Transplantation Publish Ahead of Print
DOI: 10.1097/TP.0000000000001484

Intervertebral disc repair by allogeneic mesenchymal bone marrow cells: a randomized controlled trial

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Clinical trial registries: EudraCT 2012-004444-30, ClinicalTrials.gov: NCT01860417

Funding and Support: Financial support from the *Red de Terapia Celular* (RD12/0019/0036, RD12/0019/0001 and RD16/0011/0003), Instituto de Salud Carlos III, Ministerio de Economía y

Competitividad, and the *Centro en Red de Medicina Regenerativa de Castilla y León* is gratefully acknowledged.

Conflicts of interest: The authors declare no conflicts of interest

FOOTNOTES:

Footnotes to the *Title:*

Author Contributions: Javier García-Sancho (JGS) had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. DCN, AS and JGS participated in the conception and design of the article. DCN, FA, RHR and MAMF were primarily responsible for the clinical work, and AS and MA and VG for the cell production. ISL and BT were responsible for MRI. JMM provided advice. All authors participated in analysis, discussion and interpretation of data, revision of the article, and gave final approval of the version to be published. JGS put together all data, did meta-analysis and wrote the final form of the manuscript.

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Footnotes to the *Text:* None

ABBREVIATIONS:

MSC, Mesenchymal stromal cells, also called mesenchymal stem cells; DDD, degenerative disc disease; VAS, Visual analogue scale ODI, Oswestry disability index; SF-12, Short form-12 life quality questionnaire; MRI, Magnetic resonance imaging. GMP, good manufacturing practice

ABSTRACT

Background: Degenerative disc disease often causes severe low-back pain, a public health problem with huge economic and life quality impact. Chronic cases often require surgery, which may lead to biomechanical problems and accelerated degeneration of the adjacent segments. Autologous mesenchymal stromal cells (MSC) treatments have shown feasibility, safety and strong indications of clinical efficacy. We present here a randomized, controlled trial using allogeneic MSC, which are logistically more convenient than autologous cells.

Methods: We randomized 24 patients with chronic back pain diagnosed with lumbar disk degeneration and unresponsive to conservative treatments into 2 groups. The test group received allogeneic bone marrow MSCs by intradiscal injection of 25×10^6 cells per segment under local anesthesia. The control group received a sham infiltration of paravertebral musculature with the anesthetic. Clinical outcomes were followed for 1 year and included evaluation of pain, disability, and quality of life. Disc quality was followed by Magnetic Resonance Imaging.

Results: Feasibility and safety were confirmed and indications of clinical efficacy were identified. MSC-treated patients displayed a quick and significant improvement in algofunctional indices versus the controls. This improvement seemed restricted to a group of responders that included 40% of the cohort. Degeneration, quantified by Pfirrmann grading, improved in the MSC-treated patients and worsened in the controls.

Conclusions: Allogeneic MSC therapy may be a valid alternative for the treatment of DDD that is more logistically convenient than the autologous MSC treatment. The intervention is simple, does not require surgery, provides pain relief, and significantly improves disc quality.

INTRODUCTION

Intervertebral disc degeneration is a very common disease that can lead to axial skeletal pain, radiculopathy, and myelopathy. Combined physical and medical therapies are successful in relieving pain in approximately 90% of the cases. However, the remaining 10% become chronic and generate a serious public health problem, as chronic low-back pain ruins both the life quality and the labour capacity of the patient, and increases use of health services [1,2].

The efficacy of the existing treatments is limited (see our recent meta-analysis in [3]. Surgery is the recommended gold standard when the back pain chronifies, and its analgesic value is beyond question [1,4], but it has several drawbacks [5,6]. Cell therapy has produced exciting results both *in vitro* and *in vivo* [7], and animal studies with mesenchymal stem cells (MSC) have been particularly promising [8]. On the other hand, in a recent pilot trial in humans, treatment with autologous MSC produced strong indications of clinical efficacy, with pain improvement approaching 71% of optimal during the first year. In addition, although disc height was not recovered, water content was significantly elevated [3]. This outcome compares favourably with the results of other interventions such as spinal fusion or total disc replacement, suggesting that MSC treatment could be a valid alternative treatment for chronic back pain caused by degenerative disc disease (DDD).

Even though autologous MSCs are an excellent therapeutic option, the need for cell expansion makes the procedure slow and expensive. Allogeneic cells would be logistically, much more convenient, but they may have important drawbacks, the most obvious the possibility of host immune rejection of the transplanted cells. MSC, however, are *immune privileged* [9] or *immune*

evasive [10] and inhibit immune responses in a manner not restricted by the HLA system. As a result, non-matched MSC are much better tolerated than other cell types. In fact, there are no reports of rejection in animal experiments [8,11-14] and studies of transplanted MSC persistence in the host organism show the same values for autologous and allogeneic cells [10,15]. In humans, excellent tolerance to allogeneic MSCs has been reported in many clinical trials. For example, in a recent meta-analysis of 87 lupus erythematosus patients, no transplantation-related adverse events were found after 4-years of follow up [16]. Similarly, no transplantation-related adverse events occurred in MSC-treated patients with breast cancer [17], left ventricular dysfunction [18-20], ankylosing spondylitis [21], graft versus host disease [22], and other autoimmune diseases [23]. We have demonstrated recently the safety of autologous MSC for treatment of knee osteoarthritis [24], an application very similar to the 1 proposed in the present paper

Here, we present a randomized controlled study to assess the feasibility and safety of using good manufacturing practice (GMP)-compliant bone marrow MSCs [3,25]. Additionally, we present evidence to suggest that intradiscal injection of allogeneic cells has a therapeutic value. The intervention proposed does not require surgery, does not produce anatomical modifications and does not hinder further interventions, should they be required.

MATERIALS AND METHODS

Patients and procedures

This phase I-II trial was approved by Ethics Committee at Valladolid University Hospital and by the Spanish Agency of Medicines (EudraCT 2012-004444-30). The study was also registered at ClinicalTrials.gov (NCT01860417). The design of the trial was based on our

previous 1, performed with autologous MSC [3]. We recruited 24 patients (17 male and 7 female; mean age ± SE = 38±2 years) with Pfirrmann grade II-IV DDD [26], that had been unresponsive to conventional treatments (physical and medical) for at least 6 months prior to recruitment. Recruitment was performed between July 2013 and March 2014. Detailed inclusion and exclusion criteria are reported in Table 1. The recruited patients were block randomized by the quality control manager of the cell production facility, who was blinded, to receive either the control or the experimental treatment. The allocation ratio was 1:1. Clinical, analytical, and imaging evaluations were performed to ensure compliance with the inclusion criteria. Patients were informed of the protocol design before providing written informed consent.

