INTRODUCTION:
While osteoarthritis (OA) is generally assessed using standard radiographic images in clinical practices, biochemical markers can be employed to detect the disease and determine the severity. For monitoring of progression of OA, no marker has yet gained unrestricted acceptance in the clinical routine.

Osteopontin (OPN), an extracellular matrix (ECM) glycoprotein, is a potential inflammatory cytokine and modulates a variety of pathological conditions. OPN is also expressed in synovial tissue and cartilage from patients with rheumatoid arthritis (RA) and OA, suggesting an involvement in the pathogenesis of inflammatory arthritis [1]. Proteolytic modification of OPN by thrombin cleavage reveals cryptic binding sites for α9β1 and α1β1 integrins, preferentially expressed by neutrophils and by monocytes and lymphocytes, respectively. The presence of thrombin-cleaved form of OPN is well correlated with various inflammatory disease activities [2]. We measured the synovial fluid levels of the N-terminal half of thrombin-cleaved osteopontin (OPN N-half) in RA directly, and demonstrated that OPN N-half levels were elevated in RA compared with OA [3].

The purpose of the cross-sectional study was to determine the levels of OPN N-half and non-thrombin-cleaved osteopontin (OPN full-length) in synovial fluid from patients with OA and to evaluate a possible correlation between these levels and radiographic grading of knee OA.

METHODS:
Synovial fluid samples (n = 139) were obtained from 40 men and 99 women with primary OA of the knee. The knees were divided into 2 groups based on the radiographic grading of the OA severity described by Kellgren and Lawrence: early (grade 1 and 2) and advanced (grade 3 and 4). All patients gave informed consent, and this study was approved by the local ethics committee. The early group consisted of 57 patients (25 men and 32 women; mean age 72 years; mean body mass index [BMI] 23 kg/m²) and the advanced group consisted of 82 patients (15 men and 67 women; mean age 74 years; mean BMI 25 kg/m²). Both % women and BMI were significantly higher in the advanced group than in the early group (p<0.01). Synovial membrane samples were obtained at the time of surgery in 23 patients with OA of the knee.

Immunoblotting of synovial fluid was done to examine the presence of OPN N-half. We used antibody (O-17), which can bind both OPN full-length and OPN N-half.

An enzyme-linked immunosorbent assay (ELISA) was applied to quantify the levels of OPN full-length and OPN N-half as previously described [3]. For the OPN N-half ELISA, two antibodies, O-17 and 34E3, were used. The OPN N-half ELISA system does not recognize full-length OPN and detects OPN after thrombin cleavage. To quantify the levels of non-thrombin-cleaved osteopontin (OPN full-length), human osteopontin assay kit was used with two antibodies (O-17 and 10A16). The percentage of OPN N-half (% N-half) was expressed as a percentage (OPN N-half divided by all OPN [OPN N-half plus OPN full-length]).

Expression of thrombin-cleaved OPN in synovial membranes was determined by immunohistochemistry using the avidin-biotin complex method with anti-OPN N-half (34E3) mouse monoclonal antibody.

Statistical analyses were performed using the Mann-Whitney U-test and a multiple regression test. P values < 0.05 were considered significant.

RESULTS:
The antibody O-17 reacted with full-length and N-half OPN with molecular weights of 48 kDa and 30 kDa, respectively, in synovial fluids from patients with early OA and advanced OA (Fig. 1).

ELISA results showed the median OPN full-length level in the advanced group was not statistically different from that in the early group (Fig. 2A; p = 0.26). In contrast, the median levels of OPN N-half and % N-half were significantly higher in the advanced group than in the early group (Fig. 2B, 2C; p < 0.01). After adjusting the levels of OPN N-half as well as % N-half for BMI and sex, % N-half showed statistically significant levels in the advanced group (p = 0.01). However, levels of OPN N-half showed no difference between the groups (p = 0.20).

DISCUSSION:
In this study, we demonstrated increased levels of OPN N-half in synovial fluid of advanced OA. Limitations of this study include the cross-sectional design of the trial and lack of analysis of serum levels of OPN N-half.

In conclusion, the present study showed local generation of thrombin-cleaved OPN was increased during OA progression, indicating that the percentage of OPN N-half may be a useful biochemical marker of OA progression. Further investigations are needed regarding the relationship between synovial fluid and serum levels of OPN N-half.

REFERENCES: