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Research Article

A novel view of the problem of Osteoarthritis in experimental rat model

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Abstract

Introduction: The article presents the results of the functional tests to improve the assessment of MIA-induced osteoarthritis development and the effectiveness of NSAID therapy.

Materials and methods: In the study, 26 male SD rats were used. MIA-induced osteoarthritis was simulated in the right knee joint. After an intra-articular injection of MIA, the animals were treated with ibuprofen and meloxicam. Pain assessment was studied in the following functional tests: incapacitance (hind limb weight bearing) test, von Frey test (mechanical allodynia), grip strength test, and knee diameter measurement. At the end of the study, a histological analysis of the knee joint was performed.

Results and discussion: An intra-articular MIA injection reduced 1.5 times the paw withdrawal threshold. In the rats that suffered MIA-induced osteoarthritis, the difference between the diameters of the intact and injected joints was 1.05 mm, compared to 0.03 mm difference in the control group. Hind limb weight bearing asymmetry was 89.5% when simulating MIA-induced osteoarthritis. The muscular hind limb grip strength in rats with MIA-induced osteoarthritis was significantly reduced on 3rd and 7th days after simulating osteoarthritis. Ibuprofen and meloxicam showed significant efficacy in all the above tests, although ibuprofen effectiveness was more pronounced than that of meloxicam.

Conclusion: The following functional tests were identified as the most significant and sufficient to assess the development of MIA-induced osteoarthritis and analgesic efficacy of NSAIDs: incapacitance test, allodynia test (von Frey filaments), measurement of hind limb grip strength and measurement of the diameter of the inflamed knee joint. The histological analysis made it possible to confirm the correspondence of the physiological response and pathological changes in the knee joint.

Keywords

in vivo model, monoiodoacetic acid (MIA), osteoarthritis, pain assessment, rat.

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Introduction

Osteoarthritis (OA) is the most noticeable form of the synovial joint disease, characterized by joint degeneration and pain. In addition to structural defects, there is increasing evidence that approximately 30% of OA patients have neuropathic pain (Teeple et al. 2013; Wang and Regatte 2015; Rey-Rico et al. 2016; Jacobs et al. 2017; Jin et al. 2017; Philpott et al. 2017). The mechanisms of persistent pain development are not yet clear, but most of modern OA treatment strategies are based on pain management (Ma et al. 2009; Van Velden et al. 2015; Philpott et al. 2017; Zhang et al. 2017; Kalamegam et al. 2018). Pain and disease progression are difficult to treat in many patients with OA due to the multi-factorial nature of the disease (Philpott et al. 2017). Chronic pain associated with OA is a serious problem for which there are few effective treatments (Johnson and Greenwood-Van Meerveld 2016; Hoshino et al. 2018; Zhang et al. 2018; Grundström et al. 2019).

A chemical model of MIA-induced OA develops clinically relevant OA pain symptoms, and it is most often used to test the effectiveness of pharmacological agents in the pain treatment. The MIA-induced OA model generates a reproducible stable phenotype that can be evaluated by changing the dosage of the administered substance (Horváth et al. 2016; Pitcher et al. 2016). MIA is a metabolic inhibitor that breaks down the cellular aerobic glycolysis pathway. Intra-articular injection of MIA disrupts the glycolysis in chondrocytes by inhibiting glyceraldehyde-3-phosphatase dehydrogenase, which leads to chondrocytes death, neovascularization, necrosis and destruction of the subchondral bones, as well as inflammation (Bozimowski 2015; Steinmeyer et al. 2018; Anindya et al. 2019). Besides joint damage, MIA injection induces mechanical sensitivity of the ipsilateral hind paw and weight bearing deficit. Histological observation and pain-related behavior in rat models together resemble human degenerative OA (Jacobs et al. 2017).

The development of OA has various etiologies and is accompanied by a number of complex pathological processes, resulting in changes in physiological states of the body. Nowadays it is important to optimize the pain sensitivity testing methods in animals in order to study OA and effectively develop therapeutic strategies. The histological analysis used to describe the models is a laborious and long-time stage, resulting in the determination of the morphological state of the tissues, rather than the physiological reactions of the body (Anindya et al. 2019). Pain sensitivity tests on animals make it possible to assess the nature of the inflammation development and its suppression by the most obvious and complex parameters, which result from inflammation processes at the molecular, cellular and tissue levels. Studying the natural behavior of animals during painful conditions and the underlying molecular mechanisms can facilitate the introduction of new analgesics into the clinic (Nagy et al. 2017; Chakrabarti et al. 2018; Abd ElHafeez et al. 2019).

