Differences in the osteoarthritic synovial fluid composition and rheology between patients with or without flare-up: a pilot study

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Abstract

Objective

To study the influence of the inflammatory status (flare or not) on hyaluronic acid (HA) and protein composition and on the intrinsic viscosity of the synoviol fluid (SF) from patients with knee osteoarthritis (KOA)

Methods

Patients with KOA were classified as having flare (F+) when they fulfilled the 4 following clinical criteria: 1) sudden aggravation of knee pain, 2) whose beginning was identifiable, 3) causing nocturnal awakenings 4) with clinical evidence of knee effusion. Patients were classified F- (no flare) if they do not fulfill any of the 3 first criteria. Forty-four SF were obtained by arthrocentesis and assayed using steric exclusion chromatography, which allows HA to be separated from the proteins and to determine both molecular weight (Mw) and and concentration (C) of both HA and proteins. SF rheology was determined using a rheometer at 25°C using a cone and plate geometry. Steady-state viscosity was determined in Pa.s, as a function of the shear rate at 1s-1. Correlations between (Pa.s) and HA and Pr (Mw, C and Mw x C) were calculated.

Results

Among the 44 assayed SF, 25 were classified F- and 19 F+. There were statistically significant differences between F- and F+ for most of the studied variables: HA concentration and Mw (p=0.01 and 0.001 respectively), protein concentration and Mw (p=0.02 and 0.001 respectively), product Mw x C of the proteins (p<0.0001) and viscosity ή (p=0.0005). The product [(Mw xC) HA x (MwxC) proteins] was highly discriminating between F+ and F- (p<0.0001). The steady state viscosity was highly related to HA concentration (p= 0.0002) and HA Mw (p=0.01) and was negatively correlated with (Mw x C) proteins (p=0.0005), protein concentration (p=0.0007) and protein Mw (p=0.03).

Conclusion

This pilot study shows significant differences of SF composition in patients having a flare-up compared to that of patients who do not have flare. These differences relate to both protein and HA composition and suggest that SF analysis makes possible to distinguish patients with and without flare-up

Key words

hyaluronic acid, knee osteoarthritis, synovial fluid, flare, proteins, rheology

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Introduction

Osteoarthritis (OA) is the most frequent musculoskeletal condition, with a worldwide distribution. Management of knee OA, which is the first cause of visit to the doctor for OA related symptoms, requires the combination of pharmaceutical and non-pharmacological strategies that have been recommended in several guidelines (1-3). The aim of the treatment is to control pain, improve function and health-related quality of life, while avoiding therapeutic toxicity. Knee OA natural course is usually that of a chronic and slow progressive arthropathy, punctuated by "flare-up", more or less frequent and more or less severe, whose recurrences are suspected to hasten cartilage degradation. The OARSI recommendations spécify that the treatment of knee OA should be tailored to the individual patient, taking into account factors such as age, co-morbidity, and the presence of inflammation (3).

Now although new flare clinical criteria have been proposed (4), unbiased criterion able to prove formally inflammatory flare is still lacking. In knee OA one of the main clinical signs of inflammation is the presence of a synovial fluid (SF) effusion, despite hydarthrosis can be present in osteoarthritic knee without any symptom of flare (4, 5). In One hundred and sixty-four knees of 82 ultra-sound examinations showed: joint effusion in 43.3% of the patients, synovial proliferation in 22.1%, and power doppler signal in 2.9% (6). Synovial fluid is the joint lubricant and shock absorber as well as the source of nutrition of articular cartilage. SF is a blood plasma dialysate which contains hyaluronic acid (HA), proteins, electrolytes and small molecules such as glucose and urea. In healthy patients, SF protein concentration is substantially lower than that of plasma (7). In inflammatory joint diseases, such as rheumatoid arthritis, the SF protein content and the amount of larger proteins (α2 macroglobulin, fibrinogen, Tissue Inhibitor of Metalloproteases…) are increased and SF viscosity is decreased, compared to healthy and osteoarthritic SF. In OA, SF is claimed to be characterised by the decrease of both the HA concentration, as a result of exudation, and the amount of high molecular weight (Mw) HA molecules (8, 9). These changes lead to reduce the viscoelasticity of the SF and its ability to protect the joint. However conflicting results were reported regarding both HA Mw and concentration, and proteins composition of the osteoarthritic SF (6); for example, HA concentration ranged from 0.32 mg/mL to 3.61 mg/mL and that of proteins from 17 to 56.8 mg/mL (10). We assume that these discrepancies might be explained by the fact that in the studies assessing the OA SF composition, the notion of "flare-up" has never been taken into account.

