, interleukin 6, interleukin 10, interleukin 1α, interleukin 1β, tumour necrosis factor α (TNFα), and interferon γ.

Vascular endothelial growth factor (VEGF) and endothelial growth factor (EGF) had decreased after 30 minutes. The trend continued until three hours after the treatment and returned near to basal values after 24 hours. Similar behaviour was observed for chemokine (C-C motif) ligand 2 (CCL2), but the decrease in concentration was evident only three hours after the treatment (table 1). Analysis of variance (one way analysis) performed with MedCalc software (Mariakerke, Belgium) showed a statistically significant change in EGF and CCL2, but not in VEGF, because of high interindividual variability, which has also been reported during exercise.<sup>4</sup>

VEGF concentrations were high because we used serum, which is characterised by higher concentrations than those of plasma, because of release from aggregated platelets.<sup>4</sup> The relation between the local increase in VEGF and

EGF and systemic concentrations of the molecule during tendon repair is not known. It should be noted that EGF shows significant differences, whereas VEGF is not influenced by the treatment. This must be related to the synthesis of growth factors from the tissues; VEGF is widely produced by human tendon cells in culture, whereas EGF is decreased when cells are treated with supernatants of PRP.<sup>5</sup> Moreover, the decrease in CCL2, usually induced by TNFα during acute and subchronic inflammation, is interesting. TNFα was not modified by the treatment, so the origin of CCL2 released from mononuclear cells must be linked to the engagement of the Fc receptor for IgG. The decrease in the concentration of this variable must be important in reducing the risk of thrombogenesis.<sup>6</sup>

The limits of this study are the lack of controls and small number of subjects treated. Further studies need to be performed in a larger series of athletes. However, these preliminary data suggest that local treatment with PRP for sport tendon pathologies may have systemic effects, possibly influencing homeostasis and antidoping evaluations. This treatment seems to affect anti-inflammatory reactions, but the effect is temporary, whereas the local effect is clearly longer.

*Br J Sports Med* 2006;40:816.  
doi: 10.1136/bjism.2006.029934

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Published Online First 18 August 2006

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**Table 1** Serum concentrations of variables before and various time intervals after treatment with platelet rich plasma

	Before	30 min after	3 hours after	24 hours after
VEGF (pg/ml)	140 (20–302)	123 (25–392)	65 (26–232)	119 (47–232)
EGF (pg/ml)	130 (22–182)	85 (3–156)	40 (3–153)	68 (7–153)
CCL2 (pg/ml)	285 (107–466)	286 (70–385)	185 (101–385)	256 (96–373)

Values are mean (range). The ranges are reported because the distribution of values was not normal (D'agostino-pearson test,  $p < 0.05$ ).  
VEGF, Vascular endothelial growth factor; EGF, endothelial growth factor; CCL2, chemokine (C-C motif) ligand 2.