Follow-Up Controls

The protocol included 6 visits (V0-V5). The V0 visit involved a final compliance check using the inclusion criteria, performance of the complementary evaluations and tests needed, and scheduling of dates for the next visit. At V1, treatments were administered, either MSCs ($25x10^6$ MSC in 2 ml of saline per disc) under local anesthesia or sham infiltration of paravertebral musculature close to the affected disc(s) with 2 ml of 1% mepivacaine. The V2-V5 visits (8 days, and 3, 6, and 12 months after implantation) included clinical evaluation and routine analyses, pain evaluation using visual analogue scale (VAS) [27], Oswestry Disability Index (ODI) [28] and short form-12 (SF-12) life quality questionnaire [29]. Outcomes were expressed using a 0–100% scale in all cases. Quantitative MRI exploration was performed at V0, V4, and V5. The patients, radiologists, care providers, and persons assessing the outcomes of the assay were blinded after assignment.

Cell isolation and expansion

Bone marrow was obtained from 5 healthy donors and processed using GMP conditions in the IBGM Cell Production Unit as described previously [3,25]. Isolations were carried out with the following parameters (mean ± SD; n = 5, 4 males and 1 woman): bone marrow volume = 105 ± 5 ml, average number of mononuclear cells obtained = 1.23 ± 0.25×10^9, expansion time = 27 ± 2 days, number of MSC injected into each disc = 25 ×10^6, suspended in Ringer-lactate at 12.5×10^6 cells/ml, and viability >98±1%. A serum sample from each donor was obtained to screen for human immunodeficiency, hepatitis B, and hepatitis C virus by Nucleic Acid Amplification Technology [30]. The cells obtained from each donor were used for 1-3 recipients. Immune matching was not attempted

Statistics

Data are reported as mean ± SD or SE, as indicated in each case. Significant differences were assessed by either Student's t-tests, by 2-way repeated measures analyses of variance (ANOVA), or with appropriate corresponding nonparametric tests. We used GraphPad Instat3 package software version 3.06 (GraphPad Software, La Jolla, CA) for all calculations.

RESULTS

Patients

This pilot study included 24 patients (see details in Methods) diagnosed of DDD with preserved external annulus fibrous and persistent low-back pain. Additionally, all 24 patients did not respond to conservative treatment (physical and medical) lasting at least 6 months. The

patients had 1 or 2 affected discs, with the lesion located at L1-L2 (n=1), L2-L3 (1), L3-L4 (3), L4-L5 (18), or L5-S1 (15). They were informed of the protocol design before providing written informed consent. The protocol included 6 visits (V0-V5), as described in Methods. All patients were treated at the Valladolid University Hospital. No major adverse events occurred. Eleven patients (8 controls and 3 cell-treated) required brief treatments with NSAID-type analgesics for minor pains and 2 (1 control and 1 cell-treated) required opioids (morphine sulphate tablets or traumadol).

Evolution of pain, disability and life quality

Table 2 summarises the distribution of pain and disability indexes throughout the observation period. The baseline values of pain and disability were quite homogeneous in the cohort. On average patients felt intense lumbar pain (65 ± 5 in the VAS scale) and had moderate disability (ODI of 29 ± 4) (mean ± SE; n=24). Both lumbar pain and disability were significantly reduced at 3 months after MSC transplantation, and the improvement was maintained at 6 and 12 months (Figure 1A and 1C). Compared to the basal level of pain and disability, improvement was statistically significant at all time points except at 8 days (see details at the Figure 1 legend). The pattern of improvement between VAS and ODI was parallel and resulted in global displacement of the whole distribution towards smaller values, with a strong decrease of the medians (P50% in Table 2; see also Figure S1, **SDC, http://links.lww.com/TP/B347**). Note that the effect was virtually complete at the 3th month. In the sham-treated controls the effects at 3, 6 and 12 months were not statistically significant. A fast decrease of pain was detected at the 8th day in the control group, but there was not any tendency to further improvement thereafter (Figure 1B and 1D, and

Figure S1B, **SDC, http://links.lww.com/TP/B347**). The ODI values tended to increased at 3, 6 and 12 moths in the control group (Fig. 1D)

In Figure 2 we have plotted lumbar pain relief at the end of the treatment, assessed by VAS, as a function of the initial pain score [27] (Figure 2; circles). As shown before [3], the improvement of the disability index as a function of the baseline ODI value exhibited the same relationship and can be included in the same plot (Figure 2, triangles). The efficacy of the treatment is equal to the slope of the dotted lines, fitted either for the control patients (open symbols, in black) or for the cell-treated patients (filled symbols, in red). The "perfect treatment" with a slope of 1 is also shown (continuous line, in blue). The controls fitted to a line with slope of (mean ± SEM) 0.15 ± 0.10, not significantly different from 0 ($p=0.13$), whereas the cell-treated patients fitted to a line with slope 0.28 ± 0.07, which is significantly different from 0 ($p<0.001$). There was considerable scatter not only in the control group, but also in the experimental group. In the last case, however the cohort seemed to divide into 2 groups, 1 group of 5 patients close to the blue line, with a high relief index (responders) and the remaining patients, which show little improvement (nonresponders).

In our previous study with autologous MSCs we found a slight but significant improvement of the physical component score in the SF-36 life quality questionnaire [3]. Although this tendency was also observed in the present work, the SF-12 life quality questionnaire did not reveal significant improvements of either the physical or the mental component scores (Table S1; **SDC, http://links.lww.com/TP/B347**). This is not surprising, as these indexes are less sensitive than the pain tests in the inflammatory diseases [25,31,32].

