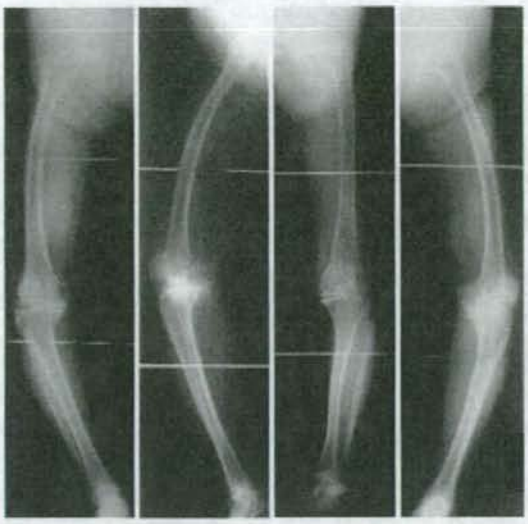
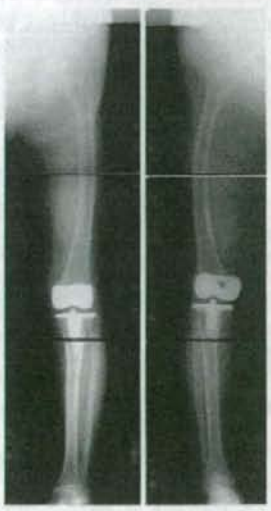


A B C D

Fig. 1. A Anteroposterior (AP) radiograph of the right leg showed a varus deformity. **B** Lateral radiograph of the right leg demonstrates strong bowing of the femur at the middle third. **C** AP radiograph of the left leg showed a varus deformity. **D** Lateral radiograph of the left leg demonstrates strong bowing of the femur at the middle third



A B

Fig. 2. A AP radiograph of the left knee after total knee arthroplasty (TKA) showed excellent alignment. **B** AP radiograph of the right knee after TKA showed excellent alignment

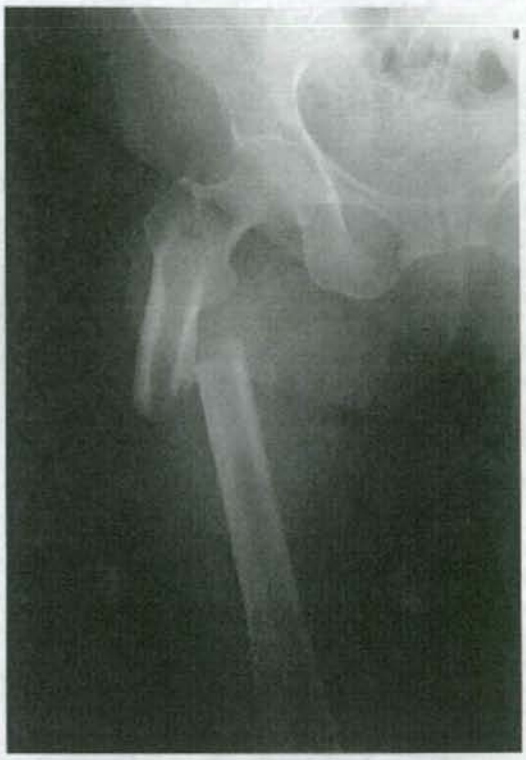


Fig. 3. Radiograph showed a displaced transverse fracture

performed. Complete relief of pain and recovery of walking ability was achieved. Subsequent roentgenograms demonstrated bone union.

At 37 months after the left TKA, the patient complained of left thigh pain that decreased at rest and increased with physical activity. There was no history of trauma. Radiographs showed an undisplaced femoral shaft transverse fracture where lordosis and bowing were the strongest, consistent with an insufficiency fracture (Fig. 4). She was treated with rest and anti-inflammatory drugs. Three months after the stress fracture (40 months after the left TKA), the fracture united and she was becoming more mobile. However, 42 months after the left TKA, she fell again and could not walk owing to severe left thigh pain. Radiographs revealed a displaced transverse fracture of the left femoral shaft in accordance with the preceding fracture (Fig. 5). Open reduction and internal fixation with an intramedullary nail was performed. Twelve months after the second operation (54 months after the left TKA), clinical and radiological examination of both arthroplasties were satisfactory (Fig. 6).

