Percutaneous autologous bone marrow concentrate for knee osteoarthritis: patient-reported outcomes and progenitor cell content

Christopher J. Centeno1,2 · Dustin R. Berger2 · Brandon T. Money1 · Ehren Dodson2 · Christopher W. Urbanek1 · Neven J. Steinmetz2

Received: 23 December 2021 / Accepted: 18 July 2022 © The Author(s) 2022

Abstract
Purpose Knee osteoarthritis (OA) is a common, progressively debilitating joint disease, and the intra-articular injection of autologous bone marrow concentrate (BMC) may offer a minimally invasive method of harnessing the body’s own connective tissue progenitor cells to counteract accompanying degenerative effects of the disease. However, the extent to which the progenitor cell content of BMC influences treatment outcomes is unclear. We sought to determine whether patient-reported outcome measures associated with BMC treatment for knee OA are related to the concentration of progenitor cells provided.

Methods In the present study, 65 patients (72 knees) underwent treatment for knee OA with autologous BMC and self-reported their outcomes for up to one year using follow-up questionnaires tracking function, pain, and percent improvement. A small fraction of each patient’s BMC sample was reserved for quantification with a haematological analyzer and cryopreserved for subsequent analysis of potential connective tissue progenitor cells using a colony-forming unit fibroblast (CFU-F) assay.

Results Patients reported significant increases in function and overall percent improvement in addition to decreases in pain relative to baseline levels following treatment with autologous BMC that persisted through 12 months. Patients reporting improved outcomes (46 of 72 knees) received BMC injections having higher CFU-F concentrations than non-responding patients (21.1×10^3 ± 12.4×10^3 vs 14.3×10^3 ± 7.0×10^3 CFU-F per mL). A progenitor cell concentration of 18×10^3 CFU-F per mL of BMC was found to best differentiate responders from non-responders.

Conclusion This study provides supportive evidence for using autologous BMC in the minimally invasive treatment of knee OA and suggests that increased progenitor cell content leads to improved treatment outcomes.

Trial registration ClinicalTrials.gov Identifier: NCT03011398, 1/7/17

Keywords Knee osteoarthritis (OA) · Bone marrow concentrate (BMC) · Colony-forming unit fibroblast (CFU-F) · Connective tissue progenitor cells

Introduction
Osteoarthritis (OA) is a progressive joint disease that presents with chronic degeneration of articular cartilage and other deleterious bone-related changes, and conservative management of the disease is largely ineffective at reducing pain and increasing function [1]. Orthobiologic therapi...
plasma (PRP) and hyaluronic acid (HA) [7, 8]. Meanwhile, some have reported arthritic knees treated with BMC to have similar outcomes to those treated with HA [9] or even saline placebo [10]. Connective tissue progenitor cells, also referred to as mesenchymal stem/stromal cells (MSCs), are thought to be a key component of BMC associated with favourable clinical outcomes [11]. Positive relationships with respect to CFU-F concentration, measured using the colony-forming unit fibroblast (CFU-F) assay, and clinical outcome have been reported for orthopaedic procedures. A nearly threefold increase in CFU-F concentration was found in tibia nonunion fractures successfully treated with BMC compared to failures [12]. Similarly, patients treated with BMC for moderate to severe discogenic low back pain reported greater reductions in pain when receiving BMC containing higher concentrations of colony-forming cells [13].

Currently, the clinical importance of progenitor cells when treating knee OA with BMC is unknown. The aim of the present study was to identify individuals with moderate to severe knee OA that were treated with autologous BMC and monitored for changes in self-reported function, pain, and percent improvement up to one year post-treatment using a patient registry. Cryopreserved BMC samples were used to obtain an estimation for the progenitor cell content of the injectates used for treatment, and the resultant sample cellularity was compared with patient-reported outcomes. Other investigators have found a positive relationship between patient-reported outcomes and autologous BMC injections containing higher concentrations of progenitor cells for other orthopedic conditions [12, 13]; thus, we hypothesized a similar relationship exists for treating knee OA with BMC.

Materials and methods

Patients treated for symptomatic knee OA at an outpatient orthopedic clinic were enrolled into an IRB approved patient registry (OHRP #IRB00002637) designed to track clinical outcomes and adverse events for autologously derived, musculoskeletal treatments. Informed consent was provided prior to entering the registry, and upon enrollment, patients were prospectively tracked using an electronic data capturing system (Dacima Software, Montreal, Quebec) that administers pre-treatment baseline and follow-up questionnaires at one, three, six, 12, and 24 months and annually thereafter. Patient-reported outcomes of interest include the Lower Extremity Functional Scale (LEFS), the Numeric Pain Scale (NPS), and a modified Single Assessment Numeric Evaluation (SANE) score (percent improvement). For the study cohort, we only utilized outcome questionnaires up to the 12-month time point.