Imaging

Magnetic Resonance Imaging (MRI) was used to assess disc height and water content of the discs. The height of the affected discs decreased by (in mm; mean ± SE) 0.38 ± 0.19 mm the controls (n=16 discs) and by only 0.04 ± 0.19 mm in the cell-treated patients (n=17 discs), but the difference was not significant. In neighbour healthy discs (n=24) the heights decreased by 0.07±0.10 mm (n=24 discs). These values were not significantly different. Water content of the discs, determined from T2-weighted sagittal images, was measured in the affected disc segment and in the contiguous 3-5 segments (see *Methods* and [3]). In some patients the water content of the affected discs improved after treatment with the MSC with little changes of the normal discs (Figure S2, **SDC, http://links.lww.com/TP/B347**). In order to homogenize the results of different patients, the water content values of the affected discs were normalised to the values obtained from the healthy discs in the same individual; for these purposes, the density of the affected segments was divided by the average value of the healthy discs. Finally, the value after the treatment was divided by the baseline value. In the cell-treated discs the image density increased by (mean ± SE) 22 ± 11 % at 12 months, compared to only 6 ± 8 % in controls (Figure S3; Table S2, **SDC, http://links.lww.com/TP/B347**). The differences, however, were not statistically significant ($p<0.07$; 1-sample paired t-test, 2-tailed value). In our previous trial with autologous MSC the difference was very similar, 18% increase in water content, but it was statistically significant because the dispersion was smaller [3]. Evolution of Pfirrmann staging, which takes into account several MRI disc parameters [26] was clearly different in the control and in the experimental groups. Results are shown in Figure 3A y 3B. In controls (B) there was a deterioration from (mean ± sem; n=20) Pfirrmann stage 3.15 ± 0.15 to stage 3.78 ± 0.16 ($p<0.001$; Wilcoxon matched-pairs signed-ranks test), whereas in the cell-treated patients (A) there was an improvement from stage 3.68 ± 0.13 to 3.18 ± 0.17 ($p<0.01$).

DISCUSSION

Previous studies have demonstrated that autologous MSCs provide an excellent therapeutic alternative for treating DDD [3]; however, allogeneic cells, which have been extensive and successfully tested for safety (see *Introduction*), would be logistically much more convenient than autologous for these treatments. This trial is, to the best of our knowledge, the first to test therapeutic efficacy of allogeneic MSC in DDD. Our results show that allogeneic MSC transplantation is feasible and safe, and does not originate any major adverse outcome. Occasional mild pain reactions were, if anything, more frequent in the control than in the cell-treated patients and responded well to the usual analgesic treatments.

The parallel improvement of pain and disability effected by the MSC treatment was quick but not immediate; it reached about 30% of the maximum at 8 days after the intervention, was nearly complete at 3 months and was maintained at 6 and 12 months (Figure 1). In the controls, not treated with MSC, the time course was very different. There was a sudden decrease of VAS during the first 8 days, but no further improvement along the following year was observed. As a matter of fact, the disability index worsened (although not statistically significantly) along the first year after the intervention. We do not know the reason for this early improvement of pain in the controls; it could be due to a placebo effect or be the result from the anesthetic infiltration, but in any of these cases it should also happen in the cell-treated group, and there is not indication of this extra-fast early improvement in the group of cell-treated patients.

Efficacy of the treatments was quantified by the slope of the pain relief-initial pain score relationship [27]. This gives values between 0 (no effect) and 1 (perfect treatment). Values of *effect size*, a primary measurement of significance (see Table 2 legend), were also computed. There was

a clear analgesic effect of the allogeneic MSC, resulting in an average 28% improvement in pain and disability 1 year after the intervention. This compares to only 15% recovery in the sham-treated controls (Figure 2). The improvement was statistically significant in the cell-treated group but not in the control group.

Quantification of the slope of the pain relief/baseline pain score relationship permits comparison of efficacy among different trials [27]. The efficacy of allogeneic treatment found in the present trial, 0.28, was smaller than the reported for autologous cells, 0.71 [3]; yet, direct comparisons are difficult because the previous study was uncontrolled. In fact, it would be most interesting to directly compare autologous with allogeneic cells in different arms of the same trial, and we are moving in this direction for future studies.

Close inspection of Figure 2 is suggestive of bimodal distribution of the cell-treated patients in the Huskisson plot; a responders subgroup of 5 patients is close to the blue line that represents perfect treatment, whereas the other 7 (nonresponders) distribute very similarly to the control patients, with no indication of effectiveness. If we were able to understand the reasons for this different behavior, that might help to understand the action mechanism of the healing effect and, eventually, to improve efficacy by choosing the most adequate cells among different available donors.

In any case, the results published here are the first to demonstrate the feasibility and safety of the allogeneic MSCs treatment while providing also indications for their efficacy for relief of chronic low back pain. As allogeneic cells are more logistically convenient than autologous, the results presented here may contribute to more widespread use of MSC treatments. In addition all the

studies coincide on the safety of allogeneic MSCs (see *Introduction*), which also encourages clinical use. However, the transition from autologous to allogeneic MSCs should be made with extreme caution to ensure safety. Allogeneic MSC treatments will benefit from further research, not only clinical, but also basic, addressing how MSCs relieve pain, promote regeneration, and become immune evasive [10]. In recent times the importance of HLA matching to determine kidney allograft survival has been questioned [33-35]. However, a recently published exhaustive investigation (a 189,141 cohort) concludes that every mismatch between donor and recipient increases risk by 11% (hazard ratio from 1.13 for 1 mismatch to 1.64 for 6 mismatches) [36]. The present case is not comparable as the accessibility inside the disc is severely limited and, as pointed out above, MSC are not only immune evasive but do also depress immune reactions (see *Introduction*). In any case, it would be very interesting to investigate the relationship between HLA mismatch and efficiency in the patients treated with allogeneic MSC in a further study.

We can only speculate regarding the mechanism by which the beneficial effect of the treatment occurs. Animal studies have shown that MSC injected in the NP area are able to survive and proliferate [37,38] and to induce beneficial effects in degenerative disc disease [39,40]. Nucleus pulposus cells induce differentiation of co-cultured MSC into nucleus pulposus-like cells with a chondrocyte phenotype [41-43]. Even more importantly, MSC stimulated nucleus pulposus cells to proliferate and to synthesise extracellular matrix [44,45]. This action may be important *in vivo* as very few MSC are required to trigger this effect [45]. In addition, MSC display a well-known immunomodulatory effect and express Fas-ligand when implanted in the spinal discs of dogs [39]. These data indicate that MSC may help analgesia by reducing inflammation. Additionally, MSC can induce the production of anti-inflammatory cytokines [45,46]. Importantly, MSCs have also been shown to stimulate co-cultured cells to proliferate and synthesize extracellular matrix [47-49]. In

fact, transplanted MSCs engrafted into the joint in mouse are activated and express Indian hedgehog and other genes. These genes in turn promote expression of collagen II and other chondrogenic genes by host cells [50]. Because of these *hit and run* effects, tracing MSC action may be elusive.

