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Exploratory data analysis on the effects of non pharmacological treatment for knee osteoarthritis

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ABSTRACT

Objective. *Osteoarthritis (OA) is a chronic rheumatic disease characterised by progressive cartilage destruction mediated by cytokines and other molecules. Chondrocyte activity and metabolism have attracted interest as targets of drug intervention, and spa-therapy can influence the serum levels of several cytokines. We investigated the effects of spa-therapy on clinical and ultrasonographic (US) findings and serum levels of cartilage oligomeric matrix protein (COMP) and several cytokines, chemokines, and growth factors in a prospective cohort of patients with symptomatic knee OA.*

Methods. *Patients (n=53) with primary symptomatic knee OA were treated for 12 consecutive days with locally applied mud-packs. Assessments were made at baseline, immediately after completion of the treatment cycle, and 6 and 12 months after completion of treatment. They included visual analogue scale (VAS) ratings of pain, the Lequesne algofunctional index for knee OA, and US with calculation of a semi-quantitative score that expressed the severity of the local inflammatory process. Serum levels of 27 cytokines (including interferon-γ-inducible protein-10 [IP-10]), chemokines, and growth factors were measured with multiplex bead-based immunoassays, and COMP levels were determined by ELISA.*

Results. *US scores, VAS pain ratings, and Lequesne indexes indicated significant improvement after spa-therapy and at the 6- and 12-month follow-ups. Serum IP-10 levels also dropped significantly (p=0.0035), and this reduction was positively correlated with improvement of the Lequesne index (p=0.031).*

Conclusions. *In patients with knee OA, spa-therapy can modulate serum levels of proinflammatory cytokines/chemokines and produce improvements in joint pain and function that persists for up to 1 year.*

Introduction

Osteoarthritis (OA) is a chronic rheumatic disease characterised by the progressive and frequently disabling destruction of articular cartilage (1). Biomechanical and biochemical aspects of the local *milieu* lead to chondrocyte activation and disruption of homeostasis (2). Several chemokines, cytokines, and growth factors have already been implicated in the inflammation and cartilage degradation associated with OA, influencing the metabolic state of chondrocytes. In the local *milieu*, the imbalance between pro- and anti-inflammatory cytokines may determine the progression of cartilage damage (3-5).

According to recent findings, pro-inflammatory cytokines such as the recently described interferon-γ-inducible protein-10 (IP-10), may be responsible for the synovial-cell and chondrocyte responses that ultimately cause tissue-damage in OA. Interest is also in the cartilage oligomeric matrix protein (COMP) that was found at increased serum levels in patients with severe, active OA correlating with radiographic progression of joint damage. Thus, modulation of chondrocyte activity and metabolism has attracted growing interest as potential mechanisms for novel anti-OA drug therapies. Previous studies have shown spa-therapy to affect serum levels of several cytokines (6). Although spa-therapy is not included among those recommended by the European League against Rheumatism (EULAR) for OA, it is one of the most frequently prescribed and it could provide beneficial effects lasting up to 6 months (7, 8). In this prospective cohort study, we investigated the short- and longer-term influence of spa-therapy on sonographic findings in knee OA, as well as on clinical manifestations and serum levels of several cytokines, chemokines and growth factors.

Materials and methods

We consecutively enrolled 53 patients with primary OA of one or both knees diagnosed according to the clinical and radiographic criteria of the American College of Rheumatology (9). Inclusion and exclusion criteria were previously described in Forestier *et al*. (8). Patients were also excluded if they had received non-steroidal anti-inflammatory drugs or other analgesics within the 2 days before enrolment.

Demographic data, histories, and clinical data of each participant were re-

corded on standardised forms. A complete physical examination and rheumatologic evaluation was performed by the same physician before the patient initiated spa-therapy. Spa-therapy consisted of a total of 12 daily mud-pack applications to the knee(s) at the Tivoli Terme Spa in Italy. Patients were treated after an overnight fast. A 10-cm layer of "mature" thermal mud (40°C) was applied to the affected knee(s). Twenty minutes later, the mud was removed, and the knee was bathed for 10-12-min in warm water (37–38°C) (10). Each patient was re-evaluated (as described above) after the end of the 12-treatment cycle, and 18/53 were also evaluated after 6 and 12 months.

