Research Article

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Regenerative Medicine

The role of osteoarthritis severity, BMI and age on clinical efficacy of bone marrow aspirate concentrate in the treatment of knee osteoarthritis

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Aim: The aim of this study was to assess whether BMI, severity of knee osteoarthritis, age and gender have any influence on the final clinical results of bone marrow aspirate concentrate injection. **Method:** A total of 111 study participants with painful knee osteoarthritis and different characteristics concerning before mentioned factors underwent bone marrow aspirate concentrate (BMAC) therapy and were followed up for 1 year. **Result:** Significant pain and functional improvement were observed in all participant groups. Participants' age and BMI did not influence the clinical outcome, but there was an influence of OA severity, especially among older patients. **Conclusion:** This study shows that BMAC therapy is effective. Younger patients with milder OA changes could be better candidates for long-lasting and more efficient BMAC therapy.

Clinical Trial Registration: NCT03825133 (ClinicalTrials.gov)

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Keywords: body mass index • bone marrow aspirate concentrate • knee osteoarthritis • osteoarthritis severity • patient's age • regeneration

Osteoarthritis (OA) is a disorder involving inflammation and subsequent damage and deterioration to bone and joint cartilage, which causes progressive pain, loss of function and disability. The destruction of the tissue and symptoms of osteoarthritis were previously thought to be caused by constant and chronic pressure on the bones and biomechanical damage, also known as 'wear and tear'. However, today, it is known that the actual etiology of the disease is much more complex, and includes inflammation, as the main drive in the pathogenesis of the disease [1,2]. Osteoarthritis is one of the main global causes of disability, pain, social and psychological distress, with knee and hip osteoarthritis being ranked as the 11th highest contributor to global disability [3]. Moreover, the burden of OA on individuals and healthcare systems is likely to increase due to growing incidence caused by the aging population and higher prevalence of obesity [4], which are two well-known risk factors for the development of osteoarthritis. Other risk factors that can influence to a greater or lesser extent the incidence of osteoarthritis have previously been classified as local (such as trauma, joint injury and inactivity) and systemic, with obesity, diet and bone metabolism being modifiable, and age, sex, ethnicity and genetics being non-modifiable risk factors [5].

Taking into consideration the complexity of the etiology and pathogenesis of osteoarthritis, as well as the variability of risk factors, establishing a single clinical presentation of the disease can be demanding. A recent review [6] has

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defined six different clinical phenotypes of knee OA: chronic pain phenotype, with a central disruption in pain regulation changing the pathophysiology, inflammatory OA, with overexpression of genes coding for inflammatory cytokines, metabolic phenotype, which is strongly influenced by metabolic factors such as obesity, dyslipidemia, diabetes and hypertension, OA as a consequence of changes in the metabolism of bone and cartilage, mechanical overload as a mechanism responsible for the disease and finally OA as a result of minimal joint damage over a longer period of time. Osteoarthritis can be classified based on radiological findings using the Kellgren-Lawrence (KL) [7] scale into four grades with increasing severity based on the presence of osteophytes, periarticular ossicles, sclerosis of bone subsequently causing narrowing of joint cartilage and presence of pseudocysts. Recently, studies have also focused on other types of OA that cannot be recognized by using the KL scale, such as early osteoarthritis or post-traumatic with emphasis on different clinical and radiological signs [8,9].

Treatment of osteoarthritis can be broadly divided into surgical and non-surgical treatment modalities. Surgical management can be further classified into joint preserving procedures such as arthroscopy or joint replacement like total knee arthroplasty which has been the most effective [10]. Non-surgical treatment modalities include pharmacological treatments like the use of paracetamol, NSAIDs, corticosteroid injections, different innovative therapies or injections [11], as well as physical therapy and lifestyle modifications as symptomatic treatments [12]. In the past decade, the use of biological and regenerative therapeutic options for the treatment of OA has been increasing, especially because of the growing knowledge on the role of inflammation, and how it leads to the development of OA. The imbalance in immune and metabolic mediators in the joint environment is thought to affect the pathogenesis of the disease. It has been observed that inflammatory mediators are elevated in various osteoarthritic (joint) tissue including synovial fluid and membrane, cartilage and subchondral bone [13]. The cellular damage and tissue injury lead to the release of damage-associated molecules, which is followed by an inflammatory reaction. In addition, activation of the inflammatory response causes the release of mediators that further promote inflammation and disrupt tissue homeostasis [13]..

In today's practice, the use of bone marrow aspirate concentrate (BMAC) for the treatment of osteoarthritis is increasing. BMAC is a type of autologous cell-based therapy composed of different varieties of cells, including mesenchymal stem cells (MSCs), hematopoietic stem cells, platelets, and active molecules such as cytokines and various growth factors [14,15]. MSCs are multipotent adult stem cells that have tremendous therapeutic potential because of their ability to self-renew, differentiate into different cell types, regulate signaling pathways through paracrine and autocrine actions, and also have a role in immunomodulation [16,17]. Even though mesenchymal stem cells make up only between 0.001 and 0.01% of the BMAC, they play an important role in regulating various signaling pathways and stimulating surrounding tissue to repair itself [15]. The therapeutic potential of BMAC is in the combined action of the cellular components and bioactive molecules to promote regeneration, anti-inflammatory activities and modulate the immune response [18]. Moreover, since one of the leading causes of OA is inflammation, using MSCs for therapy could improve cartilage repair [19,20]. According to previous case studies treatment of symptomatic OA with BMAC showed promising results and great improvement in the patients' condition [21]. Additionally, BMAC therapy is widely recognized as a safe treatment option.

The aim of this study was to evaluate how certain risk factors such as BMI, age, and the clinical severity of OA measured through the KL scale, influence the efficacy of BMAC injections for the treatment of osteoarthritis over time. Our hypothesis was that BMAC therapy is more efficient for younger patients, with lower BMI and KL grade. We have formulated a hypothesis that revolves around the potential higher biological potency of younger patients in relation to biological treatments, as well as their potentially stronger response in joints affected by milder stages of OA. Additionally, we included the direct influence of obesity on the development and progression of OA in our consideration. We believe that this study is necessary to elucidate a patient specific factors that could affect BMAC therapy results.

Materials & methods

Study design

This prospective clinical study was performed at the Clinical Center of Vojvodina, Clinic for orthopedic surgery between April 2016 and December 2017. After being selected, all patients were subjected to a clinical examination and necessary diagnostic procedures, informed about the study protocols and signed an informed consent form in accordance with Helsinki Declaration for medical research including human subjects. An approval by the Research Ethics Committee has been obtained (EC KCV 319–321/16). The study was registered in ClinicalTrials.gov

Table 1. Patients' summary statistics divided by Kellgren-Lawrence score.

