Study protocol

Needling Techniques for Tonifying Kidneys and Dredging Meridians for Knee Osteoarthritis: A Randomized Clinical Trial

NCT05014542

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The randomised control open-label study supported by recent scholarly peer-reviewed literature was originally designed and aimed to evaluate the effectiveness and safety of acupuncture with a predefined acupuncture protocol in KOA participants over 50. Due to the experiment's nature, participants and the researcher will not be blinded, but an independent observer who collected participants' answers wil be. The control group (group C) will continue with taking analgesics from before the study, while the acupuncture group (group A) will take acupuncture as an adjunct to the same analgesic regimen. All participants will take analgesics in an "as needed" manner to avoid the disadvantages to their health when taking them regularly.

Used methods reflect the need to answer the research question: *Could acupuncture on patients with KOA decrease pain, improve the function of the knee joints, and additionally achieve positive effects on debilitating symptoms of a Kidney deficiency as a root cause of KOA, estimated by recognized questionnaires?* The research was directed towards adding new findings to the body of knowledge. KOA is based on a tangible and frequently cited condition. The research protocol was confirmed by NJUCM board committee, and any deviations from the protocol will be documented and approved.

The KOA may be diagnosed easily and confidently with only clinical conclusions in patients with painful knees above 50 years if recent trauma is excluded. Typically pain aggravates with motion and load, alleviates with rest, and the persistence of morning stiffness is less than 30 minutes. Atypical presentation of KOA (inflamed knees, marked pain at rest or night, rapidly progressive pain, or systemic symptoms) supposes the need

for clinical differentiation. A typical presentation does not need confirmation with additional investigations. Moreover, there is no consistent correlation between clinical symptoms of KOA and radiographic or blood findings. Altman constructed applicable KOA diagnosis criteria, recommended by the American College of Rheumatology (ACR) which are incorporated as the study criteria.

Participants mutually substantially varied by comorbidity, emotional response, habits, beliefs, and expectations. Because all suffered from KOA and KD the acupuncture protocol was so designed to enable uniform treatment to all. Comorbidities could affect symptoms and make treatment less or more effective. The researcher estimated that with the equally provided treatments, no one participant could be harmed and the protocol may be sufficiently effective for all included.

The study proposal was accepted in 2020. The approval of the Ethical Committee of Health Center Zagreb-East was received on June 7, 2021, REF.NO 01-830-1/21. The study is registered on ClinicalTrials.gov PRS under ID NCT05014542.

Treatments will be performed in the researcher's private family medicine practice in Zagreb. The collection of participants was enabled by open access to data of four thousand patients from two family medicine practices connected by the group contract. The Central Health Information System of the Republic of Croatia (CEZIH) enabled the data search. The researcher got agreement from the other physician to approach all population data for experimental purposes. Also, the target population represents the general population regarding the explored topic involving all age groups besides preschool children. KOA prevalence in the general population enabled sufficient enrollment. The severity of KOA symptoms guarantees patients' motivation to participate and finish the envisioned protocol. A researcher who is a certified acupuncturist with over twenty years of experience provided TCM-style acupuncture.

Berman et al. study in 1999 serves for sample size calculation: the calculated 32 was doubled and 64 participants made the sample size from the expected greater attrition of participants from a longer study period. The CEZIH's database enabled searching the target population by the default

criteria of 50 years and above and M17 diagnosis criteria from four thousand patients. The International Classification of Diseases (ICD-10) encrypts KOA by M17. 180 patients made the study population. They were contacted by telephone, and if they showed a willingness to participate in the trial, they were invited to the researcher's office for more detailed arrangements. Those unavailable, with incorrect diagnosis, or unwilling to participate fell off and the first 64 who satisfied enrollment criteria were enrolled, Figure 1.

