Patient-reported health outcomes for severe knee osteoarthritis after conservative treatment with an intra-articular cell-free formulation for articular cartilage regeneration combined with usual medical care vs. usual medical care alone: A randomized controlled trial

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Abstract. Osteoarthritis (OA) is a major public health problem characterized by joint pain, fatigue, functional limitation and decreased quality of life of the patient, which results in increased use of healthcare services and high economical costs. A promising novel bioactive cell-free formulation (BIOF2) for cartilage regeneration has recently been tested in pre-clinical and clinical trials, and has demonstrated a success rate similar to that of total joint arthroplasty for the treatment of severe knee OA. The present study evaluated the efficacy of treatment with BIOF2, by including it within a conservative regimen of 'usual medical care' of knee OA, and whether its efficacy was affected in subgroups of patients presenting with comorbidities that exacerbate OA. A prospective, randomized, 2-arm parallel group phase III clinical trial was conducted, which included 105 patients in the 'usual medical care' group (paracetamol/NSAIDs and general care provided by the family physician) and 107 patients in the BIOF2 group (usual medical care + intra-articular BIOF2 application at 0, 1 and 2 months). Two aspects were evaluated at 0, 6 and 12 months: i) Minimal clinically important improvement (MCII), based on 30% improvement of pain from the baseline; and ii) the Patient Acceptable Symptom State (PASS), a questionnaire that determines patient well-being thresholds for articular pain and function. Adverse effects and regular NSAID use were registered. At 12 months, BIOF-2 treatment produced MCII in 70% of the patients and >50% achieved PASS. Excluding the patients with class 2 obesity or malalignment conditions (*genu varum* or *genu valgum* >20 degrees), the experimental treatment produced MCII and PASS in 100 and 92% of patients, respectively, compared with 25 and 8% in the group of usual medical care ($P<0.001$). No patient with malalignment and treatment with BIOF2 achieved PASS. Notably, there were no serious adverse effects. To conclude, BIOF2 is a safe therapeutic alternative that is easy to implement together with usual medical care for knee OA. Trial registration: Cuban Public Registry of Clinical Trials (RPCEC) Database RPCEC00000277. Retrospectively registered June, 2018.

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Introduction

Osteoarthritis (OA) is recognized as a major public health problem, characterized by joint pain, fatigue, functional limitation, and decreased quality of life of the patient, leading to increased use of healthcare services and consequent economic burden (1). In older persons, the knee is the joint most commonly affected by pain usually attributed to OA. In a survey of adults 50 years of age and older, nearly half of them reported having pain for a period of one year (2). Its incidence is rising due to increasing obesity and the ageing of the population (3). The inability to walk due to symptomatic knee OA has been associated with all-cause mortality (4). The high prevalence of such a condition and its impact in terms of disability, mortality, and economics, make the search for effective therapeutic alternatives of easy implementation a priority.

Apart from education and exercise, the only available nonsurgical treatments are directed at symptoms, primarily to alleviate pain and enhance daily activities and quality of life (5,6). In cases of advanced disease or ineffective conservative therapy, a recommended option is total joint arthroplasty (TJA), which consists of replacing the articulation with a prosthesis. However, such surgical treatment is costly (7-9), and there is frequently a long waiting list for patients utilizing public healthcare systems.

A promising novel, bioactive cell-free formulation (BIOF2) for articular cartilage regeneration, has recently been tested in preclinical and clinical trials (10,11). The intra-articular application of BIOF2 significantly increased cartilage thickness (12-38%) in different OA animal models, compared with articular cartilage treated with saline solution (11). BIOF2 is a mixture whose main components are a corticosteroid and organic acids (10). Corticosteroids are bioactive substances that possibly facilitate tissue atrophy and joint destruction when acting alone (12). On the other hand, in *in vitro* trials with articular cells, different organic acids, such as retinoic acid or ascorbic acid, have been shown to increase the expression of genes related to chondrogenesis (13,14) and osteogenesis (15). Even though it has been proven that those acids promote differentiation into bone cells (15-18), their capacity to generate, on their own, a morphologic differentiation into cartilage cells is a topic of debate (19,20). However, when those acids are combined with other co-factors, they aid in the process of differentiation into chondrocytes (14,21-23). According to previous reports of *in vitro* trials on animal models and human patients (11,24), the combination of the compounds present in BIOF2 act in synergy to modify the intra-articular microenvironment and stimulate articular regeneration by producing molecular and morphologic alterations in synovial fluid cells and chondrocytes (11).

The results of a previous clinical trial showed the intra-articular application of BIOF2 to be well-tolerated, with a success rate similar to that of total arthroplasty for the treatment of severe knee OA. Success was correlated with an average 22% increase in articular cartilage (24). However, the present study is the first to evaluate treatment with BIOF2 in patients with severe knee OA that are treated within the public healthcare system and receive conservative 'usual medical care' (paracetamol/NSAIDs and general care provided by the family physician) before entering into a TJA program or other therapeutic option. In the general population, there are subgroups of patients with comorbidities that exacerbate knee OA, such as *genu varum* or *genu valgum* malalignment greater than 20 degrees and/or class 2 obesity [body mass index (BMI) of 35-39]. To determine the limits of this new treatment, it is important to know whether BIOF2 is effective when included as part of a conservative usual medical care regimen and if its efficacy is affected in subgroups of patients with comorbidities.

Therefore, the present study was designed to randomly select patients undergoing usual medical care, for the addition of treatment with BIOF2 and compare them with a control group that only underwent usual medical care. The utilized regimen was that most frequently carried out at the majority of public healthcare centers in Mexico and other countries, which consisted of nonsteroidal anti-inflammatory drugs (NSAIDs) and/or paracetamol prescription. That is the therapy generally given to patients with severe knee OA, while they wait for other therapeutic options.

Patients and methods

Study design. A prospective, single-blind, 2-arm, parallel group, randomized phase III clinical trial was conducted between March 2016 and March 2018. The study was carried out according to the 'CONSORT statement' guidelines for randomized controlled trials.

The present study was approved by the ethics committee of the *Cancerology State Institute* of the Colima State Health Services, Mexico, and written informed consent was obtained from all the participants. The present clinical trial was registered as ARTROTX-II/III: RPCEC00000277 in the Cuban Public Registry of Clinical Trials (RPCEC) database.

