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INTRA ARTICULAR INJECTION OF AUTOLOGOUS MICROFAT AND PLATELET-RICH PLASMA IN THE TREATMENT OF KNEE OSTEOARTHRITIS: A DOUBLE BLIND RANDOMIZED COMPARATIVE STUDY.

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MLL, JV, EJ, FS, JM contributed to the design of the work

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MLL, MC, NR performed the follow up of the patients

EJ, FG, FS, JM drafted the manuscript

All authors revised the manuscript and agreed for all aspects of the work

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1 **INTRA ARTICULAR INJECTION OF AUTOLOGOUS MICROFAT AND PLATELET-**
2 **RICH PLASMA IN THE TREATMENT OF KNEE OSTEOARTHRITIS: A DOUBLE**
3 **BLIND RANDOMIZED COMPARATIVE STUDY.**

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26 **ABSTRACT**

27 Purpose: Compare a single abdominal microfat (MF) injection mixed or not with PRP
28 Low Dose (LD) or High Dose (HD) in order to improve MRI parameters and alleviate
29 pain and enhance functional capacity in knee osteoarthritis (OA).

30 Methods: Patients with symptomatic grade 2 to 4 knee OA according to the
31 International Cartilage Repair Society MRI classification were selected. They were
32 prospectively assessed at baseline, at 3 and 6 months of follow-up. The primary
33 endpoint was the change in maximum of value of cartilage relaxation time in T2
34 mapping sequences (T2max) at 3 months. Secondary endpoints were MRI grade
35 severity and joint space assessment, WOMAC score, pain evaluation, knee range of
36 motion and patient's satisfaction. Adverse events were also collected. The complete
37 cell counts and growth factors content of injected products were assessed to analyze
38 their potential relationship with MRI/clinical outcomes.

39 Results: Three groups of 10 patients received a single injection of 10 cc of a mix (1:1)
40 containing either MF-Saline, MF-PRP LD or MF-PRP HD. T2max did not change
41 significantly over the time for any of the groups. All treatments significantly improved
42 knee functional status and symptoms relief at 3 and 6 months. All patients were
43 responders in the MF/PRP HD at 3 months and significantly higher compared to
44 MF/PRP LD. Half of the injected PRP in the MF/PRP LD group displayed RBCs
45 contamination over 8% which was correlated with an impairment of T2max.

46 Conclusion: A single intra articular injection of MF with or without PRP is safe and
47 may offer a significant clinical improvement in patients with OA.

48

49 Level of evidence: 2 ; Randomized double-blind comparative parallel-group trial

50 Keywords : PRP, knee arthrosis, growth factors, microfat

51 INTRODUCTION

52 Osteoarthritis (OA) is a common cause of disability characterized by joint pain,
53 swelling and loss of function with consequently a negative impact on patients' quality
54 of life, that affected 303 million people globally in 2017¹. Knee OA now accounts for
55 greater than 80% of the burden of disease^{1,2}, having doubled in prevalence over the
56 last 70 years³. Available non-surgical conservative treatments include non-
57 pharmacological methods and pharmacological therapies. These options are
58 effective but with limitations : pharmacological therapies (including analgesics, non-
59 steroid anti-inflammatory drugs (NSAID) drugs and corticosteroid or hyaluronic acid
60 (HA) injections) provide only temporary benefits and sometimes are associated with
61 side effects⁴⁻⁶. This situation has led to the emergence of injectable "biologic"
62 medication. Among them, platelets rich plasma (PRP) continues to see growth in
63 popularity. It is defined as an autologous plasma suspension of platelets,
64 characterized by a higher platelet concentration than in physiological blood⁷. Once
65 injected, platelets are activated after they encountered physiological activators
66 (collagen, calcium) and release high levels of growth factors (GFs) involved in
67 reparative and regenerative mechanisms. These GFs act at various levels to restore
68 the joint homeostasis as several pre-clinical models describe chondrocytes
69 anabolism and chondral remodeling, increased HA secretion and downregulation of
70 inflammation and apoptotic pathways^{8,9}. However, despite promising clinical results
71 on pain and articular knee function¹⁰, studies failed to observe difference in articular
72 thickness when assessed by magnetic resonance imaging (MRI)^{11,12}. Thus, the high
73 abundance of adipose tissue within the body, its high surgical accessibility, and the
74 demonstrated multipotency of mesenchymal stem cells (MSCs) it contains, especially
75 towards the chondrogenic lineage^{13,14}, highlighted the potential of adipose tissue-

76 derived products for cartilage regeneration. Microfat (MF) is simply obtained through
77 dedicated fat harvesting using a dedicated multi perforated cannula with 1 mm holes
78 allowing to collect small lobules of fat (around 600 μm)¹⁵. These small lobules of
79 adipose tissue present the ideal size to promote cells survival¹⁶ and have a greater
80 trophic and regenerative qualities than adipose tissue harvested according to
81 “standard” technique¹⁷. The association of MF and PRP could be interesting in order
82 to potentiate the trophic and regenerative effects on damaged cartilage sites. The
83 combination of these two products, which are respectively rich in autologous
84 multipotent stem cells and GFs, aims to create an optimal environment for cartilage
85 cells regeneration. Furthermore, MF could play the role of a biologically active
86 scaffold as suggested by in vitro experiments¹⁸. The safety profile of autologous
87 microfat and PRP as a mixed regenerative product injected was previously described
88 in the wrist of patients presenting grade 3 or 4 OA according Kellgren Laurence
89 scale¹⁹.

90

91 The purpose of this study was to compare a single abdominal MF injection mixed or
92 not with PRP Low Dose (LD) or High Dose (HD) in order to improve MRI parameters
93 and alleviate pain and enhance functional capacity in knee OA. We also analyzed the
94 relationship between MRI/clinical results and quality of the injected products through
95 a precise biological characterization. We hypothesized that addition of PRP would
96 improve MRI and clinical results compared to MF alone up to 6 months.