Inclusion criteria for the present study were patients aged 35–85, having a physical examination consistent with moderate to severe knee OA, based on a Kellgren-Lawrence or Park classification grade II or greater on radiographs or MRI, respectively [14, 15], providing responses to baseline and six month and/or 12-month outcome questionnaires from the patient registry, and having a cryopreserved BMC sample available for laboratory analysis. Exclusion criteria included any knee injections within three months or knee surgery within six months of the BMC treatment, the presence of inflammatory or autoimmune joint-affecting diseases, quinolone- or statin-induced myopathy/tendinopathy, current involvement in health-related litigation, condition related to worker’s compensation case, bleeding disorders, taking anticoagulants or immunosuppressive medications, and/or history of chronic opioid use.

Processing, counting, and injection of bone marrow concentrate (BMC)

Utilizing ultrasound or fluoroscopic guidance, bone marrow was aspirated from the posterior superior iliac spine into heparinized syringes using a small-volume, multi-site technique [16, 17]. The autologous bone marrow aspirate (BMA) (60 to 120 mL in total) was manually processed into BMC (1.5 to 6.2 mL) by trained laboratory personnel, as previously detailed [18]. A component of the processing quality assurance (QA) protocol requires a small fraction of BMC (< 0.2 mL) to be set aside for cellular analysis and subsequent cryopreservation. An automated haematology analyzer (ABX Micros 60, Horiba Medical, France) was used to obtain a complete blood count for each BMC sample, and the white blood cell parameter was used as a representative measurement for the concentration of total nucleated cells [19]. The remaining portion of reserved BMC was cryopreserved at a concentration of ten million cells per mL using cryopreservation medium containing 30% fetal bovine serum (FBS) and 5% dimethyl sulfoxide (DMSO) by volume and a controlled rate freezing process [20].

All patients were encouraged to cease the use of non-steroidal anti-inflammatory drugs two weeks prior to and several weeks following BMC treatment. Under sterile conditions, patients received an intra-articular or intra-articular plus intraosseous injection(s) of BMC into the affected joint space via imaging guidance. Additionally, based on the patient’s clinical presentation and imaging findings, they may have received further injections into the supporting structures (i.e., ligaments, tendons, meniscus) if these structures were also diseased, damaged, or injured. Further, intra-articular injections of prolotherapy and concentrated leukocyte poor PRP were performed two to four days prior to (prolotherapy) and following (PRP) BMC treatment [21]. Patients with suspected ligamentous instability based on clinical indications were fitted for a hinged unilateral unloader knee brace or a
patellar stabilizer brace (Breg, Inc., Carlsbad, CA, USA) and instructed to wear the brace during weight bearing activity for four weeks. If patients experienced substantial post-procedural pain, opioid rescue medication was prescribed for up to five days. Patients were advised to avoid activities that caused a worsening of pain throughout their stepwise rehabilitation protocol, which began with rest and household/community ambulation. Progression of physical activities consisted of pool or low impact exercise, followed by walking, resistance training and jogging, and ultimately advancing to full functional activity. While face-to-face physical therapy sessions were encouraged, they were not required.

Colony-forming unit fibroblast (CFU-F) assay

Cryopreserved BMC samples were removed from cryogenic storage and rapidly warmed at 37°C for two minutes. Thawed BMC samples were promptly diluted tenfold in pre-warmed complete culture medium (CCM) containing 10% FBS and 1 ng per mL human fibroblast growth factor, counted using an automated cell counter (TC20, BioRad, Hercules, CA, USA) and trypan blue, and directly plated within standard six well tissue culture plates at two separate cell densities, 10×10³ and 30×10³ viable nucleated cells per cm². Following 72 hours of culture, non-adherent cells were removed by washing, and the remaining adherent cells were maintained at 37°C and 5% CO₂ with biweekly replacement of CCM.

After a 14-day culture period, the plates were washed and stained for colonies using crystal violet in methanol. A CFU-F was defined as any colony greater than 1 mm in diameter and containing a minimum of 100 cells. All colonies were counted by two independent observers. To determine CFU-F frequency, colony counts were averaged across wells and divided by the number of plated cells per well, and the CFU-F concentration was calculated by multiplying the CFU-F frequency of the cryopreserved sample by the nucleated cell concentration, as measured prior to cryopreservation [20].

