Nonrandomized Clinical Trial

Treatment of Femoral Head Osteonecrosis with Ozone Therapy: Pilot Trial of a New Therapeutic Approach

Jian-Xiong An, MD[1-3], Guo-Ping Wu, MD[4], Kun Niu, MD[1,2], You-Ping Wei, MD[5], Hui Liu, MD[1], Xin-You Gao, MD[1], Jian-Ping Wu, MD[1], Yong Wang, MD[1], Harald Renz, MD[6], and John P. Williams, MD[7]

From: [1]Department of Anesthesiology, Pain & Sleep Medicine, Aviation General Hospital of China Medical University and Beijing Institute of Translational Medicine, Chinese Academy of Sciences, Beijing, China; [2]Savaid Medical School, University of Chinese Academy of Sciences, Beijing, China; [3]School of Medical Science and Engineering, Beijing Advanced Innovation Center for Biomedical Engineering, Beihang University, Beijing, China; [4]Department of Orthopedics, Aviation General Hospital of China Medical University and Beijing Institute of Translational Medicine, Chinese Academy of Sciences, Beijing, China; [5]Department of Radiology, Aviation General Hospital of China Medical University and Beijing Institute of Translational Medicine, Chinese Academy of Sciences, Beijing, China; [6]Institute of Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, University Giessen and Philipps-Universität Marburg, Marburg, Germany; [7]Department of Anesthesiology, University of Pittsburgh School of Medicine, Pittsburg, PA, USA

Address Correspondence:
Kun Niu, MD
Department of Anesthesiology, Pain and Sleep Medicine, Aviation General Hospital of Medical University Beiyuan Rd 3#
Beijing, 100012, China
E-mail:
niukun18@mails.ucas.ac.cn

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Background: Osteonecrosis of the femoral head (ONFH) is a progressive and painful disorder due to impaired blood supply to the femoral head, yet little is known about the effect of ozone therapy in femoral head necrosis.

Objectives: We aimed to evaluate the clinical and radiographic outcomes of ozone therapy in the treatment of ONFH.

Study Design: Nonrandomized clinical trial.

Settings: The study was conducted in a single-center, academic institution.

Methods: A total of 71 patients (107 hip joints) with Association Research Circulation Osseous (ARCO) stage-I, II, III, and IV ONFH were included and assigned to undergo either intraarticular O_2-O_3 mixture hip injections with ozonated autohemotherapy (ozone therapy group, n = 39, 58 hip joints) or protected weight bearing (control group, n = 32, 49 hip joints). The primary outcomes included the Visual Analog Scale (VAS) for pain intensity and Harris Hip Score (HHS) for hip function. The secondary outcomes included bone marrow edema examination, and conversion to total hip arthroplasty (THA).

Results: Ozone therapy effectively improves VAS for pain intensity and HHS during the follow-up period compared to the control group. Ozone therapy showed a significant resolution of bone marrow edema of the femoral head compared to the control group ($P < 0.001$). Thirteen of the 49 hips (26.53%) in the control group underwent THA, whereas only 6 hips (10.34%) in the ozone therapy group required THA during a 30-month follow-up ($P = 0.041$). The cumulative analysis revealed a low rate of conversion to THA in the ozone therapy group (logrank test; $P = 0.022$).

Limitations: The study is limited by a single treatment protocol in addition to the lack of a randomized design.

Conclusions: Ozone therapy was associated with significant pain relief, improvement in hip function, and bone marrow edema resolution that may delay the need for THA in patients affected by ONFH.

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Clinical trials registration number: ChiCTR1900023449.

Key words: Osteonecrosis of the femoral head, ozone therapy, hip injection, pain intensity, hip function, differential expressed genes, gene ontology, transcriptomics

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Osteonecrosis of the femoral head (ONFH), also called avascular necrosis of the femoral head, is a progressive, refractory bone disease that stems from vascular insufficiency within the femoral head. In the early stages, patients usually experience a temporary but painful condition after lifting , whereas persistent pain and claudication occur in the later stage of ONFH (1). The prevalence of ONFH is estimated at more than 8 million patients in China (2,3), and several studies from the late 1990s have shown that 10,000 to 20,000 new patients are detected each year in the United States (4,5).

The precise pathogenic mechanisms of ONFH are not well understood. The underlying mechanism involves impairment of blood flow from capillaries and arterioles to the femoral head that is induced by either primary (idiopathic ONFH) or secondary causes, including iatrogenic osteonecrosis related to the use of steroid hormones for the control of asthma, rheumatoid arthritis, and systemic lupus erythematosus.

Ozone is an allotropic form of the element oxygen, and it has been used for various therapeutic purposes since the beginning of the last century. Intraarticular injection of an oxygen-ozone mixture (O_2-O_3 mixture) at concentrations around 30 μg/mL leads to an increase in the amount of oxygen released to the tissues via the stimulation of 2,3-diphosphoglycerate, which improves the oxygenation and metabolism at the ischemic site. Ozone may also stimulate the production of antioxidant enzymes that act as free radical scavengers in alleviating tissue ischemia and edema that are reported to be involved in pain severity (6-8). Another application method of ozone therapy is ozonated autohemotherapy (O_3-AHT). It is shown that O_3-AHT causes an increase in the red blood cell glycolysis rate and stimulates the production of adenosine triphosphate, which is associated with fewer adverse events and more rapid therapeutic results secondary to the activation of antioxidant defense systems (9). To date, however, there have been no studies to determine the therapeutic effect of ozone therapy on ONFH. We therefore undertook the current study to evaluate the effects of an intraarticular O_2-O_3 mixture hip injection in combination with O_3-AHT for patients with ONFH.