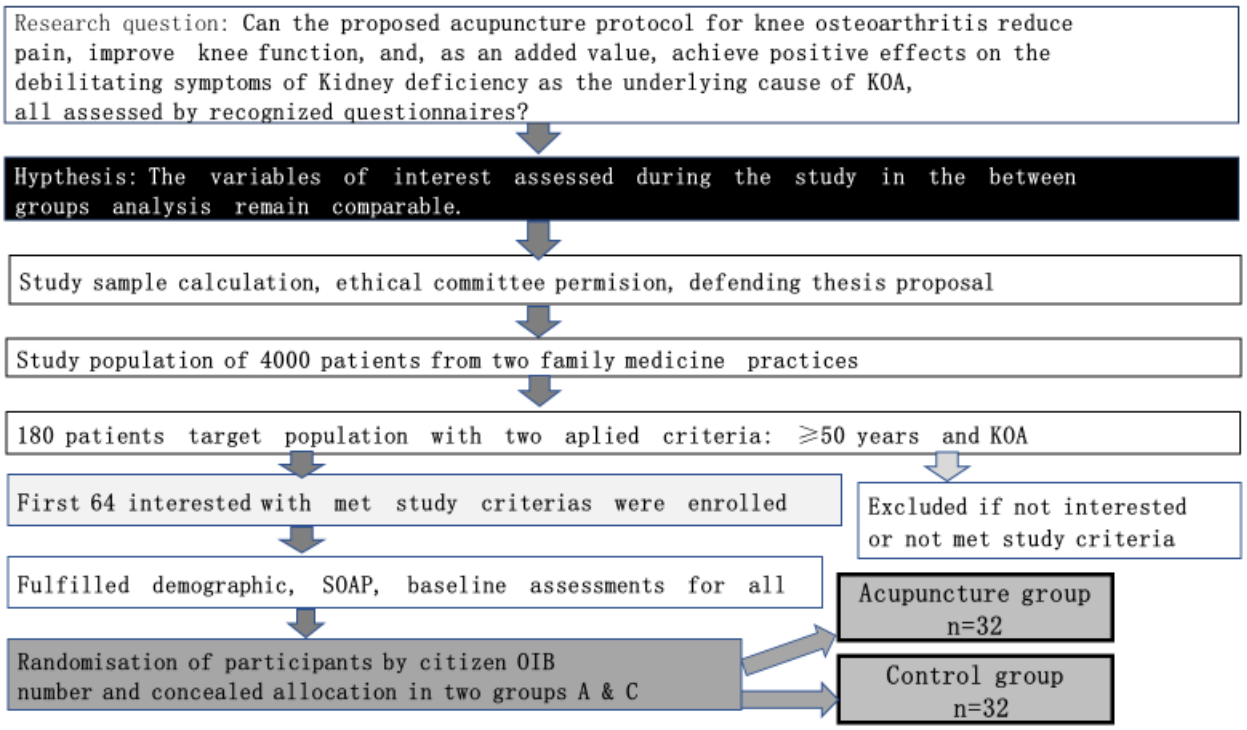

Figure 1 Pre-experimental research part

KOA is confirmed with "knee pain and three out of six associated characteristics: patient of any gender over 50 years, less than 30 minutes of morning stiffness, crepitus on active motion, bony tenderness, bony enlargement, and no palpable warmth ".[97,145] For the recruitment process, radiographic or laboratory findings were not essential because clinical criteria alone reached 95% sensitivity in diagnosis confirmation, while the X-rays had a specificity of only 69%.[9] Those who lacked a knee radiogram were referred to obtain it. In the pre-experimental week, 64 enrolled participants were examined, and baseline assessments were filled in with

the assistance of a blinded independent assessor. Participants were randomised and in a concealed manner allocated to interventional group (A) or control (C), Figure 1.

On September 6, 2021, the experiment will start. Group A will receive acupuncture and analgesics, and group C analgesics only. All in “as needed” regimen and free to change the dose. At Week 1 Group A should start with the first of three acupuncture treatment cycles, each consisting of nine acupunctures, and two adjacent cycles will be separated by a 3-week treatment-free period. Thus the entire treatment period of Group A covers 15 weeks, followed by a 9-week treatment-free period to assess short-term therapeutic effects. The first 24 weeks comprises the acupuncture treatment of group A and nine successive assessments of both groups, while all participants took analgesics identically, according to their needs. The same acupuncture treatment protocol should be offered to group C in Week 25 as a reward for participation as a control if interested. The identical acupuncture treatment of Group C will be provided from Week 25 to Week 39.