The aim of the study was to investigate whether there are differences between the OA SF obtained during a flare and that collected out of a flare.

Patients

Patients were referred to the rheumatology unit for symptomatic knee OA, and all of them were considered by the physician as requiring intra-articular injection with corticosteroid or HA derivatives. All patients had both radiographic evidence of knee OA and no other painful joint that could suggest any other rheumatologic condition, such as inflammatory, infection or metabolic diseases. None of the patients had radiographic features of crystal deposition disease. However SF were not specifically assayed for the detection of pyrophospahte, urate or hydroxyapatite crystals and the protocol did not include any blood examination.

Synovial fluid was obtained by sterile aspiration of the OA affected knee according to the usual procedure (infra patellar lateral approach on fully extended knee). After SF was obtained by arthrocentesis and treatment was administered, patients were asked to give an informed consent to allow for joint fluid assay, in accordance with the protocol approved by the direction of research and innovation ethic board of the Hospices Civils de Lyon. In case of non-acceptance by the patient the liquid was thrown away, but none of the patient had refused.

Patients were classified as having flare (F+) when they fulfilled the 4 follow-

ing clinical criteria: 1) sudden aggravation of knee pain, 2) whose beginning was identifiable, 3) causing nocturnal awakenings 4) with clinical evidence of knee effusion. Patients were classified F- (no flare) if they do not fulfill any of the 3 first criteria.

Arthrocentesis was performed in all patients by a single experienced rheumatologist (TC) using a 40 or 50-mm 20-gauge needle to remove as much SF as possible before corticosteroid or hyaluronic acid intra-articular injection. The SF was collected in sterile tubes. Each SF sample was immediately divided in three aliquots and frozen at -25°C.

Table I. Characteristics of 44 patients with knee osteoarthritis and synovial fluid effusion, classified as having (F+) or not (F-) a flare.

Item	All	F + (n=19)	F- (n=25)	*p*-value
Sex (Male/Female)	21/23	8/11	13/12	ns
Age (median, range)	67.6	73.2 (54- 83)	74.3 (39-86)	ns
BMI [kg/m²] (median, range)	27.8 (19.5, 41)	27.4 (21.3-35)	28.1 (19.5, 41)	ns
Disease duration (years) (median, range)	6.8 (0.5-24)	6.4 (0.5-24)	7.2 (2-14)	ns
KL grade I/ II/ III/ IV) (N)	0/3/20/21	0/0/8/11	0/3/13/9	ns
NSAIDs consumption (Yes/No)	17/27	9/10	8/17	ns

N: number; F+: patients with flare; F-: patients without flare; BMI: body mass index; KL grade: Kellgren-Lawrence radiographic grade; NSAIDs: non-steroidal anti-inflammatory drugs.

Table II. Differences in the composition and rheology of synovial fluid depending on whether or not an inflammatory flare.

Item units	Flare Mean (SD)	No flare Mean (SD)	*p*-value
C HA g/L	1.11 (0.53)	1.54 (0.54)	0.018
Mw HA Da x 10^6	2.96 (1.39)	1.39 (0.69)	0.0016
C proteins g/L	29.80 (5.37)	25.27 (6.04)	0.024
Mw proteins Da x 10^4	9.96 (1.69)	6.38 (4.27)	0.0016
[MwxC] HA	3.15 (1.89)	2.11 (1.49)	0.044
[MwxC] proteins	301.64 (99.69)	147.89 (97.94)	<0.0001
Viscosity Pa.s	0.214 (0.328)	0.874 (0.89)	0.0005