Pain was assessed with a 100-mm visual analogue scale (VAS), and the Lequesne algofunctional index was used to measure the severity of the patient's knee OA (11). Each patient underwent ultrasonographic (US) examinations of both knees performed by a rheumatologist experienced in sonography. Examinations were carried out in accordance with EULAR guidelines for ultrasonography in rheumatology (12) with a Philips/HP Image Point HX scanner and a 10-MHz linear probe. Sonographic signs of synovitis (synovial hypertrophy and joint effusion) were recorded, and vascularisation of the synovial membrane was assessed with power Doppler (pD) (settings: prf 1000 Hz, gain 18-30 db, low filter). Synovial hypertrophy and synovial fluid were defined in keeping with Outcome Measures in Rheumatoid Arthritis Clinical Trials (OMERACT) recommendations (13), observed on grey-scale and scored according to severity (0 to 3). Perfusion revealed by pD was scored according to intensity (0 to 3). The presence or absence of a Baker's cyst was indicated respectively with a score of 1 or 0. The sum of these scores (total US score) (range 0-10), was used as an indicator of global change of single joint score; the sum of the single-joint scores was used as an indicator of total joint involvement in each patient (range 0-20) (14).

Blood samples for laboratory assays were collected 30 min before the first mud-pack, 2 h after the last one. Commercially available multiplex bead-based immunoassay kits (Human 27-plex, Bio-Rad laboratories, Hercules, CA) were used to measure concentrations of interleukine (IL)-1B, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, basic fibroblast-growth factor (FGF-Basic), granulocyte-colony stimulating factor (G-CSF), granulocyte monocyte-CSF, interferon-γ, IP-10, monocyte chemoattractant protein-1 (MCP-1), macrophage-inhibiting protein-1α and 1β (MIP-1α and MIP-1β), platelet-derived growth factor (PDGF), regulated on activation normal t cell expressed and secreted (RANTES), tumor necrosis factor-α, and vascular endothelial growth factor (VEGF). Assays were performed according to the manufacturer's instructions. Each sample was assayed in duplicate. Briefly, the appropriate cytokine standards and samples diluted in serum diluents (50 μl/well) were added to a 96-well filter plate and incubated for 30 min at room temperature. Following washes, premixed streptavidin-phycoerythrin was added to each well and incubated for 10 min. After 3 more washes, the beads were resuspended in 125 ml of assay buffer, and the cytokines in the reaction mixture were quantified with the Bio-Plex protein array reader. Data were analysed with Bio-Plex manager software, version 4.1.1 (Bio-Rad laboratories). Values with a coefficient of variation >12% were excluded from the final data analysis. The concentrations (pg/ml) of different analytes in the plasma samples were determined with the aid of standard curves generated in the multiplex assays. Serum COMP levels were measured with a commercially available ELISA kit (Wieslab Hcomp quantitative kit, Euro-diagnostica, Malmö, Sweden).

Statistical analysis

Data were analysed with Wilcoxon's matched pairs test and paired *t*-test. Analyses were carried out with version 13.0 of the Statistical Package For Social Sciences, Chicago, IL, USA. Bonferroni correction was performed for multiple comparisons (p_c). Spearman's test was used for correlations. Data are expressed as means±SD or means and ranges. Only two-tailed *p*-values <0.05 were considered significant.

Results

At baseline, the 53 patients with knee OA had a mean US score of 4.1±2.7 (range 0-13), a mean VAS pain rating of 52.6±16.4 mm (range 18-90 mm), and a mean Lequesne index of 9.5±4.5 (range 1.5-19). Shortly after completion of spa-therapy, all 53 patients displayed statistically significant improvement in all 3 parameters, as shown in figure 1. In the 18 patients with 6- and 12-month follow-up data, clinical and US improvement persisted compared with baseline and end-of-treatment findings (Fig. 1).

Comparison of baseline and end-of-treatment laboratory findings revealed a statistically significant decrease in IP-10 levels after spa-therapy (baseline 849±485 pg/ml versus 662±417 pg/ml after spa-therapy, p=0.0035, p_c=0.021). These changes were positively correlated with improvements in the Lequesne index (Spearman's test) (p=0.031). COMP levels also dropped after spa-therapy, but this change was not significant (baseline 20.0±5.3 pg/ml versus 18.8±5.4 pg/ml after spa-therapy, p=NS). Serum levels of the other cytokines, chemokines, and growth factors analysed were not significantly different after spa-therapy (data not shown).

Discussion

OA has generally been considered a non-inflammatory degenerative disease, but recent findings indicate that several stages of the disease can be associated with inflammatory changes sustained by cytokine storms and growth-factor release (15, 16).

In our cohort of 53 patients with knee OA, spa-therapy significantly reduced pain and disease activity as measured by VAS and the Lequesne index. This improvement was also accompanied by a decrease in the US score and by changes in chemokine and cytokine serum levels that included significant decreases in IP-10, which displayed positive correlation with clinical changes. These preliminary results, although in a relatively small cohort, represent an

exploratory data analysis to assess the efficacy of spa-therapy alone in knee OA and relations with US findings and serum cytokines levels.