The whole experiment lasted 39 weeks, the assessment at Week 39 was introduced after the participants of group C mostly showed interest in acupuncture treatments; this deviation from the study protocol was approved and accepted by the authority. Add-assessment at Week 39 will assess available participants of group A 24 weeks after acupuncture ended to observe medium effects and immediate effects of acupuncture in group C, Figure 2.

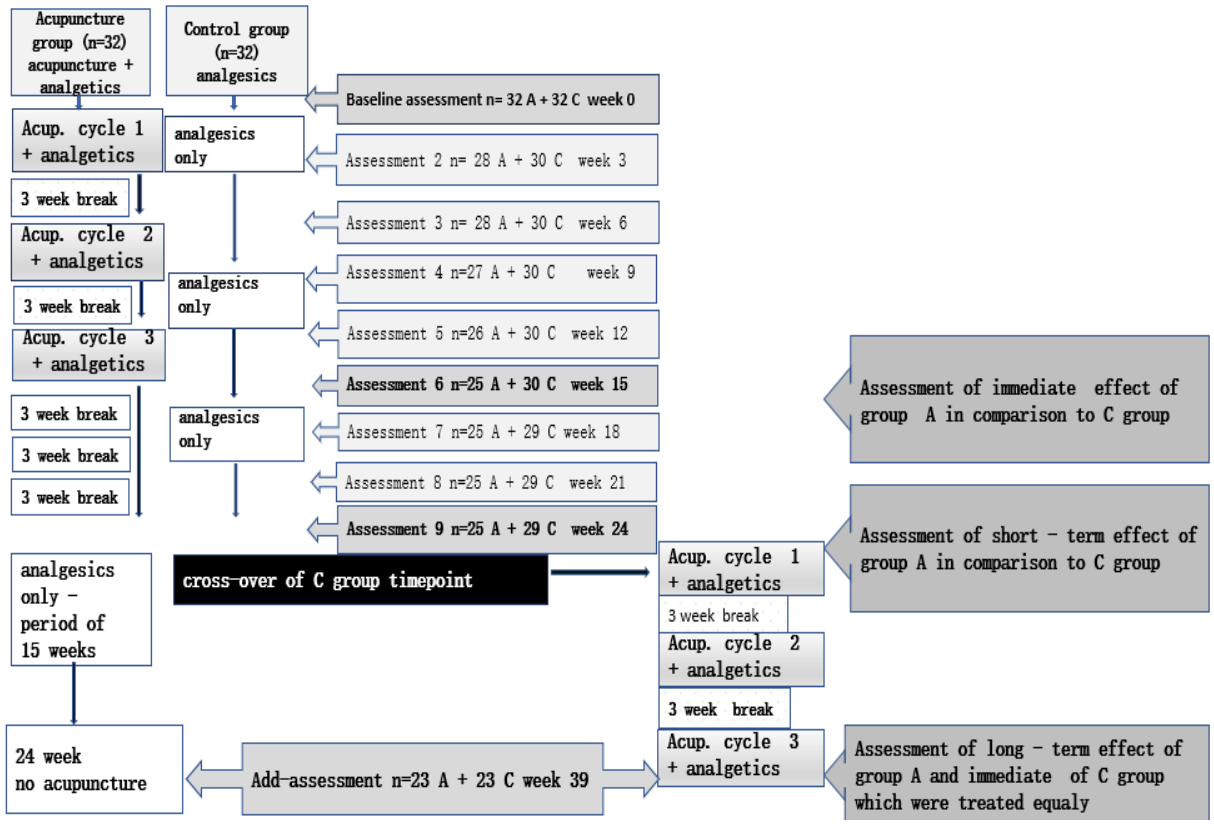

Figure 2 Experimental research part

Figure 2 The First study part lasted until Week 24. The Additional study part lasted from the Week 25 to 39. Each of three acupuncture cycle was 3-week long each, participants received acupuncture 3 times weekly, cycles were separated by 3-week period. At all 10 assessments, participants fulfilled WOMAC, NRS, KDSQ and DRUG. Assessments 6, 9, and Add evaluated immediate-, short-, and medium-term effects. The number of existing participants at specified time points was presented from n=n(A)+n(C), where the total number is equal to the sum of those in both groups. Add-additional, No-number, pt - participant, n - number of participants