Patients and Methods

Study Design

This manuscript adheres to the applicable Strengthening the Reporting of Observational Studies in Epidemiology guidelines. This work was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the Aviation General Hospital of China Medical University (approval number HK2013-01-10) and registered at http://www.chictr.org.cn/index.aspx (registration number: ChiCTR1900023449). All patients signed an informed consent. We designed and conducted this nonrandomized controlled clinical trial in patients who suffered from ONFH stages I to IV in compliance with the Association Research Circulation Osseous (ARCO) classification (10). Exclusion criteria were age younger than 18; severe cardiac disease; severe impairment of hepatic or renal function; serious current infection or hematologic disorder; microvascular complications; pregnant or lactating women; mental health disorders; nerve damage accompanied by dyskinesia; and severe diabetes mellitus with peripheral neuropathy and infection at the site of puncture.

Treatment Protocol

After applying inclusion and exclusion criteria, a research fellow performed an in-person interview to record data on patient demographics and disease characteristics. They also explained in detail the experiment and answered the patient's questions. The involved hips of patients were nonrandomly assigned to either the ozone therapy group or the control group, based on patient preference. Patients in the ozone therapy group underwent ozone therapy as well as 6 months of protected weight bearing. Patients in the control group underwent only protected weight bearing for 6 months. The protected weight bearing regimen was chosen because it is currently recommended as a nonsurgical alternative for pain relief. The flow diagram of the therapeutic process is shown in Fig. 1.

Ozone therapy is defined as an intraarticular O_2-O_3 mixture hip injection in combination with O_3-AHT. All patients were treated in our pain clinic. In each treatment course, patients who were treated as inpatients received ozone therapy once daily on a 5-day-per-week schedule for 2 consecutive weeks, repeated at 3-month intervals, and continued for a total of 3 treatment courses. The ozone therapy regimen is shown in Fig. 2.