Statistical analysis

Patient-reported outcomes were compared over time by fitting a mixed-effects model with Geisser–Greenhouse correction and Tukey’s multiple comparison tests. Patients reporting overall favorable outcomes (responders) to autologous BMC therapy were characterized as meeting or exceeding both the minimal clinically important difference (MCID) of nine points for LEFS and 40% improvement for SANE at the 6-month follow-up (the 12-month follow-up was used for three patients not reporting 6-month functional outcomes), based on previous studies [13, 22, 23]. Patient demographics and BMC cellularity were compared between responders and non-responders using unpaired t tests with Welch’s correction. A receiver operating characteristics (ROC) curve analysis was used to determine the concentration of CFU-F for best differentiating responders from non-responders [24]. Reported mean outcomes of knees treated both above and below the identified target CFU-F concentration were compared against the LEFS MCID and SANE > 40% thresholds using one-sample t tests. Results were considered significant at P < 0.05. All statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, La Jolla, CA, USA).

Results

Patient-reported outcomes following treatment with autologous BMC

Sixty-five patients (29 females and 36 males) aged 62.2 ± 8.2 years with moderate to severe knee OA (16.7% II, 47.2% III, 36.1% IV) were treated non-surgically using image guided, percutaneous injections of autologous BMC (Fig. 1). Seven patients were injected bilaterally for a total of 72 treated knees. Depending on the presence of bone marrow lesions on diagnostic imaging, a subset of patients (27 of 72 knees) received intra-articular as well as intraosseous BMC injections, as determined by the treating physician. An average volume of 2.9 ± 1.3 mL of BMC, containing 440 ± 155 million nucleated cells per mL, was injected into the index knee(s). The CFU-F frequency of cryopreserved BMC samples ranged from 4 to 105 CFU-Fs per million nucleated cells with an average of 43.3 ± 23.9 (0.00433%
± 0.00239%). After accounting for the range of nucleated cell concentrations measured within the BMC samples, the average concentration of colony-forming cells was $18.6 \times 10^3 \pm 11.2 \times 10^3$ CFU-F per mL of BMC, ranging from 925 to 57.1 $\times 10^3$. A summary of patient demographics, BMC cellularity, and reported outcomes is presented in Table 1.

Patients treated with autologous BMC reported a considerable and sustained reduction in pain, gain in function, and overall percent perceived improvement (Fig. 2). There were no differences in reported outcomes ($P > 0.05$) between knees receiving intra-articular injections and knees receiving intra-articular plus intraosseous injections (Supplementary Fig. 1). Consequently, all outcomes were analyzed as a single cohort. Baseline pain levels (NPS) decreased from $4.1 \pm 2.1$ to $2.1 \pm 2.0$ and $2.0 \pm 1.8$ at the six month and 12-month follow-ups, respectively (Fig. 2A), while the knee functional scores (LEFS) increased from $45.4 \pm 14.0$ to $59.4 \pm 14.3$ and $59.1 \pm 14.3$ over the same period (Fig. 2B). Similarly, reported percent improvement (SANE) increased from $34.8\% \pm 30.9\%$ at one month to $55.4\% \pm 34.4\%$ at 12 months (Fig. 2C). Patient-reported outcomes (LEFS, NPS, SANE) were significantly improved ($P < 0.001$) over baseline (or 1 month for SANE) at all subsequent follow-ups.

While baseline and six month SANE data were collected from every patient, follow-up rates varied at other time points, ranging from 96% (69 of 72 knees) at the one month follow-up to 85% (61 of 72 knees) at the 12-month follow-up. A majority of patients (44 or 72 knees) reported engaging in some form of post-treatment physical therapy. Moreover, ten patients reported receiving an additional injection, in the form of PRP (6), prolotherapy (2), concentrated serum proteins (1), and Hoffa’s fat pad hydrodissection (1), within the 12-month period following BMC treatment.

