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Only prolonged time from abstraction found to affect viable nucleated cell concentrations in vertebral body bone marrow aspirate

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Abstract

BACKGROUND CONTEXT: Vertebral body–derived bone marrow aspirate (BMA, with an appropriate carrier) is a potential alternative to traditional iliac crest bone graft for use in spinal fusion surgery. No studies have looked at the effect of different temporary handling/storage conditions on the osteoprogenitor potential of BMA. This is especially important because aspirate, as with cancellous and/or cortical grafts, may be extracted some time before actual implementation in regular clinical use.

PURPOSE: To characterize factors that affect BMA cell concentration during routine spinal instrumentation, this study examined whether cell counts change significantly between the second pedicle aspirated and the first pedicle harvested at the same vertebral level. This study also aims to examine the optimal perioperative storage conditions for BMA obtained from the vertebral body.

STUDY DESIGN: In vitro concentrations of viable cells were determined in BMA harvested from the first and second pedicles on every vertebral level, and after 1 hour of storage in different perioperative conditions.

PATIENT SAMPLE: BMA was harvested from 28 pedicles from seven patients undergoing lumbar instrumented fusion surgeries.

OUTCOME MEASURES: The outcome measure included viable nucleated cell concentrations in BMA.

METHODS: After obtaining HIC approval from our institution, 28 vertebral marrow aspirates (obtained from seven patients) were evaluated. Based on prior work, 4-mL aspirates from each pedicle were evaluated. BMA was aspirated from both pedicles of two vertebral levels per patient. Samples were divided and placed in different storage conditions to examine the effect of laterality (first versus second pedicle aspirated per level), temperature, media, and time, on nucleated cell counts. No funding was received for this study, and the authors disclose no study specific conflicts of interest.

RESULTS: Cell count was not significantly different between the first or second side aspirated for each vertebral level. Similarly, no significant differences were found for samples after 1 hour of storage at different temperatures (0°C, room temperature, or 37°C) or media (none, saline, essential media). Of the conditions examined, time from aspiration was the only variable found to have an impact on nucleated cell counts ($p=.003$). The viable cell count decreased to less than half by 4 hours.

CONCLUSION: As vertebral BMA is increasingly considered as a bone grafting option, the field would be remiss not to consider factors that could affect cell viability after abstraction and before

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implementation. We expected a greater effect of perioperative storage conditions than was observed. Although the variables evaluated might show small effects on cell viability in a larger study, this would not be expected to be significant. In the current study, only prolonged time from abstraction could be shown to have a significant effect on cell viability.

Keywords: Biologics; Bone marrow aspirate; Lumbar fusion; Marrow stromal cells; Perioperative storage; Vertebral body BMA

Introduction

Spinal arthrodesis is a common procedure for treating degenerative, traumatic, and deformity conditions of the spine. The rates of spinal fusions have been increasing over the past 30 years, with more than 400,000 surgeries performed annually [1]. Although instrumentation has evolved to facilitate provisional stabilization, biologic fusion of adjacent vertebrae is crucial for the long-term success of such procedures.

Autogenous iliac crest bone graft is still considered the ''gold standard'' bone graft material. However, this entails an additional procedure during surgery, a new incision site, and longer surgery time. These, along with morbidities associated with its harvest, has resulted in a decline in its use [2–5]. Alternatives, such as local bone, allograft, synthetics, recombinant proteins, and bone marrow aspirate (BMA) are being increasingly used individually or in combination.

Traditionally, BMA for spinal fusion has been obtained from the iliac crest. Although not an extensive procedure, its harvest is both unfamiliar to many spine surgeons and requires accessing an additional anatomic site. Accordingly, there has been increased interest in obtaining BMA from the vertebral body at the time of pedicle instrumentation, as the source for aspiration is already accessed as part of the regular instrumentation process.

BMA from the vertebral body has been shown to have comparable osteoprogenitor potential as that from the iliac crest. This osteogenic potential of BMA can be measured in a number of ways in the laboratory. Keeping in mind that most of the cells in BMA are anucleated, nonosteogenic red blood cells, nucleated cell counts are often the focus of studies. Cell surface markers, such as CD29, CD34, and gene expression of factors, such as Runx-2 and type I collagen, can be used to precisely separate and identify cell types, but quantified levels of these markers have generally been found to correlate with nucleated cell counts. Additionally, studies have found that the osteogenic potential of aspirate measured by these factors is comparable in vertebral body and iliac crest aspirates [6,7]. Perhaps the most definitive method to identify cells of osteogenic lineage is to culture them and determine the number of colony-forming units (CFUs) producing alkaline phosphatase, an early marker for osteoblast differentiation. This method also has been found to correlate with nucleated cell counts [8,9]. Consequently, it is not surprising that the nucleated cell concentration of BMAs has been correlated with clinical outcome, measured by volume of mineralized callus and achievement of bone union [10,11].

From a clinical standpoint, efforts have been made to optimize BMA cell counts by focusing on aspirated volume. From the ileum, studies have suggested that the number of nucleated cells decreased after 2 mL, as the return becomes venous blood as opposed to actual marrow [12]. From the vertebral body, it has been shown that the concentration of cells dropped below half of that of the first milliliter after 4 mL of aspiration [13]. Nonetheless, despite the importance of viable nucleated cell count to the function of autogenous products (local bone, iliac crest bone, BMA, or other), the handling of such tissues between harvest and use has received relatively little attention. This is especially concerning in spine surgery, where the time from harvest to implantation can be on the order of hours.

In light of this, some studies have looked at optimal storage conditions for autologous bone graft perioperatively [14,15]. These studies examined cell counts and cell metabolism and found that immediate grafting on harvest was superior to all forms of preservation. Additionally, these studies suggested that some of the loss in cell count and metabolism over time could be minimized by placement of the graft in saline solution. However, we found no similar studies that examined the optimal storage conditions of bone marrow aspirate perioperatively.

In an effort to characterize factors that affect BMA cell count during routine spinal instrumentation, this study examined whether cell counts change significantly between the second pedicle aspirated and the first pedicle harvested at the same vertebral level. Additionally, the effect of perioperative controllable factors that could affect BMA cell viability (temperature, media, and time) was evaluated.

Materials and methods

Twenty-eight vertebral body aspirates were analyzed from seven patients. Two men and five women undergoing lumbar instrumented fusion for degenerative conditions at our institution provided written informed consent to participate in the study. Mean age at the time of surgery was 52.6 years (range 23 to 71). Patients were excluded if they had previous spinal surgery with instrumentation; suffered from