Discussion

Stress fractures occur in characteristic locations in specific age groups and certain occupational situations. The most frequent locations of stress fractures are the meta-

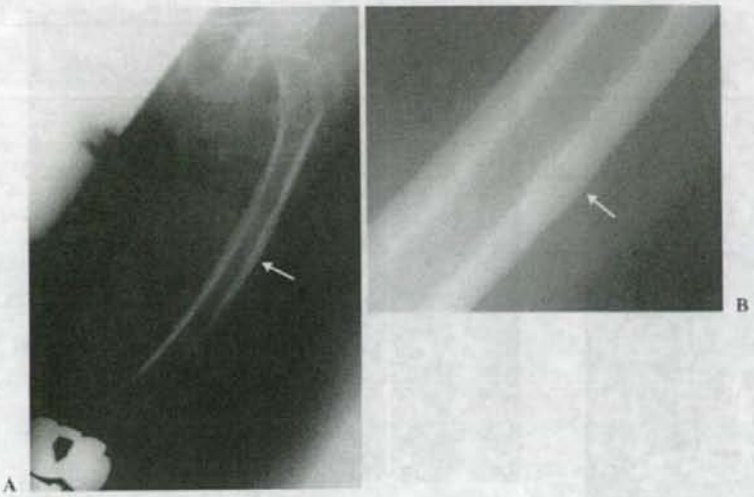


Fig. 4. **A** AP radiograph showed a lateral cortical crack perpendicular to the cortex of the mid-femoral shaft (*arrow*). **B** AP radiograph showed enlargement of the cortical crack (*arrow*)

tarsals, femoral neck, and proximal tibia.[13] Stress fractures of the femoral shaft are relatively rare. Among all stress fractures, the reported proportions of femoral shaft stress fractures, including undisplaced and displaced fractures, range from 3% to 43% in military recruits and from 3% to 21% in athletes.[10] In terms of displaced femoral shaft fractures, the incidence in military recruits has been 1.5 per 100 000 person-years in military service.[10]

As defined by Salminen et al.,[10] participation in the following activities during fracture onset are indicative of a stress fracture: (1) ordinary movement such as walking, running, or bending the knee or the hip; (2) straining or struggling; (3) slipping and stumbling at ground level; or (4) a fall from a height less than 1 m. In our patient, femoral shaft fractures occurred three times, and the right femoral shaft fracture fulfilled Salminen's criteria. The two left femoral shaft fractures were diagnosed as insufficiency fractures.

To our knowledge, only 21 femoral fractures associated with TKA have been reported in the literature[1–9]: 20 occurred in the femoral neck/subcapital[1–8] and 1 occurred in the subtrochanteric region.[9] A review of the literature failed to reveal a case in the femoral shaft area or bilateral fractures associated with TKA. Stress fracture after TKA occurs mainly in patients with considerable deformity of the knee and osteoporosis. The risk of fracture is directly related to the tissue stress/tissue strength ratio, which in turn is dependent not only on tissue composition but also on tissue geometry and the direction and magnitude of loading. Joshi et al.[8] reported four cases of femoral neck fracture after TKA in patients with large varus deformities of the knee and concluded that the main etiology of this fracture is the changes in the biochemical axis of the hip resulting from correction of large knee deformities with the hip in valgus. In contrast, Kumm et al.[9] reported a subtrochanteric fracture and concluded the main etiology of this fracture is a decrease in the tension band effect of the iliotibial tract in combination with coxa vara and changes in static and dynamic forces of the femur.

In our patient, changes in the weight-bearing axis of the extremity were likely after TKA in patients with large deformities of the knee. In addition, because of the relief of the pain following TKA, she was undoubtedly putting greater stress on the bilateral legs owing to more active use and weight bearing than before surgery. In addition, the strong lordosis of the femur and bowing at the middle third were unique and likely increased tension stress strains at the anterolateral side of the middle third of the femoral shaft, leading to this rare fracture. In past studies, knee deformity and hip geometry are risk factors for stress fracture of the femur.[8,9]