In summary, we propose that cell therapy with expanded allogeneic bone marrow-derived MSC should be considered as a putative treatment for chronic DDD. GMP handling and expansion of these cells is reproducible, and quality control tests are satisfactory. The clinical procedure is feasible and safe, and requires minimally invasive intervention without surgery or hospitalization. The procedure results in significant relief of pain and disability, and quantitative MRI evidence suggests partial disc healing. Advantages of allogeneic over autologous treatments include lower cost, higher homogeneity and the possibility of using them in seropositive patients. The healing effects appear to be smaller than those reported for treatment with autologous MSC, but this should be confirmed in future studies designed to directly compare both cell types within the same trial. These studies will track the long-term evolution as well as investigate the anatomical and functional changes that occur in the intervertebral spaces and will increase the number of patients, which is an important limitation of the present study.

ACKNOWLEDGMENTS

We thank Ms. Sandra Güemes, and Ms. Virginia Gordillo for technical support.

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FIGURE LEGENDS

Figure 1. Temporal evolution of pain and disability over time after mesenchymal stem cells (MSC) treatment.

(A, B) Graph showing changes of lumbar pain over time for MSC-treated patients (**A**) and control patients (**B**). VAS, Visual Analog Scale. (**C, D**) Graph showing changes of disability over time as measured by Oswestry disability index (ODI) for MSC-treated (**C**) and control patients (**D**). Represented values are mean ± SE. Statistical significance assessed by ANOVA for paired populations, Bonferroni test. Comparisons to t=0; ns, non-significant, *$p<0.05$; ** $p<0.01$

Figure 2. Pain and disability improvement as a result of MSC treatment.

Improvement 1 year after intervention is plotted as a function of the initial pain score or disability index [27]. Results for the relief of lumbar pain (circles) and Oswestry disability index (triangles) are all included for both, control (open symbols) and cell-treated patients (filled symbols) The continuous blue line with a slope of 1 represents the perfect treatment, in which complete pain or disability relief was achieved. The dotted lines correspond to the linear fit of controls (black) and treated data (red). The values of the slopes obtained from the best fit of the data (n=24; linear regression forced through the origin) were (mean±SE) were: control, 015 ± 0.10 (not significantly different from 0; $p=0.13$), and MSC-treated 0.28 ± 0.07 (significantly different from 0; $p<0.001$).

Figure 3. Assessment of nucleus pulposus evolution by Pfirrmann grading.

Pfirrmann grading (1 to 5) takes into account the structure of the disc, the distinction of Nucleus Pulposus and Annulus Fibrosus, the signal intensity and the height of the disc. The values (mean ± SE) at baseline (open) or 6 months (crosshatched) and 12 months after treatment (filled) are shown for control (**B**) and MSC-treated patients (**A**). Comparisons to baseline were performed by repeated measures ANOVA, Bonferroni multiple comparisons; *$p<0.01$; **$p<0.001$.

Fig. 1.