Study subjects. The inclusion criteria were: Patients ≥40 years of age, with a BMI ≤39 kg/m^2 and knee OA, according to the diagnostic criteria of the American College of Rheumatology (25). The target knee was defined as the more symptomatic knee (with a pain score of at least 5 on the 0-10 Visual Analog Scale [VAS] for at least 6 months before enrollment in the study). The patients had to be under usual medical care, based on paracetamol/NSAIDs, prescribed by their family physician. In short, they were patients with significant symptoms and/or functional limitations associated with reduced health-related quality of life. The exclusion criteria were: having received some type of intra-articular treatment within the 12 months prior to the study, a history of knee surgery, inflammatory polyarthritis, fibromyalgia, chronic fatigue syndrome, thromboembolic disease, hemorrhagic blood disease, Hb <80 g/l, neuromuscular disease, cancer, metabolic bone disease, alcoholism, drug addiction, or class 3 obesity (BMI of 40 or higher) (26). Participants were recruited from primary and secondary healthcare centers in the State of Colima, Mexico. The efficacy evaluations and intra-articular BIOF2 application were performed at the *Centro Hospitalario Unión* (a Secondary Healthcare Center) located in the State of Colima, Mexico.

A total of 237 patients were randomly allocated to the intra-articular BIOF2 group or the control group of usual medical care (paracetamol/NSAIDs) prescribed by the family physician. Randomization was performed using

computer-generated random allocation cards, and patients were assigned to one of the 2 groups. The process was conducted by researchers that did not participate in the evaluation of the results. It should be made clear that before their inclusion in the study, all the patients were under the care of their family physician and receiving the standard paracetamol/NSAID-based treatment for OA control. It was explained to all the patients that they were candidates for other established treatments, such as arthroplasty or viscosupplementation, and could exit the present study at any time to receive another treatment, whether through a government public healthcare program or through private resources.

BIOF2 administration. BIOF2 is a patented formulation for cartilage regeneration whose main components are a corticosteroid and organic acids (10). The BIOF2 manufacturing process was performed by Esteripharma Mexico (Mexico City, Mexico), according to the GMP (Good Manufacturing Practices) for pharmaceutical products for use in clinical trials.

BIOF2 was administered on three occasions at 1-month intervals (at month 0, 1, and 2). It was an outpatient procedure performed at a traumatology or orthopedics consultation office, as previously described. With the patient in a seated position and the treatment knee flexed at 0 degrees, BIOF2 was injected into the knee joint space, under sterile prep conditions. The area of injection was inferior lateral to the patella, at the lateral level of the joint line. The injection was performed with a 1.5-inch 20-gauge needle, passing through the fat pad to the firm surface of the intercondylar notch. Following the withdrawal of the needle, a cotton ball soaked in alcohol was placed with pressure at the injection site, after which the site was covered with a sterile dressing (BandAid). The patient could carry out his or her normal activities immediately after the procedure, with no special indications. All patients continued to be seen by their family physician for general care, healthy lifestyle promotion, and if necessary, continued taking the paracetamol/NSAID-based treatment regimen, with no intervention from the researchers in relation to drug prescription or lifestyle indications. In addition, the patients were referred to the physiotherapy and rehabilitation service. Those with *genu varum* or *genu valgum* malalignment were prescribed a 6-mm external or internal insole, respectively, as part of their treatment.

Usual medical care. That group of patients continued with the usual treatment prescribed by their family physician. It consisted of paracetamol/NSAID use and the promotion of a healthy lifestyle. The researchers did not intervene in relation to drug prescription or lifestyle indications. The patients were also referred to the physiotherapy and rehabilitation service. A 6-mm external or internal insole was prescribed to the patients with *genu varum* or *genu valgum* malalignment, respectively, as part of their treatment.

Outcome measures and follow-up. There were 3 co-primary endpoints, assessed as the change from the baseline, or more exactly, the difference between the values at enrollment and at 6 and 12 months. One endpoint was the maximum pain upon movement during the week before the follow-up visit, measured on the 0-10 Visual Analog Scale (VAS) (27). Intensity of joint pain was recorded, from 'no pain' (score of 0) to 'worst imaginable plain' (score of 10). The VAS was selected because it is currently the validated scale that best evaluates pain in diseases presenting with arthralgia (28,29), and it has also been used as a primary endpoint in other clinical trials on OA (30). Another endpoint was the number of patients achieving minimal clinically important improvement (MCII), defined as the smallest change in measurement that signifies important improvement in a patient's symptom (27). It was calculated through a dichotomous score per outcome, based on 30% improvement of pain from the baseline, as previously described in different clinical trials (27,31-34). The third endpoint was the Patient Acceptable Symptom State (PASS), defined as the value of symptoms the patient considers to be the thresholds of well-being for pain and function. Our study incorporated the most widely used anchoring question to identify PASS cut-off points, which was: 'Taking into account all your daily activities, do you consider your current state satisfactory in relation to pain level and functional impairment?' The response options were 'Yes' or 'No' (34). Treatment success was defined as the MCII or PASS questionnaires answered in the affirmative at month 12 of follow-up. The secondary endpoints were change in daily NSAID use at month 12 of follow-up and the register of all adverse events, monitored by the researchers through anamnesis, and abnormal routine laboratory test results.

Blinding. Only the researchers that evaluated treatment effectiveness through the VAS, MCII, and PASS instruments answered by the patients, those that carried out the anamnesis in relation to NSAID consumption, and those that performed the statistical analyses were blinded.

Sample size. Sample size was calculated based on a 100% increase in the number of patients with MCII at 12 months in the BIOF2 group, compared with the control group (paracetamol/NSAIDs=20% vs. BIOF2=40%). Eighty-one patients from each group were required to reach the required power (0.8), when the statistical analysis was performed at the level of the 2-tailed alpha (0.05). That calculation was made using the Sample Size Calculator for two independent study groups with binomial primary endpoints (ClinCalc LLC; http://clincalc.com/stats/samplesize.aspx).

Statistical analysis. Data were presented as the mean ± standard deviation (VAS) or percentages (MCII and PASS). For the inferential statistics, normal data distribution was first determined using the Kolmogorov-Smirnov test and the equality of variances was confirmed using the Levene's test. The VAS pain quantification and other numerical data (BMI or age) were compared between groups using the Student's t test. The categorical values were compared using the Fisher's exact test. The relative risk (RR) and 95% confidence interval were calculated to determine the probability of achieving PASS or of habitually using paracetamol/NSAIDs (at least once a day), comparing the usual medical care group vs. the BIOF2 group. The statistical analyses were performed on the patient total and compared between specific substrates to assess treatment efficacy in patient subgroups [i.e., excluding patients with *genu varum* or *genu valgum* malalignment greater than 20 degrees and/or class 2 obesity (BMI of 35-39)]. The statistical