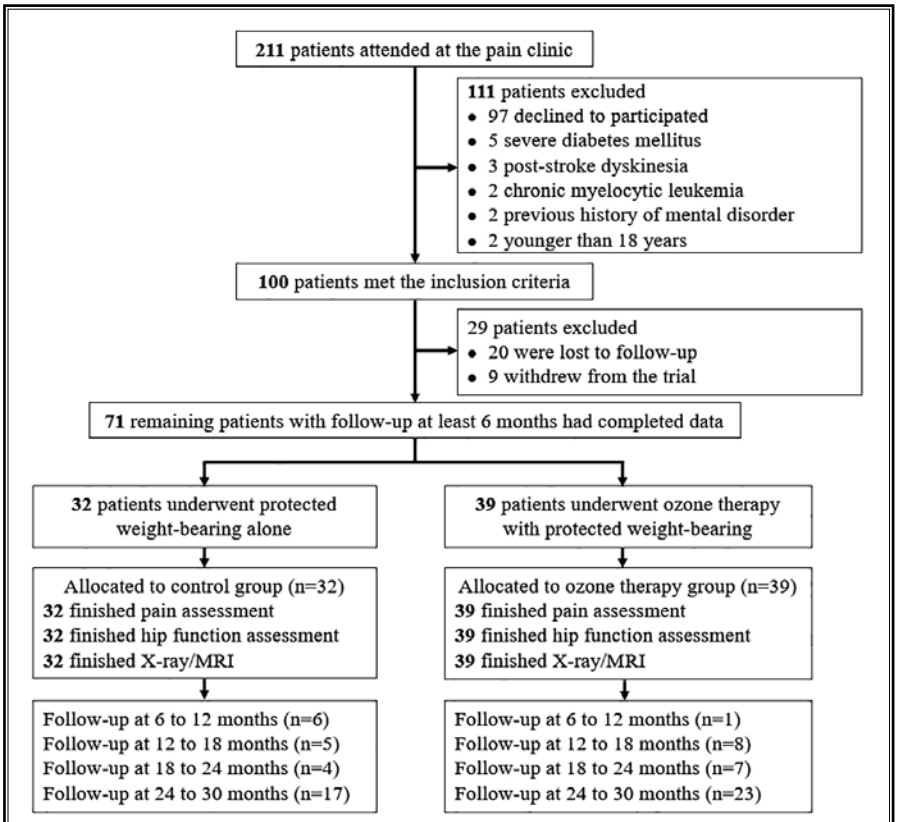

Fig 1. *Patient flow chart.*

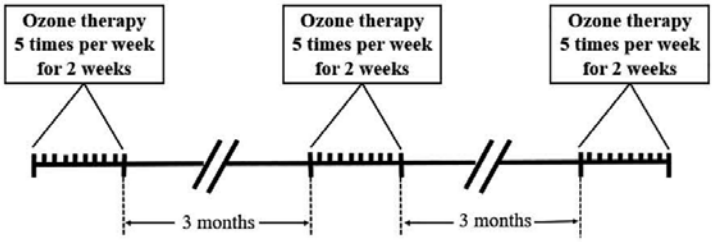

Fig 2. *Ozone therapy regimen.*

Before ozone therapy commenced, standard monitoring was undertaken, which included heart rate and pulse oximetry for each patient. The ultrasound-guided technique and intraarticular ozone hip injection procedures were carried out by 2 board-certified physicians. All hip injections were performed with the use of an anterior-inferiorly longitudinal approach (11). Specifically, with patients lying supine, the transducer probe (Vivid-I, GE Healthcare Bio-Sciences Corp., Piscataway, NJ) was placed longitudinally, aligned with the long axis of the femoral neck in a parasagittal plane over the hip so that we could obtain a femoral head/neck junction image (Fig. 3). Once the femoral neuromuscular structures and the path of the needle were identified, the skin was disinfected. Following this, a 22-gauge spinal needle was placed using a freehand technique using a long axis approach into the hip joint. This path allows the echogenic needle to be within the plane of the transducer probe and visualized in real time. Confirmation of needle placement into the intraarticular space was ensured by echo locating the needle tip proximate to the surface of the femoral head/neck junction (Fig. 3).

The dosage of O_2-O_3 mixture was 30 mL at a concentration of 30 µg/mL, generated by an ozone therapy device (Ozomed® basic, Kastner-Praxisbedarf-GmbH, Rastatt, Germany) in which the ozone concentration was measured and self-checked by titration according to the rules established by the International Ozone Association. All patients were observed for 20 minutes after the procedure.

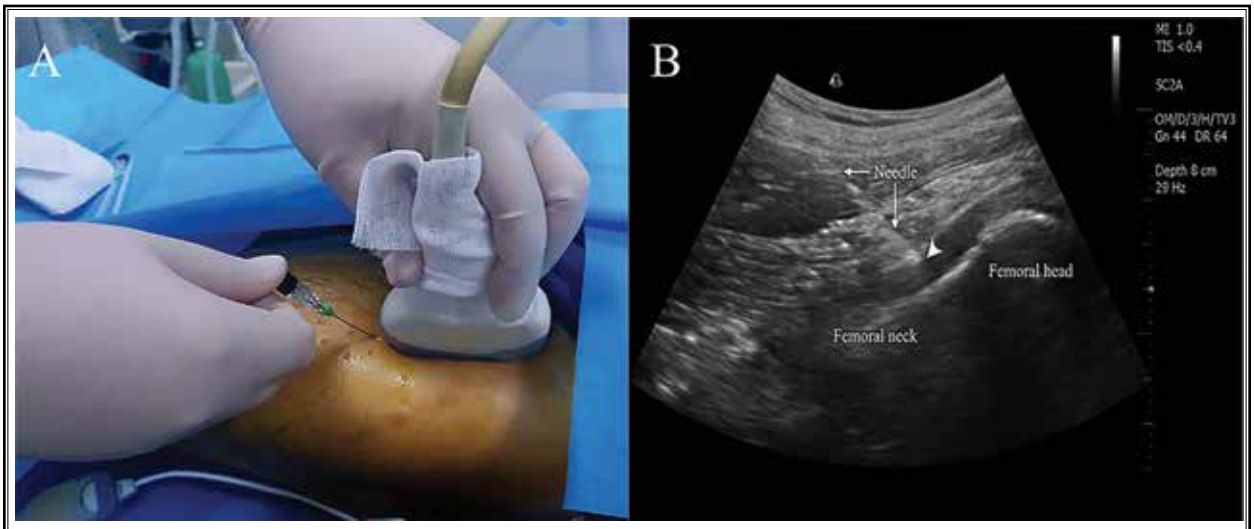

Fig 3. *Ultrasound-guided ozone intrarticular hip injection for ONFH.*

Bocci (12) has described the method of making an O_3-AHT mixture. Briefly, 100 mL of blood from the antecubital vein was injected into a plastic blood bag with an anticoagulant (normally sodium citrate 3.8%). After that, a 100 mL O_2-O_3 mixture at a concentration of 30 μg/mL was extracted from the ozone therapy device described previously and introduced into the same blood bag. The exposure of blood to the O_2-O_3 mixture lasted at least 5 minutes because mixing of blood must be gentle to avoid foaming. At the end of this process, the blood was transfused back into the antecubital vein within 20 minutes.

Outcome Measurements

Primary Outcomes

The primary outcomes were measured by the visual analog scale (VAS) for pain intensity and the Harris Hip Score (HHS) for hip function and were assessed preprocedure and at 3, 6, 9, 12, 18, 24, and 30 months following ozone therapy. The assessment of pain intensity with the VAS was recorded as numbers, where 0 indicated no pain and 10 indicated severe pain. The HHS is a questionnaire that is used to assess a patient's hip function, normal daily activities, and objective range-of-motion movements (13). The total score of an HHS is 100; patients with an overall rating of 90 or higher are considered "excellent"; 89 to 80 are considered "good"; 79 to 70 are considered "fair"; those less than 70 are considered "poor." Physicians blinded to the patient assignment conducted the HHS in the pain clinic.

The length of the follow-up period was calculated from the starting date of ozone therapy to the time of the last follow-up visit or conversion to total hip arthroplasty (THA).

Secondary Outcomes

1) Magnetic resonance imaging (MRI) examinations were made preprocedure and at 6, 12, 24, and 30 months after ozone therapy. MRI was used to evaluate bone marrow edema of the femoral head (14) with the use of the staging system from the ARCO (Table 1). Grades of bone marrow edema were defined as: no bone marrow edema (grade 0), perinecrotic bone marrow edema (grade 1), bone marrow edema extending into the femoral head (grade 2), bone marrow edema extending into the neck of the femur (grade 3), and bone marrow edema extending into the intertrochanteric region (grade 4).
2) Cumulative curves revealed the number of patients who underwent a conversion to THA as the endpoint during the entire follow-up period.
3) Any adverse event was recorded during the treatment course and at each follow-up time point.