### Improved outcomes are reported by patients having higher CFU-F concentrations

A majority of patients (66%, 43 of 65) reported meeting or exceeding the LEFS MCID (9 points) and SANE threshold (> 40%) with respect to one or more treated knees at the six month follow-up and thus were considered responders. However, a subset of knees (36%, 26 of 72) failed to respond to autologous BMC treatment, and approximately one-third of the non-responding knees (9 of 26) reported

---

**Table 1** Summary of demographics, BMC sample composition, and patient-reported outcomes for all patients and knees

| Demographics |  |
|--------------|  |
| Patients ($n$) | 65  |
| Gender (female, male) | 29F, 36M  |
| Age (years) | 62.2 ± 8.2  |
| Body mass index (BMI) | 26.9 ± 4.8  |
| Bilateral patients ($n$) | 7  |
| Total knees treated ($n$) | 72  |
| OA severity grade |  |
| II (%) | 12 (16.7%)  |
| III (%) | 34 (47.2%)  |
| IV (%) | 26 (36.1%)  |
| BMC composition |  |
| BMC vol. (mL) | 2.9 ± 1.3  |
| [BMC] ($\times 10^6$ cells / mL) | 440 ± 155  |
| CFU-F frequency (%) | 0.0043 ± 0.0024  |
| [CFU-F] ($\times 10^3$ CFU-F/mL) | 18.6 ± 11.2  |
| Reported outcomes |  |
| $\Delta$ NPS at 6 months ($n$) | $-2.0 \pm 2.0$ (70)  |
| $\Delta$ NPS at 12 months ($n$) | $-2.1 \pm 2.0$ (62)  |
| $\Delta$ LEFS at 6 months ($n$) | $14.0 \pm 13.7$ (69)  |
| $\Delta$ LEFS at 12 months ($n$) | $13.6 \pm 13.8$ (61)  |
| SANE at 6 months ($n$) | 55.3 ± 32.1 (72)  |
| SANE at 12 months ($n$) | 55.4 ± 34.4 (61)  |

---

**Fig. 2** Patient-reported outcomes following treatment with autologous BMC for knee OA. Violin plots of reported **A** pain (NPS), **B** function (LEFS), and **C** percent improvement (SANE) at 1-, 3-, 6-, and 12-month follow-ups from patients receiving intra-articular (black) or intra-articular and intraosseous (blue) injections. Lines represent mean values. One symbol $P < 0.05$, two symbols $P < 0.01$, three symbols $P < 0.001$ versus baseline (*) and 1-month follow-up (†).
deteriorating function ($\Delta$LEFS $\leq 0$) and no improvement (SANE $\leq 0$) at the six month follow-up. Injectate cellularity, patient age, and BMI were compared between responding and non-responding patient cohorts (Fig. 3). The mean CFU-F concentration, frequency, and nucleated cell concentration were significantly greater ($P = 0.004, 0.030, 0.046$) within the BMC of responding knees (Fig. 3A–C), yet no differences ($P > 0.05$) were observed between responding knees and non-responding knees with respect to BMC volume, biological age, or BMI (Fig. 3D–F). Further, no differences in average knee OA severity grades were observed between responding and non-responding knees ($P > 0.05$). High interpatient variability in CFU-F size, density, and frequency were observed in both groups (Fig. 3G–H). A summary of patient demographics, BMC cellularity, and reported outcomes from responders and non-responders is presented in Table 2.

Fig. 3 Responders to autologous BMC therapy for knee OA tend to have more progenitor cells in their injectates than non-responders. The A CFU-F concentration, B CFU-F frequency, C nucleated cell concentration, D volume, E age, and F BMI of patients and their BMC when separated based on outcome (non-responder = N, responder = R). Images of multi-well CFU-F assay plates representative of patients having (enlarged symbols in A–F) G lower and H higher CFU-F concentrations. Horizontal lines represent mean values. * $P < 0.05$, ** $P < 0.01$
Patient-reported outcomes were compared against the CFU-F concentrations of their respective autologous BMC treatments (Fig. 4). Scatterplots of CFU-F concentration versus percent improvement and change in LEFS at both 6- and 12-month follow-up time points indicate patients having higher concentrations of colony-forming cells generally report improved outcomes (Fig. 4A–B). The receiver operating characteristic (ROC) curve (AUC = 0.677, \( P = 0.013 \)) revealed a maximum sensitivity and specificity of 63.0% and 76.9%, respectively, at a CFU-F concentration of 18\( \times 10^3 \) CFU-F per mL of BMC (Supplementary Fig. 2). When separated into two cohorts based on this CFU-F concentration cutoff, knees treated with autologous BMC having > 18\( \times 10^3 \) CFU-F per mL reported outcomes, on average, that were significantly greater than the MCID for LEFS (6-month \( P = 0.004 \), 12-month \( P = 0.025 \)) and SANE (6-month \( P < 0.001 \), 12-month \( P = 0.005 \)) threshold (Fig. 4C–D). In contrast, patients treated with BMC having fewer than 18\( \times 10^3 \) CFU-F per mL reported average outcomes that failed to exceed the LEFS MCID and SANE threshold.