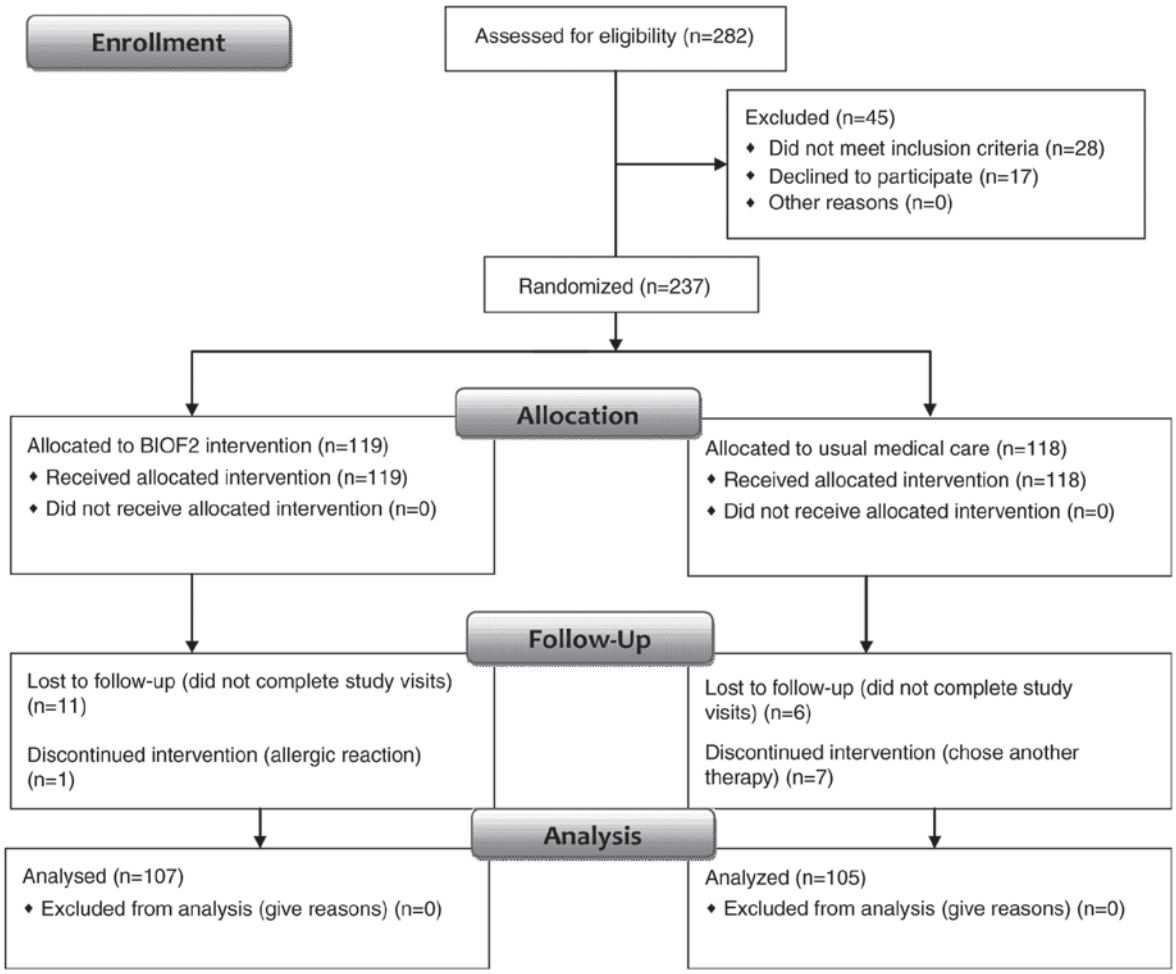

Figure 1. Consort 2010 flow diagram showing the number of patients screened, included, eliminated, and analyzed in the study.

analysis was carried out using the SPSS, version 20, software (IBM Corp., Armonk, NY, USA), with the exception of the RR, which was calculated using MedCalc v17.7.2 software (MedCalc Software bvba, Ostend, Belgium) and the sample size, which was calculated using the online ClinCalc software (ClinCalc LLC; http://clincalc.com/stats/samplesize.aspx). A 2-sided P<0.05 was considered statistically significant.

Results

From the 282 patients screened, 237 were randomized into one of the two study groups. A total of 107 patients in the BIOF2 group and 105 patients in the usual medical care control group completed the study and were analyzed (Fig. 1). The clinical characteristics of the patients are shown in Table I.

Tables II-IV shows the clinical evaluations of knee OA throughout the 12-month follow-up. Only 14% of the patients in the usual medical care group (paracetamol/NSAIDs) achieved MCII in 12 months. In contrast, treatment with BIOF2 produced important clinical improvement in 70% of the patients and >50% of the patients achieved acceptable symptom state, which was significantly higher than that found in the usual medical care group. Treatment with BIOF2, in relation to usual medical care, was associated with a 5-fold increased probability of achieving MCII (RR=4.90, 95% CI: 3.0-7.9, P<0.001), and an 11-fold increased probability of achieving PASS (RR=11.18, 95% CI: 4.6-26.7, P<0.001). Furthermore, there was greater therapeutic success with BIOF2 when patients with class 2 obesity and *genu varum* or *genu valgum* malalignment greater than 20 degrees were excluded, resulting in MCII in 100% of the PASS in 90% (Figs. 2 and 3). Even though BIOF2 treatment significantly reduced pain in the patients with class 2 obesity and *genu varum* or *genu valgum* malalignment, its efficacy in those subgroups was drastically reduced, given that none of the BIOF2 group patients with malalignment achieved acceptable symptom state and only 42% of the patients with class 2 obesity treated with BIOF2 did (Fig. 2).

At the beginning of the study, all the patients required daily paracetamol/NSAID use. The drug most frequently used by each patient was distributed as follows: 40% diclofenac, 32% naproxen, 12% ketorolac, 9% paracetamol, and 7% celecoxib. 11% of the patients combined one of those drugs with tramadol. At the 12-month follow-up, treatment with BIOF2 significantly reduced daily NSAID use (RR=0.42, 95% CI 0.34-0.53, P<0.001), compared with the usual medical care group. Upon study completion, only 42% of the BIOF2 group required habitual NSAID use, whereas 100% of the patients in the usual medical care group required paracetamol/NSAID use daily. Only 13% of the patients in the subgroup that had no class 2 obesity and no malalignment that were treated with

Table I. Distribution of the main clinical characteristics of the study subjects.

Clinical characteristic	NSAIDs	BIOF2	P-value
Women (%)	60.0%	58.0%	0.43[a]
Age (years)	61.5±8.2	60.7±6.7	0.41[b]
BMI	31.9±4.0	32.7±3.3	0.12[b]
Varus/valgus[c]	39.0%	32.7%	0.20[a]
Diabetes	22.8%	25.2%	0.40[a]
High blood pressure	32.4%	29.9%	0.40[a]

Percentages or averages and standard deviation are shown. BMI, body mass index. [a]Fisher's exact test analysis. [b]Student's t test analysis. [c]Varus/valgus deformity at the knee greater than 20 degrees.