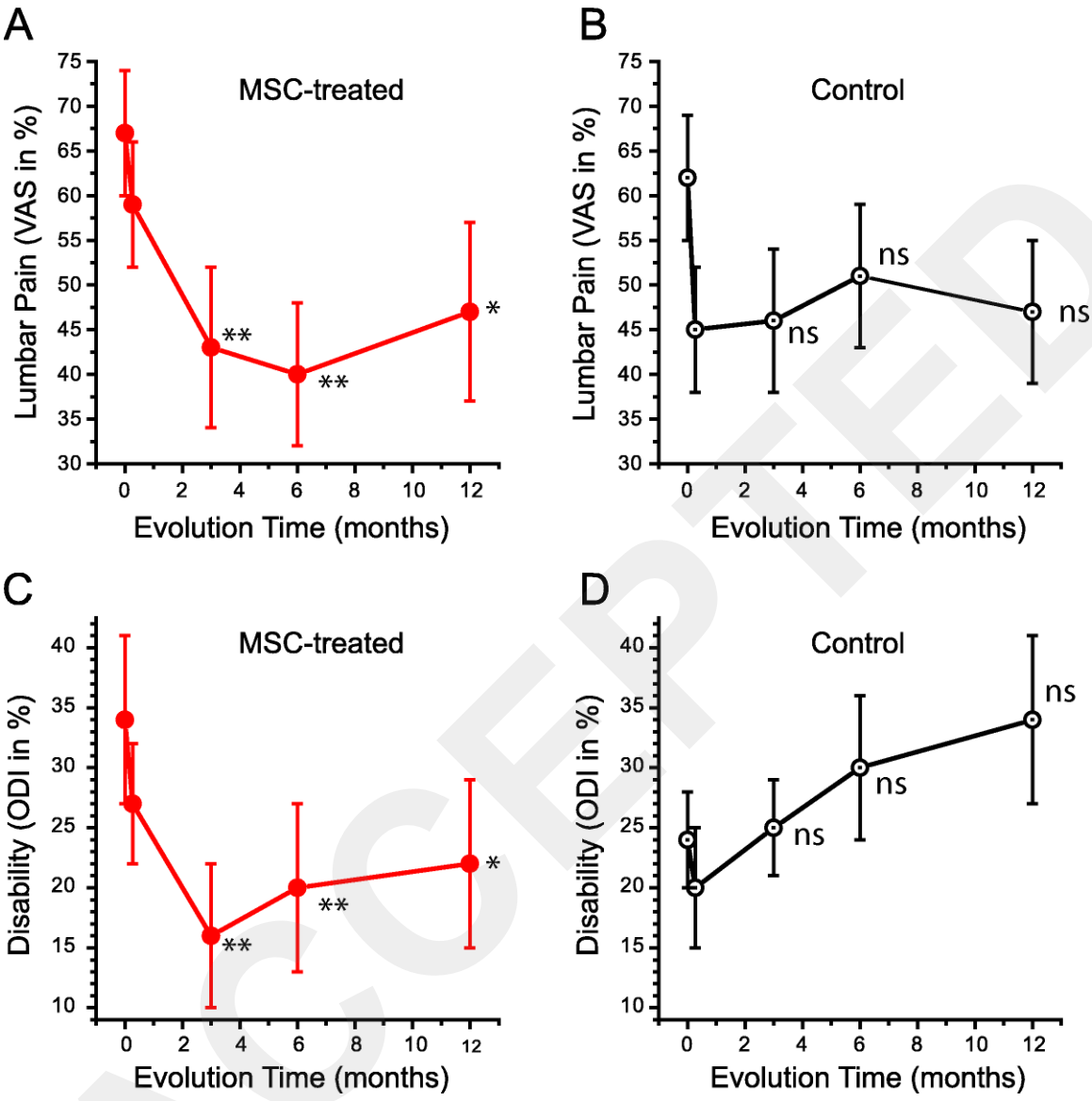


Fig. 2

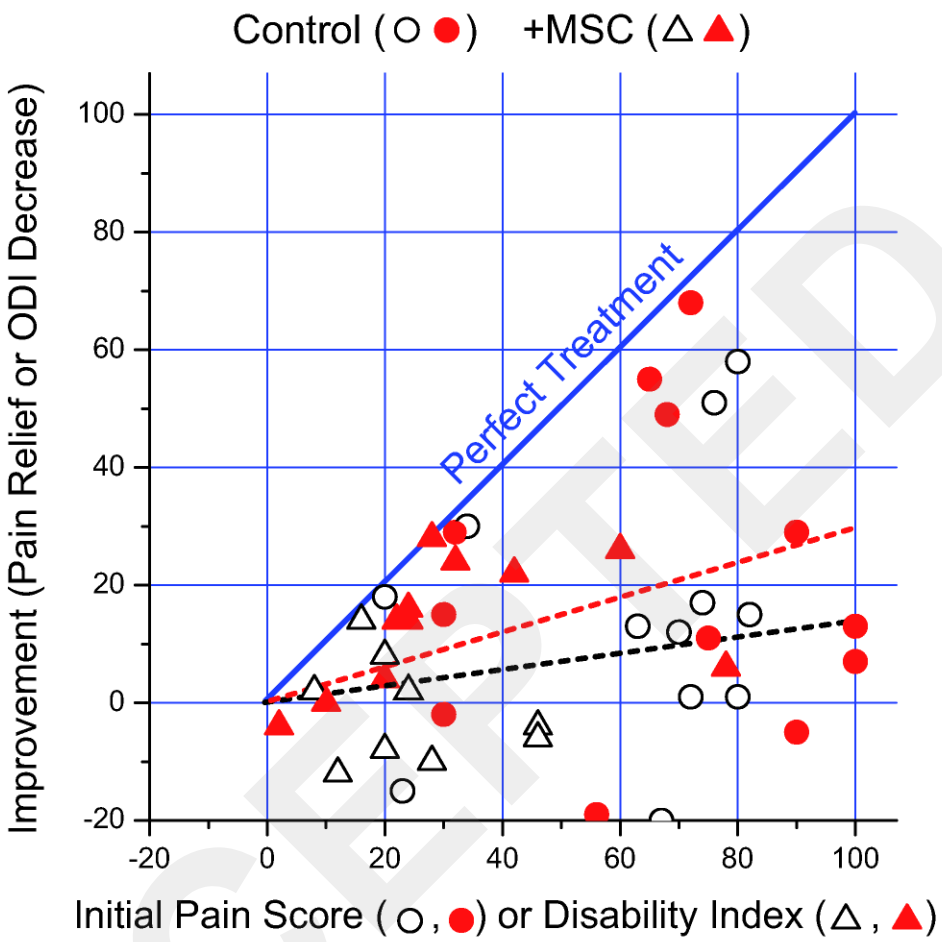


Fig. 3

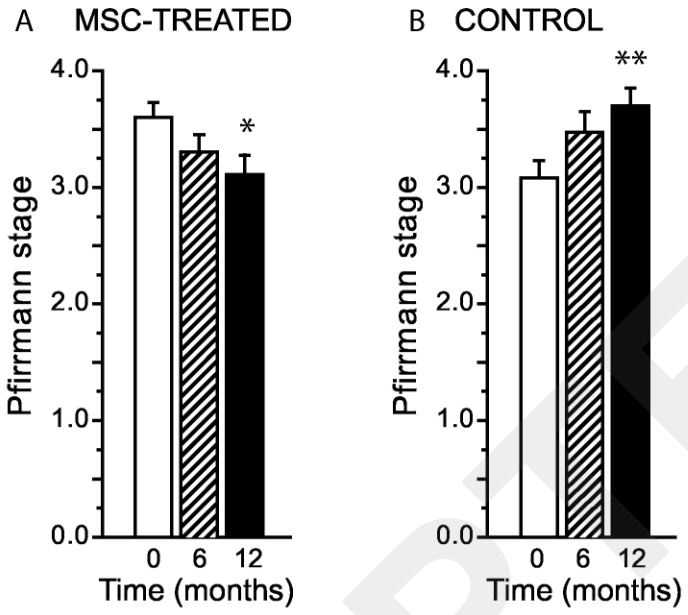


Table 1. Inclusion and exclusion criteria

Inclusion criteria:
1. Degenerative disease of 1 or 2 lumbar discs with predominant back pain after conservative treatment (physical and medical) for over 6 months.
2. Fibrous ring capable of holding the cell implantation, demonstrated by RMI image (stages 2, 3 and 4 of Pfirrmann).
3. Decrease of disc height of more than 20 % (radiographic measurement in side image).
4. Absence of spinal infection.
5. Haematological and biochemical analysis wit no significant alterations that contraindicates intervention.
6. The patient is able to understand the nature of the study.
7. Informed written consent of the patient.
8. In fertile women, negative pregnancy test result and acceptance of adequate contraceptive methods
Exclusion criteria:
1. Age over 75 or under 18 or legally dependent
2. Allergy to gentamicin, or to bovine, cattle or horse serum.
3. Congenital or acquired diseases leading to spine deformations that may upset cell application.
4. Spinal segmental instability, spinal canal stenosis, isthmus pathology and other conditions that may compromise the study
5. Modic III changes on MRI images [51].
6. Overweight with body mass index (mass in Kg/size in m2) greater than 35 (obesity grade II).
7. Pregnancy or breast-feeding
8. Neoplasia
9. Immunosuppression
10. Hypersensitivity to amide-type local anaesthetics or other known contraindications or interactions of mepivacaine
11. Participation in another clinical trial or treatment with another investigational product within 30 days prior to inclusion in the study.
12. Other conditions that may, according to medical criteria, discourage participation in the study.