### Discussion

Patients report considerable reductions in pain, increases in function, and general overall improvement up to one year following treatment with autologous BMC for moderate to severe knee OA, consistent with our previous results and those of others [6–8, 25]. The most important finding of the present study is the increased cellularity (nucleated cell concentration, CFU-F frequency, and CFU-F concentration) measured within the BMC injectates provided to patients responding favorably compared with those that did not. Some have suggested that the progenitor cell content of BMC, as measured using a CFU-F assay, contributes to positive clinical outcomes. Patients treated with BMC injections having > 2\( \times 10^3 \) CFU-F per mL reported faster and greater reductions in discogenic pain associated with lumbar degenerative disc disease [13], and a higher average CFU-F concentration, > 1.5\( \times 10^3 \) CFU-F per mL, was found in the BMC of successfully treated tibial nonunion compared to treatment failure [12]. The present study determined a concentration of 18\( \times 10^3 \) CFU-F per mL to best differentiate responders from non-responders for knee OA treatment.

Recently, our group detailed an alternative approach to the CFU-F assay that utilizes cryopreserved BMC in lieu of freshly obtained BMC, enabling the establishment of a biobank for the retrospective analyses of potential connective tissue progenitor cells for comparison with patient-reported outcomes [20]. To our knowledge, this is the first time the CFU-F concentration within BMC, obtained retrospectively from cryopreserved patient samples, has been reported with respect to outcomes associated with treatment for knee OA.

### Table 2

Summary of demographics, BMC sample composition, and patient-reported outcomes when separated into responding and non-responding patient cohorts. *Reported outcomes were anticipated to be significantly different based on bifurcating the patient population based on the LEFS MCID (9) and SANE (> 40%) thresholds.

|                         | Non-responders | Responders | \( P \) value |
|-------------------------|----------------|------------|--------------|
| **Demographics**        |                |            |              |
| Patients (n)            | 22             | 43         | -            |
| Gender (female, male)   | 9F, 13M        | 20F, 23M   | -            |
| Age (years)             | 61.4 ± 9.7     | 62.6 ± 7.5 | 0.622        |
| Body mass index (BMI)   | 26.3 ± 4.7     | 27.2 ± 4.9 | 0.476        |
| Bilateral patients (n)  | 3              | 4          | -            |
| Total knees treated (n) | 26             | 46         | -            |
| **OA severity grade**   |                |            | 0.812        |
| OA severity grade       | 3.2 ± 0.7      | 3.2 ± 0.8  |              |
| **BMC composition**     |                |            | 0.795        |
| BMC vol. (mL)           | 3.2 ± 1.5      | 3.0 ± 1.2  |              |
| \([\text{BMC}] (\times 10^6 \text{ cells/mL})\) | 395 ± 117     | 464 ± 169  |              |
| CFU-F frequency (%)     | 0.0036 ± 0.0017| 0.0048 ± 0.0027| 0.030       |
| \([\text{CFU-F}] (\times 10^3 \text{ CFU-F/mL})\) | 14.3 ± 7.0    | 21.1 ± 12.4|              |
| **Reported outcomes**   |                |            |              |
| \(\Delta\) NPS at 6 months (n) | −0.7 ± 1.6 (26) | −2.7 ± 1.8 (44) | <0.001       |
| \(\Delta\) NPS at 12 months (n) | −1.0 ± 1.6 (19) | −2.6 ± 2.0 (43) | 0.002        |
| \(\Delta\) LEFS at 6 months (n) | 1.4 ± 7.3 (26) | 21.7 ± 10.5 (43) | <0.001       |
| \(\Delta\) LEFS at 12 months (n) | 1.1 ± 10.1 (19) | 19.3 ± 11.3 (42) | <0.001       |
| SANE at 6 months (n)    | 21.5 ± 26.7 (26) | 74.3 ± 14.1 (46) | <0.001       |
| SANE at 12 months (n)   | 23.1 ± 34.5 (18) | 68.9 ± 23.9 (43) | <0.001       |
Reported differences in CFU-F concentrations between our group and others is likely attributed to several laboratory factors. For example, BMC prepared by trained laboratory processors in our facility is three to five times more concentrated with respect to nucleated cells than BMC prepared by standard bedside devices [26]. Further, our group utilizes a low plating density to obtain the highest number of CFU-F, as recommended by others [27]. In contrast, similar studies report their plating density to be eightfold higher, which may be suboptimal for quantifying CFU-F [12, 28]. Both laboratory factors contribute to BMC with greater CFU-F concentrations than those reported by others to date.