In order to improve the completeness of follow-up, a reminder was sent to all patients who had been involved in the study prior to their next treatment course. All telephone interviews were performed by research fellows.

Sample Collection

To explore the potentially essential genes and pathways involved in response to ozone therapy, we collected blood samples from 7 patients (14 samples total) from the ozone therapy group and divided them into 2 subgroups: 4 patients (R1, R2, R3, and R4) with pain relief and hip function improvement of more than 50% after ozone therapy (responders); 3 patients (NR1, NR2, and NR3) had less than 50% reduction of pain and improvement of hip function after ozone therapy (nonresponders). Blood samples were collected preprocedure and 6 months after the first treatment course of ozone therapy. Briefly, 8 mL peripheral venous blood samples from patients were obtained in the morning following overnight fasting for at least 12 hours.

RNA Sequencing Analysis

RNA extraction, library construction, and sequencing have been previously described elsewhere (15). For the RNA-sequence data, the annotation of the transcriptome was defined by hg38 downloaded from the University of California Santa Cruz. Then the cleaned reads were aligned to the hg38 reference genome using TopHat 2.0.9 (Johns Hopkins Center for Computational Biology, Baltimore, MD) with the default parameters. Hisat2 version 2.0.6 (Johns Hopkins Center for Computational Biology, Baltimore, MD) (16), samtools 0.1.19 (Genome Research Institute, United Kingdom) (17), and HTseq-python 2.7.13 (Illumina Inc., San Diego, CA, USA) (18) were used to estimate the number of mapped reads in each annotated gene based on the mapping results. Then, we used edgeR R-3.3.3 (www.bioconductor.org) to further calculate the differential expressed genes (DEGs) by comparing the expression values between responders and nonresponders.

Genes identified to be differentially expressed were searched against the database of STRING (http://string-db.org/) by BlastX with a cut-off E-value of $10e^{-5}$. The results were then annotated using ANNOVAR (www.openbioinformatics.org) to generate Gene Ontology (GO) annotations and mapped to the categories of the GO database. Functional annotation enrichment was performed at $P < 0.05$ for DEGs using GO terms for biological processes in the topGO package (version 3.5.1 The R Foundation for statistical computing, Vienna, Austria).

Statistical analysis

All statistical analysis was performed using IBM SPSS Statistics 19.0(IBM Corporation, Armonk, NY). Continuous data were presented as mean ± standard deviation (SD), and categorical data were presented as a percentage. The Student's t-test and the nonparametric Kruskal-Wallis test were used to compare the control group and the ozone therapy group values assuming both normal and nonnormal distribution. The χ^2 test was used for comparison of categorical variables. Fisher's exact test was used for tests of proportions. The cumulative curves were used to compare the 2 groups in conversion to THA. The log-rank test was used to assess the equality of survival functions. A P value < 0.05 was considered significant.

Table 1. *The stages of ARCO classification.*

ARCO Stages	Definition
Stage I	Positive MRI and/or bone scintigraphy with normal radiograph or CT
Stage II	Radiographic changes in the femoral head including sclerosis, cysts, or osteoporotic changes of the femoral head
Stage III	Radiographic sign of subchondral fracture (crescent sign)
Stage IV	Radiographic sign of flattening of the femoral head and osteoarthritic changes such as decreased joint space and acetabular changes

Abbreviations: ARCO = Association Research Circulation Osseous; MRI = magnetic resonance imaging; CT = computed tomography

Results

From April 2013 through October 2018, a total of 100 patients were contacted and met eligibility criteria: 71 patients (107 hip joints) completed the assessment of pain intensity and hip function (Fig. 1). Baseline parameters were well balanced between the control group and the ozone therapy group. The demographic and baseline data for all patients are shown in Table 2.

Primary Outcomes

There was a significant decrease in pain intensity in both the control group and the ozone therapy group during each follow-up time point compared to prior to therapy (all $P < 0.05$; Fig. 4A); however, the VAS for pain intensity in the ozone therapy group was statistically lower than that in the control group (95% confidence interval [CI] 1.0 to 2.3; $P < 0.001$). There was a significant improvement in the HHS in both the control group and the ozone therapy group during each of the follow-up time points compared with that before therapy (all $P < 0.05$; Fig. 4B); however, the HHS in the ozone therapy group was statistically higher than that in the control group (95% CI -17.0 to -6.8; $P < 0.001$).

Table 2. *Baseline characteristics of patients and osteonecrosis.*

Control		Ozone Therapy	*P* value
Characteristics of Patients			
Number of patients (hips)	32 (49)	39 (58)	0.315
Gender (hips)			
Men/women	22 (33)/10 (16)	32 (47)/7 (11)	0.265
Age (mean ± SD)	42.91±14.182	44.64±10.442	0.555
Characteristics of osteonecrosis			
Etiologic Factors, no. of patients (%)			
Alcohol	7 (21.88)	18 (46.15)	0.251
Corticosteroids	10 (31.25)	9 (23.08)	
Idiopathic	2 (6.25)	1 (2.56)	
Trauma history	8 (25.05)	5 (12.82)	
Negative	5 (15.63)	6 (15.38)	
Pre- VAS for pain intensity	4.620± 1.49	4.74 ± 1.67	0.653
Pre- Harris Hip Score	64.02 ± 12.92	63.66 ± 11.68	0.878
ARCO stage, no. of patients (hips)			
I	6 (7)	5 (5)	0.492
II	4 (5)	6 (9)	
III	16 (22)	25 (31)	
IV	10 (15)	11 (13)	
Average length of follow-up (mo)	20.06 ± 9.38	22.46 ± 7.63	0.239

Data are presented as mean ± standard deviation (SD), mean (range) or n.
Abbreviations: ARCO = Association Research Circulation Osseous; Pre- = before ozone therapy; VAS = visual analog scale.