Table 2. Total sumscore of Visual Analogue Scale (VAS) measurements for lumbar pain and Owestry disability index (ODI).

VAS_CONT	C or E	N	MEAN	DS	SE	SMD	MIN	P25%	P50%	P75%	MAX	Conf 95	Conf 99
V0	C	12	62	23	7	N.A.	20	56	71	77	82	13	17
V2 (8d)	C	12	45	25	7	0.75	9	22	49	61	81	14	19
V3 (3M)	C	12	46	27	8	0.71	0	24	50	62	85	15	20
V4(6M)	C	12	51	29	8	0.49	6	26	52	79	84	17	22
V(12M)	C	12	47	28	8	0.67	2	24	54	68	87	16	21
VAS_EXPTL	**C or E**	**N**	**MEAN**	**DS**	**SE**	**SMD**	**MIN**	**P25%**	**P50%**	**P75%**	**MAX**	**Conf 95**	**Conf 99**
V0	E	12	67	26	7	N.A.	30	50	70	90	100	15	19
V2 (8d)	E	12	63	26	7	0.17	16	51	67	82	99	14	19
V3 (3M)	E	12	43	30	9	0.94	7	16	40	63	98	17	23
V4(6M)	E	12	40	29	8	1.07	3	12	47	60	93	17	22
V(12M)	E	12	47	36	10	0.80	3	14	47	78	95	21	27
ODI_CONT	**C or E**	**N**	**MEAN**	**DS**	**SE**	**SMD**	**MIN**	**P25%**	**P50%**	**P75%**	**MAX**	**Conf 95**	**Conf 99**
V0	C	12	24	14	4	N.A.	4	15	22	30	46	8	10
V2 (8d)	C	12	20	16	5	0.30	0	10	16	31	50	9	12
V3 (3M)	C	12	25	15	4	-0.10	2	16	24	31	52	8	11
V4(6M)	C	12	30	20	6	-0.43	2	14	28	45	60	11	15
V(12M)	C	12	34	25	7	-0.76	2	20	29	51	94	14	19
ODI_EXPTL	**C or E**	**N**	**MEAN**	**DS**	**SE**	**SMD**	**MIN**	**P25%**	**P50%**	**P75%**	**MAX**	**Conf 95**	**Conf 99**
V0	E	12	34	23	7	N.A.	2	22	26	47	78	13	17
V2 (8d)	E	12	27	17	5	0.31	2	18	21	29	60	10	13
V3 (3M)	E	12	16	20	6	0.76	2	6	9	16	72	11	15
V4(6M)	E	12	20	24	7	0.62	0	7	12	19	88	13	18
V(12M)	E	12	22	24	7	0.53	0	8	10	24	72	14	18

Abbreviations: P25%, P50%, and P75% represent 25th, 50th (median), and 75th percentiles, respectively. Conf 95 and Conf 99 = confidence interval at 95 and 99% significance. Min= minimum value, Max= maximum value. SMD= standardized mean difference, computed as improvement

(baseline value minus value at the end of treatment) divided by the SD of the baseline value. SMD is used here as an estimate of effect size. The correlation between effect size and magnitude of the change are: 0= null, 0.20= small, 0.50= medium, 0.8= large. Details on effect size can be found in [52].

In all cases, the scale was from 0 to 100%. Measurements performed before cell transplantation (baseline), 8 days, 3, 6 and 12 months afterwards (12 M) are shown. C = control patients (mepivacaine), E= experimental cell-treated patients, VAS= Visual Analogue Scale for pain, ODI, Oswestry Disability Index. N.A.= Does not apply.

SUPPLEMENTAL DIGITAL CONTENT

Intervertebral disc repair by allogeneic mesenchymal bone marrow cells: a randomized controlled trial

by

David C Noriega[1], Francisco Ardura[1], Ruben Hernández-Ramajo[1], Miguel Angel Martín-Ferrero[1], Israel Sánchez-Lite[1], Borja Toribio[1], Mercedes Alberca[2], Veronica García[2], José Maria Moraleda[3], Ana Sánchez[4] & Javier García-Sancho[4,5]

CONTENTS:

SDC, Table S1. Results from the SF-12 life quality questionnaire.

	Baseline		8 days		3 months		6 months		1 year	
Arm	**PCS**	**MCS**	**PCS**	**MCS**	**PCS**	**MCS**	**PCS**	**MCS**	**PCS**	**MCS**
Control (n=12)	40±3	52±3	43±3	50±2	43±3	46±3	39±3	48±3	42±3	50±3
+MSC (n=12)	39±2	46±3	39±2	47±3	47±3	50±2	46±3	52±2	45±3	48±3

PCS, Physical Component Summary. MCS, Mental Component Summary. Values are given as mean±SE

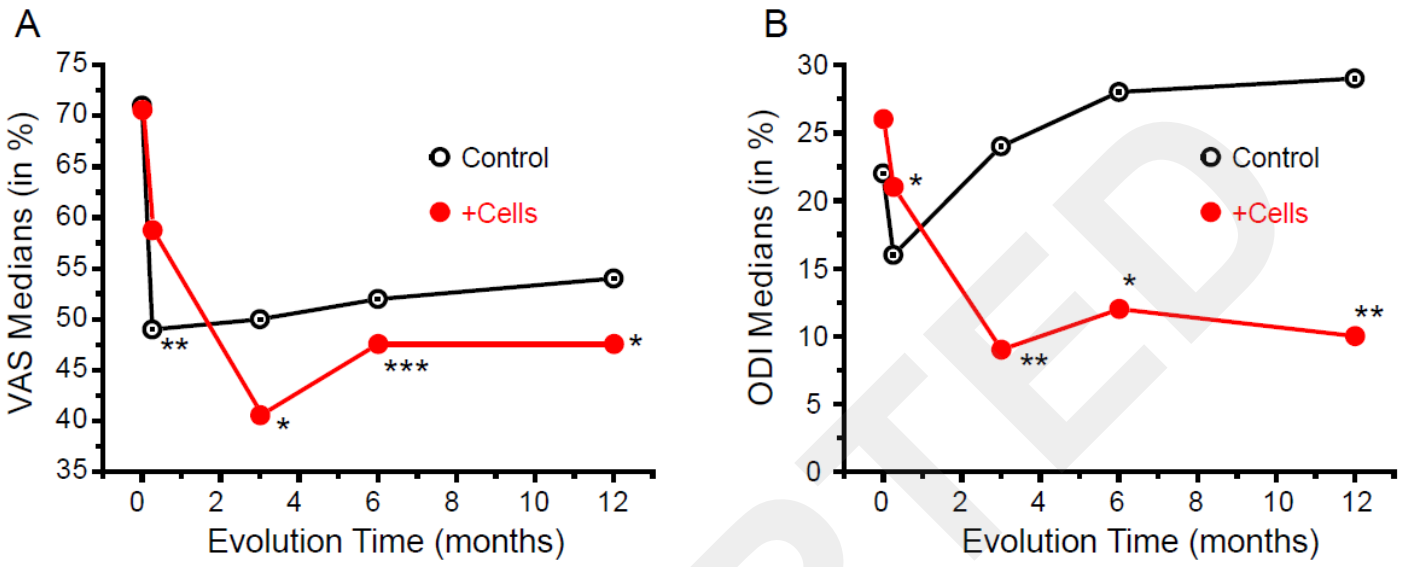
SDC, Table S2. Fluid contents of the discs along the clinical trial period.

