Reply to comments of Carmona et al. to the article: Regenerative medicine for the treatment of musculoskeletal overuse injuries in competition horses

Paola Torricelli · Giuseppe Filardo · Elizaveta Kon ·
Milena Fini

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In a recently published study, we documented our experience using platelet-rich plasma (PRP) and concentrated bone marrow mononucleated cells (BMMNCs) for the treatment of musculoskeletal injuries (ligament and tendon overuse injuries) in competition horses refractory to other therapies [1]. We evaluated number of cells and presence of growth factors (GFs) injected, and performed follow-ups on horses weekly, up to one year. The results of our study showed a marked improvement in the degree of lameness, with 84.6% of horses returning to competition, and a direct correlation between number of platelets injected and clinical outcome.

The main complaint of Carmona et al. was regarding the lack of a control group. In fact, this is an important limitation, as we clearly stated in the discussion of the manuscript. We agree that this combined treatment approach can not be recommended for a wide clinical use just from this case series, and further randomised controlled studies on a greater number of horses are needed to confirm these results. However, while on the one hand we recognise the lack of scientific basis on which recommend the treatment, on the other hand we intend to increase the knowledge on this biological treatment by documenting and analysing our results. As for athletes, it is extremely

difficult to have Level I studies in competition horses because of their enormous value and the high interest among their performance, but they also have the advantage of representing a particular study population subjected to mechanically demanding activities with highly stressed musculoskeletal tissues. They have been strictly followed-up, thus allowing us to document some interesting findings. First of all, the study showed the absence of major adverse events, i.e. only two horses developed minor reactions with self-limiting swelling after the treatment, however, that gradually resolved within one month. A marked improvement was observed both clinically and, with ultrasound examination, the healing time was reported, thus allowing the readers to better understand the potential of this biological approach. More interestingly, even a correlation between biological properties and final outcome could be documented. This is an important finding, since GFs are potent molecules and small dose variations of the same factors may exert completely opposite effects in the regulation of the complex regeneration process, even impairing tissue healing, as already documented in the literature [2]. For example, Weibrich et al. [3] reported that the platelet (PLT) concentration required for a positive PRP effect on bone regeneration spanned a limited range. An advantageous biological effect seemed to occur with a certain PLT concentration. At lower concentrations, the effect was suboptimal, whereas higher concentrations had a paradoxically inhibitory effect. Therefore, one of the important findings of our study is that we could document that the range of PLT concentration used for the treatment of ligaments and tendons was safe, and we also demonstrated a direct correlation between PLT amount and clinical outcome. Another aspect to consider is the presence of numerous products commercially available, which differ considerably for the number of PLTs obtained and injected

P. Torricelli · M. Fini
Research Institute Codivilla-Putti,
Laboratory of Preclinical and Surgical Studies,
Rizzoli Orthopaedic Institute,
Bologna, Italy

G. Filardo · E. Kon (✉)
Laboratory of Biomechanics and Technology
Innovation - III Clinic, Rizzoli Orthopaedic Institute,
Bologna, Italy
e-mail: e.kon@biomec.ior.it

[4]. Our study helps to explain the controversial results found in the literature when comparing different procedures, suggesting the importance of the stimulation with a proper dose in order to have better results.

We also disagree with the concern on the use of calcium to activate PLTs and its potential deleterious effect on BMMNCs. Studies investigating the effects of high extracellular calcium concentration on mesenchymal stem cells are reported in literature [5–7]. Nevertheless, very high concentrations of calcium chloride are considered antiproliferative [8]. We calculated the mean amount of calcium chloride in cell culture medium and the mean amount of calcium chloride added to PRP/BMMNCs, in order to avoid toxic concentration.

Moreover, the low number of horses evaluated did not allow detection of a clear dose-response curve, but the two horses with worst results received both the lower amount of PLTs and BMMNCs, thus suggesting a role in the healing process also for BMMNCs and an influence of their concentration on the final outcome.

Finally, unlike the doubts on the BMMNCs efficacy in the treatment of musculoskeletal lesions, literature shows that stem cells are a promising tool in regenerative medicine, and are also increasingly being successfully applied in equine medicine especially for tendon and ligament injuries, as in this study, alone or in association with PRP [9–11]. PRP may play a double role when combined with BMMNCs. Once activated, PLT gel represents a three-dimensional scaffold that can support cells and allow them to be delivered and maintained in the lesion site, and PLTs may act synergistically with mesenchymal stem cells, thereby potentially increasing the healing potential. Currently, the clinical effect of combined therapy and in particular the use of stem cells is controversial, and we agree with other authors [12, 13] of the need for further investigation and controlled and well-designed studies to confirm these preliminary findings and better understand mechanisms of action, risks of this procedure, and cost-benefit analysis prior to wide, indiscriminate clinical use.

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