97

98 **MATERIALS AND METHODS**

99 **Study Design, Randomization and Intervention**

100 This monocentric prospective randomized, comparative, double blinded, parallel
101 group trial lasted from November 2017 to July 2019 and was performed in
102 collaboration between the orthopedic department of **ANONYMIZED** and the cell
103 therapy department of **ANONYMIZED**. After a first screening, the suitability for
104 inclusion was assessed by a senior orthopedic surgeon (MLL), according to the
105 inclusion and exclusion criteria mentioned in table 1. Included patients were
106 randomly divided into 3 treatment groups: i) single injection of MF mixed with saline
107 (MF/Saline group), ii) MF mixed with a pure PRP containing 1 billion of platelets
108 (MF/PRP LD group), iii) MF mixed with a pure PRP containing 3 billion of platelets
109 (MF/PRP HD group). The study blinding was ensured by blood collection to obtain
110 PRP for each patient, whatever their group. The randomization of patients was done
111 by the cell therapy department after PRP preparation based on platelets
112 concentration level using a randomization list provided by an independent statistician.
113 A block randomization was generated by a computer with a 4-seized-block random-
114 number list. The cell therapy department which is not involved either in patient's
115 injection or follow-up, was in charge of the preparation of MF and PRP or saline,
116 according to randomization. Final syringes were covered to prevent patients and
117 senior orthopedist surgeon (MLL) from identifying the presence of PRP.

118 The protocol was approved by a national ethics committee and national health
119 regulatory authority (**ANONYMIZED**). The study was carried out according to the
120 Declaration of Helsinki and with the principles of Good Clinical Practice. All patients
121 gave written informed consent before their participation. The study was registered at
122 clinicaltrials.gov (NCT **ANONYMIZED**). The original study protocol is available as
123 supplemental file.

124

125 **PRP Preparation Method**

126 After a seven-fold skin decontamination (antiseptic foaming solution, rinsing with
127 sterile water, drying, antiseptic foaming solution, rinsing with sterile water, drying, and
128 alcoholic dermal antiseptic) performed inside the operating room, 45 mL of peripheral
129 blood was collected by venepuncture using a 21-gauge needle filling one 50 mL
130 syringe containing 5 mL of anticoagulant citrate dextrose solution-A (ACD-A) (Fidia,
131 Abano Terme, Italy). The syringe was immediately packed in a sterile bag while the
132 adipose tissue was harvested, before transfer of both products to the good
133 manufacturing practices (GMP) cell therapy facility where all manipulations were
134 conducted under a class A microbiological safety cabinet. The blood was transferred
135 into the Hy-tissue 50 PRP device (Fidia, Abano Terme, Italy) before centrifugation
136 using the Omnigrafter 3.0 (Fidia, Abano Terme, Italy) and PRP Large Volume Cycle
137 (3200 rpm for 10 minutes). All plasma was recovered using a 20 mL syringe through
138 the Push-out system. 300 µL of whole blood and each autologous PRP preparation
139 were sampled to determine platelets, leukocytes and red blood cells (RBCs)
140 concentrations. Based on the level of platelets concentrations and the randomization
141 group, a second spin at 3500 rpm for 10 minutes in a Multifuge Heraus 3 S-R
142 centrifuge (Thermo Scientific, Indianapolis, IN) was performed on residual plasma to
143 obtain platelets poor plasma (PPP) necessary to dilute and obtain 7 mL of PRP at a
144 final concentration of 200 G/L (LD) or 600 G/L (HD). Two mL were used for quality
145 control analysis including a complete cell count (RBCs, leukocytes and platelets
146 concentrations), sterility testing and GF quantification released by the mix. The
147 remaining 5 mL of PRP were transferred in a 10-cc syringe blinded using a sticker.
148 Batch release criteria for PRP production were: absence of macroscopic platelets
149 aggregation, final PRP volume equal to 5 mL, platelets purity > 90%, RBCs

150 contamination < 8%, leukocytes contamination <2%. These criteria were checked by
151 a senior pharmacist before delivery of the products in the operating room.

152

153 **Microfat preparation method**

154 After a seven-fold skin decontamination, harvesting of the adipose tissue was
155 performed in the abdominal area using a Hapifat® kit (Benew Medical, Melesse,
156 France). Entry points were made with a 14-gauge needle, prior to insertion of the
157 infiltration/harvesting St'rim cannula (14 gauge, 2-mm external diameter, 8 holes of
158 0.58 mm square). The infiltration was performed in a closed system between a bag
159 containing an anesthetic solution (200 mL of physiologic serum and 40 mL of
160 lidocaine 1% and epinephrine (1 mg/L)) and the cannula using the 3-way valve Fat
161 Lock System® (Benew Medical, Melesse, France). Diffusion of the anesthetic
162 solution during 10 minutes was waited before MF harvesting. MF was harvested with
163 the same St'rim cannula connected to both a 10-mL syringe and a purification
164 Puregraft 50 (Bimini, Solana Beach, CA) bag through a Fat Lock System® under a
165 low vacuum keeping the plunger in contact with the fat. Approximately 45 mL of
166 MF was collected and packed in a sterile way with whole blood harvested before
167 transfer to the GMP cell therapy facility. Adipose tissue was then purified two times
168 using 1:1 rinsing with saline solution²⁰ allowing for the elimination of fluid excess, lipid
169 phase, blood cells, and fragments through filtration by the Puregraft bag membrane.
170 Finally, 7 mL of MF were obtained: 2 mL dedicated to quality control and 5 mL
171 transferred in a 10-cc syringe blinded with a sticker. Batch release criteria for MF
172 production were: final MF volume equal to 5 mL volume, macroscopic absence of
173 bloody/oily phase. These criteria were checked by a senior pharmacist before
174 delivery of the products in the operating room.

175

176 **Injection**

177 The two 10 mL syringes were connected and mixed by gently shaking back and forth
178 ten times to obtain a final product homogeneously mixed product of 10 mL. The intra
179 articular knee injection was performed by an experienced senior orthopedist surgeon
180 (MLL) well trained to ultrasound guided injections with a 16-gauge needle after a
181 conventional skin aseptic decontamination and using a lateral patellofemoral
182 approach (SonoSite Edge II Ultrasound System, Fujifilm Sonosite Europe,
183 Amsterdam, Netherlands). After the injection, patients were sent back home with
184 instructions to limit the overuse of the leg without range of motion restriction for at
185 least 48 hours and to use paracetamol or ice on the injected area to relieve pain if
186 necessary. NSAID drugs were prohibited during 7 days following the injection. During
187 the follow-up, no treatment restriction was applied and subsequently a gradual
188 resumption of normal sports or recreational activities was tolerated.

189

190 **Biological characterization of injected products**

191 *Cell count.* Whole blood and each PRP preparation were sampled to determine
192 platelets, leukocytes and RBCs counts using automated hematologic blood cell
193 analyzers Sysmex XN-10 (Sysmex, Kobe, Japan) in accordance with recently
194 published guidelines²¹.