	Values are given as mean ± SE (n)			
	Density[a] before Transplantation	**Density[a] 6 months afterwards**	**Density[a] 12 months afterwards**	**Ratio[b] 12 months/6months**
Control	0.48 ± 0.05 (20)	0.51 ± 0.05 (20)	0.49 ± 0.05 (20)	1.06 ± 0.08 (20)
+MSC	0.46 ± 0.05 (18)	0.42 ± 0.05 (18)	0.52 ± 0.06 (18)	1.22 ± 0.11 (18)

[a]Image densities were measured in the T2-weighted MRI images and are normalized to 1 with regard to the healthy discs. The values of density at 6 and 12 months did not significantly differ from baseline ANOVA, Bonferroni test).

[b]Ratio was performed for each individual disc and then the ratio values used for calculations of mean and SE. The ratio values did not differ significantly from 1.

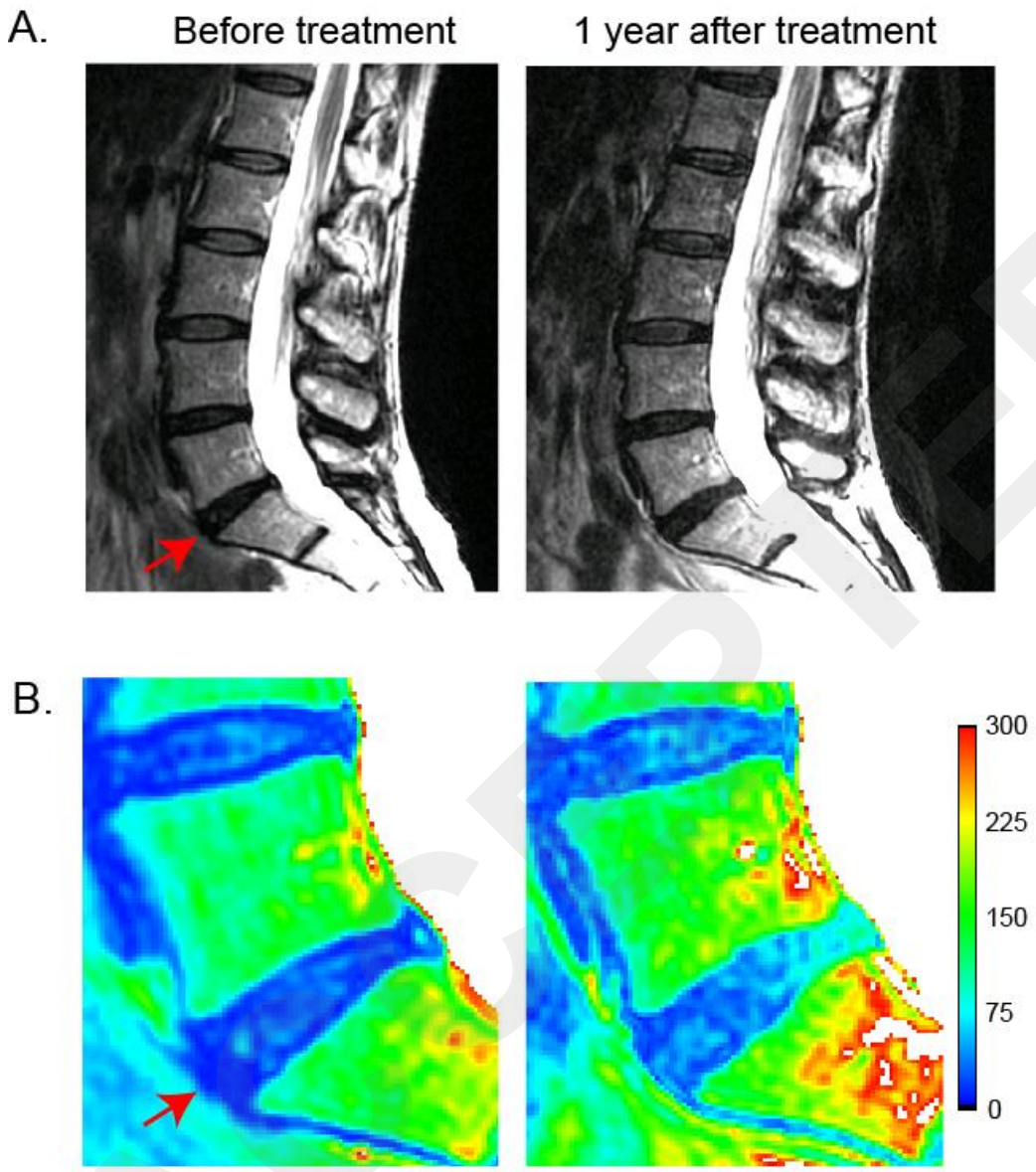
SDC, Figure S1.



SDC, Figure S1. Evolution of medians of lumbar pain (A) and disability (B) in the control (black empty circles) and the MSC-treated cohorts (red filled cycles) one year after the intervention.

