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LETTER TO THE EDITORS

Reply to the letter by Dhillon and colleagues

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Dear Editors,

We would like to thank Prof. Dhillon and colleagues for the opportunity to revisit and explore some interesting and very controversial aspects of this innovative biological treatment approach. In a previous study [5] and in the subsequent analysis "platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis" [3] cited by Dhillon et al., we aimed to analyze our preliminary results with the use of intra-articular knee injections of platelet-rich plasma (PRP) as minimally invasive treatment for knee degenerative pathology. Our findings suggested the potential to reduce pain and improve both knee function and quality of life with short-term efficacy, especially in younger patients with chondral degenerative lesions or early osteoarthritis (OA).

First of all, we would like to answer the observations made about the PRP preparation method. Our procedure involved a 150-ml venous blood sample, which was centrifuged twice to produce a 20-ml unit of PRP. The PRP was then divided into 4 small units of 5 ml each. All of the open procedures were performed in an A-class sterile hood. As correctly underlined, once prepared, the first PRP sample was injected within 4–6 h. The remaining samples were immediately stored at −30°C, in order to avoid the risks of bacterial proliferation and accumulation of pyrogenic cytokines, and towed after 3 and 6 weeks for the other cycle injections. We disagree with the doubts about the storing procedure. Freezing them gives us the time to proceed with the quality analysis. This has to be considered as an advantage, not a disadvantage, with respect to the open procedures for fresh PRP application. In fact, now in our clinical practice, we are also freezing the first sample; this delays the first injection but increases the safety procedure, ensuring a controlled not contaminated intra-articular delivery of the product.

The alteration of the morphology and decrease in platelet functional properties, which includes degranulation of alpha-granules, after storing platelets in freezing conditions, is well known. On the contrary, there are no data on the effect of freezing on the clinical results of platelet injections and freeze-thawing is even one of the methods used for releasing intracellular GFs [2, 9]. Some studies, as well as some of our preliminary unpublished data, show a not significant influence of freezing on GF release, and frozen platelets have also been used by other authors [2, 8].

Regarding methods that increase the storage life of platelets in freezing conditions, such as the mentioned dimethylsulfoxide (DMSO), they involve further platelet manipulation and carry some risks, too (not excluding severe adverse events such as neurological toxicity), and removing it before patient administration also means losing cells and bioactive molecules [4]. Having freshly harvested PRP might preserve all the platelet functions better, but currently the data are still controversial and we cannot say that freeze-thawing adversely affects their properties to the point of impairing their clinical efficacy.

Cellularity is another debated aspect of not secondary importance when evaluating PRP properties and the results obtained with its application. In fact, not only platelets but also leukocytes, monocytes, macrophages, and mast cells are contained in many platelet concentrates. Some authors define PRP as only platelets and attribute better results to leukocyte depletion, because of the deleterious effects of proteases and reactive oxygen released from white cells;

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others consider them as a source of important cytokines and enzymes that may be important also for the prevention of infections and report that PRP significantly inhibits the growth of *Staphylococcus aureus* and *Escherichia coli* [1, 6, 7]. We agree on the importance of assessing the potentially morbid effects of leukocyte-contaminated PRP, and we are trying to determine the role of other cells in both in vitro and in vivo studies.

The intra-articular delivery of PRP is performed through a classic knee injection approach that cannot guaranty directly reaching the lesion site. Bending the knee allows the PRP to be distributed in the joint and therefore to the lesion site, too, before it becomes a gel. Thus, we consider this approach preferable to immobilizing the joint immediately after injection. Anyway, we also believe that part of the effect of PRP is due to the influence on the overall joint homeostasis, through the reduction in synovial membrane hyperplasia and modulation of the cytokine level, and for this mechanism, the intra-articular distribution of the platelet concentrate is even more desirable.

In our study, we evaluated both degenerative chondral lesions and early or advanced OA. As pointed out by Dhillon and colleagues, the use of the WOMAC scoring system would have been a better option in place of IKDC for osteoarthritic knees, making the study more comparable with traditional treatment strategies, too. However, we preferred IKDC subjective score as primary outcome measure, because is for cartilage lesions and not OA that we expected better results and a higher indication for this treatment, and actually, this is what the results confirmed. This score is approved and recommended by the ICRS (International Cartilage Repair Society) evaluation package for the evaluation of cartilage lesions. A more OA-oriented scale might better suit patients affected by OA, but not for degenerative cartilage lesions, thus being less indicated for our study. Now, we are performing a randomized study using both IKDC and KOOS scores to favor comparison with other traditional treatment strategies, too.

With regard to the need for controls, we agree on their fundamental importance in every study related to intra-articular injections of knee joint, in order to determine the role of placebo. In fact, this study lacks a control group and we do not think it will turn out to be a landmark study defining the role of PRP in osteoarthritis of the knee. There is a need for well-designed randomized trials and also preclinical and clinical studies focusing on defining the optimal formulation, the proper dosage and timing of application, and determining which patients, type and phase of pathology may better respond to this biological promising minimally invasive approach.

Nevertheless, we also believe that it was important to report our preliminary findings, too, in order to avoid an unconsidered excessively wide clinical application. As for every new treatment, clinical use should be approached cautiously. On the contrary, PRP injections risk becoming considered as a "miracle cure" and currently they are already widely used in a multitude of clinical applications. Documenting our results aimed on the one hand at showing patient categories that clearly cannot benefit from this treatment approach, thus limiting its use to patients who "apparently" might benefit and on the other hand helping to clarify the expectations offered by this procedure, until high-level clinical evidence confirming the efficacy of platelet-rich plasma is available.

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