Methods

Steric exclusion chromatography

Each SF was assayed twice by steric exclusion chromatography (SEC) using an Alliance GPCV2000 system (Waters Corp, Boston, MA) equipped with three detectors online: a differential refractometer, a viscometric detector, and a multiangle laser light scattering detector from Wyatt Technology Corp (Santa Barbara, CA) (11, 12). The concentration of HA polymer in the sample injected was lower than 0.5 g/L with an injection volume of 108 µL using two columns in series (Shodex OH-pack 805 and 806; Showa Denko KK, Tokyo, Japan); these columns allow separation of high-hydrodynamic-volume HA from the lower-hydrodynamic-volume proteins. Knowing the volume injected and the refractive index increment dn/dc, we could determine the polymer concentration eluted. Before injection, all samples were filtrated on a 0.2-µm-pore cellulose acetate filter (Sartorius AG, Göttingen, Germany) to retain large aggregates (or flocculated proteins). The eluent used was a 0.1-mol/L NaNO3 aqueous solution at an elution temperature of 30°C and a flow rate of 0.5 mL/minute. Molecular weight distribution and weight-averaged molecular weight of HA and proteins could be determined separately from light scattering detection eliminating the need for molecular weight calibration. The signal of the differential refractometer is related to the polymer concentration eluted through the coefficient dn/dc [0.153 for HA (13) and 0.190 for the protein pool (14)].

Only soluble proteins were detected in this assay. Because of the large dilution used, we assumed no protein-HA complexes perturbed the quantitative determination of the polymers. The amount of soluble proteins was determined using the Bradford titration after dilution of the SF (ratio 1:20). The interference with HA was taken into account in a calibration curve performed with bovine serum albumin. The HA content of SF is much lower than that of proteins and its contribution does not exceed 2% in the protein determination.

Rheology

The rheologic behavior of SF was determined using an AR 1000 Rheometer (TA Instruments, New Castle, DE) at 25°C using a cone and plate geometry (4-cm-diameter plate with 3.59° cone). Dynamic experiments were performed in the linear viscoelastic region where G´ and G˝ are independent of the stress applied at a given frequency. Dynamic moduli (storage modulus G´ and loss modulus G˝) and complex viscosity |η*| were determined as a function of the angular frequency (ω) expressed in Hertz. Steady-state viscosity η was determined as a function of the shear rate γ covering the range from 0.1 to 50 s^{-1}.

Dynamic moduli (storage modulus G´ and loss modulus G˝, complex viscosity |η*|, and steady-state viscosity η were measured.

Statistical analysis

The statistical analysis was performed using Statview® 5.0 software (SAS Institute, Cary, NC, USA). Inter-group comparisons (F+ *vs*. F-) were performed using Mann-Whitney test. Correlations between variables were calculated using Spearman test. All statistical tests were carried out two-tailed at the 5% level of significance.

Results

Among 109 consecutive patients referred to the department of rheumatology with knee OA and needing an intra-articular injection, forty-four patients (23 women/ 21 men; mean age, 67.6 years; mean body mass index, 27.8) were included in the trial. In 33 cases it was impossible to determine whether the patient had flare or not (one or more of the required criteria

was missing). In 32 cases the volume of SF obtained was too low to achieve both the rheology and SEC (at least 5 mL were needed) or was unusable because of bleeding puncture. Among the 44 assayed SF, 25 were classified F- and 19 F+. Characteristics of patients are summarised in Table I.

There were statistically significant differences between F- and F+ for most of the studied variables: HA concentration and Mw (p=0.01 and 0.001 respectively), protein concentration and Mw (p 0.02 and 0.001 respectively), product Mw x C of the proteins (p<0.0001) and viscosity ή (p=0.0005).

The product [(Mw xC) HA x (MwxC) proteins] was highly discriminating between F+ and F- (p<0.0001), while the product (Mw x C) HA was not statistically different between groups.

The steady state viscosity was highly related to HA concentration (p= 0.0002) and HA Mw (p=0.01) and was negatively correlated with (Mw x C) proteins (p=0.0005), protein concentration (p=0.0007) and protein Mw (p=0.03).

All the data are shown in Table II and Figures 1–3.