At the final follow-up, there was a significant improvement in the VAS for pain intensity and the HHS, but this did not reach statistical significance in the ozone therapy patients with ARCO stage I and II compared to that prior to ozone therapy (Figs. 4C, 4D).

Secondary Outcomes

At the final follow-up, all patients completed an assessment utilizing MRI imaging. It showed a significant overall improvement of bone marrow edema ($P < 0.001$) in the ozone therapy group compared with the control group (Table 3). In the ozone therapy group, there was a resolution of bone marrow edema among patients with grades 3–4 to 0–2 after ozone therapy (Fig. 5). The cumulative analysis demonstrated that conversion to THA is significantly associated with ozone therapy (Fig. 6). The control group patients showed a statistically higher probability of conversion to THA versus the ozone therapy group patients. Of note, 3 patients (6 hips) in the ozone therapy group underwent THA within the 12-month follow-up. Two (4 hips) of whom had a recurrence of pain symptoms due to sustained alcohol consumption, and one (2 hips) experienced excessive weight-lifting exercise. They were not followed after conversion to THA.

Side Effects

Fifty patients (46.7%) in the ozone therapy group reported a transient distending pain at the site of the injection during the administration of the O_2-O_3 mixture hip injection; however, these symptoms spontaneously resolved in 3 hours. One patient reported a mild fever after the O_2-O_3 mixture injection that spontaneously resolved in 24 hours. One patient reported tachycardia, chest pain, and shortness of breath that was not associated with changes in blood pressure or oxygen saturation during the O_2-O_3 mixture hip injection. These symptoms resolved within 10 minutes after treatment utilizing an oxygen mask and psychological counseling. We suspect that this patient suffered from a pulmonary embolism caused by direct entry of the O_2-O_3 mixture from each hip joint into the arterioles of the femoral head.

Identification of DEGs in Patients With ONFH After Ozone Therapy

To identify DEGs between responders and nonresponders of patients with ONFH, we performed the transcription profile data obtained from the GO database based on the 4 responders (R1, R2, R3, and R4) and the 3 noresponders (NR1, NR2, and NR3) who underwent ozone therapy. According to the cutoff criteria, 368 DEGs were identified in responders with ONFH compared with nonresponders, including 126 upregulated and 242 downregulated DEGs. DEGs in ONFH samples were determined using volcano plots and hierarchical cluster analysis of the data (Fig. 7A-B).

Enrichment Analysis of DEGs Between Responders and Nonresponders

To identify the aberrant cellular functions in peripheral blood leukocytes between patients with ONFH who had a good response and those who were nonresponsive after ozone therapy, GO analysis was performed on the DEGs. The significantly enriched clinical outcomes related to GO terms of the upregulated DEGs mainly included the following: "type I interferon"; "cytokine stimulus"; "innate immune response"; "defense

