Scand J Med Sci Sports 2012: 22: 147–148
doi: 10.1111/j.1600-0838.2011.01427.x



SCANDINAVIAN JOURNAL OF
MEDICINE & SCIENCE
IN SPORTS

# Editorial

## We cannot take oranges for apples in the field of platelet-rich plasma products

We have read the editorial by Michael Kjaer and Monica Bayer, "*The use of platelet rich plasma in sports medicine: a quick fix or medical doctors on shaky ethical ground?*" (Kjaer & Bayer, 2011) with interest. We were kindly invited by Prof. Engebretsen to take part in consensus, which aimed to shed light on some of the current discussions regarding the safety and therapeutic potential of platelet-rich plasma therapies (Engebretsen et al., 2010). We appreciate the importance of the initiative promoted by Prof. Engebretsen and the efforts of all those who were involved during two days discussing the state of the art and future directions in the field. Sincerely, the task was not easy. In our opinion, the consensus paper reflects more an initial aim to clarify and answer some of the arising questions about this field more than a consensus paper by itself.

Our research team started working in this field more than 15 years ago. In fact, the plasma rich in growth factors technology (PRGF-Endoret) is the pioneering autologous approach for accelerating wound healing and tissue regeneration (Anitua, 1999). We also reported for the first time in the scientific literature the use of this autologous technology in orthopedics and sports medicine (Sánchez et al., 2003). Since then, we have made many efforts toward understanding the molecular mechanisms underlying this technology, addressing the safety and regulatory issues for its clinical application and optimizing the protocols for the suitable clinical translation (Anitua et al., 2010).

We agree with the editorial that more basic, animal, and clinical studies are needed to fully understand the potential of the platelet and plasma-based approaches (and in this way to cast light on the biological program of tissue regeneration). However, it is also our opinion that these challenges are not necessarily roadblocks that limit the potential of the field but constitute the necessary steps that a therapeutic approach must overcome. The aim of the present letter is to clarify some of concerns expressed by Kjaer & Bayer.

One initial topic of controversy and confusion is the terminology for platelet-rich preparations. The potential scientific and economic interest in the field together with the lack of a suitable standardization and definition for these products has provoked the appearance of a wide range of biological preparations and a jungle of terms easily confused by mistakenly being used interchangeably, which is a fallacy. We can not take orange for apples. In many cases, the term "platelet rich plasma" is used to identify these preparations even if they are obtained using different protocols and differ from a qualitative and quantitative point of view. Therefore, it is easy to understand that differences in some key properties of these "platelet rich plasmas" including the platelet concentration, the type of anticoagulant and clot activator, the number of centrifugations and centrifugation speed, the presence of pro-inflammatory leukocytes, and the level of activation among others can markedly influence the final biological effects. In addition, the latter is not only a consequence of the qualities of the product, which are directly related to the preparation process, but also of the critical application procedure in the patient, when? how? and where?

Maybe some of these arguments could help to explain the results obtained by De Vos (2010) in the randomized control trial evaluating the therapeutic potential of platelet-rich plasma in the treatment of chronic Achilles tendinopathy. Even though the clinical trial is methodologically perfect, the type of intervention used may lead in our opinion to incomplete or false conclusions. First, it is fully necessary to address the qualitative and quantitative characteristics of the product under investigation. The authors did not clearly report any of these parameters in the study. Of note is that the product used in the clinical study contained high levels of leukocytes, specifically neutrophils that may express matrix-degrading enzymes, such as matrix metalloproteinases-8 (MMP-8) and MMP-9, and release reactive oxygen species that may destroy surrounding injured or healthy cells. Second, the dosage used in the trial was surprising. The idea of considering the platelet-rich plasma as a magic bullet for treating a chronic disease is far from being optimal. Our protocol for PRGF-Endoret preparation and application in chronic tendinopathy contrast from the De Vos protocol and so does the clinical efficacy. In our hands, patients receiving three consecutive injections of PRGF-Endoret technology separated by no more than 15 days recovered their range of motion earlier and took less time to take up gentle running and training (Sánchez et al., 2007). Ultimately in biology and medicine as well, recovery of the function is of paramount importance.

Third, the basic science in the field is also progressing rapidly. For example, it has been demonstrated that platelet and plasma-based products induce a potent anti-inflammatory effect in chrondocytes by reducing the transcriptional-activation function of the *nuclear factor kappa-light-chain-enhancer of activated B cells* (NF-κB), a regulator of the inflammatory process, and by decreasing expression of the C-X-C chemokine receptor type 4 (CXCR4) gene (Bendinelli et al., 2010). Furthermore, the cocktail of proteins released by these products could help recruit the patient's own cells, including stem or progenitor cells (Kajikawa et al., 2008).