Boden et al.[14] classified stress fractures as low-risk or high-risk injuries. Low-risk stress fractures have a favorable prognosis when treated with activity restriction. In contrast, high-risk stress fractures are prone to delayed union or nonunion, especially if the diagnosis is delayed. Femoral shaft stress fracture is classified as a low-risk fracture, whereas femoral neck stress fracture is a high-risk fracture.[14] Femoral shaft stress fractures in athletes and military recruits are undoubtedly considered low risk because most cases are not displaced and can be treated nonoperatively. Considering the clinical course of this case, femoral shaft stress fractures after TKA may be considered high-risk fractures because of fracture dislocation and prolonged morbidity. Therefore, special attention to stress fracture is warranted when a

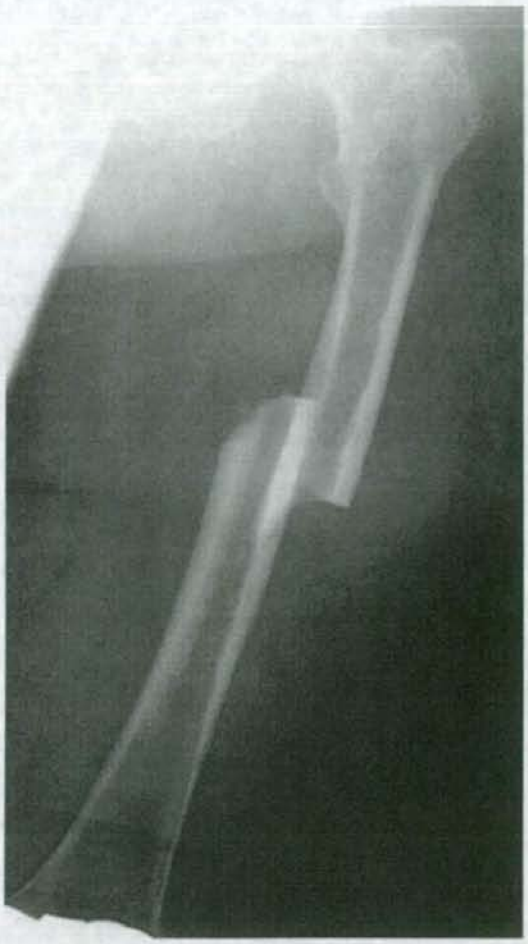


Fig. 5. Radiograph showed a displaced transverse fracture in accordance with the preceding fracture

patient with considerable deformity of the knee and eccentric geometry undergoes TKA and later complains of severe thigh pain.

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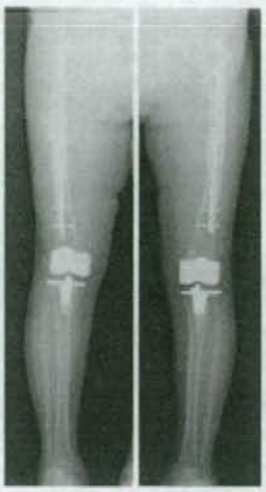

A B

Fig. 6. A AP radiograph of the right leg after open reduction and internal fixation showed good alignment and bone union. **B** AP radiograph of the left leg after open reduction and internal fixation showed good alignment and bone union

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ORTHO SUPERSITE

Course of Avascular Necrosis of Femoral Head Without Collapse of Femoral Head at First Examination: Minimum 8-Year Follow-up

By Rui Niimi, MD; Akihiro Sudo, MD; Masahiro Hasegawa, MD; Atsumasa Uchida, MD
ORTHOPEDICS 2008; 31:755

August 2008

Abstract

Seventeen hips in 13 patients with avascular necrosis (AVN) of the femoral head without collapse of the femoral head at first examination were followed for at least 8 years of conservative treatment. Long-term outcomes of hips with AVN were divided into 3 groups: (1) hips without collapse, (2) hips with progression but cessation of collapse, and (3) hips with progression of collapse. In groups 1 and 2, good clinical results are expected. Hips with femoral head collapse >3 mm at 3 years from the onset of hip pain progressed to osteoarthritis.