Variable	Osteoarthritis severity		
	Kellgren-Lawrence = 2	Kellgren-Lawrence = 3	Kellgren-Lawrence = 4
Sex			
Male	27 (55.1%)	23 (50%)	9 (56.2%)
Female	22 (44.9%)	23 (50%)	7 (42.8%)
BMI			
Mean BMI	27.49	29.56	29.34
SD BMI	4.52	3.92	5.62
Age			
Mean age	57.16	65.06	67.94
SD age	10.54	8.98	10.78
Visual analog scale pain (VAS p) scale			
Mean pre op VAS p	6.90	7.41	7.31
SD	1.53	1.64	1.74
KOOS pain scale			
Mean	48.43	47.4	50.06
SD	18.50	16.16	20.08
KOOS overall			
Mean	43.39	42.80	43.62
SD	17.34	16.58	16.80
WOMAC			
Mean	41.78	47.62	42.77
SD	19.32	16.51	22.09
IKDC			
Mean	36.31	36.21	35.77
SD	14.66	13.30	16.01

(NCT03825133) and the results presented in this paper are an excerpt from this registered study. The study was performed by CONSORT guidelines.

Patient characteristics

A total of 111 patients were selected to be included in the study. In order to participate in the study, patients needed to meet inclusion criteria. After careful review of all the test results, we set inclusion criteria for this study: adult patients with symptomatic knee OA, KL grade from 2 to 4 (2) with symptoms for at least 12 months. Exclusion criteria for the study were as following: knee instability, severe misalignment (valgus/varus over 10 degrees), inflammatory arthritis such as rheumatoid arthritis and ankylosing spondylitis and underlying diseases such as hematologic disorders, septicemia, coagulopathy, neoplasm, active infection and immune deficiency or other chronic diseases such as COPD or cardiomyopathy.

Patient's summary statistics divided by KL score is presented in Table 1.

BMAC sampling & processing

With the patient lying in the supine position, the region on and around the tibial tuberosity was carefully draped and cleaned with an antiseptic solution. After the area was prepared, local anesthesia (Lidocaine 2 ml) was infiltrated from the skin and subcutaneous tissue to the periosteum. A minimal incision using a blade was performed, and a trocar was placed. Next, using two 50 ml syringes previously filled with 10 ml of anticoagulant acid citrate dextrose formula A (ACD-a), about 60 ml of autologous bone marrow was aspirated in each syringe. During the aspiration, the trajectory of the needle was moved several times in order to achieve an easier and better aspiration of the bone marrow. The obtained bone marrow from the syringes was placed into the Angel separating system (by Arthrex®), centrifuged and processed, after which 5–6 ml of BMAC was obtained and combined with 4–5 ml of autologous plasma. Finally, 10 ml of BMAC and autologous plasma (combined content) was carefully injected into the treated knee as single dose trough three most used portals: suprapatellar lateral and infrapatellar medial and lateral [22,23].

Data collection & patient follow-up

Before the intervention, patients were asked to fill out questionnaires regarding the symptoms and subjective findings they experience on their knees. Immediately following the intervention, patients were asked to describe the subjective pain they felt during the administration of BMAC, using the Visual Analog Scale Pain (VAS p). The same was asked of patients on days 3, 7, 14 and 21 post-intervention. The questionnaires used were KOOS pain and KOOS overall scale, WOMAC and IKDC scale. Patients were then asked to come back for a check-up after 1, 3, 6, 9 and 12 months. During follow-ups, patients were examined and asked to fulfill questionnaires. Furthermore, they were also asked to note any unwanted side effects that may have appeared related to the intervention, as well as the use of pain medication and physical therapy.

Statistical analyses

As the data is consisted of repeated pain measures in the same set of patients, linear mixed models (LMMs) were used for analysis. LMMs are an extension of the linear regression models, that allow for correlation between observations and the use of longitudinal data. Most importantly, LMMS can incorporate both fixed and random effects. In this analysis, covariates such as BMI, sex, age and the severity of osteoarthritis were modelled to have fixed effects, whereas a random effect was included to account for the patient-level variability in the model line intercept.

First, exploratory analysis was completed in order to explore any possible correlation between the variables used in the models, as well as understand further the underlying distributions of the variables used. For better visual representation, graphs showing the variation in values of all 5 pain scales for each patient characteristic of interest were shown. For this purpose, continuous variables have been converted into categorical. BMI and age were split up into three categories: <25, 25–30, >30, and <55, 55–65, >65, respectively. Second, five LMMs have been fitted, where an outcome variable for each model was a different pain scale measurement. Once the models were fitted, effect sizes were reported, and statistical significance of the variables was assessed using the p-values. We also calculated the power of the observed results for the statistically significant variables. Statistical analysis was performed using R version 4.2.2.

Results

Summary statistics

The flow chart showing patient exclusion criteria and the final number of patients in the dataset can be seen in Figure 1.

In total, 111 patients were treated and screened over 12 months and fulfilled the pre-intervention tests. There were 57 men (51.4%) and 54 women (48,6%). We diagnosed 49 patients as KL grade II (44.1%), 46 patients as KL grade III (41,4%) and 16 patients as KL grade IV (14.4%). In Table 1, summary statistics of for the dataset can be seen, split into three different groups depending on the KL value. The variables of interest that are reported are sex, age, BMI and the pre-operation scores of the five pain scales used.

From the table, it can be concluded that participants with more severe OA tend to be older. There are no other observable trends for the rest of the variables in the table, with the values randomly varying across the three subgroups. In terms of sex, there is an equal number of both males and females in the subgroup when KL = 3, whereas there is a slightly higher number of males in the other two subgroups.

VAS pain scale

In Figures 2, the distribution of VAS pain scores can be seen for four different measurements: before operation, and then after 3, 7, 14 and 21 days. Participants are divided by their KL score, BMI and age. From the graph it can be concluded that there is a clear improvement in pain scores. However, as the boxes for all groups in the three graphs overlap, it cannot be said that there is a greater improvement for a specific group. Second, in Table 2, the summary results for Model 1 can be seen, where VAS pain scale scores were used as the outcome variable. The variables that have been included in the model are age, BMI, sex, KL and time, where time can either be pre operation, 3, 7, 14 or 21 days after. As seen from the table, time is the only variable that is statistically significant. The calculated statistical power for the time variable was 100%.