195 *Mixed products GFs Release Measurement.* 500 μ L of MF and PRP or saline were
196 mixed together and then maintained in a 48-well collagen coated plate (CellAffix,
197 APSciences, Columbia, MD, USA) and incubated for 30 minutes at 37°C with 5%
198 CO₂ to allow contact with collagen. After this incubation, 500 μ L of non-
199 supplemented Dulbecco's modified eagle medium (DMEM) (Gibco, Thermo Fisher

200 Scientific, Waltham, MA, USA) was added to the mixture and incubated for 24 hours
201 at 37 °C with 5% CO₂. After 24 hours the samples were centrifuged (Multifuge
202 Heraus 3 S-R centrifuge, Thermo Scientific, Indianapolis, IN) at 1500 rpm for 5
203 minutes to remove MF and platelets, and conditioned media were stored at -80 °C
204 until used for analysis. A combination of 12 cytokines and GFs classified as
205 inflammatory (Interleukin-1 β , Tumor Necrosis Factor- α (TNF- α), Interleukin-6 (IL-6),
206 Inteferon γ (IFN γ), anti-inflammatory (Interleukin-1 Receptor antagonist (IL1-Ra),
207 Interleukin-10 (IL-10)) and regenerative (Platelet Derived Growth Factor (PDGF) AA-
208 BB or AB-BB, Vascular Endothelial Growth Factor (VEGF), Nerve Growth Factor
209 (NGF), Epidermal Growth Factor (EGF), Fibroblast Growth Factor 2 (FGF2),
210 Transforming Growth Factor β 1 (TGF- β 1) were measured using a Magpix instrument
211 (Luminex xMAP Technology, Luminex Inc., Austin, TX, USA) allowing simultaneous
212 measurement of the different analytes in small sample volume.

213 *Microbiological assay.* 250 μ L of PRP or MF were sampled in Bactec culture bottles
214 (Peds Plus Aerobic/F and Plus Anaerobic/F culture vials, containing each 40 mL of
215 medium). The Bactec method (Becton Dickinson, Sparks, MD, USA) used a
216 computer-controlled incubation/detection system. The media used contained
217 proprietary factors designed to inactivate a wide variety of antibacterial and antifungal
218 agents. Bactec culture bottles were incubated at 37 °C for a total of 10 days, and
219 automated readings were taken every 10 minutes. Detection of organisms resulted in
220 an audible alarm and automatic recording of time to detection.

221

222 **MRI Analysis**

223 MRI examination was performed on Magnetom Aera 1.5 T Siemens MRI scanner
224 with a 15-channels transmitter/receiver knee coil with patients in supine position knee
225 extended. The following MRI protocol was applied for each patient :

226 *Anatomical images.* Proton density weighted turbo spin echo sequences with fat
227 saturation (DP TSE FS 2D) sequences in sagittal, coronal and transverse planes
228 (Supplemental Table 1).

229 *T2 quantification.* Multi-echo spin echo sequences to measure the relaxation
230 transverse rate T2 (whose change is related to the modification of hydration and
231 structure of tissues) in at least one plane. The T2 parametric map was calculated
232 inline by pixel wise analysis using Siemens Syngo MapIt solution. It used the log-
233 linear least square algorithm to fit the signal as a function of echo time (TE) values.

234 *Post processing.* All measurements were performed by an experimented engineer
235 from Siemens ® and double controlled by i) an experimented radiologist and ii) the
236 senior orthopedist in charge of the follow-up of the patients. The femoro-tibial (medial
237 and lateral) and femoro-patellar compartments were assessed and following region
238 of interest (ROI) were considered: femoro-tibial medial at the level of femur (FTM F)
239 and tibia (FTM T) levels; femoro-tibial lateral at the level of femur (FTL F) and tibia
240 (FTM T) levels; femoro-patellar at the level of trochlea (FP T) and patella (FP P).
241 Corresponding International Cartilage Regeneration Society (ICRS) grades were
242 measured at each follow-up. Maximum of T2 mapping and joint spacing were
243 measured only for the most damaged ROI for each patient. A manual segmentation
244 using the free-hand tool has been done to measure maximum value of cartilage
245 relaxation time on one 3 mm-slice focused on the chondral defect (T2max
246 corresponding to maximum value of the signal over at least 15 voxels analyzed in the
247 ROI) on T2 maps series. A threshold for the normal T2max was set below 40 ms with

248 healthy cartilage ranging from 20 to 39 ms. The joint space was measured manually
249 after x20 zooming and using the distance line tool on anatomical series. A measure
250 of 0.05 cm was considered as a significant increase according Siemens® engineer
251 based on the inherent measurement error defined by the pixel size (0.04 x 0.04 cm²).
252 Figure 1 describes the process to measure MRI based outcomes.

253

254 **Evaluation Tools and Follow-up**

255 Patients were prospectively assessed at baseline and at 3, and 6 months after
256 injection. The primary endpoint measure was defined as the mean improvement in
257 T2max on MRI sequences. MRI evaluation also included assessment of ICRS grade
258 for all ROI and measure of joint space in the most damage area for each patients.
259 Clinical evaluation included the Western Ontario and McMaster Universities Arthritis
260 Index (WOMAC)²², pain Visual Analog Scale (VAS), measures of knee joint range of
261 motion. Patients' satisfaction was rated on a 5-level scale: Very Unsatisfied, Not
262 satisfied, Neutral, Satisfied and Very satisfied. Responders to treatments were
263 defined as patients presenting a decrease of at least 10 points of WOMAC total score
264 defined as the Minimum Clinically Important Difference^{23,24}. Adverse events were
265 recorded.

266

267 **Statistical analysis**

268 *Sample Size.* Sample size was based on previous results of the T2max on MRI
269 sequences²⁵ (mean T2 relaxation time 34.9 ± 5.4 ms). An improvement of at least 10
270 ms was considered clinically relevant. The standard deviation used was 5.4 ms.
271 Using a two-sided α level of 0.05 with a power of 80%, the number of patients was of

272 6 per group. Based on this estimation, the sample size was fixed at 10 patients per
273 treatment group.

274 *Results Analysis.* Efficacy and safety were assessed on intention-to-treat population.
275 Per-protocol analyses were also done as sensitivity analysis. Continuous data are
276 summarized by mean and 95% confidence interval and categorical variables by
277 frequency, number and percentage of patients. Normality were graphically examined
278 and tested using Shapiro-Wilk test. Treatment groups were compared using Analysis
279 of variance (ANOVA) or Kruskal-Wallis test (3 groups), Student t test or Mann-
280 Whitney test (biological characteristics of PRP) and Chi-square test or Fisher's exact
281 test. P-values <0.05 were considered significant. When significant differences
282 between treatment groups were observed, pairwise comparisons were done with
283 adjusted p-values (Tukey). Changes at M6 from baseline were tested to zero using
284 Wilcoxon signed rank test. Pearson correlation coefficient were calculated between
285 biological parameters and clinical changes. Statistical analyses were performed
286 using SAS Version 9.4 (SAS Institute, Cary, NC).