The predictive power of the progenitor cell concentration within BMC to discriminate patient outcomes, as calculated using the area under the ROC curve is, 68%, indicating that false negative and false positive results are expected. Notably, a considerable portion of responding knees (17 of 46, 37%) were treated with BMC having fewer than $18 \times 10^3$ CFU-F per mL (false negative). There

![Image](image-url)
may be factors present within BMC other than connective tissue progenitor cells, which contribute to the healing response. Elevated levels of the anti-inflammatory molecule, interleukin-1 receptor antagonist protein (IRAP), which is thought to improve OA through the inhibition of pro-inflammatory interleukin-1β (IL-1β) signaling have been found at increased levels in BMC [29]. Others have suggested the ratio of connective tissue progenitor to mononuclear cells within BMC may be another important and overlooked factor that contributes to healing [30]. In comparison, a smaller number of non-responding knees (6 of 26, 23%) received BMC injections having more than $18 \times 10^3$ CFU-F per mL (false positive). Additional investigation is necessary to better determine patient candidacy for BMC treatment of knee OA to help exclude those patients unlikely to respond, regardless of progenitor cell content.

Being a retrospective analysis of clinical treatment registry data from a single multi-physician, interventional orthopaedic pain practice, the present study is characterized by inherent heterogeneity. Variation in patient knee OA severity, BMC volume and cellularity, primary injection type (intra-articular and/or intraosseous), unilateral vs bilateral knee treatment, whether supporting structures (i.e., ligaments, tendons, meniscus) warranted concurrent treatment, participation in post-treatment physical therapy, and receiving additional injections, among others, must be considered when interpreting the results. The present study is small and preliminary in nature; no sample size or power calculations with respect to progenitor cell content were undertaken beforehand, as currently available CFU-F data, using our laboratory approach is limited. Some patients in the cohort were lost to follow-up (up to 15% at 12 months), resulting in missing data at that time point, and while a strong correlation between fresh and cryopreserved BMC with respect to the number of CFU-F has been previously demonstrated, it is possible that the cryopreservation and cryorecovery processes adversely affect the outgrowth of some colonies [20, 31]. Additional, well-controlled prospective studies are needed and would be essential to determining how the various biological components of BMC, including colony-forming connective tissue progenitor cells, contribute to tissue healing in the context of moderate to severe OA of the knee.

In conclusion, this is the first study to retrospectively assess CFU-F concentrations from autologous BMC samples for the purpose of relating progenitor cell content with patient-reported outcomes for knee OA. Overall, responders to treatment had greater concentrations of nucleated cells and colony-forming progenitor cells. Further investigation is warranted to more accurately classify patients that will be responders to treatment based on the cellularity of their BMC.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00264-022-05524-9.

Author contribution All authors contributed to the study design. Material preparation, data collection, and analysis were performed by DRB, ED, and NJS. Main manuscript preparations were written by DRB, BTM, and NJS, and all authors commented on previous versions of the manuscript. CWU contributed to new grading and data analysis for requests associated with requirements for resubmission. All authors read and approved the final manuscript.

Funding Funding was provided by the Centeno-Schultz Clinic and Regenexx.

Data availability Data may be made available upon request.

Code availability Not applicable.

Declarations

Ethics approval Approval of the clinical registry was obtained through the International Cellular Medicine Society Institutional Review Board (OHRP #IRB00002637).

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Consent to publish has been obtained from all participants.

Conflict of interest One or more of the authors has declared a potential conflict of interest. DRB, ED, and NJS are employed by Regenexx, LLC. CJIC is a shareholder, patent holder, and chief medical officer of Regenexx, LLC, and an owner of the Centeno-Schultz Clinic. BTM and CWU are employed by the Centeno-Schultz Clinic.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Crawford DC, Miller LE, Block JE (2013) Conservative management of symptomatic knee osteoarthritis: a flawed strategy? Orthop Rev (Pavia) 5(1):e2. https://doi.org/10.4081/or.2013.e2

2. Vora A, Borg-Stein J, Nguyen RT (2012) Regenerative injection therapy for osteoarthritis: fundamental concepts and
evidence-based review. PM&R 4(Suppl):S104–S109. https://doi.org/10.1016/j.pmrj.2012.02.005

3. Centeno CJ, Al-Sayegh H, Freeman MD, Smith J, Murrell WD, Bubnov R (2016) A multi-center analysis of adverse events among two thousand, three hundred and seventy two adult patients undergoing adult autologous stem cell therapy for orthopaedic conditions. Int Orthop 40(8):1755–1765. https://doi.org/10.1007/s00264-016-3162-y