KOOS pain, KOOS overall, WOMAC & IKDC

The same analysis was applied to the KOOs pain, KOOS overall, WOMAC and IKDC pain scales. In Figures 3–5 the distribution of the four pain scores with standard deviations can be seen for the 6 repetitions of measurement:

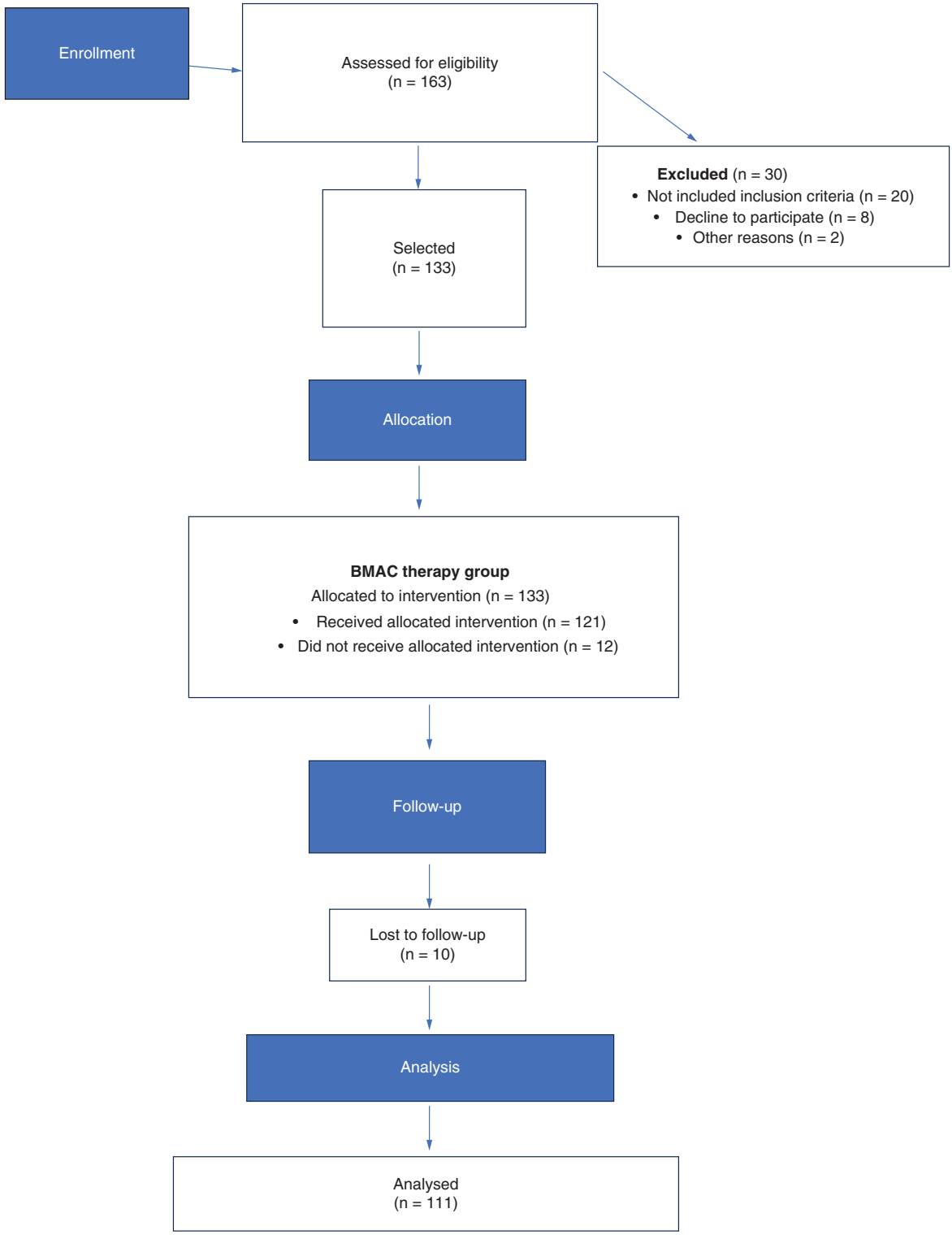


Figure 1. Flow diagram of the study.
BMAC: Bone marrow aspirate concentrate.