The acupuncture treatment protocol envisioned 27 treatments per participant. The number of performed treatments was recorded. Acupuncture treatment was uniform for all participants. The design embraced the basic theoretical concept of the causal connection between KOA (knee Bone Bi syndrome) and KD. The selection of points consisted of 8 local and 3 aimed to affect KD, which were needled bilaterally with prescribed techniques and the intention to elicit *de Qi.*[139]

In total 10 assessments were provided at weeks 0, 3, 6, 9, 12, 15, 18, 21, 24, and 39. The time and day of treatments or assessments were

adapted to the patient's possibility to participate as much as possible. The observer was independent and blinded to which group a participant belonged.

WOMAC total and subscales, KDSQ, NRS, and DRUG were evaluated. DRUG was calculated from the average daily dose of analgesics in the last 3 days before assessment, according to the participant's statement. Different types of analgesics were converted into comparable doses of ibuprofen as the most prescribed one and expressed in IBU (ibuprofen milligrams units).

Methods

1 Sample size calculation

Calculation was performed from the Berman[23] study with a similar research design, which achieved significant differences by acupuncture between the experimental and standard medication groups. Berman used a shorter treatment period than the one envisioned for the present study (8 vs. 15 weeks). Otherwise, the protocols were similar. From Berman's study, WOMAC total scores in the A and C groups were comparable at the baseline, after the treatment period of 8 weeks WOMAC total scores were in group A 28,08 with standard deviation (SD) 17,96 and in group C 50,11 (SD=14,52). The sample size was calculated using the PASS software package[146] envisioning a two-sided test with unequal variances: type I error 0,05 and 0,95 power. Group sample sizes of 16 and 16 (i.e. the calculated sample size of 32 subjects) achieve 95,78% statistical power to reject the null hypothesis (H_0) of equal means when the population mean difference is $\mu 1-\mu 2=28,1-50,1=-22,0$ with SD(group A)=18,0 and SD(group C)=14,5, and with a significance level $\alpha=0,050$ using a two-sided two-sample unequal-variance t-test.[146–150] Taking into account that in the study the treatment period will be significantly longer compared to Berman's and that a certain proportion of subjects is expected to be lost to follow-up, the study population was doubled to a total of 64 subjects. The sample size was large enough to control the risk of false-negative or false-positive findings, to increase the precision of the results, and to provide 95% power of the study for conclusion.

2 Inclusion and exclusion criteria

Study criteria were predetermined for the enrollment of participants. Inclusion criteria were patients aged 50 years or older with confirmed KOA for at least six months, at least moderate knee pain in the last month, taking analgesics for pain control during that period, by the radiographs confirmed KOA's Kellgren-Lawrence (K-L) grade 2 or higher, and signed informed consent. Exclusion criteria were receiving knee intra-articular injection three months before the participant entered the study, unstable chronic or uncontrolled concomitant disease, bleeding diathesis or using anticoagulants. Those who satisfied all criteria were assigned to the study.

The knee radiographs and K-L grading served as additional conformation of KOA. Participants were not asked to renew existing images because radiographs do not follow the clinical presentation unambiguously.[7] New ones were done only for those who did not have them before. Because of the researcher's confidence in recognizing KOA from rheumatoid arthritis, psoriatic arthritis, rheumatic fever, and other knee conditions, the conditions were not listed as excluding factors. Two participants were enrolled with unilateral total knee endoprosthesis.

3 Randomisation and concealed allocation

Patients were assigned to the study after the enrolment criteria were applied. A population of 64 patients was evenly randomly allocated into two groups: the experimental or acupuncture (A) group and the control (C) group. Randomisation and concealed allocation were achieved using the participants' permanent unique randomly generated and coincidentally assigned Personal Identification Number (OIB). The acronym OIB stands for "*Osobni identifikacijski broj*" in Croatia; all citizens receive it at birth. OIBs mutually differ according to their size, but all possess eleven digits.