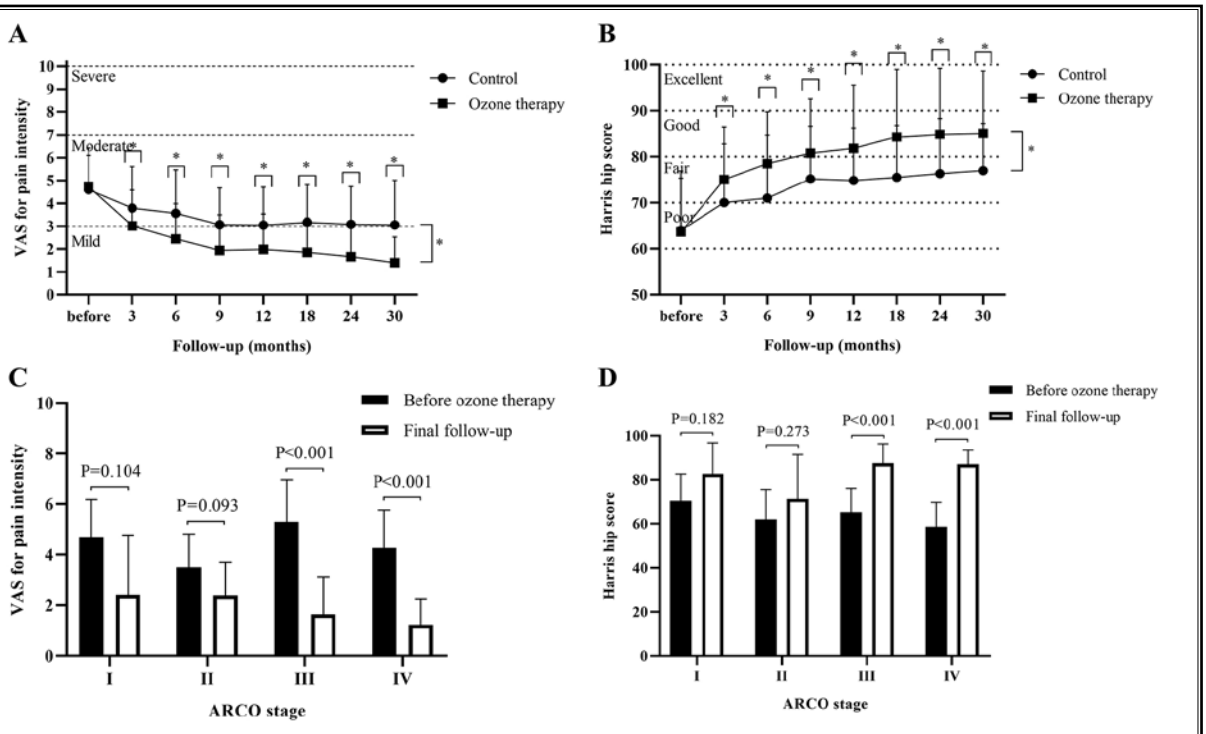

Fig 4. *The changing trend of VAS for pain intensity and HHS for hip function.*

response"; "erythrocyte differentiation"; "immune system process"; "oxygen transport"; and "cellular response to interleukin-1". The downregulated DEGs mainly included: "mitotic cell cycle"; "organelle fission"; "chromosome segregation"; "regulation of transcription"; and "cell cycle process" A bubble plot offers a visual representation of the pathways (Figs. 7C-D).

Discussion

In the present study, we performed clinical outcomes, imaging examinations, and transcriptomic analysis of patients with ONFH who were diagnosed with various ARCO stages to investigate the efficacy of ozone therapy.

There is growing evidence that the intraarticular administration of an O_2-O_3 mixture effectively reduces pain intensity and provides protective immunomodulatory effects on cartilage (8,19-21). When injected into a joint capsule, it can stimulate fibroblastic joint repair, reduce inflammation, and promote new cartilage growth (22,23). However, these referenced studies (8,19-21) were focused on pain control for patients with knee osteoarthritis; the therapeutic effect of ozone therapy on hip disease was not explored. It is well known that treatment for ONFH can be challenging. The goals of ONFH treatment are to relieve pain, restore hip function, and to arrest or slow the progression of the disease (24). Because the hip joint capsule attaches to the base of the surgical neck of the femur, once the needle tip is inserted into the neck, the O_2-O_3 mixture will be deposited within the capsule.

The primary outcomes showed a significant reduction in the average VAS for pain intensity and an enhancement in the average HHS for hip function at each follow-up time point compared with that before ozone therapy. However, the changing trend for VAS and HHS over the follow-up period revealed that the ozone therapy group achieved greater improvement in the analgesic effect and joint function of hips compared to the control group ($P < 0.05$). There were no significant differences noted before and at the final follow-up when comparing the VAS or HHS patients in ARCO stage I and II who underwent ozone therapy. (Figs. 4C-D). We suspect that the small sample size (5 hips in ARCO stage I, and 9 hips in ARCO stage II, compared with 31 hips in ARCO stage III and 13 hips in ARCO stage IV) may have caused this phenomenon. Alternatively, it may also be that there is less damage

Table 3. *Clinical and imaging outcome data at final follow-up.*

Variable	Control	Ozone Therapy	*P* value
No. of patients	32	39	
No. of hips	49	58	
Primary Outcome			
VAS for pain intensity	3.38 ± 1.94	1.92 ± 1.47	<0.001
Harris Hip Score	72.51 ± 14.05	84.21 ± 12.46	<0.001
Secondary Outcome			
Bone marrow edema			
Before ozone therapy			
Grade 0	0	2	0.333
Grade 1	4	5	
Grade 2	16	19	
Grade 3	18	13	
Grade 4	11	19	
At final follow-up			
Grade 0	2	25	<0.001
Grade 1	9	11	
Grade 2	18	10	
Grade 3	14	9	
Grade 4	6	3	
Conversion to THA (%)	13 (26.53)	6 (10.34)	0.041

Grades of bone marrow edema were defined: no bone marrow edema (grade 0), peri-necrotic bone marrow edema (grade 1), bone marrow edema extended into the femoral head (grade 2), bone marrow edema extended into the neck of the femur (grade 3) and bone marrow edema extended into the intertrochanteric region (grade 4).
Abbreviations: VAS = visual analog scale; THA = total hip arthroplasty.

done to the joints in earlier and less severe forms of this illness, thus making it harder to discern a positive response.

From the secondary outcomes, patients in the ozone therapy group at the final follow-up examination revealed a significant decrease in bone marrow edema from grades 3-4 to 0-2 compared to the control group ($P < 0.001$). A patient with rheumatoid arthritis in this study showed a significant improvement in the clinical and imaging outcomes (Fig. 5). He had an abnormally elevated rheumatoid factor (RF), C-reactive protein, and erythrocyte sedimentation rate (ESR) levels before ozone therapy. In comparison, both the RF and ESR were decreased to normal levels during each follow-up time after one treatment course of ozone therapy (data not shown). These clinical lab indexes suggest that ozone therapy may restore immunological parameters to normal levels, which is consistent with a previous report (25). The cumulative analysis is a valuable method that allows one to assess the conversion rate to THA over time when comparing the control and ozone therapy groups. Using this technique we found that the ozone therapy group patients showed a higher probability of not converting to THA versus the control group over the entire follow-up period (log-rank test; $P = 0.022$; hazard ratio = 5.217) (Fig. 6). This result was also confirmed at the final follow-up when comparing the 2 groups (10.34% vs 26.53%, $P = 0.041$) in Table 3.