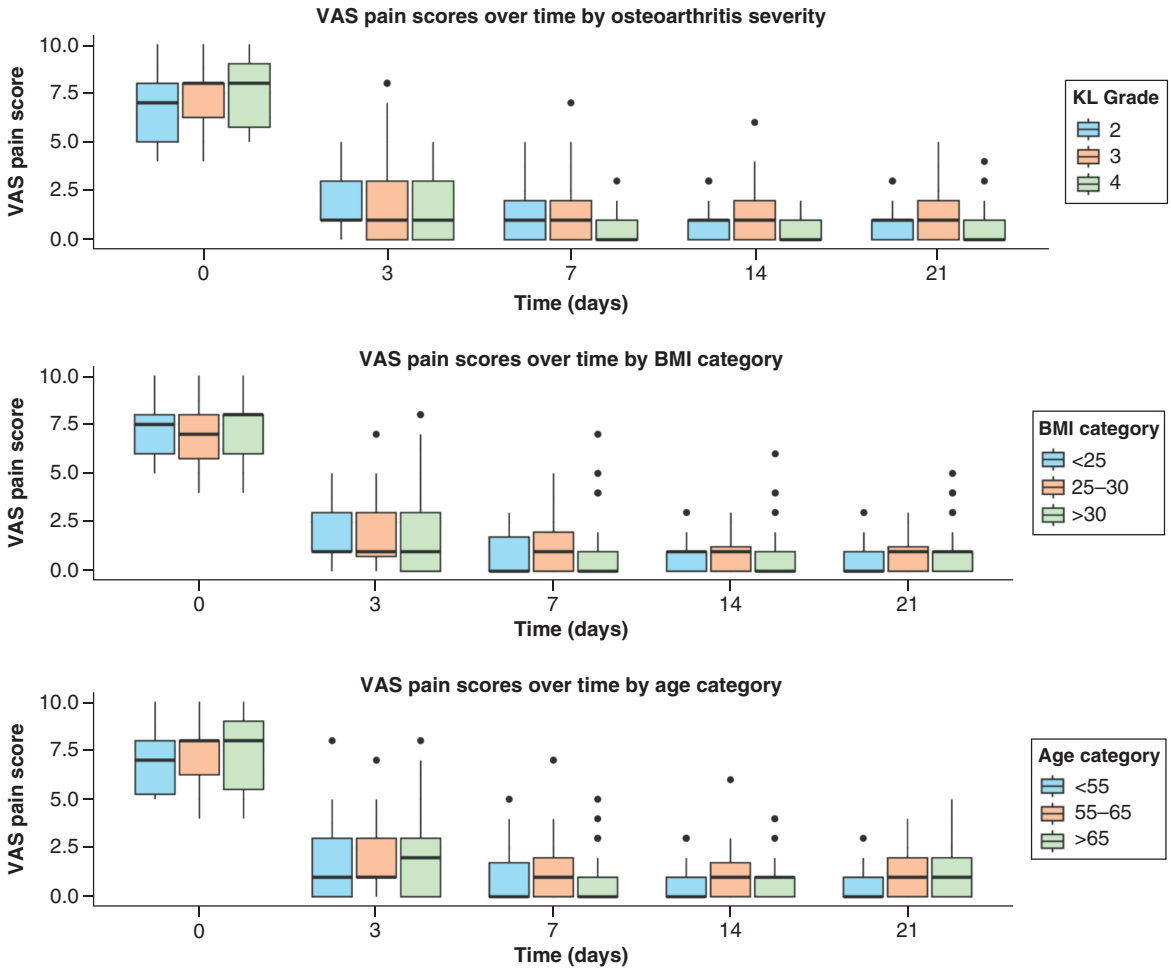


Figure 2. Visual analog scale pain score over time by Kellgren-Lawrence score, BMI and age.
VAS: Visual analog scale.

Table 2. Effect sizes and p-values for Model 1 with visual analog scale pain score as the outcome variable.

Variable	Model 1	
	Effect size	p-value
Age	0.02	0.09
BMI	-0.01	0.64
Sex – Female	0.23	0.25
Time – 3 days	-5.36	<2e–16
Time – 7 days	-6.08	<2e–16
Time – 14 days	-6.28	<2e–16
Time – 21 days	-6.25	<2e–16
KL = 3	0.25	0.28
KL = 4	-0.29	0.38

before operation and then after 1, 3, 6, 9 and 12 months. The patients were split into KL, BMI and age categories. As for the VAS pain scale, it is clear that there are improvements in the pain levels, however, there are no clear differences in the improvement for specific groups.

The results from the analysis using LMM models can be seen in Table 3, Table 4, Table 5 and Table 6. In Model 2 the KOOS pain scale was used as the outcome variable. The same variables were included in the model as specified for Model 1, except the time scale was now either pre-operation, 1, 3, 6, 9 or 12 months after. Age and

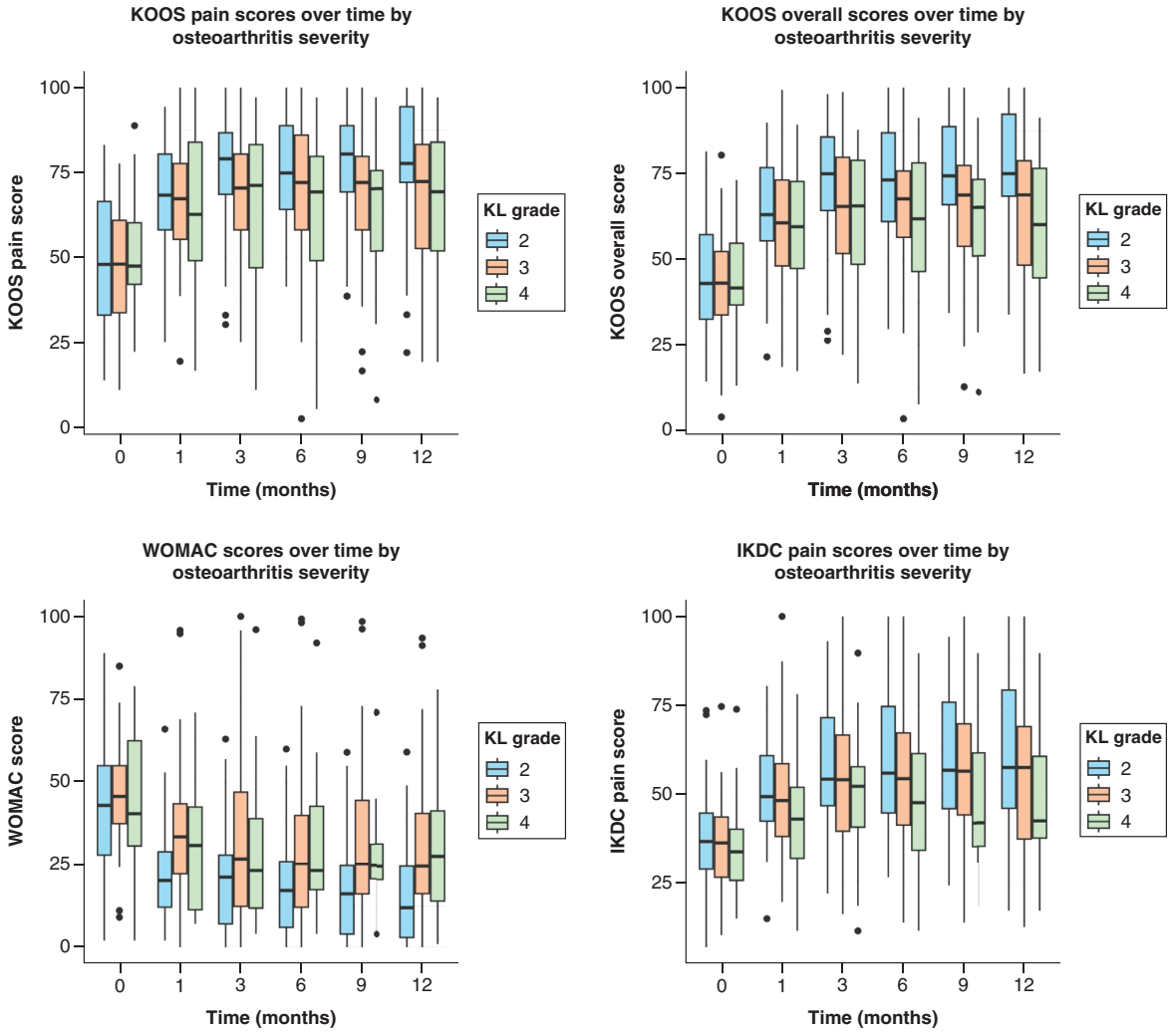


Figure 3. KOOS pain, KOOS overall, WOMAC and IKDC pain score over time by Kellgren-Lawrence score.

time were found to be statistically significant in this model, with age having a negative effect on the pain score and time a positive effect. Statistical power for the age variable was 74 and 100% for the time variable.