OIBs were sorted from the greatest to the smallest and attached to numbers from 1 to 64, the biggest OIB was linked to the smallest number. Those scheduled under the even numbers were allocated to group A, while odd numbers formed group C, according to the alphabet order of the first letter of the involved word (i.e. "A" with "even" and "C" with "odd"). The

experimenter could not know to which group any participant would be allocated and the criterion of concealment was satisfied.

4 Safety assessment

The adverse events were spontaneously reported and recorded successively throughout the experiment. The practitioner addressed open questions to the patient at every session to increase the possibility of side effect reporting. Identified side effects were monitored and followed until resolution. Reporting serious adverse reactions to the competent authority was obligatory. The study presented side effects in detail in Section 11.

Also, participants' radiographs were not renewed to prevent unnecessary radiation of patients for a more precise KOA staging because X-ray images and clinical presentation do not correlate directly in KOA.[9]

5 Study protocol

From the need to preserve Essence and restrain the Ming Men Fire tonifying the Kidney from the adult age is important. Yin and Yang are opposed poles but exist inseparably; deficiency of one depletes the other, and one becomes produced from the other in favourable circumstances. Yin can be obtained from Yang, and Yang can be enriched with the tonifying of Yin in the balanced treatment.[137] According to Xun Zi," myriad beings form when the heaven and earth meet together, and all changes and transformations start when Yin and Yang meet together ".[138] The quote emphasises the clinical reasoning of simultaneous mild tonifying of Kidney Yin and Yang while treating KD. Another saying from the Essential Reading in Medicine: "Without Yang, Yin cannot engender, without Yin, Yang cannot transform ",[124] confirms the previous statement.

The Bi syndrome could be superficial (tendons, muscles) as arises from the interaction of localised weaknesses (local Qi and Blood flow are impeded) and sufficiently strong pathogenic factor invasion. The treatment strategy requires removing obstructions and stimulating the local Qi and Blood flow only.[119]

Knee Bone Bi syndrome has a more complicated evolution. Bones and cartilage share the same developmental lineage, their formation and maintenance relate to Kidney Essence. It is a chronic and degenerative

condition which primarily originates from KD while external factors invasion contributes to symptomatic manifestation formation. Both amplify each other; unresolved pathogenic factor lingers and weaken internal organs, and vice versa depleted organs exhaust the organ-representative distinct parts and make them prone to further pathogens invasion.[119] In combined patterns, an obligatory strategy is to tonify the deficient root and drain the excess because a strong root provides Qi and Blood for related regions and enables excess to be expelled, further if only the root has been nourished it will be difficult to expel the excess, or if only the excess becomes drained disorder will reoccur with more intensity.[133,140,141] Therefore, KOA should be treated with simultaneous tonifying of KD and expelling pathogens with mild reinforcing-reducing or even technique on local points to treat manifestation by support local Qi and Blood flow but not in a dispersive way rather to derive excess toward the deficiency.[79,142] When the described methods act, the reinforcing-reducing effect should be produced by the regulation of Qi on combined full-empty conditions such as knee Bone Bi syndrome and KD as a requirement for treatment success.

Points selection (poins are descibed at Attachment 1):

- local points are *Dubi* (ST-35), *Neixiyan* (EX-LE-4), *Heding* (EX-LE-2), *Xuehai* (SP-10), *Yanglingquan* (GB-34), *Yinlingquan* (SP-9), *Zusanli* (ST-36), and *Sanyinjiao* (SP-6),
- points based on KD are *Taixi* (KI-3), *Shenshu* (BL-23), and *Guanyuan* (CV-4).

Needle selections: Disposable sterile acupuncture needles 0.30 × 40 mm of the brand Green Nature, manufactured by Suzhou Zhong Jing Life & Science Technology Co., Ltd. Wujiang, China were used.

The depth and angle procedure: perpendicular and 1-1.5 inches deep for SP-10, EX-LE-2, GB-34, SP-9, ST-36, SP-6, BL-23, and CV-4 and perpendicular and 0.5-1 inch deep for KI-3. Points ST-35 and EX-LE-4 should be needled in the horizontal insertion and up to 2 inches deep.

Acupuncture techniques: reinforcing method for KI-3, BL-23, and CV-4, while the mild reinforcing-reducing or even technique for all other local points.