The concentrations and doses of the O_2-O_3 mixture were based on previous clinical experience (20). Specifically, 20 mL to 40 mL of an O_2-O_3 mixture alters the pressure around the hip joint and allows ozone to diffuse into the tissue spaces of the joint capsule, thus reaching

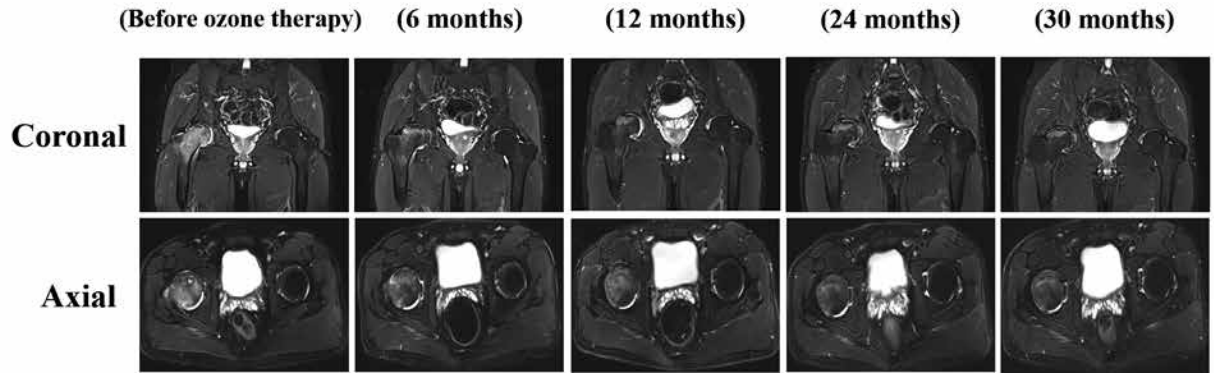

Fig 5. *Follow-up of a 39-year-old male with ARCO stage III osteonecrosis of the femoral head (steroid usage due to rheumatoid disease) and an additional bone marrow edema.*

several relevant areas, such as the osteonecrotic lesion, edema in the tissue, inflammatory synovitis, and joint effusions. We found 30 mL of an O_2-O_3 mixture at a concentration of 30 µg/mL in combination with O_3-AHT was effective in pain relief and hip function of patients with ONFH (Table 3).

It was reported that one therapeutic effect of O_3-AHT is increasing the generation of endogenous vasodilators, such as nitric oxide and carbon monoxide (26). Another benefit of O_3-AHT is the enhanced glycolysis and concentration of 2,3-diphosphoglycerate in erythrocytes, which increases the oxygen supply for ischemic tissues and improves the microcirculatory disturbance, resulting in promoting repair of bone necrosis (27).

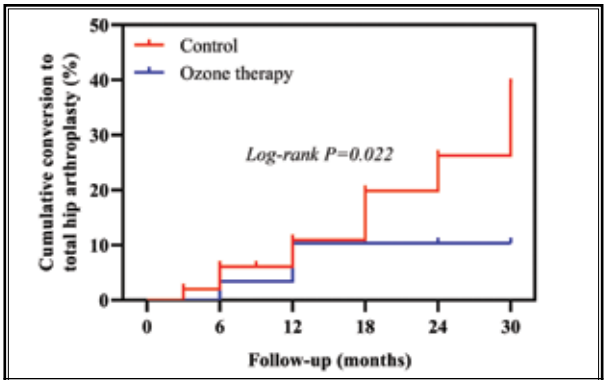

Fig 6. *Results of the cumulative analysis of the control group and ozone therapy group, with the conversion to THA as the endpoints during the entire follow-up period.*