Currently, various functional tests are being developed on rodents in order to create tools for basic and applied research. Functional tests and models enabled rapid progress on the anatomical and molecular basis of physiological and pathological pains, although they have not completely switched to new painkillers yet. The quality of pain perception assessment methodologies is a cornerstone of preclinical studies targeting at creating new analgesics. In the variety of methods for assessing pain and analgesic effect, it is difficult to choose methods most adapted to specific pathological conditions, including the study of the effectiveness of NSAIDs (Botz et al. 2017; Chakrabarti et al. 2018).

In view of the above, the aim of the study was to review the performance of functional tests to improve the assessment of MIA-induced osteoarthritis development and the effectiveness of NSAID therapy.

Materials and methods

The animals were kept in the biological testing laboratory of the Institute of Biological Control of the Russian Academy of Sciences accredited by AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International). Routine animal care was performed in accordance with standard laboratory operating procedures. The animals were kept in the controlled environment of two-corridor barrier rooms: temperature 20–24 °C, relative humidity 30–60%, and 12-hour light cycle. Temperature and humidity were controlled by the EVL monitoring system. The procedures with the animals were reviewed and approved of by the Bioethical Committee of the Institute of Bioorganic Chemistry of the Russian Academy of Sciences (RAS), the experimental protocol code is no. 688/19.

A total of 26 male SD rats (6–8 weeks) were randomly assigned to four treatment groups before the study began. Three experimental groups of animals were given a single dose of 3 mg of MIA (Monosodium Iodoacetate) in a 50-µl volume, using a 29-G needle inserted through the patellar ligament into the intra-articular (IA) space of the right knee. The control rats were injected with a single dose of an equivalent volume of saline (50 µl). The increased volume of the hind limbs, limited mobility, and gait change were the signs of inflammation that appeared 3–4 days later. The second group with simulated OA (n = 5) and the control group (n = 5), starting from the 3rd day of the study, were injected with 0.9% NaCl solution subcutaneously (1 ml/kg). The third (n = 8) and fourth (n = 8) groups with simulated OA were administered with ibuprofen (40 mg/kg orally) and meloxicam (0.5 mg/kg intramuscularly), respectively. The injections were performed daily, at the same time of the day, from the 3rd to the 14th day. Inflammation was described by the hind limb grip strength test, incapacitance test, and mechanical allodynia test. The diameters of the inflamed and healthy hind paws were measured, using an electronic caliper. On the 3rd, 7th and 14th days of the study, behavioral tests were performed one hour after the introduction of the substances.

Weight asymmetry, or Static Weight Bearing, was measured by using a SWB-Touch unit (Bioseb, France).

Changes in the weight distribution of the hind paws between the left (MIA) and right (contralateral) limbs were used as an indicator of discomfort in the knee joint that received MIA. During the test, the animal was comfortably secured in the holder, and the hind paws were placed on two separate sensor plates. The animal adjusted the weight distribution on its both hind paws. During the test, a weight applied to each sensor was recorded. The test was repeated three times, and the measurement data were averaged.

The threshold of pain sensitivity of rat paws in case of mechanical impact was determined be means of the von Frey method using a BIO-EVF4 device (Bioseb, France) with hard Eppendorf tips. A hard Eppendorf tip, mounted on the sensitive element, was used to assess the pain sensitivity. The animals were placed in individual cages with a metal grating floor, and allowed a 30-minute adaptation before the start of the test. Increasing force was applied to the central plantar region of the hind paw to induce reflecting flexion of the joint. The test was repeated three times.

Hind limbs grip strength was measured on a Grip Strength Meter (Columbus Instruments, Columbus, OH, USA). The tensile force of the dynamometer in kilograms (Chatillon DFIS-10, AMETEK, Inc/Columbus Instruments) was used to record the muscle strength of the hind limbs. The measurements were repeated three times for each rat.