287

288 **RESULTS**

289 **Characteristics of patients**

290 A total of 45 patients were screened and 30 were randomized. One patient treated in
291 the MF-PRP LD could not be included in the analysis due to incomplete data at 3
292 months (**Figure 2**). Patient groups were homogeneous for all the parameters (**Table**
293 **2**).

294 **Biological characteristics of mixed products**

295 The **Table 3** summarizes biological characteristics of experimental products. The
296 PRP final volume was systematically of 5 mL with a very pure PRP extraction (92
297 [89.5;94.4] and $93 \pm [91.5;94.2]$ % of platelets for MF/PRP LD and MF/PRP HD
298 groups, respectively). An expected significant difference ($p < 0.001$) was observed in
299 the number of injected platelets (1.1 [1.0;1.2] and 3.0 [2.8;3.1] billion platelets for
300 MF/PRP LD and MF/PRP HD groups, respectively). The presence of high dose
301 platelets was associated with a significantly higher amount of 3 from the 4
302 inflammatory cytokines quantified (Interleukin-1 β , Tumor Necrosis Factor- α ,
303 Interleukin-6) compared to MF/Saline and/or MF/PRP LD. MF/PRP HD was also the
304 mix releasing the highest quantity of IL-10 anti-inflammatory cytokine. Finally,
305 MF/PRP HD also released a significantly higher amount of PDGF, EGF and TGF- β
306 regenerative factors compared to MF/Saline and/or MF/PRP LD. MF/Saline released
307 a higher amount of NGF and FGF2 than MF/PRP LD ($p = 0.036$ and $p = 0.029$)
308 whereas MF/PRP LD released higher concentrations of EGF compared to MF/saline
309 ($p < 0.001$). Production conformity was complete in 18 batches. The main non
310 conformity observed was the contamination in RBCs higher than 8% and retrieved in
311 7 PRP productions (5 in LD group and 2 in HD group).

312

313 **Comparison of MRI assessments**

314 MRI efficacy endpoints are detailed in Table 4a. T2max significantly decreased at 6
315 months compared to baseline in the MF/Saline group (-8.1 [-15.1;-1.1] $p = 0.03$). No
316 difference was observed between groups regarding the change on T2max at 3 or 6
317 months. Only one patient from 7 of the MF/saline group with abnormal T2max was
318 able to reach the normal value below 40 ms. None of the 9 patients in the MF/PRP

319 LD or HD groups were able to normalize their T2max. The relative proportion of
320 grade 4 damaged areas were higher than 40% initially and decreased below 30% at
321 6 months in all groups. While this was combined to an increase in grade 3 damaged
322 areas in the groups MF/Saline or MF/PRP HD and an increase of grade 2 damaged
323 areas in MF/PRP LD group, the number of area improved and the associated level
324 showing improvement were similar between experimental groups. No progression of
325 any of the initial damaged areas in grade 1 was observed (Figure 3 and
326 supplemental Figure 1). Finally, the proportion of patients with an increase in joint
327 space ≥ 0.05 cm in at least one area treated at 6 months reached 70% in MF/Saline
328 vs 44% and 40% in MF/PRP LD and HD respectively ($p=0.42$, Figure 4).

329

330 **Comparison of clinical assessments**

331 Clinical efficacy endpoints are detailed in Table 4b. All treatments were effective in
332 improving knee functional status and reducing symptoms, with a significant decrease
333 observed at 6 months on all scores, except for MF/PRP LD with WOMAC function
334 and MF/PRP HD with WOMAC stiffness .The mean changes from baseline to 3
335 months in WOMAC total score were -22.6 [-35.8;-9.4] , -10.3 [-23.5;2.8]and -21.9 [-
336 31.1;-12.7] for MF/Saline, MF/PRP LD and MF/PRP HD groups respectively
337 ($p=0.13$). The mean change at 3 months in pain VAS was not statistically different
338 ($p=0.55$) between the groups (-28.0 [-46.4;-9.6], -22.2 [-37.5;-7.0] and -33.5 [-49.5;-
339 17.5] for MF/Saline, MF/PRP LD and MF/PRP HD groups, respectively).The
340 satisfaction rate was of 80.0% in the MF/Saline and MF/PRP HD groups and 66% in
341 the MF/PRP LD group at 3 months ($p=0.75$). Majority of the patients with initial
342 abnormal flexion or extension of the knee improved at 3 months without difference
343 between the groups. Globally, the improvement was maintained at 6 months without

344 significant difference over experimental groups. The only significant difference
345 observed concern the percentage of responders at 3 months which was higher in
346 MF/PRP HD (100%) than MF/PRP LD group (44.0%) ($p=0.01$) but this was not
347 maintained at 6 months (80% for MF/saline, 70% for MF/PRP HD and 44 % for
348 MF/PRP LD; $p=0.30$).

349

350 **Correlation between biological characteristics and clinical/MRI outcomes**

351 Supplemental Table 2 presents the correlation between biological characteristics of
352 injected mixed products and clinical (WOMAC and pain VAS change) and MRI
353 outcomes (T2max). The percentages of platelet purity and RBCs contamination were
354 respectively associated to an improvement and impairment in change of T2max over
355 the time ($r=-0.34$, $p=0.05$ and $r=0.33$, $p=0.05$).

356

357 **Safety**

358 None of the adverse events reported was judged as related to the study treatment.
359 The mean pain VAS during injection was similar between the experimental groups
360 (23.0 [0.6;45.4], 33.7 [14.1;53.3] and 29.0 [6.0;52.0] in MF/Saline, MF/PRP LD and
361 MF/PRP HD groups; $p= 0.74$). Respectively 4, 3, and 3 patients reported a pain > 30
362 during injection in MF/Saline, MF/PRP LD and MF/PRP HD groups ($p=1$). None of
363 the production non conformity observed was correlated with adverse events.