BIOF2 required daily paracetamol/NSAID use at the end of the study.

With respect to adverse effects, 90% of the patients treated with BIOF2 presented with local joint pain of 8.0±0.9 intensity (0 to 10 on the visual analogue scale) after BIOF2 application. It lasted 98±45 sec and subsided spontaneously. In some cases, pain radiated to the pelvis. One patient had an allergic reaction to BIOF2, which was resolved with the use of oral antihistamines. That patient was eliminated from the study after the first application. Routine laboratory testing identified no significant abnormalities in either group. Abdominal pain/discomfort was another frequently reported adverse event (74.3% in the usual medical care group and 17.7% in the BIOF2 group), for which the family physician of the majority of the patients added H2-blockers or proton pump inhibitors to prevent severe acute NSAID-related gastroduodenal damage.

Discussion

In patients with severe knee OA that were conservatively treated with usual medical care based on paracetamol/NSAIDs, the intra-articular application of a cell-free bioactive formulation, BIOF2, produced clinically important and statistically significant benefits. At 12 months, 70% of the patients treated with BIOF2 achieved MCII, and in patients with no lower limb malalignment, that figure was 100%. The best PASS result was produced in 92% of the patients treated with BIOF2 that had no class 2 obesity or malalignment. BIOF2 efficacy was reduced in the patients with class 2 obesity, with PASS achieved in only 42%. None of the patients with *genu varum* or *genu valgum* malalignment achieved a state of well-being.

The results of the present study are congruent with those of a previous clinical trial demonstrating a similar success rate of BIOF2 treatment to that of TJA (75%) at one year of treatment (24). Prior preclinical and clinical trials showed that BIOF2 was capable of increasing articular cartilage and simultaneously reducing the histologic abnormalities caused by OA (11). Joint cartilage was increased through the elevated expression of SOX9, a transcription factor that is essential for chondrocyte differentiation and cartilage formation (11). The present study produced new data with respect to the subgroup of patients that most benefitted from treatment with BIOF2 and the limitations in patients with OA-exacerbating comorbidities, such as obesity and lower limb malalignment. With such data, family physicians can have a better idea of

Table II. Comparison of VAS scores between patients treated with NSAIDs and BIOF2 at 6 and 12 months following the intervention.

A, All patients

Timepoint	NSAID, n=105	BIOF2, n=107	P-value
Baseline	9.0+1.0	9.0+1.0	0.893
Month 6	8.5+1.2	3.9+3.3	<0.001
Month 12	8.7+1.4	4.1+3.5	<0.001

B, Patients with no class 2 obesity or malalignment

Timepoint	NSAID, n=47	BIOF2, n=53	P-value
Baseline	8.8+1.2	9.1+1.1	0.211
Month 6	8.0+1.4	1.3+1.6	<0.001
Month 12	8.2+1.7	1.4+1.6	<0.001

C, Patients with class 2 obesity and malalignment

Timepoint	NSAID, n=29	BIOF2, n=27	P-value
Baseline	9.1+0.7	8.9+0.8	0.230
Month 6	8.8+0.7	8.3+0.7	0.016
Month 12	9.2+0.6	8.4+0.6	<0.001

D, Patients with class 2 obesity and no malalignment

Timepoint	NSAID, n=17	BIOF2, n+19	P-value
Baseline	9.2+0.9	9.3+0.9	0.814
Month 6	8.8+1.0	4.1+2.5	<0.001
Month 12	8.7+0.9	4.4+3.4	<0.001

E, Patients with malalignment and no class 2 obesity

Timepoint	NSAID, n=12	BIOF2, n=8	P-value
Baseline	9.3+0.9	8.3+1.4	0.089
Month 6	9.0+1.1	6.1+1.2	<0.001
Month 12	9.2+1.4	7.6+0.5	0.007

Data is presented for all patients (A) and divided per group according to absence or presence of obesity and/or malalignment (B-E). Data were presented as the mean ± standard deviation. A Student's t-test was used for statistical analysis. VAS, maximum pain upon movement during the week before the follow-up visit, measured on the 0-10 visual analog scale; NSAIDs, usual medical care with prescription of paracetamol/nonsteroidal anti-inflammatory drugs; BIOF2, usual medical care plus new therapeutic formulation; Malalignment, genu varum or genu valgum malalignment greater than 20 degrees; N, sample number.

Table III. Comparison of the percentage of patients reaching MCII among patients treated with NSAIDs and BIOF2 at 6 and 12 months following the intervention.

A, All patients

Timepoint	NSAID, n=105 (%)	BIOF2, n=107 (%)	P-value
Month 6	12.4	72.9	<0.001
Month 12	14.3	70.1	<0.001

B, Patients with no class 2 obesity or malalignment

Timepoint	NSAID, n=47 (%)	BIOF2, n=53 (%)	P-value
Month 6	23.4	100	<0.001
Month 12	25.5	100	<0.001

C, Patients with class 2 obesity and malalignment

Timepoint	NSAID, n=29 (%)	BIOF2, n=27 (%)	P-value
Month 6	0.0	0.0	-
Month 12	0.0	0.0	-

D, Patients with class 2 obesity and no malalignment

Timepoint	NSAID, n=17 (%)	BIOF2, n=19 (%)	P-value
Month 6	0.0	100	<0.001
Month 12	11.7	100	<0.001

E, Patients with malalignment and no class 2 obesity

Timepoint	NSAID, n=12 (%)	BIOF2, n=8 (%)	P-value
Month 6	16.6	75.0	0.019
Month 12	8.3	37.5	0.255

Data is presented for all patients (A) and divided per group according to absence or presence of obesity and/or malalignment (B-E). Fisher's exact test was used for statistical analysis. MCII, Minimal clinically important improvement (denoted by 30% improvement from baseline pain; categorical value: 'Yes' or 'No'); NSAIDs, usual medical care with prescription of paracetamol/nonsteroidal anti-inflammatory drugs; BIOF2, usual medical care plus new therapeutic formulation; Malalignment, genu varum or genu valgum malalignment greater than 20 degrees; -, undetermined due to absence of positive data for MCII or PASS in both groups; N, sample number.

Table IV. Comparison of the percentage of patients reaching PASS among patients treated with NSAIDs and BIOF2 at 6 and 12 months following the intervention.