The biomaterial was collected for histological examination. Bone and cartilage tissues of the joint were fixed and decalcified. The knee joint was incised in the sagittal plane, and embedded in paraffin. Paraffin sections 5–7 μ thick were stained with hematoxylin and eosin and studied using conventional light microscopy. The histological analysis assessed the following morphological features: inflammatory infiltration of the synovial membrane (synovitis), synovial hyperplasia, cartilage destruction and bone destruction. To assess an intensity of a morphological feature, the following scale was used: 0 points – within normal range, 1 – minimal, 2 – low, 3 – moderate, 4 – high, and 5 – very high intensity.

Descriptive statistics were applied to all quantitative data: average values and standard deviations were calculated. To establish intergroup differences, the data were analyzed using appropriate statistical methods. The statistical analysis was carried out by STATISTICA 7.1. The differences between the groups were considered significant at $P<0.05$, $P<0.01$ and $P<0.001$.

Results and discussion

It was shown (Fig. 1A) that an intra-articular MIA injection significantly reduced the von Frey thresholds of the injured paw compared with those of the non-osteoarthritic rats. The allodynia test showed significant differences between the saline-treated animals and the MIA-injected rats. The paw withdrawal threshold in the group of rats with MIA-induced osteoarthritis increased 1.5 times on the 3rd, 7th and 14th days of the experiment compared to that in the control group. Ibuprofen or meloxicam introduction showed no statistical significance in the rats without osteoarthritis throughout the experiment. Significant differences in sensitivity to pain were observed between the groups of rats with osteoarthritis: animals after MIA injection and subsequent treatment with saline solution had pain sensitivity 39–43% higher than in the groups with a subsequent therapy by meloxicam or ibuprofen administered on day 3, by 35–42 % higher by day 7 -and by an average of 34% –by the end of the experiment.

Three days after the MIA injection, a significant increase was observed in the rats' knee diameter in the groups with a MIA injection (Fig. 1B). In the control group, the difference between injected and non-injected joint diameters was 0.03 mm, while in the rats with MIA-induced osteoarthritis, the difference increased to 1.05 mm. Ibuprofen and meloxicam introduction in the osteoarthritic groups 3 and 4 showed their efficacy on the 3rd test day: the differences between the joint diameters were to 0.88 and 0.91 mm, respectively. In the groups receiving ibuprofen and meloxicam, the joint diameter significantly decreased on the 7th day of the experiment in comparison with that in the groups with osteoarthritis, which had been injected with saline solution, and the diameter differences were 0.23 mm and 0.65 mm, respectively. On the 14th day, the difference in the diameters of the right and left joints decreased in all groups, though a significant decrease was observed in group 4 with meloxicam introduction, which was 0.7mm less compared to that in control group. Ibuprofen introduction in group 3 intensified a reduction in the knee joint diameter: on the 7th day of the experiment, the difference between the group of rats with osteoarthritis and the group treated with ibuprofen was 74%, whereas the meloxicam treatment resulted in the difference of only 40%.

The data obtained (Fig. 1C) show the results of weight bearing changes that are associated with MIA injection into the knee joint. In this experiment, the results differed from the dynamics of changes in the knee joint diameter. The saline-treated animals showed no weight bearing changes. On the 3rd day of the experiment, weight bearing asymmetry with regard to weight was most significant only in the rats that had MIA-induced osteoarthritis, and was 89.5% higher than that in the saline-treated animals. Weight bearing asymmetry was also observed in the groups treated with meloxicam and ibuprofen. Weight bearing asymmetry in the ibuprofen-treated animals was 60.2% higher compared with that in the control group, which was lower than that in the meloxicam-treated animals (82.4%). The weight bearing asymmetry insignificantly increased in the groups on the 7th day of the experiment, through on the 14th day weight redistribution values in the MIA-, ibuprofen- and meloxicam-treated animals did not differ from those in the control group. In this test, the effectiveness of ibuprofen was also more pronounced than that of meloxicam. Ibuprofen introduction reduced weight bearing asymmetry by 4 times on the 3rd day of the experiment, whereas meloxicam introduction reduced weight bearing asymmetry by 2 times by the 7th day of the experiment.