Points were needled bilaterally, so the acupuncture treatment included 21 points. Complete treatment lasted 30 minutes, and reinforcing and reducing manipulation were provided every 10 minutes, three times in

total, except at BL-23, which was reinforced briefly three times in a sitting position and needles were withdrawn immediately after manipulation to allow the participants to lie down in supine position for treatment continuation. From ST-35 and EX-LE-4 position (directly in the knees) an even milder reinforcing-reducing manipulation form was required. The needles were placed at the intended depth to elicit de Qi.

Providing acupuncture treatments in 15 weeks: The complete treatment period includes three therapeutic cycles three weeks long, and two adjacent cycles were separated by 3 weeks without acupuncture. Treatments were attended 3-time weekly during any of the treatment cycles. Thus, the complete treatment protocol contained 27 possible acupuncture sessions.

The points were selected to treat the KOA and KD simultaneously. Envisioned treatment follows syndrome differentiation and understanding of the theoretically substantiated genesis of KOA. The points for KOA treatment are determined without individual adjustment because all participants suffer from KOA and KD. It was concluded that not a single point could harm any patient concerning the assessed state of health when the exclusion criteria for enrollment were applied. Knee Bone Bi syndrome and KD are interconnected in the onset of the disease and the recovery process. Treatment should target the pathology from the evolution of Bone Bi syndrome: excess of Damp-Cold pathogen and deficiency of Blood, Qi, Yin, Yang, and Essence. By the treatment, tonifying of the Kidney enables nourishing of related knee region and meridians to allow meridian-dredging by deep activation of Qi and Blood as a requirement for leaching out Damp supported with manipulation of local points on Spleen's transportation and transformation processes to solve local Dump congestion and improve body nourishment, to invigorate Blood, and release consequential joint constriction. [79]

This acupuncture protocol was designed by textbook exploration[19,79,118,124,139] and literature mining. [54,115,119,133,140,142] supported by supervisor *** and approved by the *** committee during the thesis proposal defence.

6 Needling techniques

A reinforcing method of KD was performed with slow needle insertion to strengthen the intention of stimulation at the predicted depth. Simultaneously the experimenter inhaled, intending to obtain *de Qi*. Six slow and short needle rotations formed one manipulation. Three manipulations were provided in total, every 10 minutes in one treatment. The rotation includes both directions with amplitude <180° by the speed one-time in two seconds; one direction lasted one second. The withdrawal was quick with the exhalation of the experimenter to strengthen the mild form of tonifying, firstly under the skin surface, then from the skin with an immediate covering of the hole.[143]

A mild reinforcing-reducing method at local points was performed with an even speed of inserting and withdrawing the needle. After the needle reached the desired depth, six up-and-down vertical needle movements of <10 mm in depth made one manipulation. One up-and-down movement was performed with a frequency of both needle directions in one second.[143] One treatment was supposed three manipulations, every 10 minutes.

ST-35 and EX-LE-4 points required even milder manipulation to be applied from their position. Also, in the case of participants with a weaker constitution, a slightly milder form of local points manipulation was performed to prevent the appearance of weakness. That supposes lowering of vertical needle movement depth and decreasing the manipulation frequency.

From a TCM perspective, the agreed-upon strategy for knee Bone Bi treatment is to calmly tonify the Kidney and Liver Blood, nourish Qi and Blood, invigorate them, and expel the Damp-Cold pathogen from obstructed channels and collaterals without harming the vital Qi.[54,119,120] The expelling of the pathogens from KOA is assured by reinforcing Kidney Qi and dredging the local meridians by even technique, not to disperse pathogens but rather to process and transfer them toward existing deficiences to achieve balance.

From so designed treatment of the knee Bone Bi syndrome is expected:

1. by reinforcing Kidney, KD and knee Bone Bi syndrome should be treated,

2. when knee Bone Bi syndrome is nourished systematically with Qi and Blood, a stronger and more persistent treatment effect could be expected compared to local treatment only,

3. additional improvements in KD's scope of symptoms which intensity decreased, but improved the patient's general health. The unintentional improvements of general body conditioning arise when general Qi is reinforced, Blood moved, and Yin and Yang are produced.