In Model 3, KOOS overall pain scale was the outcome variable, where age and time were also the only statistically significant variables, as seen from Table 4. Similar to the results from the previous model, statistical power for the age variable was 74.8 and 100% for the time variable.

In Model 4, WOMAC scale was used as the outcome variable. Time and KL grade 3 are statistically significant, with KL = 3 shown to lead to worse pain scores. However, in this model age was not found to be statistically significant, although the p-value is close to 0.05 significance level. The statistical power for the KL variable was 54.70 and 100% for the time variable.

Lastly, in Model 5, the outcome variable was the IKDC pain scale. As in models 2 and 3, age and time were found to be statistically significant, where age was associated with a worse pain score. The calculated statistical power for the age variable was 98% and 100% for the time variable.

Discussion

This study showed that the effect of BMAC application in OA treatment is correlated with age, however, there was no correlation between clinical results and BMI. Only one model showed that there was a correlation with OA severity. It can be concluded that there is sufficient evidence supporting our hypothesis that the BMAC injections have a greater efficacy among younger patients. Model 4 would also suggest that the treatment is more efficient

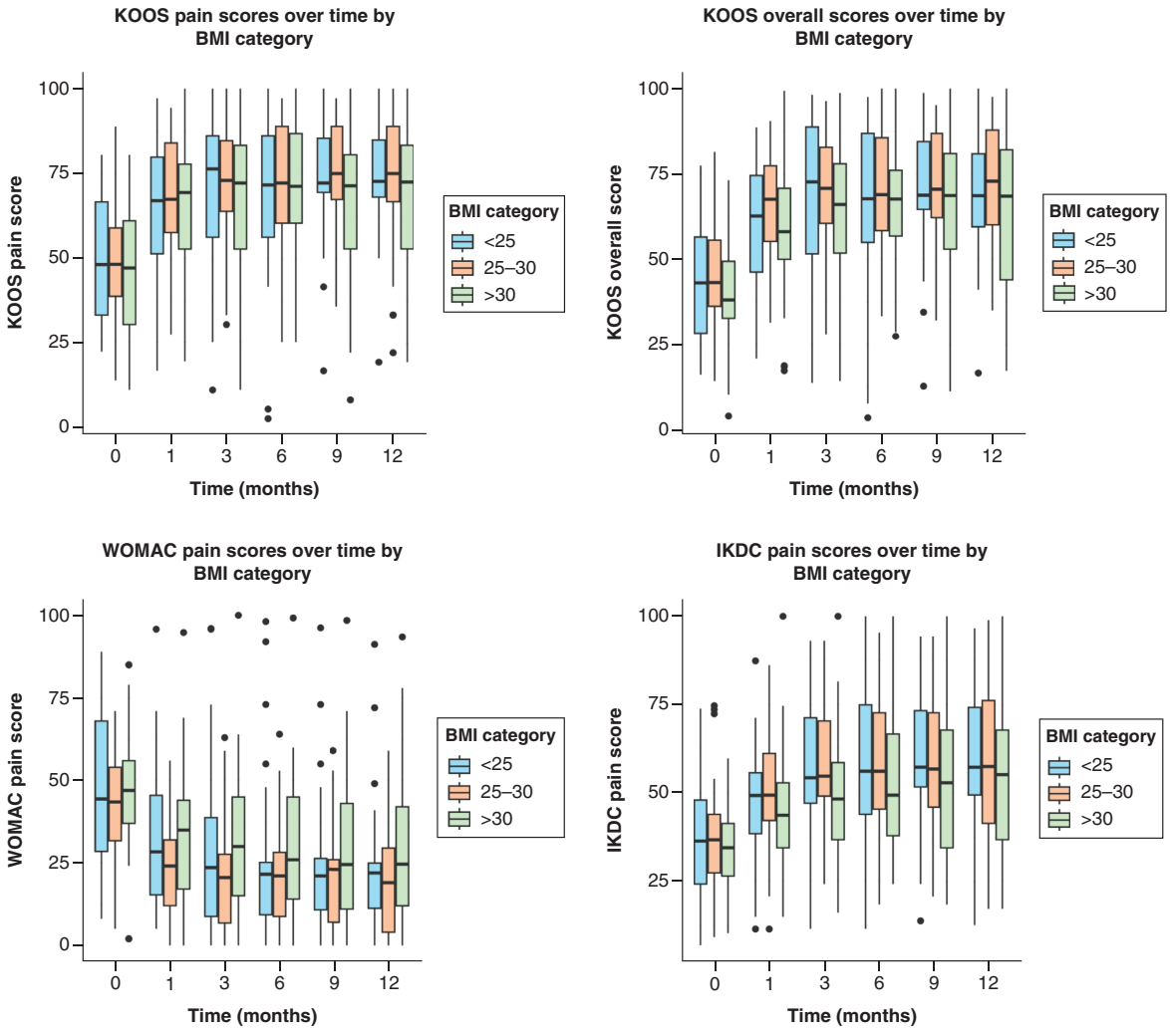


Figure 4. KOOS pain, KOOS overall, WOMAC and IKDC pain score over time by BMI category.

in patients with lower OA severity, however, this should be further confirmed in a larger dataset, where there would be greater statistical power. From these results, we can conclude that BMAC is a promising option for OA treatment. During the procedure, harvested cells from bone marrow are concentrated by performing density gradient centrifugation, providing higher concentrations of cells as well as secreted growth factors [17]. Moreover, injections were administrated trough 3 most often used knee portals, which was shown to have no effect on the final clinical result [23].