4. Hendrich C, Franz E, Waertel G, Krebs R, Jager M (2009) Safety of autologous bone marrow aspiration concentrate transplantation: initial experiences in 101 patients. Orthop Rev (Pavia) 1(2):e32. https://doi.org/10.4081/or.2009.e32

5. Kim JD, Lee GW, Jung GH, Kim CK, Kim T, Park JH, Cha SS, You YB (2014) Clinical outcome of autologous bone marrow aspirates concentrate (BMAC) injection in degenerative arthritis of the knee. Eur J Orthop Surg Traumatol 24(8):1505–1511. https://doi.org/10.1007/s00590-013-1393-9

6. Centeno C, Sheinkop M, Dodson E, Stempeter I, Williams C, Hyzy M, Ichim T, Freeman M (2018) A specific protocol of autologous bone marrow concentrate and platelet products versus exercise therapy for symptomatic knee osteoarthritis: a randomized controlled trial with 2 year follow-up. J Transl Med 16(1):355. https://doi.org/10.1186/s12977-018-1736-8

7. El-Kadiry AE, Lumbao C, Salame N, Rafei M, Shammara R (2022) Bone marrow aspirate concentrate versus platelet-rich plasma for treating knee osteoarthritis: a one-year non-randomized retrospective comparative study. BMC Musculoskelet Disord 23(1):23. https://doi.org/10.1186/s12913-021-04910-5

8. Dulic O, Rasovic P, Lalic I, Kecjovic V, Gavrilovic G, Abazovic D, Maric D, Miskulin M, Bumbasirevic M (2021) Bone marrow aspirate concentrate versus platelet rich plasma or hyaluronic acid for the treatment of knee osteoarthritis. Medicina (Kaunas) 57(11). https://doi.org/10.3390/medicina57111193

9. Boffa A, Di Martino A, Andriolo L, De Filippis R, Poggi A, Kon E, Zaffagnini S, Filardo G (2021) Bone marrow aspirate concentrate injections provide similar results versus viscosupplementation up to 24 months of follow-up in patients with symptomatic knee osteoarthritis. A randomized controlled trial. Knee Surgery, Sports Traumatology, Arthroscopy. https://doi.org/10.1007/s00167-021-06793-4

10. Shapiro SA, Kazmerchak SE, Heckman MG, Zubair AC, O’Connor MI (2017) A prospective, single-blind, placebo-controlled trial of bone marrow aspirate concentrate for knee osteoarthritis. Am J Sports Med 45(1):82–90. https://doi.org/10.1177/0363546516662455

11. Muschler GF, Midura RJ (2002) Connective tissue progenitors: practical concepts for clinical applications. Clin Orthop Relat Res 395:56–80. https://doi.org/10.1097/00002089-200206000-00008

12. Hernigou P, Poignard A, Beaupatan F, Rouard H (2005) Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. J Bone Joint Surg Am 87(7):1430–1437. https://doi.org/10.2106/JBJS.D.02215

13. Pettine KA, Murphy MB, Suzuki RK, Sand TT (2015) Percutaneous injection of autologous bone marrow concentrate cells significantly reduces lumbar discogenic pain through 12 months. Stem Cells 33(1):146–156. https://doi.org/10.1002/stem.1845

14. Kellgren JH, Lawrence JS (1957) Radiological assessment of osteo-arthritis. Ann Rheum Dis 16(4):494–502. https://doi.org/10.1136/ard.16.4.494

15. Park HJ, Kim SS, Lee SY, Park NH, Park JY, Choi YJ, Jeon HJ (2013) A practical MRI grading system for osteoarthritis of the knee: association with Kellgren-Lawrence radiographic scores. Eur J Radiol 82(1):112–117. https://doi.org/10.1016/j.ejrad.2012.02.023

16. Hernigou P, Homma Y, Flouzat Lachaniette CH, Poignard A, Allain J, Chevallier N, Rouard H (2013) Benefits of small volume and small syringe for bone marrow aspirations of mesenchymal stem cells. Int Orthop 37(11):2279–2287. https://doi.org/10.1007/s00264-013-1707-z

17. Friedlis MF, Centeno CJ (2016) Performing a better bone marrow aspiration. Phys Med Rehabil Clin N Am 27(4):919–939. https://doi.org/10.1016/j.pmrj.2016.06.009

18. Centeno C, Pitts J, Al-Sayegh H, Freeman M (2014) Efficacy of autologous bone marrow concentrate for knee osteoarthritis with and without adipose graft. Biomed Res Int 2014:370621. https://doi.org/10.1155/2014/370621