A, All patients

Timepoint	NSAID, n=105 (%)	BIOF2, n=107 (%)	P-value
Baseline	0.0	0.0	-
Month 6	4.7	52.3	<0.001
Month 12	4.7	53.3	<0.001

B, Patients with no class 2 obesity or malalignment

Timepoint	NSAID, n=47 (%)	BIOF2, n=53 (%)	P-value
Baseline	0.0	0.0	-
Month 6	8.5	90.5	<0.001
Month 12	8.5	92.5	<0.001

C, Patients with class 2 obesity and malalignment

Timepoint	NSAID, n=29 (%)	BIOF2, n=27 (%)	P-value
Baseline	0.0	0.0	-
Month 6	0.0	0.0	-
Month 12	0.0	0.0	-

D, Patients with class 2 obesity and no malalignment

Timepoint	NSAID, n=17 (%)	BIOF2, n=19 (%)	P-value
Baseline	0.0	0.0	-
Month 6	0.0	42.1	0.002
Month 12	0.0	42.1	0.002

E, Patients with malalignment and no class 2 obesity

Timepoint	NSAID, n=12 (%)	BIOF2, n=8 (%)	P-value
Baseline	0.0	0.0	-
Month 6	8.3	0.0	0.638
Month 12	8.3	0.0	0.638

Data is presented for all patients (A) and divided per group according to absence or presence of obesity and/or malalignment (B-E). Fisher's exact test was used for statistical analysis. PASS, Patient acceptable symptom state, (defined as the value of symptoms the patient considers to be the threshold of well-being for pain and function; categorical value: 'Yes' or 'No'); NSAIDs, usual medical care with prescription of paracetamol/nonsteroidal anti-inflammatory drugs; BIOF2, usual medical care plus new therapeutic formulation; Malalignment, *genu varum* or *genu valgum* malalignment greater than 20 degrees; - Undetermined due to absence of positive data for MCII or PASS in both groups; N, sample number.

treatment outcome and inform patients of the expectations in relation to BIOF2 treatment.

Obesity has been associated with greater pain and articular damage, due to a metabolic-inflammatory process and a mechanical effect (35). Obesity produces increased proinflammatory cytokine and collagenase production in cartilage, which is related to a systemic increase of leptin in obese individuals (36,37). Leptin and its receptor have been identified in human chondrocytes, osteophytes, synovium, and infrapatellar fat pad, and may affect growth factor synthesis and anabolism (38-40). Leptin expression has been directly associated

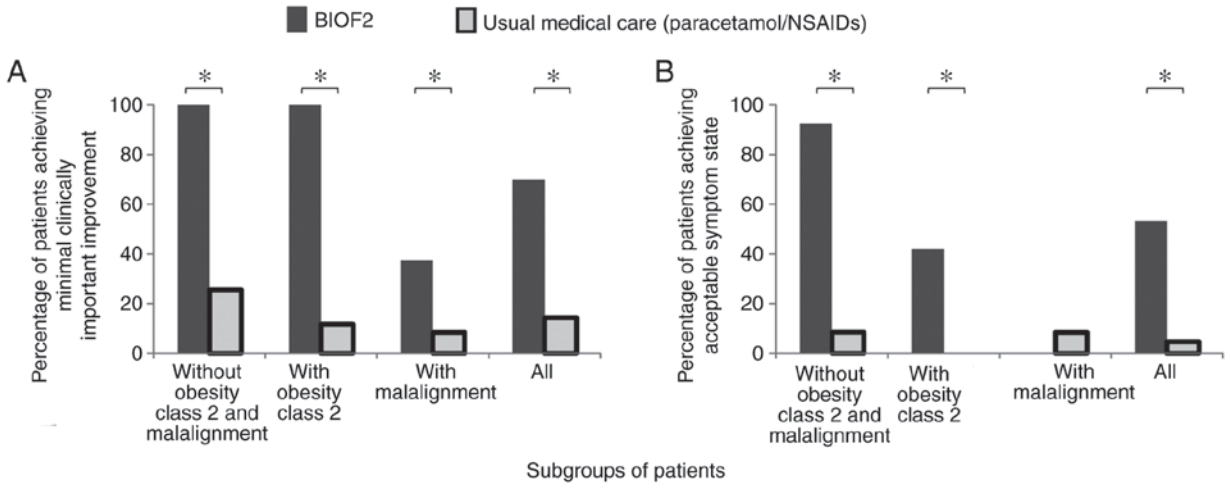

Figure 2. Percentage of patients with severe osteoarthritis that achieved minimal clinically important improvement and acceptable symptom state at 12 months of follow-up. (A) 100% of the patients with no malalignment (*genu varum* or *genu valgum* malalignment greater than 20 degrees) and treated with BIOF2 achieved MCII, whereas only 37% of the patients with malalignment had such improvement. (B) More than 90% of the patients treated with BIOF2 (with no class 2 obesity or malalignment) achieved PASS. That state was greatly reduced in the patients with class 2 obesity and unachieved in the patients with malalignment. Usual medical care with paracetamol/NSAIDs had significantly lower results than treatment with BIOF2 (*P<0.05 as indicated), except in the possibility of one patient with malalignment achieving PASS. In that situation neither of the two treatments were effective. Class 2 obesity: BMI of 35-39. BIOF2, bioactive cell-free formulation; MCII, minimal clinically important improvement; PASS, patients and acceptable symptom state; BMI, body mass index.