In general, there is a lack of published studies with the aim of correlating factors that could influence the results of BMAC treatment in the knee OA. Our results corroborate those of a previous study, where it was observed that BMAC therapy has a better effect in younger patients, with lower KL grade and BMI [24]. According to the study performed by Gursoy *et al.* applying BMAC injections in the early or moderate stage of osteoarthritis has shown better results [25]. Same findings were found by GB Kim [26] with confirmation of BMAC efficacy at KL grade II rather than KL gr III or IV. On the other hand, results from another study couldn't find a statistically significant correlation between better clinical outcome and patients' KL grade, after the BMAC therapy [27]. Although there is clear evidence about the direct effect of BMI on the development of knee OA [28], we didn't find correlation between BMI and clinical results of BMAC therapy. Conrozier *et al.* [29] recently published a study with similar findings concerning intraarticular treatment of the OA with hyaluronic acid, describing better results among patients with lower BMI but without statistical significant differences. Gobbi *et al.* [30] investigated a correlation between patients' age and the efficacy of a regenerative scaffold in the treatment of chondral lesions of the knee, where they did not

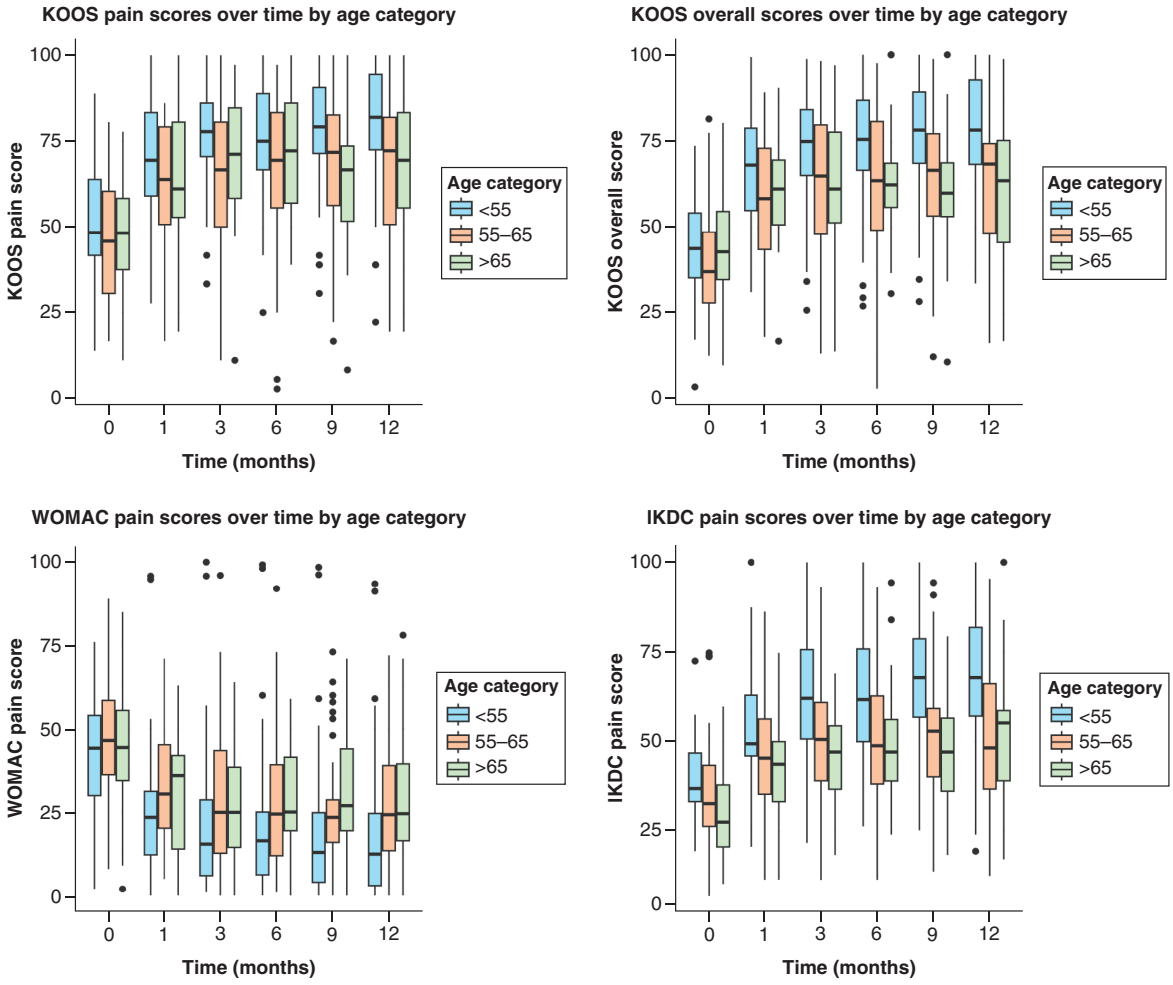


Figure 5. KOOS pain, KOOS overall, WOMAC and IKDC pain score over time by age category.

Table 3. Effect sizes and p-values for Model 2 with KOOS pain score as the outcome variable.

Variables	Model 2	
	Effect size	p-value
Age	-0.39	0.01
BMI	0.18	0.59
Sex – Female	-0.54	0.85
Time – 1 month	18.89	<2e–16
Time – 3 months	22.15	<2e–16
Time – 6 months	23.06	<2e–16
Time – 9 months	23.21	<2e–16
Time – 12 months	24.38	<2e–16
Kellgren-Lawrence = 3	-2.01	0.55

find any differences between groups of those younger than 45 and those older than 45 years of age. Migliorini *et al.* [31] stated that patients treated at earlier degeneration stages reported statistically significant greater outcomes, which is in accordance with our findings. In a broad study including a Bayesian network meta-analysis, Migliorini *et al.* [32] stated a role of different factors affecting a efficacy of intraarticular injections of biological preparations that are also observed by our study. Finally, Freitag *et al.* [33] in their recently published research, included 329

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Table 4. Effect sizes and p-values for Model 3 with KOOS overall score as the outcome variable.

Variables	Model 3	
	Effect size	p-value
Age	-0.40	0.01
BMI	0.09	0.79
Sex – Female	-0.78	0.79
Time – 1 month	19.20	<2e–16
Time – 3 months	23.85	<2e–16
Time – 6 months	24.58	<2e–16
Time – 9 months	25.66	<2e–16
Time – 12 months	25.34	<2e–16
Kellgren-Lawrence = 3	-3.08	0.38

Table 5. Effect sizes and p-values for Model 3 with WOMAC pain score as the outcome variable.

Variables	Model 4	
	Effect size	p-value
Age	0.28	0.01
BMI	-0.09	0.79
Sex – Female	1.05	0.79
Time – 1 month	-15.26	<2e–16
Time – 3 months	-17.71	<2e–16
Time – 6 months	-19.14	<2e–16
Time – 9 months	-20.10	<2e–16
Time – 12 months	-20.00	<2e–16
Kellgren-Lawrence = 3	8.32	0.38

Table 6. Effect sizes and p-values for Model 3 with IKDC Pain Score as the outcome variable.

Variables	Model 5	
	Effect size	p-value
Age	-0.57	8.78e–05
BMI	-0.09	0.77
Sex – Female	-2.33	0.40
Time – 1 month	12.94	<2e–16
Time – 3 months	18.42	<2e–16
Time – 6 months	19.99	<2e–16
Time – 9 months	20.74	<2e–16
Time – 12 months	21.42	<2e–16
Kellgren-Lawrence = 3	0.28	0.93

patients treated with adipose-derived mesenchymal stem cells and arrived at conclusions very similar to ours. They found that participants' age and weight did not influence the clinical outcome.