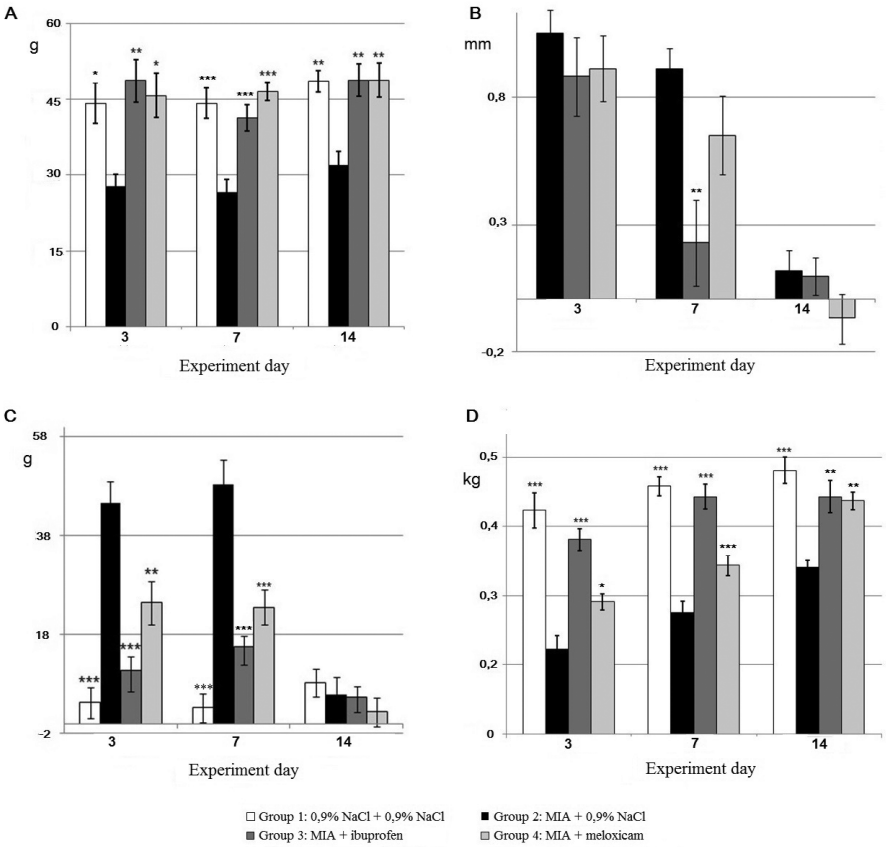

Figure 1. Comparison of ibuprofen and meloxicam analgesic and anti-inflammatory efficacy in the model of MIA-induced osteoarthritis. **Note:** (a) – hind limb withdrawal threshold in the allodynia test; (b) – the difference in diameters of healthy and sore knee joints, diameter values in group 1 are shown as 0 on *y* axis; (c) – the difference in weight bearing asymmetry between the limbs with healthy and sore knee joints in the incapacitance test; (d) – hind-limb grip strength in the Grip Strength test. Data are expressed as the mean ± s.e.m. * Differences from group 2 are significant at $p < 0.05$ in post-hoc Duncan test; ** Differences from group 2 are significant at $p < 0.01$ in post-hoc Duncan test; *** Differences from group 2 are significant at $p < 0.001$ in post-hoc Duncan test; MIA – monoiodoacetic acid.

The hind-limb grip strength (Fig. 1D) in the rats with MIA-induced osteoarthritis significantly reduced on the 3rd and 7th days after an intra-articular MIA injection into the hind-limb knee joint as compared to that in the saline-treated animal group. On the 14th day of the experiment, no significant differences were observed among any of the groups. Reduction of hind-limb grip strength reached its peak on day 3 (47%), with a slight recovery of functionality: by the 14th day, the difference was on average 28.8% compared to that in the controls without administering MIA. No significant differences in the hind-limb grip strength between the control group and the groups receiving ibuprofen and meloxicam were detected on any of the testing days. The largest improvement in grip strength was observed in the animals receiving ibuprofen, and the differences with the control group were 41.5%, 37.7%, 22.8% on the 3rd, 7th and 14th days, respectively. The animals of the group treated with meloxicam showed a 22% increased hind-limb grip strength on the test days compared to that in the animals with MIA-induced osteoarthritis.