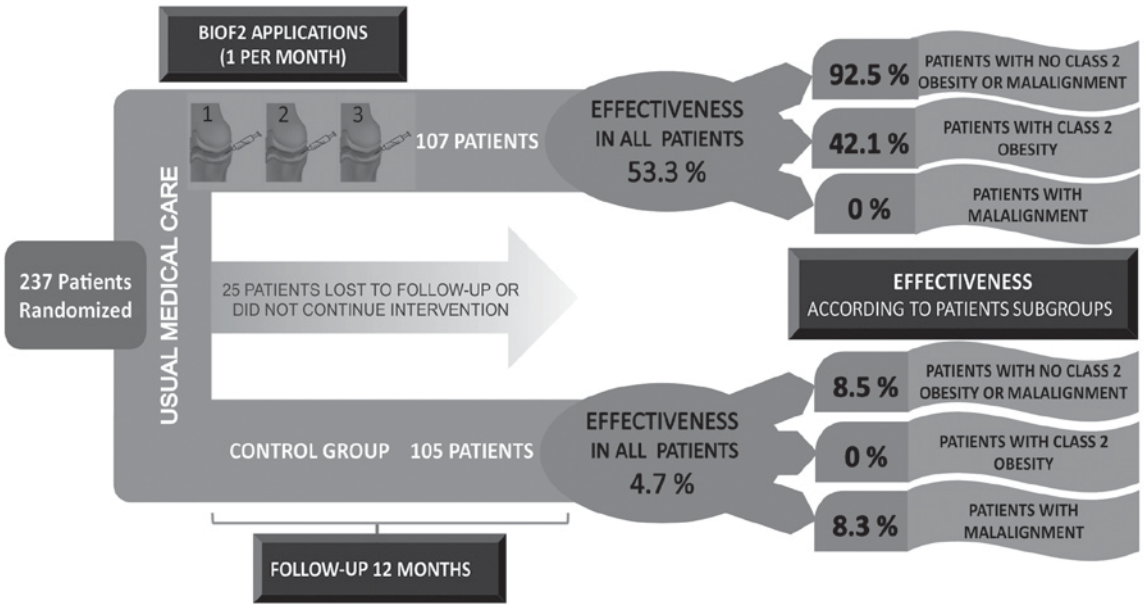

Figure 3. Diagram showing the general strategy of the project and the main results. Both groups received usual medical care, and in one group, treatment with BIOF2 was added. Treatment effectiveness is shown based on the percentage of patients that achieved a PASS at 12 months. That result was stratified according to the presence or absence of patient comorbidities. BIOF2 treatment efficacy was above 90% in patients with a BMI below 35 (with no class 2 obesity) and with no malalignment. Tables II-IV provides detailed information on the other efficacy parameters at the baseline and at 6 and 12 months after treatment. BIOF2, bioactive cell-free formulation; PASS, patients and acceptable symptom state; BMI, body mass index.

with the degree of cartilage degeneration (38). In addition, excess weight contributes to greater mechanical load on the joint (35). Those aspects may be the cause of the lower BIOF2 effectiveness in patients with class 2 obesity found in the present study. It is important to mention that all the patients with class 2 obesity had relevant clinical improvement, even though less than half achieved PASS. Most likely, patient weight reduction and/or a greater number of BIOF2 applications could increase the therapeutic response in that subgroup of patients, which is an aspect that should be analyzed in future studies.

In patients with important malalignment, BIOF2 application produced MCII in 75% of the patients at 6 months. It was reduced to 35% at 12 months, and none of those patients achieved PASS. Malalignment is a potent predictor of disease progression in patients with OA, and is a local mechanical factor in the knee that can mediate symptoms (41). The beneficial effect of BIOF2 in that subgroup of patients appears to be temporary and does not completely resolve the patient's complaints. Surgical correction of the malalignment, followed by treatment with BIOF2, could be a therapeutic strategy to be evaluated in future studies.

A relevant characteristic of the present study is that it assessed the use of BIOF2 in patients receiving usual medical care. The term 'usual care' describes the care commonly given by practitioners in a community. For more than a decade, the usefulness of evaluating new treatments against background conditions of medical practices has been postulated, considering that it is often essential, for scientific and ethical reasons, to have a usual care comparison arm in the study of a new drug (42). The use of NSAIDs and/or paracetamol has been shown in clinical trials to improve knee OA symptomatology. However, its effectiveness varies, depending on the drug used, dose (17), baseline pain, and radiologic features (43-46). Oral NSAIDs or paracetamol are the agents most frequently utilized in the treatment of arthrosis (43). However, neither the patients nor the physicians that prescribe the drugs are satisfied with their results, given that in general, adequate health states are not achieved through their therapeutic use (43). Despite that fact, the use of those drugs, together with the promotion of healthy lifestyles and rehabilitation techniques, is the usual medical care given for the treatment of knee OA in the majority of public healthcare systems in Mexico and other countries. With respect to severe knee OA, the treatment of choice could be TJA, but that option is often not available in the short term for patients within the public healthcare system and the wait for said treatment can be years. As those patients wait, the common usual medical care is the prescription of paracetamol/NSAIDs.

The low level of efficacy of paracetamol/NSAID prescription found in the present study does not concur with the good or moderate success rates reported in other studies on OA (44,45). There are several possible explanations for that. The high OA severity in the patients upon entering the present study (mean VAS for pain of 9, 0-10 scale) could have affected the results. With respect to the drugs used, it was reported in other studies that etoricoxib, celecoxib, and aceclofenac had the highest rankings for improvement, whereas in our study celecoxib was used in only 7% of the patients. In previous trials, evaluations were carried out only during active treatment. Our study reflected habitual NSAID use of the patients in the community and therefore it is likely that drug dose and treatment adherence varied considerably over a one-year period. Discontinuation rates of prescription NSAIDs have been reported to exceed 85% within six months of their use (46). Nevertheless, our results coincided with those of a study that analyzed the effect of prescription NSAIDs on knee OA. Those authors reported that NSAID prescription was not associated with MCII in the patient-reported symptoms of pain, stiffness, and function (46) in evaluations of one and two years. Therefore, we believe that our results reflect the real-life occurrence in a community of patients with knee OA receiving long-term treatment with paracetamol/NSAIDs.

The addition of BIOF2 to the usual medical care significantly increased the well-being indicators analyzed in the patients and significantly reduced NSAID use. Prolonged NSAID use can cause adverse effects, especially that of kidney damage (47). Thus, treatment with BIOF2 could also aid in reducing the risks caused by long-term NSAID intake. Other clinical trials have evaluated strategies for articular cartilage regeneration through cellular therapy or implants utilizing novel biomaterials. However, those procedures are complex, costly, and difficult to implement in medical centers. Therefore, we consider treatment with the new BIOF2 to be a promising and readily implemented option for the treatment of OA that can be incorporated into the usual medical care of patients with knee OA at a public or private healthcare center with ease. BIOF2 can be applied as an outpatient procedure in routine medical consultations, taking the customary precautions utilized in any intra-articular injection. The only adverse effect detected was pain upon application, which, albeit intense, spontaneously remitted within sec.

In conclusion, the intra-articular application of a new BIOF2, was safe and well-tolerated and resulted in a success rate above 90% in patients with no class 2 obesity and no malalignment. At 12 months, its effect was limited in the patients with class 2 obesity and was close to null in the patients with malalignment. BIOF2 is a safe and easily implemented therapeutic alternative in patients receiving usual medical care for knee OA.

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Availability of data and materials

All relevant data appear in the present study. The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

ID, AOC, ADS, JD and IPR designed the study, performed the analyses and drafted the manuscript. BP and JPG conceived the novel bioactive cell-free formulation. JV, MMH, JPR, JLC and JG participated in the clinical evaluation of the patients. MLM, CEB and AC participated in the design of the statistical analysis. JD was the clinical trial administrative coordinator. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the ethics committee of the Cancerology State Institute of the Colima State Health Services, Mexico (reference number: CEICANCL061115-O STEOART10), and written informed consent was obtained

from all the participants. All procedures performed in this protocol were in accordance with the Declaration of Helsinki. The present clinical trial was registered as ARTROTX-II/III: RPCEC00000277 in the Cuban Public Registry of Clinical Trials (RPCEC) database.