It is important to mention that almost immediately after the BMAC application, a subjective reduction in pain was observed using the VAS pain scale, in all patients, regardless of BMI, age or clinical severity. The reduction in pain was most significant in the first 3 days after the procedure, with a continued and steady decrease during the next 27 days. Our results are in accordance with a previous study, where it was found that the BMAC application provided pain relief and recovery [34]. With regards to BMI, we have not observed a statistically significant improvement in all scores. The improvements in comparison between pre-intervention values and post-intervention values stayed consisted over time for all three groups, confirming BMAC efficacy.

There are several limitations to this study. First, as all patients come from the same clinic, it is possible that there is some selection bias, and that the patient dataset is not representative of the general population. It would be greatly beneficial to repeat this study in a larger dataset with patients from multiple geographical locations, as to make the

results applicable to the wider population. Furthermore, a larger dataset would also increase the power of the study and elucidate any possible associations that were missed due to the limited size of this cohort. Another possible limitation is the use of linear mixed models. It is plausible that for associations that were not observed, such as that between the pain scale scores and BMI, there is a non-linear relationship that better fits the data. Last, it could be argued that the choice of the use of BMI in the models might have been the wrong one, as it is such a global variable that encompasses complex underlaying measurements. The use of more specific weight measurements, such as fat percentage or muscle mass, might be better explain the underlying causal relationship and show true association.

Conclusion

Considering the 12 months functional outcomes of the patients, it was observed that the application of bone marrow aspirate concentrate provided functional recovery in knee OA. We concluded that age is associated with the functional results of the treatment with BMAC, with older patients having worse pain scores. We found partial evidence suggestive of an association between the severity of osteoarthritis and the clinical results of the therapy. We did not find that there was an association between patients' BMI with final clinical result of this therapy. Further studies are necessary in order to determine the effectiveness of applications and long-term follow-up.

Summary points

- Osteoarthritis (OA) is a major cause of disability, pain and psychological distress globally, and its incidence is expected to rise due to factors like an aging population and increased obesity rates. Risk factors for OA include both modifiable factors like obesity and non-modifiable factors like age and genetics. The clinical presentation of OA varies due to its multifactorial etiology, and different clinical phenotypes have been identified.
- Bone marrow aspirate concentrate (BMAC) is a type of autologous cell-based therapy that contains various types of cells, including mesenchymal stem cells, which have the potential to promote regeneration, reduce inflammation and modulate the immune response. Previous case studies have shown promising results with BMAC therapy for symptomatic OA.
- The aim of this study was to evaluate how certain risk factors such as BMI, age, and the clinical severity of OA influence the efficacy of BMAC injections for the treatment of OA over time. The hypothesis was that BMAC therapy would be more effective in younger patients with lower BMI and milder stages of OA. The study aimed to provide insights into patient-specific factors that may affect the outcomes of BMAC therapy.
- This prospective clinical study was conducted at a clinic for orthopedic surgery. A total of 111 patients with symptomatic knee OA were included in the study. Inclusion criteria included adult patients with KL grade 2–4 OA and symptoms lasting at least 12 months. Exclusion criteria included knee instability, severe misalignment, inflammatory arthritis and underlying diseases. BMAC was obtained from the patients' bone marrow, processed, and injected into the affected knee. Patient data, including pain scores and functional outcomes, were collected before the intervention and at various follow-up time points up to 12 months. Linear mixed models were used for statistical analysis to assess the influence of BMI, age, and OA severity on the outcomes of BMAC therapy.
- The study included 111 patients, with 57 men and 54 women. The distribution of patients across different KL grades was as follows: 49 patients with KL grade II, 46 patients with KL grade III, and 16 patients with KL grade IV. The summary statistics showed that patients with more severe OA tended to be older, but there were no clear trends for other variables. The analysis of pain scores using different scales (VAS, KOOS, WOMAC and IKDC) showed improvements in pain levels over time, but no specific group showed a greater improvement. The statistical models revealed that age and time were significant factors influencing pain scores, while BMI and OA severity had mixed results across the models.
- The study findings suggest that the efficacy of BMAC therapy for OA may be influenced by age but not BMI. Younger patients may have a greater biological potency and stronger response to treatment, while older patients may experience less improvement in pain scores. The severity of OA, as measured by the KL scale, showed mixed results in its influence on treatment outcomes. These findings contribute to a better understanding of patient-specific factors that may affect the effectiveness of BMAC therapy for OA.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Data sharing statement

The authors certify that this manuscript reports original clinical trial data. Deidentified, individual data that underlie the results reported in this article (text, tables, figures and appendices), along with the study protocol will be available indefinitely for anyone who wants access to them.

References

Papers of special note have been highlighted as: ● of interest; ●● of considerable interest

1. Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). *Osteoarthr. Cartil.* 21(1), 16–21 (2013).
2. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheumatol.* 64(6), 1697–1707 (2012).
3. Cross M, Smith E, Hoy D *et al.* The global burden of hip and knee osteoarthritis: Estimates from the Global Burden of Disease 2010 study. *Ann. Rheum. Dis.* 73(7), 1323–1330 (2014).
4. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. *Lancet* 393(10182), 1745–1759 (2019).
5. Johnson VL, Hunter DJ. The epidemiology of osteoarthritis. *Best Pract. Res. Clin. Rheumatol.* 28(1), 5–15 (2014).
6. Dell'Isola A, Allan R, Smith SL, Marreiros SSP, Steultjens M. Identification of clinical phenotypes in knee osteoarthritis: a systematic review of the literature. *BMC Musculoskelet. Disord.* 17(1), 425 (2016).