364

365

366

367

368

369

370 **DISCUSSION**

371 The principle finding of this study was that single intra articular injection of MF with or
372 without PRP is safe and may offer a significant clinical improvement in patients with
373 OA. The primary objective was the reduction of T2max at 3 months but no significant
374 difference was found between experimental groups. A significant decrease in T2 max
375 was observed at 6 months in MF/Saline group without reaching a mean improvement
376 of 10 ms considered as clinically relevant. Other MRI assessments showed a
377 reduction in ICRS grade severity and improvement in joint space without reaching
378 statistical significance. All patients were considered as responders 3 months after the
379 procedure in the MF/PRP HD and significantly higher compared to LD group.
380 Interestingly, all experimental groups showed a higher improvement in WOMAC
381 mean change compared to a study with similar inclusion criteria and comparing a
382 single injection of pure PRP (-10.1 ± 15.4 and -8.1 ± 21.4 at 3 months and 6 months)
383 vs HA (-5.3 ± 12.3 and -6.2 ± 15.7 at 3 months and 6 months)²⁶. However, these
384 findings should be carefully interpreted knowing the substantial placebo effect in
385 arthrosis care, which could reach a mean improvement in WOMAC total score of
386 11.34 ^{27,28}. Furthermore, long-term comparison with more accessible and cost
387 effective injectable drugs (steroid, HA or PRP alone) will be necessary to ensure the
388 interest of MF injection. The positive impact of MF on knee OA is consistent with
389 available literature describing the use of microfragmented fat (MFAT). MFAT
390 production slightly differ from MF as it is obtained by reducing the size of the adipose
391 tissue around 300 to 800 μm after harvesting whereas MF initially selects small fat
392 lobules (600 μm) using a dedicated cannula. Interestingly, both techniques avoid
393 centrifugation step and prefer abundant washing to eliminate oily substances and

394 blood residues. This aspect could be of importance as in vitro assays previously
395 underlined that grafts prepared by washing with filtration exhibited a significantly
396 reduced blood cell and free lipid content, with a greater adipose tissue viability than
397 other methods²⁹. In 2017, Hudetz et al reported pain VAS improvement from $39.4 \pm$
398 25.6 to 11.7 ± 16.2 at 6 months after MFAT injection of 4 to 15 cc in 17 patients (32
399 knees) with symptomatic knee OA grade 3 or 4³⁰. Their study also included an
400 exhaustive MRI analysis based on delayed gadolinium-enhanced magnetic
401 resonance imaging of cartilage (dGEMRIC) index to estimate joint cartilage
402 glycosaminoglycan (GAG) content correlated with cartilage quality. They reported
403 175 dGEMRIC index values improved and only 37 impaired³⁰. Interestingly, they
404 recently reported that only 3 patients underwent knee arthroplasty 24 months after
405 the procedure and more than half of dGEMRIC measurements were still improved
406 and opposed to the expected GAG decrease over the natural course of knee OA³¹.
407 Conversely, Pintat and colleagues have injected 6 cc of adipose tissue mixed with 3
408 cc of pure PRP and did not report differences on MRI follow-up at 6 months in grade,
409 surface or T2 value of the chondral lesions³². Elongation of T2 relaxation time at later
410 degeneration stages could be linked to the progressive erosion that leads to a loss of
411 both cartilage quantity and quality, with better sensitivity to OA progression as
412 compared to arthroscopy³³. Furthermore, different studies showed significant
413 correlations between T2 mapping and patient-reported outcome measures after
414 cartilage repair surgery³⁴. These arguments guided our choice to use T2 relaxation
415 time as main criteria, which can be discussed. Indeed, literature review highlights the
416 great heterogeneity in MRI timing, analysis and results following adipose tissue
417 injection in knee OA and emphasizes the need for a consensus definition of
418 responders using MRI. One interesting perspective might be a quantitative volumetric

419 assessment of healthy/damaged cartilage which have been shown to be correlated
420 with clinical improvement³⁵. This parameter can be compared to reference data
421 generated in longitudinal studies assessing the annual loss of cartilage volume in
422 patella of OA population which is comprised between 4.1 to 8% with no significant
423 change in the healthy population^{35,36}. However, cartilage quantitative volumetric
424 assessment is still performed manually and is time consuming and should benefit
425 from artificial intelligence to circumvent these issues and become a reference
426 parameter. From a clinical perspective, the combined product used by Pintat et al
427 provided an improvement of WOMAC from 34.3 ± 24.0 to 15.7 ± 16.7 at 6 months.
428 While the obtention of adipose tissue necessitates a centrifugation step which differs
429 from our procedure, they also prepared a highly pure PRP. This choice is consistent
430 with *in vitro* data describing exposition of synovial cells with leucocyte-rich PRP and
431 RBCs that resulted in significant cell death and proinflammatory mediator
432 production³⁷. The rationale to avoid RBCs in final PRP is based on the potential
433 release of hemoglobin, hemein and iron after lysis, which can induce oxidative stress
434 and pro-inflammatory reactions in tissues³⁸. Our study is in line with these findings as
435 platelets purity and RBCs contamination were respectively correlated with
436 improvement and impairment in T2max. Interestingly, half of MF/PRP LD presented
437 production non conformity with contamination in RBCs higher than 8%. This is
438 combined to the superiority in short term responders rate of the MF/PRP HD over LD
439 and reinforce the consensus recommendations edited in 2019 by the American
440 Association of Orthopedic Surgeons (AAOS) claiming detailed and rigorous biological
441 characterization of whole blood and PRP for each injection performed in clinical
442 study³⁹. Thus, a consensus is emerging for the use of leukocyte-poor, RBC-free
443 formulations of PRP when administered intra-articularly.

444 Other adipose related strategies are more complex and consist to directly inject a
445 homogeneous suspension of MSCs obtained after weeks of expansion. From a
446 regulatory point of view, adipose derived MSCs fall under the framework of directive
447 1394/2007 from the European Parliament and the European Council, which
448 described a new category of health products referred to as “advanced therapy
449 medicinal products” (ATMPs). Conversely, the preparation of MF can be done within
450 one hour, allowing for lipoaspiration and reinjection to be performed on the same
451 procedure. This point should be considered from a cost-effectiveness perspective.

452 To date, no data are available to explain the mechanism of action of MF in knee OA.
453 The main hypothesis is linked to the presence of highly functional MSCs with
454 chondrogenic differentiation capacity⁴⁰, as they are kept intact into the adipose
455 stromal vascular niche during the preparation process⁴¹. Recent findings on MFAT
456 also reported a long-lasting secretion of anti-inflammatory cytokines (up to 28 days)
457 and the ability to inhibit inflammatory function of monocyte cell line in vitro⁴².

458

459 **Limitations**

460 The major limitation of the study is the absence of a placebo group which is an
461 important weakness knowing the substantial placebo effect in arthrosis care. The
462 limited number of treated patients resulted in an underpowered trial (10%) to assess
463 the primary endpoint which raises the question of the choice of T2 relaxation time as
464 main criteria. Other limitations that can be mentioned are : a 6 months follow-up
465 which could be considered short for OA study and the highly specific cohort limiting
466 any extrapolation.