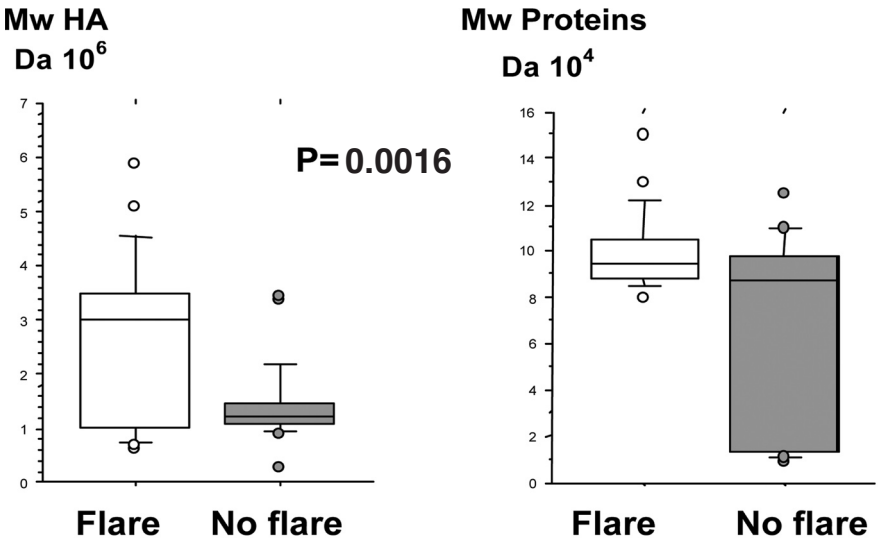

Fig. 1. Differences in the molecular weight (Mw) of hyaluronic acid (HA) and proteins of synovial fluid depending on whether or not an inflammatory flare.

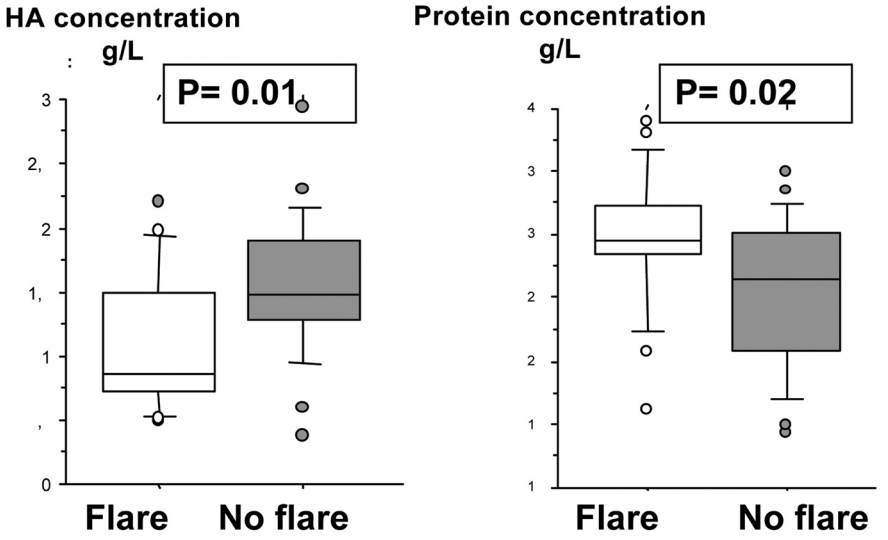

Fig. 2. Differences in the concentration of hyaluronic acid (HA) and proteins of synovial fluid depending on whether or not an inflammatory flare.

Discussion

Differences in SF composition between inflammatory and degenerative diseases are well known but at our knowledge this study is the first one showing differences in osteoarthritic SF composition depending on the inflammatory status.