One of the most promising imaging in OA techniques is US, which represents a simple, widely available tool for detecting joint effusion and synovial thickening, as recently reported in the EULAR evidence-based recommendations for the diagnosis of knee OA (evidence level: I-b) (17, 18). In our study, we used US to monitor the response to spa-therapy in patients with knee OA patients. Results were maintained at 12 months, suggesting that spa-therapy may also have positive effects in terms of the control of disease progression.

IP-10 is a pro-inflammatory chemokine (19, 20) that regulates immune responses by activating and recruiting lymphocytes into the joint and plays roles in leukocyte homing in inflamed tissues. Recent reports have shown that serum and/or tissue levels of IP-10 are increased in various autoimmune/inflammatory diseases such as rheumatoid arthritis. The observed significant decrease in IP-10 levels after spa-therapy may result in reduced pro-inflammatory *stimuli* and the positive correlation with clinical improvement suggests a pathological role for IP-10 in knee OA.

Serum COMP levels have been proposed as a molecular marker of cartilage damage in OA, and these levels displayed correlation with radiographic progression of joint damage (21). Even if we observed a reduction in mean COMP levels after spa-therapy, such reduction did not correlate with US findings.

In conclusion, the spa-therapy evaluated in this OA cohort produced substantial clinical improvement. Further studies are needed to clarify the potential effects of Spa therapy on the cytokine profile of OA patients.

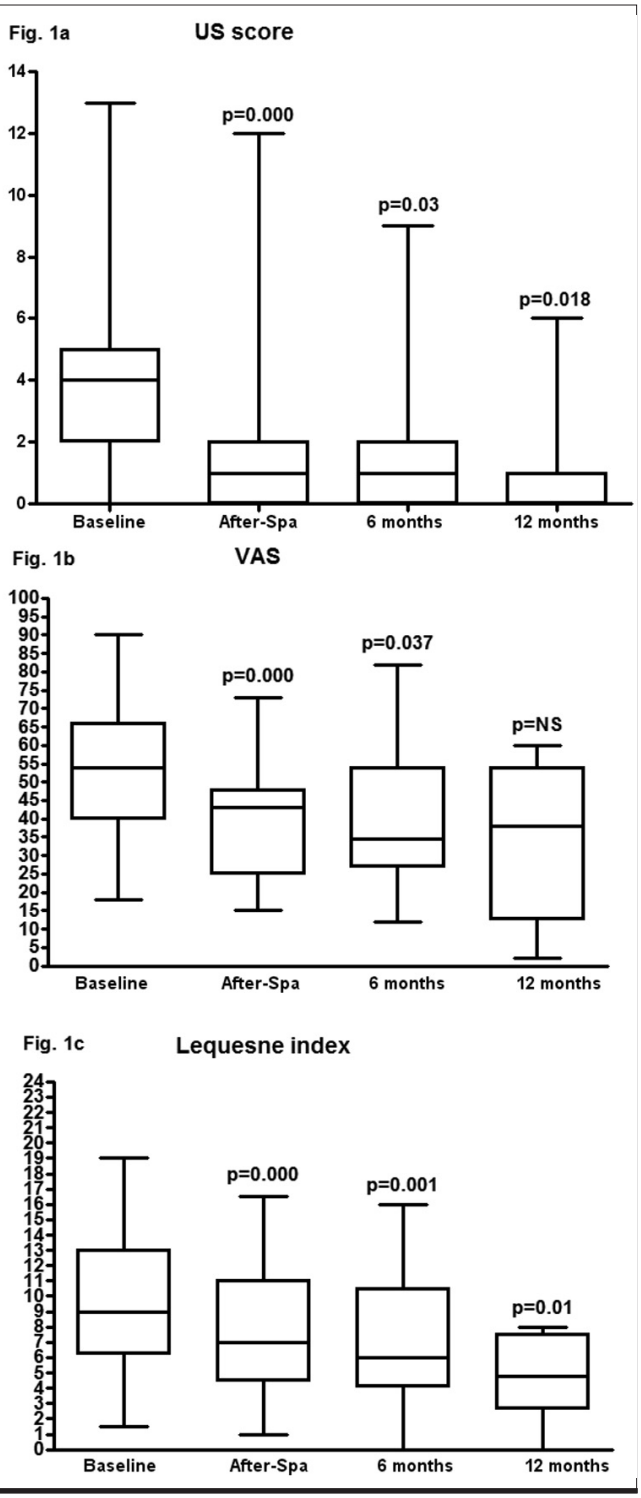

Fig. 1. Box-and-whiskers plots of the results observed before and after spa-therapy for OA: (**a**) US scores, (**b**) VAS pain ratings, and (**c**) Lequesne indexes. Patients were evaluated at baseline (n=53), immediately after spa-therapy (n=53), and 6 and 12 months after spa-therapy (n=18). P-values are given for differences *vs*. previous visit (Wilcoxon's matched pairs test).

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