Two-fold Increase in the Number of Total Nucleated Cells in the Bone Marrow Concentrate Obtained From the Bone Marrow Aspirate May Not Be Ideal: Response

Authors' Response:

The letter by Drs Atluri and Boddu regarding our study is appreciated and raises a few questions that have been and will continue to be points of debate in regenerative medicine. Regenerative medicine is very much in its infancy even though the basic science of healing and cell biology is expanding at an exponential rate. The challenge that we (the authors) see is how to balance long-held dogma against in vitro studies and translate that knowledge to clinically relevant applications. These debates are further complicated by the paucity of randomized clinical trials.

The purpose of our study was to evaluate the popular belief that a multiple-site harvest on the posterior iliac crest is superior to a single-site harvest. Our study showed that the only statistically significant finding was that of increased pain in the multiple-site harvest technique. The subject of the letter to the editor largely centers on the use of total nucleated cell (TNC) count as a predictor of mesenchymal stem cell (MSC) count. It is a commonly held belief that when one is concentrating a bone marrow aspirate sample, the higher the level of TNCs, the higher the number of MSCs because the density of MSCs is similar to that of other nucleated cells. Theoretically, if a large number of TNCs are captured, then a large number of MSCs should be captured in comparison with the capture of a smaller number of TNCs yielding a lower number of MSCs. However, the most numerous of the nucleated cells are neutrophils.

One of the features of the device that was used in this study (Arthrex Angel) is that it has the ability to reduce neutrophil (the most numerous of nucleated cells) content while still retaining a large portion of hematopoietic cells and MSCs in a bone marrow fraction when the appropriate settings are selected. In our study, we chose to use a 12% hematocrit setting. The method by Drs Atluri and Boddu is to set the device to the maximum hematocrit setting (25%), which results in capturing a much larger nucleated cell layer, yielding a higher TNC count. A higher nucleated cell count does not necessarily translate into a higher stem cell count. Based on the work our group has done, it is our opinion that an assessment for fibroblast colony-forming units (CFU-F) would not reveal a statistically significant difference in stem cell count in samples processed at 12% and 25% despite showing significant differences in TNC count. We also believe that TNC counts are not a reliable surrogate for stem cell counts.

Evidence to support this supposition is found in a paper by Cassano et al,[1] who compared 2 devices with significantly different TNC counts and found no statistically significant differences in stem cell counts. Further, studies pointing to CFU-F may be inherently flawed. Drs Atluri and Boddu cite the Hernigou et al[2] study as evidence that TNC counts and CFU-F data could reliably predict outcomes. In the study by Hernigou et al,[2] the CFU-F counts were obtained at 12 days. Although those cell counts are interesting and potentially helpful in developing dose response, they are nonetheless not indicative of what was implanted at time zero. Cell culture occurs under optimal conditions, and without further study to delineate the relationship between in vitro cell culture and in vivo implantation, the resulting cell counts cannot be used as evidence of an outcome predictor. We support the use of CFU-F and TNC counts to understand the characterization of the end product generated by the device, but again they are not a replacement for carefully controlled randomized studies and outcome data. In our physician group, we have collected outcome data on more than 1000 patients treated with the same method described in our study and have seen clinical improvement at the 3-, 6-, 9-, 12-, and 24-month time points.[3]

We thank the journal for publishing our study and Drs Atluri and Boddu for their letter to the editor, which raises important questions. We hope such questions will generate interest in development of studies to better understand which diagnostic tests are translatable to enhance clinical application of autologous biologic treatments.

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