The values are the medians of the two 12-patient cohorts. Statistical significance of the differences with the baseline values (t=0) was assessed by the Wilcoxon matched-pairs signed-ranks test; *p<0.05; **p<0.01; ***p<0.005.

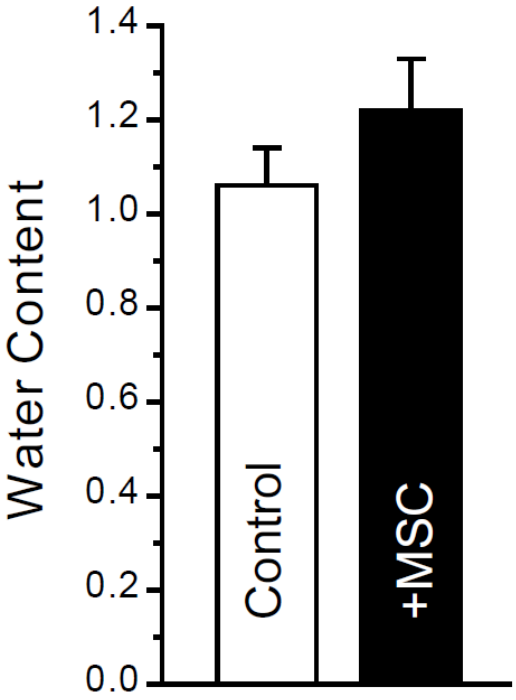
SDC, Figure S2.



SDC, Figure S2. Representative MRI changes after treatment with MSC.

(A) Grey images with red arrow pointing to the affected disc, L5S1. Note that after treatment the disc is denser. **(B)** Close-up of the affected disc and his upper neighbour. Density coded in pseudocolor with calibration scale at right. The density of L5S1 was increased by about 50 %, but the healthy discs were little affected. Data from patient 5. On average, the density of the affected discs increased by 22 % after MSC treatment (Table S2).

SDC, Figure S3.



SDC, Figure S3. Evolution of relative fluid content of the affected discs. Changes in density of T2-weigthed images in the nucleus pulposus area 1 year after the intervention were measured. Density was normalized with regard to the baseline level (1.00). Values are mean ± SE for the control (open bar) and for the MSC-treated patients (filled bar). Differences with 1.00 are not significant.

SDC, SUPPLEMENTARY METHODS.

Bone marrow harvesting, purification and culture

Puncture and bone marrow aspiration were performed in an ambulatory surgery session. The patient, in prone position, underwent light sedation. The surgical field was brushed with alcoholic povidone-iodine solution (chlorhexidine if a history of allergy to iodine exists) and delimited with sterile fields, leaving free both posterior iliac crests. After local anesthesia (20 ml of 1% lidocaine without epinephrine diluted v/v with saline), two members of the extractor team, placed on both sides of the operating table, performed several punctures with a 11-G trocar under the iliac spine, aiming towards the posterior sacroiliac joint (this is the iliac area with higher trabecular density). The technique involves sudden cortical perforation and repeated aspiration of small bone marrow volumes (2-4 ml) to minimize contamination with the peripheral blood. The aspirate was injected into a heparinized bag for transport. Two successive aspirations were performed by rotating 90 degrees clockwise the beveled trocar. The same puncture hole allows a further 1-2 mm deepening twice, repeating the same methodology with 2-4 ml suction, syringe change, 90º bezel rotation and new aspiration. Then the trocar can be removed by sliding it slightly, and a few millimeters above the cortical pelvic, puncture can be repeated, continuing on both sides of the pelvis until about 80 ml are collected. Bone marrow (sterile bag heparinized with a volume of about 80 ml of aspirate) was refrigerated to 4ºC, conditioned and shipped to the Cell Therapy Unit. Further processing should was done within 24 h.

Donor serum samples were also obtained at this time in order to carry out the required screenings for excluding VIH and hepatitis A and B contamination (Annex VIII and Directive 2004/23/EC of the European Parliament and of the Council). Tests must be performed by Nucleic

Acid Amplification Testing (NAAT) to circumvent quarantine, which would otherwise be necessary to avoid the window effect [1]. An aliquot of the serum sample must be stored to allow further analysis if required in future.

Bone marrow samples were transported to the Cell Production Unit at 4-12°C within 24 hours of harvesting. The mononuclear cell fraction was isolated by density-gradient centrifugation, resuspended, and cultured in MSC expansion culture medium [2] in 175-cm^2 tissue culture flasks, with periodic washing to remove non-adherent cells. When cells reached 80% confluence, they were trypsinized and re-plated, and the process was repeated for two more passages. At the end of this period (21-24 days), cells were harvested, resuspended in Ringer's lactate solution containing 0.5% human albumin (CSL Behring GmbH, Marburg, Germany) and 5 mM glucose, and transported at 4-20°C to the hospital application. In addition to quality-control tests, viability and flow cytometric immunophenotypic profiles [2,3] were determined at this stage.

Details on cell injection.

This intervention was performed under slight sedation and radiological control. Cells were injected by disc puncture avoiding neurovascular elements [4]. After double brushing of the region with aqueous povidone-iodine (chlorhexidine in patients allergic to iodine), the field was delimitated with sterile sheets and local anesthesia (Mepivacaine-1%) was applied to skin, subcutaneous tissue and muscle close to the puncture. With fluoroscopy in anteroposterior position, a vertical line corresponding to the projection of the spinous processes was marked on the skin with a sterile dermographic pencil. Then a perpendicular line corresponding to the projected image of a Kirschner needle aligned with the intervertebral space to be treated was drawn. At a point of located 8 to 9 cm (depending on the patient morphotype) from the midline, a 20G spinal needle was inserted with an inclination of 25 to 35 degrees towards the midline. Fluoroscopy was then changed to lateral position. This view ensures that the penetration of the needle follows the right direction until the nucleus pulposus is reached. After the correct position of the needle into the nucleus pulposus was verified in both, the anteroposterior and lateral fluoroscopic views, the suspension of MSC was slowly injected. No incidents have been recorded in none of the 10 patients using this procedure.

Following the cell infusion, the patient was generally discharged after a 2 hour-observation period. Lumbostat corset was not prescribed and moderate walking was permitted. Labour activity was suspended for one week. Exercises for tonifying paravertebral and abdominal muscles were started 1 month after intervention. Analgesic medication was adapted to the needs of each patient and anti-inflammatory drugs were not used.

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