On the 14th day after an intra-articular injection of MIA, microscopy showed typical signs of arthritis in all the animals: inflammatory infiltration of the synovial membrane with synovial hyperplasia, destructive changes in articular cartilage, destructive and necrotic changes in the menisci, total necrosis of the cruciate ligaments. Destructive changes in bone tissue were not detected (probably due to the short period of observation).

In the group of the animals that had received saline solution after simulating osteoarthritis, a pronounced inflammatory infiltration of the synovial membrane (mean score – 4.33), accompanied by synovial hyperplasia (3.67) was observed, as well as cartilage destruction (3.0).

Due to the introduction of ibuprofen in the MIA-induced osteoarthritis model, the mean score of synovitis was 2.75; synovial hyperplasia – 2.25; and cartilage destruction – 3.0. In the group of animals treated with meloxicam, these pathomorphological changes were of 2.0; 1.0 and 3.0 points, respectively.

Microscopy of the knee joint of male rats in the control group showed that 3 of 4 animals had minimal inflammatory infiltration of the synovial membrane, and 1 animal had none (average score – 0.75). Neither synovial hyperplasia, nor cartilage and bone damage was detected in any of the control animals. Microphotographs of histological preparations of the right knee joint of male rats of all the studied groups are presented in Figures 2 and 3.

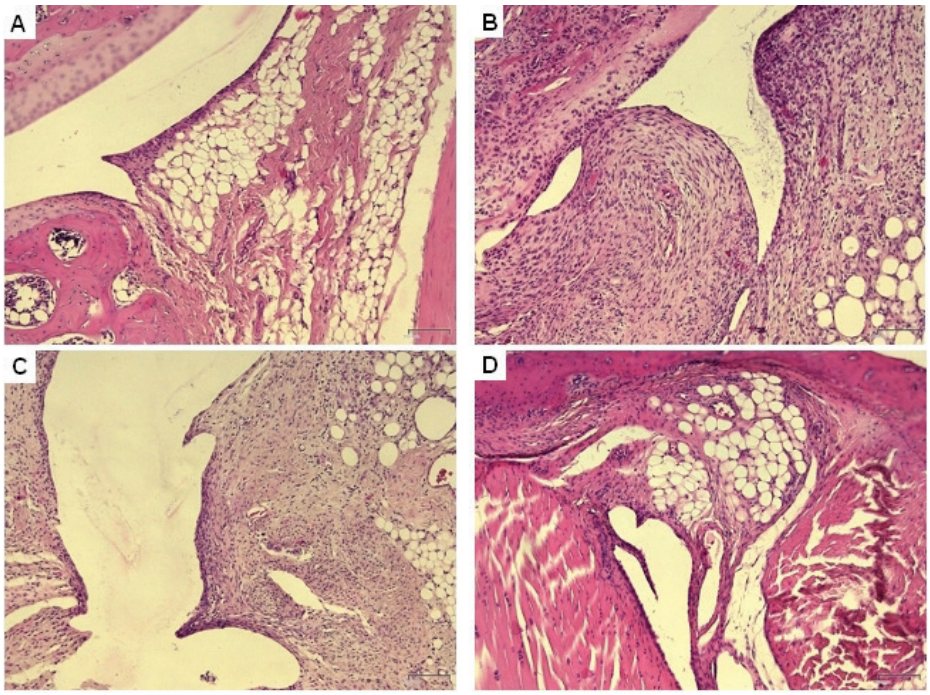

Figure 2. Fragments of the synovial membrane of the right-knee joint of male rats of the control group, as well as against the background of the introduction of a 0.9 % sodium chloride solution, ibuprofen and meloxicam in the MIA-induced osteoarthritis model. Animals were sacrificed 14 days after arthritis induction. Scoring of synovitis and synovial hyperplasia. H&E staining, 100x (scale size corresponds to 100 μm). **Note:** (a) Group 1: 0.9% NaCl + 0.9% NaCl, inflammatory infiltration: 1, synovial hyperplasia: 0; (b) Group 2: Monoiodoacetic acid (MIA) + 0.9% NaCl, inflammatory infiltration: 4, synovial hyperplasia: 4; (c) Group 3: MIA + ibuprofen: inflammatory infiltration: 3, synovial hyperplasia: 3; (d) Group 4: MIA + meloxicam: inflammatory infiltration: 2, synovial hyperplasia: 0.