Patient consent for publication

Not applicable.

Competing interests

Dr Juan Paz-Garcia and Dr Brenda Paz-Michel declare that they are the inventors of the experimental formulation (BIOF2) used in the present study (patent no. US9089580 B1). These authors did not have a role in the study design, data collection, or the analyses. The other authors declare that they have no competing interests.

References

1. Hafez AR, Alenazi AM, Kachanathu SJ, Alroumi AM and Mohamed ES: Knee osteoarthritis : A review of literature. Phys Med Rehabil Int 1: 1-8, 2014.
2. Jinks C, Jordan K, Ong BN and Croft P: A brief screening tool for knee pain in primary care (KNEST). 2. Results from a survey in the general population aged 50 and over. Rheumatology (Oxford) 43: 55-61, 2004.
3. Johnson VL and Hunter DJ: The epidemiology of osteoarthritis. Best Pract Res Clin Rheumatol 28: 5-15, 2014.
4. Liu Q, Niu J, Li H, Ke Y, Li R, Zhang Y and Lin J: Knee symptomatic osteoarthritis, walking disability, NSAIDs use and all-cause mortality: Population-based wuchuan osteoarthritis study. Sci Rep 7: 3309, 2017.
5. Davis AM: Osteoarthritis year in review: Rehabilitation and outcomes. Osteoarthritis Cartilage 20: 201-206, 2012.
6. Bannuru RR, Osani M, Vaysbrot EE and McAlindon TE: Comparative safety profile of hyaluronic acid products for knee osteoarthritis: A systematic review and network meta-analysis. Osteoarthritis Cartilage 24: 2022-2041, 2016.
7. Herrera-Espiñeira C, Escobar A, Navarro-Espigares JL, Castillo Jde D, García-Pérez L and Godoy-Montijano A: Total knee and hip prosthesis: Variables associated with costs. Cir Cir 81: 207-213, 2013 (In Spanish).
8. Ethgen O, Bruyère O, Richy F, Dardennes C and Reginster JY: Health-related quality of life in total hip and total knee arthroplasty. A qualitative and systematic review of the literature. J Bone Joint Surg Am 86-A: 963-974, 2004.
9. Robinson JC, Pozen A, Tseng S and Bozic KJ: Variability in costs associated with total hip and knee replacement implants. J Bone Joint Surg Am 94: 1693-1698, 2012.
10. Garcia JP and Paz Michel BA: Formulation for regeneration of bone, cartilage, teeth, and periodontium and treatment of tumors and cysts. US Patent No. 9089580 B1. 2015.
11. Delgado-Enciso I, Paz-Garcia J, Rodriguez-Hernandez A, Madrigal-Perez VM, Cabrera-Licona A, Garcia-Rivera A, Soriano-Hernandez AD, Cortes-Bazan JL, Galvan-Salazar HR, Valtierra-Alvarez J, *et al*: A promising novel formulation for articular cartilage regeneration: Preclinical evaluation of a treatment that produces SOX9 overexpression in human synovial fluid cells. Mol Med Rep 17: 3503-3510, 2018.
12. Farkas B, Kvell K, Czömpöly T, Illés T and Bárdos T: Increased chondrocyte death after steroid and local anesthetic combination. Clin Orthop Relat Res 468: 3112-3120, 2010.
13. Sekiya I, Tsuji K, Koopman P, Watanabe H, Yamada Y, Shinomiya K, Nifuji A and Noda M: SOX9 enhances aggrecan gene promoter/enhancer activity and is up-regulated by retinoic acid in a cartilage-derived cell line, TC6. J Biol Chem 275: 10738-10744, 2000.
14. Reiter I, Tzukerman M and Maor G: Spontaneous differentiating primary chondrocytic tissue culture: A model for endochondral ossification. Bone 31: 333-339, 2002.
15. Hadzir SN, Ibrahim SN, Abdul Wahab RM, Zainol Abidin IZ, Senafi S, Ariffin ZZ, Abdul Razak M and Zainal Ariffin SH: Ascorbic acid induces osteoblast differentiation of human suspension mononuclear cells. Cytotherapy 16: 674-682, 2014.
16. Ogston N, Harrison AJ, Cheung HF, Ashton BA and Hampson G: Dexamethasone and retinoic acid differentially regulate growth and differentiation in an immortalised human clonal bone marrow stromal cell line with osteoblastic characteristics. Steroids 67: 895-906, 2002.
17. Gentili C, Bianco P, Neri M, Malpeli M, Campanile G, Castagnola P, Cancedda R and Cancedda FD: Cell proliferation, extracellular matrix mineralization, and ovotransferrin transient expression during in vitro differentiation of chick hypertrophic chondrocytes into osteoblast-like cells. J Cell Biol 122: 703-712, 1993.
18. Skillington J, Choy L and Derynck R: Bone morphogenetic protein and retinoic acid signaling cooperate to induce osteoblast differentiation of preadipocytes. J Cell Biol 159: 135-146, 2002.
19. Benya PD, Brown PD and Padilla SR: Microfilament modification by dihydrocytochalasin B causes retinoic acid-modulated chondrocytes to reexpress the differentiated collagen phenotype without a change in shape. J Cell Biol 106: 161-170, 1988.
20. Temu TM, Wu KY, Gruppuso PA and Phornphutkul C: The mechanism of ascorbic acid-induced differentiation of ATDC5 chondrogenic cells. Am J Physiol Endocrinol Metab 299: E325-E334, 2010.
21. Zur Nieden NI, Kempka G, Rancourt DE and Ahr HJ: Induction of chondro-, osteo- and adipogenesis in embryonic stem cells by bone morphogenetic protein-2: Effect of cofactors on differentiating lineages. BMC Dev Biol 5: 1, 2005.
22. Chen H, Wang H, Li B, Feng B, He X, Fu W, Yuan H and Xu Z: Enhanced chondrogenic differentiation of human mesenchymal stems cells on citric acid-modified chitosan hydrogel for tracheal cartilage regeneration applications. RSC Adv 8: 16910-16917, 2018.
23. Mahmod SA, Snigh S, Djordjevic I, Yee YM, Yusof R, Ramasamy TS and Rothan HA: Phytoestrogen (Daidzein) promotes chondrogenic phenotype of human chondrocytes in 2D and 3D culture systems. Tissue Eng Regen Med 14: 103-112, 2017.
24. Delgado-Enciso I, Paz-Garcia J, Valtierra-Alvarez J, Preciado-Ramirez J, Almeida-Trinidad R, Guzman-Esquivel J, Mendoza-Hernandez MA, Garcia-Vega A, Soriano-Hernandez AD, Cortes-Bazan JL, *et al*: A phase I-II controlled randomized trial using a promising novel cell-free formulation for articular cartilage regeneration as treatment of severe osteoarthritis of the knee. Eur J Med Res 23: 52, 2018.
25. Ringdahl E and Pandit S: Treatment of knee osteoarthritis. Am Fam Physician 83: 1287-1292, 2011.
26. Center for Disease Control and Prevention: Defining adult overweight and obesity. Centers Dis Control Prev: pp8-9, 2016.
27. Tubach F, Ravaud P, Baron G, Falissard B, Logeart I, Bellamy N, Bombardier C, Felson D, Hochberg M, van der Heijde D and Dougados M: Evaluation of clinically relevant changes in patient reported outcomes in knee and hip osteoarthritis: The minimal clinically important improvement. Ann Rheum Dis 64: 29-33, 2005.
28. Englbrecht M, Tarner IH, van der Heijde DM, Manger B, Bombardier C and Müller-Ladner U: Measuring pain and efficacy of pain treatment in inflammatory arthritis: A systematic literature review. J Rheumatol Suppl 90: 3-10, 2012.
29. Delgado-Enciso I, Paz-Michel B, Melnikov V, Guzman-Esquivel J, Espinoza-Gomez F, Soriano-Hernandez AD, Rodriguez-Sanchez IP, Martinez-Fierro ML, Ceja-Espiritu G, Olmedo-Buenrostro BA, *et al*: Smoking and female sex as key risk factors associated with severe arthralgia in acute and chronic phases of chikungunya virus infection. Exp Ther Med 15: 2634-2642, 2018.
30. Reginster JY, Dudler J, Blicharski T and Pavelka K: Pharmaceutical-grade Chondroitin sulfate is as effective as celecoxib and superior to placebo in symptomatic knee osteoarthritis: The ChONdroitin versus CElecoxib versus placebo trial (CONCEPT). Ann Rheum Dis 76: 1537-1543, 2017.
31. van der Wees PJ, Wammes JJ, Akkermans RP, Koetsenruijter J, Westert GP, van Kampen A, Hannink G, de Waal-Malefijt M and Schreurs BW: Patient-reported health outcomes after total hip and knee surgery in a Dutch University Hospital Setting: Results of twenty years clinical registry. BMC Musculoskelet Disord 18: 97, 2017.
32. Revicki D, Hays RD, Cella D and Sloan J: Recommended methods for determining responsiveness and minimally important differences for patient-reported outcomes. J Clin Epidemiol 61: 102-109, 2008.