467

468 **CONCLUSIONS**

469 A single intra articular injection of MF with or without PRP is safe and may offer a
470 significant clinical improvement in patients with OA.

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639 **FIGURES LEGENDS**

640

641 **Figure 1.** Process measurements of MRI based outcomes. Anatomical images were
642 used in sagittal, coronal and transversal planes to define ICRS grade in the different

643 compartments and the most damaged ROI. Joint space and maximum of relaxation
644 time were measured for the most damaged ROI.

645

646 **Figure 2.** Participant flow diagram according Consolidated Standards of Reporting
647 Trials (CONSORT statement).

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649 **Figure 3.** Repartition of OA grade according International Cartilage Repair Society
650 MRI classification over the time in the different experimental groups

651

652 **Figure 4.** Left knee MRI follow up in T2 DP FAT SAT coronal (A,B,C) sequence and
653 T2 mapping sequence (D,E,F), in a 33 years old patient in supine position knee
654 extended with symptomatic grade 3 chondropathy in the femoro tibial medial
655 compartment at month 0 (A) and grade 2 at month 6 (C).The joint space increase
656 from 0,35 cm at baseline (A) to 0,40 cm at M3 (B) and 0,49 at M6 (C). The maximum
657 value of cartilage relaxation time on T2 mapping sequences decrease from 56 ms at
658 month 0 (D) to 44 ms at month 6 (F)

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664 **TABLES**

665 **Table 1.** Inclusion and Exclusion Criteria

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<p>INCLUSION CRITERIA</p> <ul style="list-style-type: none">• age between 20 and 65 years;• symptomatic knee OA from grade 2 to 4 according International Cartilages Regeneration and Joint Preservation Society (ICRS) MRI criteria;• pain visual analog scale ≥ 40;• failure of well-managed pharmacological treatment > 1 year ;• Body Mass Index between 20 and 30 ;• Hb > 10 g/dL;• negative pregnancy test ;• written informed consent.
<p>EXCLUSION CRITERIA</p> <ul style="list-style-type: none">• contraindications making MRI scanning unsafe;• thrombocytopenia < 150 G/L; thrombocytosis > 450 G/L; thrombopathy• coagulation disorders;• infectious disease or positive serology to VIH-1, HCV, HBV and syphilis;• actual chronic treatment by oral corticosteroid (or last dose taken less than 2 weeks before);• intra articular knee injection of corticosteroid or hyaluronic acid less than 8 weeks before inclusion;• platelet inhibiting agent (Aspirin, NSAID) or antivitamin K treatment completed less than 2 weeks before inclusion;• fever or recent disease;• auto immune disease;• inflammatory arthritis;• immune deficit;• history or ongoing malignant tumor• patient under guardianship or involved in another clinical trial.

667 ICRS : International Cartilages Regeneration Society; MRI Magnetic Resonance Imaging,; Hb :
668 haemoglobin; VIH : human immunodeficiency virus; HCV : Hepatitis C Virus; HBV : Hepatitis B Virus,
669 NSAID : Nonsteroidal anti-inflammatory drugs

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674 **Table 2.** Baseline Characteristics of patients

	MF/Saline (n=10)	MF/PRP LD (n=10)	MF/PRP HD (n=10)	p-value
Age (y)	49.6 [44.0; 55.2]	46.4 [36.8; 56.1]	44.5 [38.6; 50.4]	0.486
BMI (kg/m²)	25.5 [24.5; 26.4]	23.5 [23.1; 24.2]	25.0 [24.3; 25.8]	0.112
Disease history (y)	7.4 [3.0; 11.8]	6.8 [3.0; 10.5]	11.8 [5.9; 17.7]	0.371
Men, n	5	5	7	0.721
Knee history				
Traumatic, n	6	8	4	0.248
Non traumatic, n	1	1	0	0.999
Surgical history	6	8	5	0.510
ACL, n	3	4	1	0.450
Arthroscopy, n	5	4	5	0.999
Other, n	1	1	0	0.999
Previous treatment				
CTC, n	7	9	6	0.450
HA, n	10	10	9	0.999
PRP, n	5	3	3	0.709
Patients with		5	5	0.999
- 1 damaged area, n	4			
- 2 damaged areas, n	4	3	5	0.893
- 3 damaged areas, n	2	2	0	0.507
Patients with ≥1 stade IV, n	9	7	7	0.642
Patients with bone oedema, n	4	2	6	0.248
Patients with abnormal extension, n	3	4	2	0.879
Patients with abnormal flexion, n	4	5	1	0.228

675 Data represent mean [95% confidence interval],, or number of patients (n). P-value from Kruskal-
676 Wallis test and Fisher exact test. BMI : Body Mass Index; ACL : anterior cruciate ligament; CTC :
677 corticotherapy; HA : hyaluronic acid; PRP : Platelets rich plasma
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683 **Table 3.** Biological characteristics of experimental products