19. Mantripragada VP, Pizziu NS, George J, Bova W, Ng M, Boehm C, Muschler GF (2019) Reliable assessment of bone marrow and bone marrow concentrates using automated hematology analyzer. Regen Med 14(7):639–646. https://doi.org/10.2217/rme-2018-0173

20. Berger DR, Aune ET, Centeno CJ, Steinmetz NJ (2020) Cryopreserved bone marrow aspirate concentrate as a cell source for the colony-forming unit fibroblast assay. Cytotherapy 22(9):486–493. https://doi.org/10.1016/j.jcryt.2020.04.091

21. Centeno C, Cartier C, Stempeter I, Dodson E, Freeman M, Azuiké U, Williams C, Hyzy M, Silva O, Steinmetz N (2021) The treatment of bone marrow lesions associated with advanced knee osteoarthritis: comparing intraosseous and intraarticular injections with bone marrow concentrate and platelet products. Pain Physician 24(3):E279–E288

22. Binkley JM, Stratford PW, Lott SA, Riddle DL (1999) The lower extremity functional scale (LEFS): scale development, measurement properties, and clinical application. North American Orthopaedic Rehabilitation Research Network. Phys Ther 79(4):371–383

23. Rene F, Casimiro L, Tremblay M, Brousseau L, Chea P, Letourneau L, Silva M, Stockwell V, Bergeron LP (2011) Fiabilité ‘test testet et validité’ de construit de la version française: aise de L’E chelle fonctionnelle des membres inférieurs (E’FMI), partie II. Physiother Can 63(2):249–255. https://doi.org/10.3138/ptc.2010-12F

24. Centeno CJ, Al-Sayegh H, Bashir J, Goodyear S, Freeman MD (2015) A dose response analysis of a specific bone marrow concentrate treatment protocol for knee osteoarthritis. BMC Musculoskelet Disord 16:258. https://doi.org/10.1186/s12891-015-0714-z

25. Kon E, Boffa A, Andriolo L, Di Martino A, Di Matteo B, Magarelli N, Maracci M, Onorato F, Trenti N, Zaffagnini S, Filardo G (2021) Subchondral and intra-articular injections of bone marrow concentrate are a safe and effective treatment for knee osteoarthritis: a prospective, multi-center pilot study. Knee Surg Sports Traumatol Arthrosc. https://doi.org/10.1007/s00167-021-06530-0

26. Hegde V, Shonuga O, Ellis S, Fragomen A, Kennedy J, Kudryashov V, Lane JM (2014) A prospective comparison of 3 approved systems for autologous bone marrow concentration demonstrated nonequivalency in progenitor cell number and concentration. J Orthop Trauma 28(10):591–598. https://doi.org/10.1097/BOT.0000000000001113

27. Maeschi K, Rustichelli D, Calabrese R, Gunetti M, Sanavio F, Castiglia S, Risso A, Ferrero I, Tarella C, Fagiolli F (2012) Multipotent mesenchymal stromal stem cell expansion by plating whole bone marrow at a low cellular density: a more advantageous method for clinical use. Stem Cells Int 2012:920581. https://doi.org/10.1155/2012/920581

28. Veyrat-Masson R, Boiret-Dupre N, Rapateli C, Descamps S, Guilouard L, Guerin JJ, Pigeon P, Boisgard S, Chassagne J, Berger MG (2007) Mesenchymal content of fresh bone marrow: a proposed quality control method for cell therapy. Br J Haematol 139(2):312–320. https://doi.org/10.1111/j.1365-2414.2007.06786.x

29. Cassano JM, Kennedy JG, Ross KA, Fraser EJ, Goadle MB, Forliti LA (2018) Bone marrow concentrate and platelet-rich plasma
differ in cell distribution and interleukin 1 receptor antagonist protein concentration. Knee Surg Sports Traumatol Arthrosoc 26(1):333–342. https://doi.org/10.1007/s00167-016-3981-9

30. Chaput CD, Shar A, Jupiter D, Hubert Z, Clough B, Krause U, Gregory CA (2018) How stem cell composition in bone marrow aspirate relates to clinical outcomes when used for cervical spine fusion. PLoS One 13(9):e0203714. https://doi.org/10.1371/journal.pone.0203714

31. Kaplan A, Sackett K, Sumstad D, Kadidlo D, McKenna DH (2017) Impact of starting material (fresh versus cryopreserved marrow) on mesenchymal stem cell culture. Transfusion 57(9):2216–2219. https://doi.org/10.1111/trf.14192

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.