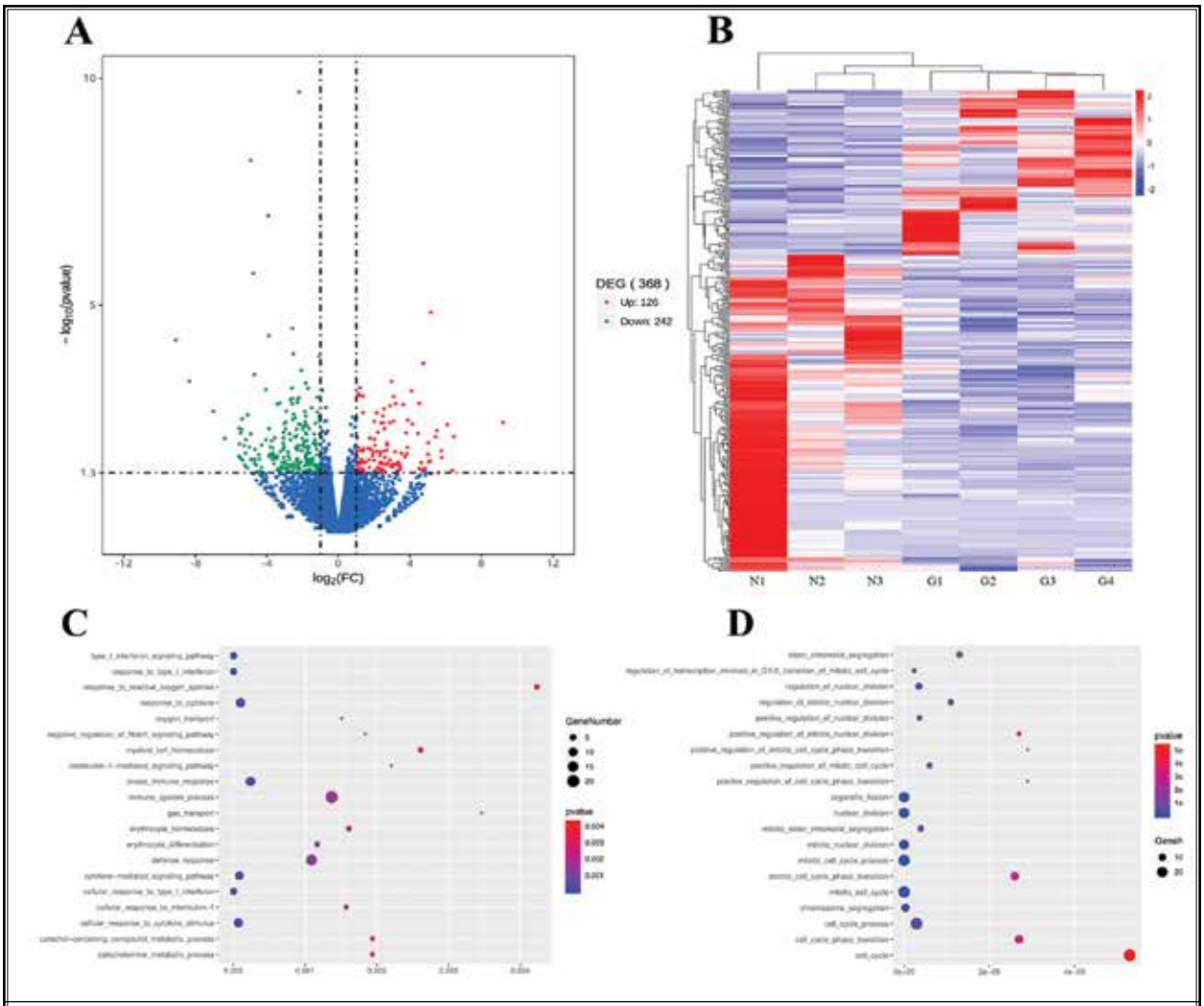

Fig 7. *The difference in gene expression between responders and nonresponders.*

While we have shown that intraarticular O_2-O_3 mixture hip injections combined with O_3-AHT were superior to protected weight-bearing in patients with ONFH, further studies are required to determine whether O_3-AHT has an additional effect on ozone therapy.

Based on our GO analysis results, an upregulation of IFN-I controlled genes could induce an inhibiting viral replication state, which plays an essential role in the regulation of both the innate and adaptive arms of the immune response (28). IFN-I also stimulates systemic immune activation as well as an antitumor effect, associated with the depletion of CD4 T cells and attenuation of antigen-specific T cell responses that are reported to be involved in the pathogenesis of synovitis as well as pain severity (29). We also found that "erythrocyte differentiation," "erythrocyte homeostasis," and "oxygen transport" were shown in the upregulated categories. It is known that red blood cells are essential in oxygen transport to the tissues, whereas hemorheological alterations may affect the blood supply in the femoral head (30). For example, it is reported that an ozone concentration of 50 μg/mL has positive effects on red blood cell deformability (31), contributing to benefits in oxygen supply and blood microcirculation at the femoral head.

The proposed mechanism of the analgesic action of ozone hip injection in the early stages mainly results from the elimination of inflammation followed by resolution of bone marrow edema, which is consistent with the potential mechanisms of the immune response and aseptic inflammation observed in the transcriptomics analysis as well as the MRI images. In contrast, the later recovery of the hip function may be due to the regeneration of hypoxic-ischemic femoral head necrosis via neovascularization and immunoregulation. However, this hypothesis needs further confirmation.

Limitations

The current study has certain limitations. First, the study was not randomized and involved a relatively small sample size. Although there was no significant difference in baseline characteristics between the 2 groups, additional randomized, placebo-controlled, multi-center studies are necessary to confirm the ideal dosing regimen, dose-response, and the optimum number of treatment courses related to the stages of ARCO in patients with ONFH. Secondly, short-term follow-up imaging remains insufficient to detect the possible effect of ozone therapy in promoting bone tissue repair and reconstruction. A more extended period of follow-up would be required.

Conclusions

Intraarticular O_2-O_3 mixture hip injection in combination with O_3-AHT appears to be an effective and acceptable method for providing long-term pain relief, functional recovery, and resolution of bone marrow edema in different ARCO stages of ONFH patients, thus mitigating the need for surgery. Immunoregulation and mitotically active pathways of lymphocytes may be involved in the differences in clinical outcomes between responders and nonresponders to ozone therapy.

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Author's participation

Jian-Xiong An: Substantial contributions to the design, analysis, and interpretation of data for the work; revising the work for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Guo-Ping Wu: Substantial contributions to the analysis and interpretation of data for the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Kun Niu: Substantial contributions to the design, acquisition, analysis, and interpretation of data for the work; drafting the work for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

You-Ping Wei: Substantial contributions to the acquisition and analysis of data for the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Hui Liu: Substantial contributions to the design and acquisition of data for the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Xin-You Gao: Substantial contributions to the acquisition of data for the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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