33. Escobar A, García Pérez L, Herrera-Espiñeira C, Aizpuru F, Sarasqueta C, Gonzalez Sáenz de Tejada M, Quintana JM and Bilbao A: Total knee replacement; minimal clinically important differences and responders. Osteoarthritis Cartilage 21: 2006-2012, 2013.
34. Kvien TK, Heiberg T and Hagen KB: Minimal clinically important improvement/difference (MCII/MCID) and patient acceptable symptom state (PASS): what do these concepts mean?. Ann Rheum Dis 66 (Suppl 3): iii40-iii41, 2007.
35. Sowers MR and Karvonen-Gutierrez CA: The evolving role of obesity in knee osteoarthritis. Curr Opin Rheumatol 22: 533-537, 2010.
36. Koskinen A, Vuolteenaho K, Nieminen R, Moilanen T and Moilanen E: 011 Proinflammatory and catabolic role of leptin in osteoarthritis. Correlation with IL-6, MMP-1 and MMP-3 in synovial fluid and effects in human OA cartilage. Osteoarthritis Cartilage 18: S13-S14, 2010.
37. Lago R, Gomez R, Otero M, Lago F, Gallego R, Dieguez C, Gomez-Reino JJ and Gualillo O: A new player in cartilage homeostasis: Adiponectin induces nitric oxide synthase type II and pro-inflammatory cytokines in chondrocytes. Osteoarthritis Cartilage 16: 1101-1109, 2008.
38. Simopoulou T, Malizos KN, Iliopoulos D, Stefanou N, Papatheodorou L, Ioannou M and Tsezou A: Differential expression of leptin and leptin's receptor isoform (Ob-Rb) mRNA between advanced and minimally affected osteoarthritic cartilage; effect on cartilage metabolism. Osteoarthritis Cartilage 15: 872-883, 2007.
39. Toussirot E, Streit G and Wendling D: The contribution of adipose tissue and adipokines to inflammation in joint diseases. Curr Med Chem 14: 1095-1100, 2007.
40. Presle N, Pottie P, Dumond H, Guillaume C, Lapicque F, Pallu S, Mainard D, Netter P and Terlain B: Differential distribution of adipokines between serum and synovial fluid in patients with osteoarthritis. Contribution of joint tissues to their articular production. Osteoarthritis Cartilage 14: 690-695, 2006.
41. Hunter DJ, Sharma L and Skaife T: Alignment and osteoarthritis of the knee. J Bone Joint Surg Am 91 (Suppl 1): S85-S89, 2009.
42. Dawson L, Zarin DA, Emanuel EJ, Friedman LM, Chaudhari B and Goodman SN: Considering usual medical care in clinical trial design. PLoS Med 6: e1000111, 2009.
43. Arboleya LR, DE LA Figuera E, García Ms and Aragón B; Grupo De Estudio Vicoxx: Tratamiento sintomático de la artrosis: Patrón de utilización de antiinflamatorios no esteroides en los centros de salud españoles. Rev Española Reumatol 29: 300-307, 2002.
44. Da Costa BR, Reichenbach S, Keller N, Nartey L, Wandel S, Jüni P and Trelle S: RETRACTED: Effectiveness of non-steroidal anti-inflammatory drugs for the treatment of pain in knee and hip osteoarthritis: A network meta-analysis. Lancet 387: 2093-2105, 2016.
45. Bannuru RR, Schmid CH, Kent DM, Vaysbrot EE, Wong JB and McAlindon TE: Comparative effectiveness of pharmacologic interventions for knee osteoarthritis: A systematic review and network meta-analysis. Ann Intern Med 162: 46-54, 2015.
46. Lapane KL, Yang S, Driban JB, Liu SH, Dubé CE, McAlindon TE and Eaton CB: Effects of prescription nonsteroidal antiinflammatory drugs on symptoms and disease progression among patients with knee osteoarthritis. Arthritis Rheumatol 67: 724-732, 2015.
47. Yaxley J and Litfin T: Non-steroidal anti-inflammatories and the development of analgesic nephropathy: A systematic review. Ren Fail 38: 1328-1334, 2016.