It is usually claimed that the main feature of the OA SF is a decrease of its viscoelastic properties due to quantitative and qualitative alterations of HA molecules characterised by a decrease in the amount of HA molecules of high molecular weight and in the concentration of HA at least partly as a result of exudation (8) These changes reduce the viscoelasticity of the SF and its ability to protect the joint (15, 16). Hyaluronic acid in healthy adults is claimed to have a molecular weight in the range of 4 to 5×10^{6}Da (16, 17). Its synovium concentration ranges from 2.5 to 4 mg/mL (17, 18) which grossly corresponds to a total amount of 4 to 8 mg HA in healthy subjects in who the volume of SF is assumed to range from 0.5 to 4 mL (19). Hyaluronic acid is considered as the primary determinant of the visco-elastic behaviour of SF. The viscosity of a solution of HA is known to be strictly related to the product of the molecular weight by the concentration of HA (CxMw) (20, 21). However, at low share rate for a given (C x Mw), the HA rheological behaviour is a Newtonian one (*i.e.* viscosity is unrelated to share rate) while that of the synovial fluid is non-Newtonian (ie, viscosity decreases when share rate increases). Furthermore, the viscosity of SF is much higher than that of a solution of HA alone at the same concentration and same Mw. All this strongly suggests that the specific behavior of synovial fluid is related to interactions between HA and proteins since HA is an anionic polyelectrolyte able to interact with positive charges on proteins (22, 23).

Our results showed that SF viscosity was decreased in patients with flare compared to that of "no flare" subjects, despite there was no major differences in the product (C x Mw) HA. Indeed, surprisingly we found significantly higher HA Mw in patients with flare, offset by a lower HA concentration. So it is not the decrease of the product (CxMw) HA that may explain the decrease of SF viscosity in case of flare,

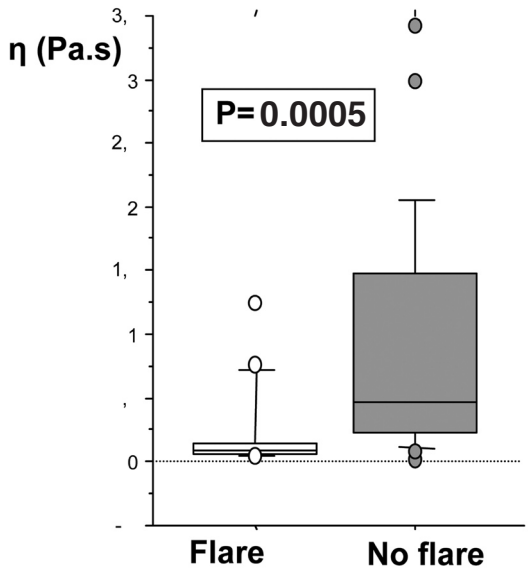

Fig. 3. Differences in steady state viscosity of synovial fluid depending on whether or not an inflammatory flare.

but, as demonstrated in this study, the SF protein composition. In patient with flare we found higher amount of proteins of higher Mw, leading to a product (C x Mw) of proteins much higher (about 50%) than that of patients without flare. This was chiefly due to the increase of the amount high Mw proteins suggesting that flare-up is a real inflammatory process, close, albeit lower, to that of inflammatory rheumatic diseases.

The main limitation of the study is of course the definition of the flare-up. Indeed, almost a third of patients could not be classified on the basis of the KOFUS criteria. To date only a clinical definition of flare is available and in a number of cases it appears impossible to accurately determine whether or not there is a flare. It is the reason why we only selected criteria that seemed the most reliable: sudden aggravation of knee pain, whose beginning was identifiable, causing nocturnal awakenings with clinical evidence of knee effusion. These criteria are of course debatable, but the fact that we have highlighted differences between the groups suggests that the clinical definition of flare is not so bad. However the present results have to be confirmed from a largest sample of patients and different flare definitions.

The other limitation is due to the SEC and rheologic procedures which require a minimal amount of liquid to about 3 mL, a quantity which is not always obtained by joint aspiration, especially apart from periods of flare-up. At last, despite none of the patients had radiographic features of crystal deposition disease, the presence of calcium pyrophosphate, urate or hydroxyapatite crystals cannot be definitively excluded, at least in some patients, since no identification of microcrystals in synovial fluid has been performed.

In conclusion, this pilot study shows significant differences of SF composition in patients having a flare-up compared to that of patients who do not have flare. These differences relate to both protein and hyaluronic acid composition and suggest that synovial fluid analysis makes possible to distinguish patients with and without flare-up. This could have a significant impact in the choice of treatment especially when this choice is between intra articular injection of steroids and viscosupplementation. Further studies are needed to characterise the high Mw proteins found in the flare-up SF (*i.e.* reelin that has been found in both inflammatory and OA SF) (24) and to investigate the influence of the protein composition on the clinical results of viscosupplementation and steroid intra articular injections.

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