7 Groups A and C

Each participant, regardless of the group, had the same number, order, and structure of treatments, and the difference between the groups was in that, in the first 24 weeks of the study, the experimental group A (henceforth, group A) received a combination of an acupuncture and an analgetic treatment, whereas the control group C (henceforth, group C) received the analgetic treatment only. After the 24th week, group C receives a combined acupuncture and analgetic treatment while group A receives analgesics only, Figure 8-2.

The crucial 24 weeks was organised so that each patient of group A underwent three three-week treatment cycles, each week consisting of three in-person treatments for a total of 27 treatments per patient. At the end of the first and second treatment cycles, patients were given a three-week break from treatment. At the end of the final cycle (15 weeks from the start of the study) patients underwent immediate-term assessment, and then the patients entered a 9-week observational period during which they did not receive acupuncture. At the end of the observational period (24 weeks from the start of the study), they underwent an assessment which gauged the short-term efficacy of their treatment, Figure 8-2.

Following the initial 24 weeks, group A stopped receiving any acupuncture treatment, whereas group C started receiving the combined acupuncture and analgetic treatment, which was promised to incentivise participation. After the ensuing 15 weeks, another assessment was administered to both groups to assess the immediate effects of group C and the medium-term effects of 24 weeks follow-up of group A (Figure 8-2). During the complete study period, all participants took analgesics according to symptom intensity without the need for prior consultation.

Recommendations to take analgesics "as needed" during the study continued from before.

All participants underwent nine assessments simultaneously up to Week 24 and the final at Week 39. Although both groups received identical acupuncture treatments, group A had four assessments along the acupuncture treatments, unlike group C.

8. Assessments

The study comprised 10 identical assessments at Weeks 0, 3, 6, 9, 12, 15, 18, 21, 24, and 39, 4 variables (WOMAC, NRS, KDSQ, and DRUG) were assessed for all available participants. The intensity of knee symptoms (WOMAC, NRS), KD intensity (KDSQ), and dose of analgesics taken (DRUG) were assessed. Fulfilling the forms by participants was observed by the blinded and independent nurse who did not know to which group participants belonged. She took care of the timing to contact participants to fill out the forms, to obtain information about the dose of analgesics, and to provide explanations if needed.

WOMAC is a renowned questionnaire with high reliability and good validity for the assessment of KOA.[151] It measures 5 items for pain, 2 for stiffness and 17 for functional limitation, and its maximal score is 96 points. WOMAC is highly responsive to clinical changes[152] and is commonly utilised for KOA treatment assessment.

The NRS continuous scale designed as 100 mm long, is a widely used and recognized scale for pain intensity assessment. The intermediate part of the scale was divided into ten enumerated parts for easier browsing and filling the scores: the maximal 100 scores signified unbearable pain, and the minimal 0 no pain. WOMAC and rating scales for pain are widely accepted, and commonly used in KOA trials.[29,30,42]

Chinese medicine's routine diagnostic methods (inspection, inquiry, auscultation, and palpation) have not been shown as reliable measures; only examination and auscultation were shown as methods from which conclusions can be drawn toward the Chinese medicine modalities research endpoints.[153] However, Chen[49] constructed KDSQ, a valid and reliable measure for assessing Kidney Yin and Yang deficiency syndromes and evaluating syndrome-specific therapies, explained and presented in Section 6.6.3 and Appendices

2 and 3. From 39 symptoms of KD from the literature, symptoms related to childhood or male gender were excluded, and not relevant to this study either.

In this study, KDSQ evaluates the therapeutic effect of acupuncture on KD symptoms intensity in urination, sexual drive, aversion to Wind and Cold, tiredness, sleep quality, hot flashes, heat in the hands and soles, tinnitus, dizziness, lower back pain, pain in knees and other joints. Also, it was confirmed valid for use in Croatia because no population or territorial circumstances exist that change the content or its usefulness. Also, KDSQ represents a valid measure for the examined topic in a population older than 50 years and is suitable for use in the study of both sexes after the question of vaginal dryness was excluded. All questions well present the grade of intensity of listed symptoms and patients clearly understood how to respond to them. Some questions conclude more about Kidney Yin or Kidney Yang deficiency, others relate to both entities.