Biological Characteristics		MF/Saline (n=10)	MF/PRP LD (n=10)	MF/PRP HD (n=10)	p-value
Blood sample	Leukocytes(G/L)		5.7 [4.8; 6.7]	6 [4.7; 7.2]	0.738
	RBCs (T/L)		4.2 [3.8; 4.5]	4.4 [4; 4.8]	0.286
	Platelets (G/L)		194 [165; 222]	223 [208; 237]	0.054
PRP	Leukocytes(G/L)		1 [0.6; 1.4]	4 [2.5; 5.5]	<0.001
	RBCs (T/L)		0.02 [0.01; 0.02]	0.04 [0.03; 0.05]	<0.001
	Platelets (G/L)		220 [203.2; 236.8]	591.7 [556.1; 627.3]	<0.001
Quantity Injected	Leukocytes x10 ⁶		5.1 [3; 7.2]	20.1 [12.7; 27.6]	<0.001
	RBCs x10 ⁶		90 [61.8; 118.2]	210 [160; 260]	<0.001
	Platelets x10 ⁹		1.1 [1.0; 1.2]	3.0 [2.8; 3.1]	<0.001
% injected	Leukocytes		0.43 [0.25; 0.62]	0.62 [0.42; 0.83]	0.133
	RBCs		7.6 [5.2; 10.1]	6.5 [5.2; 7.8]	0.374
	Platelets		92 [89.5; 94.4]	92.9 [91.5; 94.2]	0.465
Cytokines pg/mL		(n=9)*	(n=9)*	(n=9)*	(n=6)*
	IL1b	2.3 [-3; 7.5]	1 [-0.2; 2.1]	15.4 [-6.2; 37.1]	0.045 HD>(MF,LD)
	TNFα	6.7 [-1.7; 15.2] (n=6)	10.9 [7.5; 14.3]	34.1 [7.6; 60.6]	0.007 HD>(MF,LD)
	IL6	3818 [2162; 5474]	2161 [1226; 3096] (n=8)	7077 [1313; 12841]	0.029 HD>LD
	IFNγ	0	13.5 [-13; 40.1]	2.4 [-1.4; 6.1]	0.386
	IL10	4.9 [2.7; 7]	6.2 [3.2; 9.2]	14.3 [3.8; 24.9]	0.013 HD>(MF,LD)
	IL1Ra	85.4 [-19.3; 190.2]	63.4 [23.3; 103.5]	144.7 [78.2; 211.2]	0.282
	Ratio IL1Ra/IL1b	82.7 [-23.2; 188.6]	84.6 [-37.1; 206.3]	44.9 [-30.1; 119.9]	0.830
	PDGF	90.8 [40.1; 141.4]	499.7 [134.6; 864.9]	1046.3 [759.7; 1332.8]	<0.001 HD>LD>MF
	VEGFα	64.5 [11.2; 117.8]	66.2 [-5.1; 137.5]	13.3 [-12.1; 38.7]	0.332
	NGF	23.4 [8.5; 38.2]	6.5 [3; 9.9]	9.9 [1.7; 18.2]	0.036 MF>LD
	EGF	6.8 [0.5; 13]	88.6 [53.9; 123.2]	227.7 [165.7; 289.6]	<0.001 HD>LD>MF
	FGF2	10402 [4811; 15993]	3954 [2907; 5001]	6201 [3217; 9185]	0.029 MF>LD
	TGFB1	1928 [795; 3060]	4330 [3604; 5057]	9428 [4978; 13877]	<0.001 HD>(MF,LD)
Production Conformity					
MF Macroscopic aspect		10/10	10/10	10/10	
MF Volume = 5 mL		10/10	10/10	10/10	
MF Sterility		9/10	9/10	8/10	
PRP Volume = 5 mL			10/10	10/10	

PRP Platelets purity > 90%	8/10	10/10
PRP Red Blood Cells contamination < 8%	5/10	8/10
PRP Leukocytes contamination < 2%	10/10	10/10
PRP Aggregates absence	10/10	10/10
PRP Dose: 1 or 3 billion (±10%)	10/10	7/10
PRP Sterility	10/10	10/10

684 Data represent mean [95% confidence interval] or number of productions. P-value from Mann-Whitney
685 test and Kruskal-Wallis test. Tukey test for pairwise comparisons. *The cytokines assay was not
686 performed for 1 patient in MF/Saline group and for 4 patients in MF/PRP HD group.
687 RBC : red blood cells; TNF- α : tumor necrosis factor- α ; IL : interleukin; EGF, epidermal growth factor;
688 VEGF: vascular endothelial growth factor; PDGF: platelet-derived growth factor; NGF: nerve growth
689 factor; FGF2 : fibroblast growth factor 2; TGF- β 1 : transforming growth factor β 1; IL1Ra : interleukin-1
690 receptor antagonist; MF : microfat; PRP : platelets rich plasma; LD : low dose; HD : high dose; G:
691 Giga (x106); T:Tera (x109); L : Litre
692

693 **Table 4a.** Radiological outcomes

	MF/Saline (n=10)	MF/PRP LD (n=10)	MF/PRP HD (n=10)	p-value
T2 Maximum (ms)				
Baseline	49.1 [42.3 ; 55.9]	56.9 [43.9 ; 69.9]	61.9 [52.8 ; 71]	0.078
Change M3	-5.9 [-17.2 ; 5.4]	1.1 [-13.7 ; 15.9]	-3.3 [-15.1 ; 8.4]	0.845
Change M6	-8.1 [-15.1 ; -1.1]	-7.3 [-23.2 ; 8.7]	-4.2 [-14.6 ; 6.2]	0.757
p-value change at M6	0.029	0.313	0.359	
Joint Spacing (cm)				
Patients with increase \geq 0.05 cm (all areas)				
M3	3 (30)	4 (44)	2 (20)	0.540
M6	7 (70)	4 (44)	4 (40)	0.419
Number of area with improvement				
M3	2/60 (3)	1/54* (2)	1/54* (2)	1.000
M6	6/60 (10)	7/48* (15)	6/60 (10)	0.715
Level of OA showing improvement at M6				
IV	6/14 (43)	3/12 (25)	4/12 (33)	0.636
III	0/9 (0)	4/12 (33)	2/10 (20)	0.195

694 Data represent mean [95% confidence interval] deviation or number of patients (frequency). P-value
695 from Kruskal-Wallis test or Fisher exact test. MF : microfat; PRP : platelets rich plasma; LD : low
696 dose; HD : high dose; OA : osteoarthritis
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	MF/Saline (n=10)	MF/PRP LD (n=10)	MF/PRP HD (n=10)	p-value
WOMAC total score				
Baseline	38.8 [27 ; 50.6]	36.9 [20.7 ; 53.1]	42.5 [27.3 ; 57.7]	0.876
Change M3	-22.6 [-35.8 ; -9.4]	-10.3 [-23.5 ; 2.8]	-21.9 [-31.1 ; -12.7]	0.126
Change M6	-23.8 [-37.6 ; -10]	-15.1 [-30.8 ; 0.6]	-21.2 [-36.3 ; -6.1]	0.524
<i>p-value change at M6</i>	0.01	0.031	0.008	
WOMAC Stifness				
Baseline	3.3 [2.1 ; 4.5]	4.5 [3.5 ; 5.5]	3.9 [2.1 ; 5.7]	0.32
Change M3	-1.3 [-3 ; 0.4]	-1.6 [-3.1 ; 0]	-1.8 [-3.5 ; -0.1]	0.885
Change M6	-1.8 [-3 ; -0.6]	-2 [-3.3 ; -0.7]	-1.4 [-2.9 ; 0.1]	0.687
<i>p-value change at M6</i>	0.016	0.016	0.063	
WOMAC Pain				
Baseline	9.5 [7 ; 12]	8.5 [5.4 ; 11.6]	11.2 [8.2 ; 14.3]	0.507
Change M3	-5.2 [-8.4 ; -2]	-2.4 [-4 ; -0.9]	-5.5 [-7.9 ; -3.1]	0.08
Change M6	-5.1 [-8.5 ; -1.7]	-3 [-5.4 ; -0.6]	-6 [-9.2 ; -2.8]	0.279
<i>p-value change at M6</i>	0.012	0.031	0.008	
WOMAC Functional limitation				
Baseline	26 [17.5 ; 34.5]	23.9 [11.3 ; 36.5]	27.4 [16.4 ; 38.4]	0.893
Change M3	-16.1 [-24.8 ; -7.4]	-6.3 [-17.1 ; 4.4]	-14.6 [-20.8 ; -8.4]	0.126
Change M6	-16.9 [-26.5 ; -7.3]	-9.7 [-21.9 ; 2.5]	-13.8 [-24.6 ; -3]	0.286
<i>p-value change at M6</i>	0.01	0.1	0.002	
Responder 10 pointsn (%)				
Change M3	8 (80)	4 (44)	10 (100)	0.014 HD>LD
Change M6	8 (80)	4 (44)	7 (70)	0.296
Pain VAS				
Baseline	54 [43.2 ; 64.8]	60 [48.8 ; 71.2]	66 [54.7 ; 77.3]	0.241
Change M3	-28 [-46.4 ; -9.6]	-22.2 [-37.5 ; -7]	-33.5 [-49.5 ; -17.5]	0.549
Change M6	-33.3 [-52.2 ; -14.5]	-34.4 [-49.4 ; -19.5]	-36.7 [-53.4 ; -19.9]	0.894
<i>p-value change at M6</i>	0.016	0.004	0.004	
Likert = 1 or 2 (satisfied) n (%)				
M3	8 (80)	6 (66)	8 (80)	0.751