However, the basic science should also be analyzed and discussed carefully. For example, Kjaer & Bayer describe that TGF-β, one of the proteins present in the platelet-rich plasma products, may downregulate and decrease the expression of type XIV collagen and decorin in tendon cells. These results cannot be directly extrapolated to platelet-rich plasma products, as the latter contain hundreds of additional proteins and growth factors that modulate the direct effects of one unique recombinant protein. In fact, in a similar study, we observed that human keratocytes and conjunctival fibroblasts can be differentiated into myofibroblasts by adding TGF-β1. However when PRGF-Endoret is added, cells maintain their phenotype, even if the PRGF-Endoret contains significant amounts of TGF-β1. Our results show that the autologous formulation prevents and inhibits TGF-b1-induced myofibroblast differentiation (Anitua et al., 2011).

The authors of the editorial discussed how pro-collagen I levels were not affected by adding PRGF-Endoret to the fibroblasts (Anitua et al., 2009). In this study, however, we showed that VEGF and HGF levels were upregulated after treatment with the autologous pool of growth factors. The total amount of pro-collagen was also upregulated, but as the data we reported were normalized with the cell dose, the final values were not statistically different. In addition, we actually hypothesize that the 72-h assay used in that study might not be enough for the cells to properly fabricate and express the protein.

Last, but not least, we have never claimed and will not do it in the future that the clinical use of PRGF-Endoret should be justified because it is not dangerous as a treatment. We agree with you that it is unscientific to provide this statement. However, we think it is necessary for the scientific and clinical community to know that in these 15 years of basic and applied research, we have not found side effects after using PRGF-Endoret in hundreds of thousands of patients not only in oral and maxillofacial surgery, but also in orthopedics and in dermatology.

In summary, we are seeking to develop this autologous technology following the strictest scientific and clinical rules. Our efforts during the last 15 years in understanding how this technology works, how it can be applied, and how the optimum effects can be obtained clearly reflect our goal of using science as paradigm. We know much work lies ahead, but we also believe this technology will see exciting results through the next decade.

*E. Anitua[1] and M. Sánchez[2]*
*[1]Biotechnology Institute (BTI), Vitoria, Spain, [2]Unidad de Cirugía Artroscópica "Mikel Sánchez", Vitoria, Spain*
*mikel.sanchez@usphospitales.com and eduardoanitua@eduardoanitua.com*

## References

Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants 1999: 14: 529–535.

Anitua E, Sanchez M, Merayo-Lloves J, De la Fuente M, Muruzabal F, Orive G. Plasma rich in growth factors (PRGF-Endoret) stimulates proliferation and migration of primary keratocytes and conjunctival fibroblasts while inhibits and reverts TGF-{beta}1-induced myodifferentiation. Invest Ophthalmol Vis Sci 2011: 52 (9): 6066–6073.

Anitua E, Sánchez M, Orive G. Potential of endogenous regenerative technology for in situ regenerative medicine. Adv Drug Deliv Rev 2010: 62: 741–752.

Anitua E, Sánchez M, Zalduendo MM, de la Fuente M, Prado R, Orive G, Andía I. Fibroblastic response to treatment with different preparations rich in growth factors. Cell Prolif 2009: 42 (2): 162–170.

Bendinelli P, Matteucci E, Dogliotti G, Corsi MM, Banfi G, Maroni P, Desiderio MA. Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: mechanisms of NF-κB inhibition via HGF. J Cell Physiol 2010: 225: 757–766.

Engebretsen L, Steffen K, Alsousou J, Anitua E, Bachl N, Devilee R, Everts P, Hamilton B, Huard J, Jenoure P, Kelberine F, Kon E, Maffulli N, Matheson G, Mei-Dan O, Menetrey J, Philippon M, Randelli P, Schamasch P, Schwellnus M, Vernec A, Verrall G. IOC consensus paper on the use of platelet-rich plasma in sports medicine. Br J Sports Med 2010: 44 (15): 1072–1081.

Kajikawa Y, Morihara Y, Sakamoto H, Matsuda K, Oshima Y, Yoshida A, Nagae M, Arai Y, Kawata M, Kubo T. Platelet-rich plasma enhances the initial mobilization of circulation-derived cells for tendon healing. J Cell Physiol 2008: 215: 837–845.

Kjaer M, Bayer M. The use of platelet rich plasma in sports medicine: a quick fix or medical doctors on shaky ethical ground? Scand J Med Sci Sports 2011: 21: 493–495.

Sánchez M, Anitua E, Azofra J, Andía I, Padilla S, Mujika I. Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. Am J Sports Med 2007: 35: 245–251.

Sánchez M, Azofra J, Anitua E, Andía I, Padilla S, Santisteban J, Mujika I. Plasma rich in growth factors to treat an articular cartilage avulsion: a case report. Med Sci Sports Exerc 2003: 35: 1648–1652.

de Vos RJ, Weir A, van Schie HT, Bierma-Zeinstra SM, Verhaar JA, Weinans H, Tol JL. Platelet-rich plasma injection for chronic Achilles tendinopathy: a randomized controlled trial. JAMA 2010: 303 (2): 144–149.