Eight questions were selected to assess Kidney Cold syndrome and eight for Kidney Heat syndrome. Cold-type questions were about urination (night-time, day-time frequency, dripping, incontinence, increased urine volume), low energy feeling, swellings, and cold feeling in the back. Heat-type questioned the feelings of heat (hot flushes, tidal waves, five palms heat), night sweating, sleeping disturbances, high-pitch tinnitus, night thirst, and increased dreaming. KDSQ with 23 questions in total can produce a maximum of 69 points when the question about vaginal dryness was excluded.

All participants took prescribed analgesics before the study started, they were advised to reduce the dose in the period of remission or to increase it if the pain aggravated. The dose varied according to the pain intensity. The assessor recorded the dose of analgesics taken according to the participants' statements from the last three days before the assessment or DRUG, the only objective study measure. The types of analgesics participants used were presented in Table 9-1. Ibuprofen was the most prescribed one and was selected as the representative analgesic. To enable the presentation of the impact of acupuncture on medication intake, doses of all taken analgesics were recalculated in the comparable doses of the

representative one. Comparable doses were determined from the pharmacological maximal daily doses (High daily dose) and single doses (Low daily dose)[104] as presented in Table 9-1. This calculation is adopted from a clinical approach in daily practice, when a change of analgesics is required then a comparable dose represents an alternative remedy. All could not be transferred to the same analgesic due to existing affinities or intolerance. The doses are expressed as ibuprofen units (IBU), IBU correspond to ibuprofen milligrams.

Table 1 DRUG - Comparable doses of analgesics - crude approximation

Study analgesics	Low daily dose	Medium daily dose	High daily dose
Diclofenac	50 mg	100 mg	150 mg
Ketoprofen	50 mg	100 mg	200 mg
Tramadol	37.5 mg	75 mg	150 mg
Ibuprofen	600 mg	1200 mg	2400 mg
DRUG IBU*	**600 IBU**	**1200 IBU**	**2400 IBU**

Table 1 Comparable doses of ibuprofen were calculated from minimal, medium and maximal daily doses of analgesics used in the study.[104] IBU* - ibuprofen unit, the dose of taken analgesics corresponds to number of milligrams of ibuprofen.

Of all variables the higher scores imply the greater symptom intensity.

8.1 Study outcomes

The study's outcomes were formed from all assessed variables of interest WOMAC total, WOMAC pain, WOMAC stiffness, WOMAC functional disability, NRS, DRUG, and KDSQ to observe the effects of acupuncture at specified moments: the immediate-term effects at Week 15 which coincided with end of acupuncture, the short-term effects at Week 24 of nine weeks period after acupuncture treatment of group A ended, and Week 39 brought two sorts of outcomes depending on the group being observed: the medium-term effects of group A 24 week after acupuncture and the immediate term effects of group C.

The primary outcome is the WOMAC total score at Week 15 (WOMAC 15) when treatment of group A ends; it represents the sum of three measures of three disturbed KOA quality. It was selected as the primary outcome to present the most important immediate-term effect of group A produced by the designed protocol. All other outcomes were secondary and considered supportive ones.

9. Quality assurance, data storing and general terms

The study used randomisation and concealed allocation of participants to ensure the normality of data distribution and the similarity of baseline data between groups. From calculated required size the sample size was doubled to ensure the precision of inferences and decrease the probability of type I error. The participants and the researcher were not blinded due to the surgical nature of the experiment. Blinding of the independent observer was performed to reduce study bias. During the assessment week, the evaluator collected the completed forms in a room where acupuncture was not performed. He invited participants in alphabetical order, different from the order obtained by randomization. Patients were given questionnaires to fill out, and an observer was available to help them if needed. Patients who wished could submit the forms via e-mail. Side effects were thoroughly described.

The trial was overseen and monitored by the researcher. Data is stored electronically on a computer with secure and limited access. Data transfers were encrypted and all information that could identify individuals was removed. The experimenter of this study was responsible for data management and statistical analysis of the study. Approval for research was obtained from the Ethics Committee of the Zagreb East Health Centre on June 7, 2021, REF. NUMBER 01-830-1/21. All study participants signed a written informed consent. The project was fully financed by the funds of the private practice of family medicine run by the researcher.