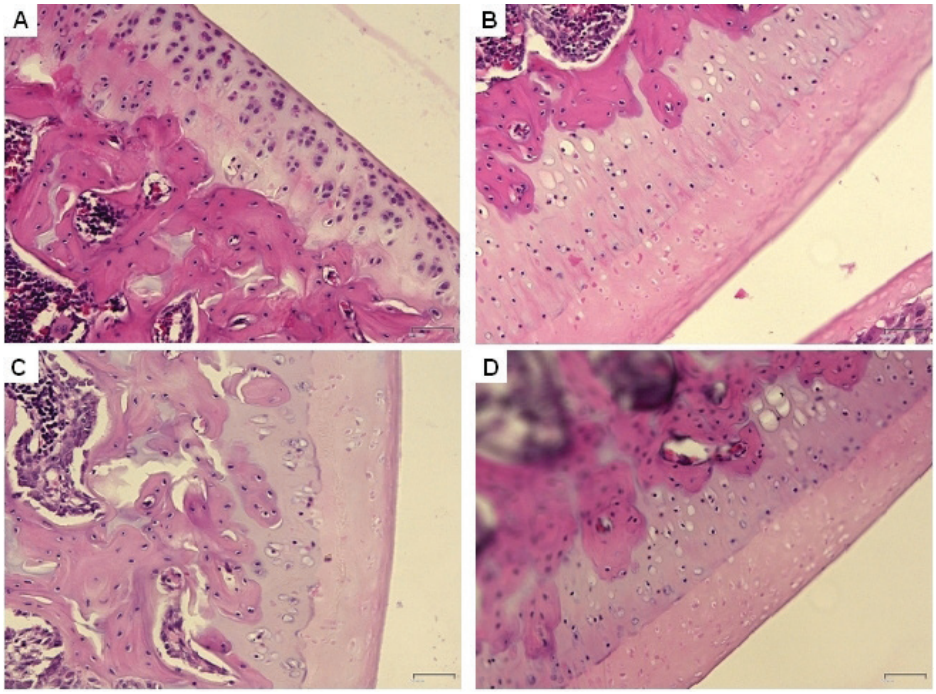

Figure 3. Fragments of the distal epiphyseal cartilage of the femur of the right-knee joint of male rats of the control group, as well as against the background of the introduction of a 0.9 % sodium chloride solution, ibuprofen and meloxicam in the MIA-induced osteoarthritis model. Animals were sacrificed 14 days after arthritis induction. Scoring of cartilage destruction and bone destruction. H&E staining, 200x (scale size corresponds to 50 μm). **Note:** (a) Group 1: 0.9% NaCl + 0.9% NaCl, cartilage destruction: 0, bone destruction: 0; (b) Group 2: Monoiodoacetic acid (MIA) + 0.9% NaCl, cartilage destruction: 3, bone destruction: 0; (c) Group 3: MIA + ibuprofen: cartilage destruction: 3, bone destruction: 0; (d) Group 4: MIA + meloxicam: cartilage destruction: 3, bone destruction: 0.

Conclusion

The assessment of pain in osteoarthritis animal models is an integral part of interpreting the utility of a model to describe the clinical condition and to ensure accurate translational medicine. This paper focuses on the model of inflammation, which allows studying the development of OA symptoms and the effectiveness of drugs from the group of non-steroidal anti-inflammatory drugs.

The tests that assess the pain sensitivity of animals allow us to assess the nature of the inflammation development and its suppression, focusing on the most obvious resulting parameters. To improve the study of the MIA-induced inflammation model, functional tests and criteria were selected to accurately describe the development of the inflammatory effect: incapacitance test, allodynia test (von Frey filaments), hind-limb grip strength, and measurement of the diameter of the knee joint of the inflamed limb. The histological analysis has shown that the MIA-induced OA model morphologically corresponds to such and can be used in experiments to study new approaches in the treatment of osteoarthritis. Using classical therapy with ibuprofen (40 mg/kg orally) and meloxicam (0.5 mg/kg intramuscularly) daily from the 3rd to the 14th day of the experiment on the MIA-induced OA model, it was possible to describe objectively and fully the changes in painful behavior of animals corresponding to the clinical data.

Conflict of interest

The authors declare no conflict of interest.

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