●● **In this study, the authors shed light on a completely new perspective of osteoarthritis (OA), opening up new horizons for clinical research in this field.**

7. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis Ann Rheum Dis 1957. *Ann. Rheum. Dis.* 16(4), 494–503 (1956).
8. Mahmoudian A, Lohmander LS, Mobasheri A, Englund M, Luyten FP. Early-stage symptomatic osteoarthritis of the knee—time for action. *Nat. Rev. Rheumatol.* 17(10), 621–632 (2021).
9. Madry H, Kon E, Condello V *et al.* Early osteoarthritis of the knee. *Knee Surgery, Sport. Traumatol. Arthrosc.* 24, 1753–1762 (2016).
10. Hussain SM, Neilly DW, Baliga S, Patil S, Meek RMD. Knee osteoarthritis: a review of management options. *Scott. Med. J.* 61(1), 7–16 (2016).
11. Sconza C, Leonardi G, Carfì C *et al.* Intra-Articular Injection of Botulinum Toxin for the Treatment of Knee Osteoarthritis: A Systematic Review of Randomized Controlled Trials. *Int. J. Mol. Sci.* 24(2), 1486 (2023).
12. Kan HS, Chan PK, Chiu KY *et al.* Non-surgical treatment of knee osteoarthritis. *Hong Kong Med. J.* 25(2), 127–133 (2019).
13. Stefik D, Vranic V, Ivkovic N *et al.* An insight into osteoarthritis susceptibility: integration of immunological and genetic background. *Bosn. J. Basic Med. Sci.* 21(2), 155–162 (2021).
14. Dulić O, Lalić I, Janjić N, Rašović P, Gavrilović G, Abazović D. In step with contemporary trends-stem-celltherapy as a key driver of regenerative orthopedics at the Clinical Center of Vojvodina-preliminary data for the treatment of knee osteoarthritis and osteochondral lesions. *Med. Pregl.* 69(Suppl. 1), 77–84 (2016).
15. Weber AE, Bolia IK, Trasolini NA. Biological strategies for osteoarthritis: from early diagnosis to treatment. *Int. Orthop.* 45(2), 335–344 (2021).
16. Han Y, Li X, Zhang Y, Han Y, Chang F, Ding J. Mesenchymal stem cells for regenerative medicine. *Cells* 8(8), 886 (2019).
17. Kim GB, Seo MS, Park WT, Lee GW. Bone marrow aspirate concentrate: its uses in osteoarthritis. MDPI AG 21(9), 3224 (2020).
18. Cavallo C, Boffa A, de Girolamo L *et al.* Bone marrow aspirate concentrate quality is affected by age and harvest site. *Knee Surgery, Sport. Traumatol. Arthrosc.* 31(6), 2140–2151 (2022).
19. Zha K, Li X, Yang Z *et al.* Heterogeneity of mesenchymal stem cells in cartilage regeneration: from characterization to application. *Nature Research.* 19(6), 1–14 (2021).
20. Dulic O, Rasovic P, Lalic I *et al.* Bone marrow aspirate concentrate versus platelet rich plasma or hyaluronic acid for the treatment of knee osteoarthritis. *Medicina (B. Aires).* 57(11), 1193 (2021).
21. Keeling LE, Belk JW, Kraeutler MJ *et al.* Bone Marrow Aspirate Concentrate for the Treatment of Knee Osteoarthritis: A Systematic Review. SAGE Publications Inc 50(8), 2315–2323 (2022).
22. Wagner BS, Howe AS, Dexter WW *et al.* Tolerability and Efficacy of 3 Approaches to Intra-articular Corticosteroid Injections of the Knee for Osteoarthritis: A Randomized Controlled Trial. *Orthop. J. Sport. Med.* 3(8), 2325967115600687 (2015).
23. Dulic O, Lalic I, Kecojevic V *et al.* Do knee injection portals affect clinical results of bone marrow aspirate concentrate injection in the treatment of osteoarthritis? A prospective randomized controlled study. *Regen. Med.* 15(8), 1987–2000 (2020).

- **This study is crucial for the purpose of this manuscript as it explores the role of knee portals in the clinical efficacy of bone marrow aspirate concentrate (BMAC) injections.**

24. Centeno C, Pitts J, Al-Sayegh H, Freeman M. Efficacy of autologous bone marrow concentrate for knee osteoarthritis with and without adipose graft. *Biomed Res. Int.* 2014, 370621 (2014).

- **This paper represents one of the pioneering research studies that establishes a foundation for clinical investigations involving bone marrow aspirate concentrate (BMAC).**

25. Gursoy S, Akkaya M, Simsek ME, Bozkurt M. Functional outcomes of bone marrow aspirate concentrate application in osteoarthritis of the knee. *Int. J. Res. Med. Sci.* 7(2), 587 (2019).

26. Kim GB, Kim J-D, Choi Y, Choi CH, Lee GW. Intra-articular bone marrow aspirate concentrate injection in patients with knee osteoarthritis. *Appl. Sci.* 10(17), 5945 (2020).

27. Tur–Kaspa I. Clinical outcome of PGD. *Reprod. Biomed. Online.* 16, S13 (2008).

28. Reyes C, Leyland KM, Peat G, Cooper C, Arden NK, Prieto-Alhambra D. Association between overweight and obesity and risk of clinically diagnosed knee, hip, and hand osteoarthritis: a population-based cohort study. *Arthritis Rheumatol.* 68(8), 1869–1875 (2016).

29. Conrozier T, Eymard F, Chouk M, Chevalier X. Impact of obesity, structural severity and their combination on the efficacy of viscosupplementation in patients with knee osteoarthritis. *BMC Musculoskelet. Disord.* 20(1), 376 (2019).

30. Gobbi A, Karnatzikos G, Mahajan V, Malchira S. Platelet-Rich Plasma Treatment in Symptomatic Patients With Knee Osteoarthritis: Preliminary Results in a Group of Active Patients. *Sport. Heal. A Multidiscip. Approach.* 4, 162–172 (2012).

31. Migliorini F, Rath B, Colarossi G *et al.* Improved outcomes after mesenchymal stem cells injections for knee osteoarthritis: results at 12-months follow-up: a systematic review of the literature. *Arch. Orthop. Trauma Surg.* 140, 853–868 (2019).

32. Migliorini F, Driessen A, Quack V *et al.* Comparison between intra-articular infiltrations of placebo, steroids, hyaluronic and PRP for knee osteoarthritis: a Bayesian network meta-analysis. *Arch. Orthop. Trauma Surg.* 141, 1473–1490 (2021).

33. Freitag J, Wickham J, Shah K, Tenen A. Real-world evidence of mesenchymal stem cell therapy in knee osteoarthritis: a large prospective two-year case series. *Regen. Med.* 17(6), 355–373 (2022).

- **This trial is one of the largest research studies on adipose-derived mesenchymal stem cell therapy. It serves as a complementary investigation that, when combined with the real-world evidence case series, provides conclusive evidence of the efficacy of adipose-derived mesenchymal stem cell therapy in treating knee osteoarthritis. In the context of this manuscript, we have found further confirmation of our findings.**

34. Kim J-D, Lee GW, Jung GH *et al.* 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 (2014).