M6	8 (80)	8 (89)	6 (60)	0.430
Patients with initial abnormal flexion or extension				
Flexion Improvement $\geq 5^\circ$, n (%)				
M3	4 (100)	4 (80)	1 (100)	1
M6	4 (100)	4 (80)	1 (100)	1
Extension improvement $\geq 5^\circ$, n (%)				
M3	1 (33)	2 (50)	2 (100)	0.524
M6	2 (66)	3 (75)	2 (100)	1

700 Data represent mean [95% confidence interval] or number of patients (frequency). P-value from
701 Kruskal-Wallis test or Fisher exact test. WOMAC : Western Ontario and McMaster Universities
702 Osteoarthritis Index; MF : microfat; PRP : platelets rich plasma; LD : low dose; HD : high dose; OA :
703 osteoarthritis; VAS : visual analog scale.
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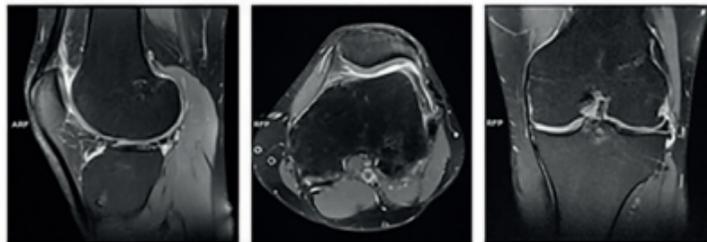
SEQUENCES

1. ANATOMICAL IMAGES :

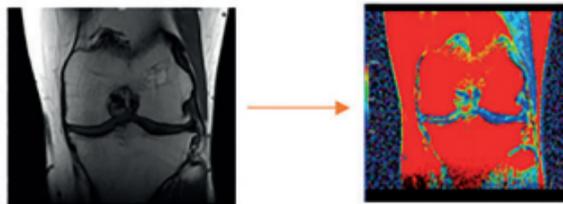
DP TSE FS 2D sequences

Acquisition in sagittal, transverse and coronal planes

(See supplemental Table 1)



2. T2 map based on multi echo spin echo sequence



VISUAL EVALUATION

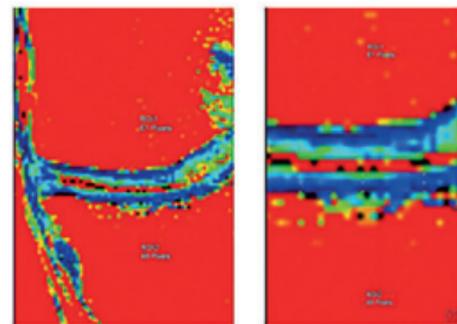
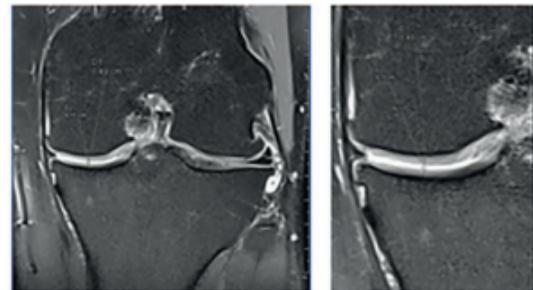
ICRS MRI grading on all compartments

Selection of the most damaged ROI
Measurement of :

- **Joint Space**
- **T2 relaxation time**

QUANTITATIVE EVALUATION

Joint Space measurement
1 slice / 3 mm



T2 relaxation time measurement
1 slice / 3 mm / > 15 voxels



MRI Magnetom Aera
1,5T Siemens

Assessed for eligibility (n= 45)

Excluded (n= 15)

- Not meeting inclusion criteria (n= 13)
 - MRI contraindication (n=6)
 - Impossibility to stop platelet inhibitor (n=5)
 - BMI <20 (n=2)
- Declined to participate (n= 2)

Randomized (n= 30)

Allocated to **MF/Saline** (n= 10)

- Received allocated product (n= 10)

Allocated to **MF/PRP LD** (n= 10)

- Received allocated product (n= 9) :
1 consent withdrawal

Allocated to **MF/PRP HD** (n= 10)

- Received allocated product (n= 10)

Lost to follow-up (n= 0)

Discontinued intervention (n= 0)

Lost to follow-up (n= 0)

Discontinued intervention (n= 0)

Lost to follow-up (n= 0)

Discontinued intervention (n= 0)

Analysed at M3 (n= 9)

Excluded from analysis (n= 1) :
1 major deviation

Analysed at M6 (n= 10)

Analysed at M3 (n= 8)

Excluded from analysis (n= 1) :
1 major deviation

Analysed at M6 (n= 7)

Excluded from analysis (n= 2) :
1 major deviation
1 missing data

Analysed at M3 (n= 8)

Excluded from analysis (n= 2) :
1 major deviation
1 missing data

Analysed at M6 (n= 10)