Disruption of the femoral head contour by collapse of necrotic segments is considered to be an important turning point in the history of avascular necrosis (AVN) of the femoral head, and in many cases, surgical treatment is required soon after the onset of symptoms.[1] However, in a few cases, conservative treatment is sufficiently effective to avoid surgical procedures for long periods.[2] For patients with early-stage AVN, it is important to accurately predict its natural course. To date, the natural course of AVN has not been well-documented or adequately described in the literature.[3]

In this study, we followed AVN in selected patients whose femoral heads were not collapsed at first examination and who were treated conservatively over 8 years. This study documents the characteristics of patients who can be treated without surgery for long periods.

Materials and Methods

Included in this study, which occurred between 1974 and 1998, were 22 hips in 16 patients with AVN who could be followed up for a minimum of 8 years with conservative treatment. Among these, 17 hips in 13 patients, which did not show collapse of the femoral head at first examination, were enrolled in this study (Table). Surgery was not undertaken for >8 years by patients with mild or no clinical symptoms, or by patients who refused to have surgery. Patients thus included 11 hips in 8 men and 6 hips in 5 women with a mean age of 37 years (range, 20-74 years). Four patients had bilateral involvement and 9 patients had unilateral involvement. Patients were followed clinically and radiographically at 3- to 12-month intervals for a mean of 13 years (range, 8-26 years). Four of 17 hips underwent total hip arthroplasty (THA) after 8 years of conservative treatment.

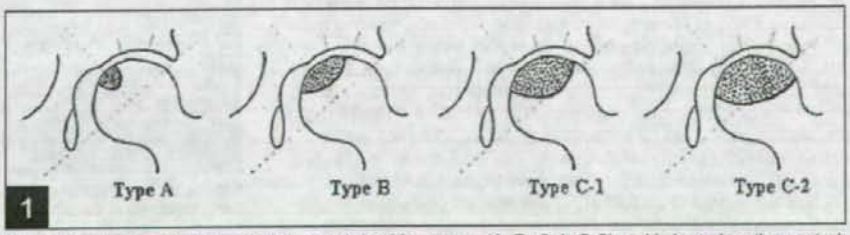

Figure 1: The classification scheme consists of four types (A, B, C-1, C-2) and is based on the central coronal section of the femoral head on T1-weighted images or the anteroposterior radiographic view. Type A lesions occupy less than the medial 1/3 weight-bearing area. Type B lesions occupy greater than 1/3 or less than 2/3 of the weight-bearing area. Type C-1 and C-2 lesions both occupy more than 2/3 of the weight-bearing area, but type C-2 lesions extend beyond the acetabular edge. The weight-bearing area is defined as the area lateral to the mid-vertical line of the line through the acetabular edge and the teardrop bottom.

Table

Patient Data

Patient no./Sex/Age (y)	Associated condition	Necrotic lesion[a]	ARCO stage at first presentation	Follow-up (mo)	ARCO stage at final follow-up	Pain score[b]	Comments
1/M/30	Steriod use	C-2	II	132	IV	1	THA, 132 months
2/F/33	Steriod use	C-2	I	98	IV	2	THA, 98 months
3/F/74	Idiopathic	C-2	II	147	IV	2	THA, 147 months
4/F/38	Steriod use	C-1	II	222	IIIA	5	Cessation of collapse
5/F/38	Steriod use	C-1	II	222	IIIA	5	Cessation of collapse
6/M/38	Steriod use	C-1	II	236	II	6	No collapse
7/F/43	Steriod use	C-2	I	180	IV	3	Progression of collapse
8/M/53	Steriod use	B	I	118	I	5	No collapse
9/M/37	Idiopathic	C-2	II	132	IIIA	6	Cessation of collapse, no symptom
10/M/37	Idiopathic	C-2	II	132	IIIA	6	Cessation of collapse, no symptom
11/M/36	Steriod use	A	I	190	IV	2	THA, 190 months
12/M/62	Idiopathic	C-2	I	103	I	6	No symptom
13/M/27	Steriod use	C-2	II	105	IV	5	Progression of collapse
14/M/27	Steriod use	A	I	103	I	5	No collapse
15/M/22	Steriod use	C-2	I	96	I	5	No collapse
16/M/22	Steriod use	C-1	I	96	I	5	No collapse
17/F/20	Steriod use	C-1	I	323	I	6	No collapse

Abbreviation: THA, total hip arthroplasty.
[a]Necrotic lesion was evaluated according to the radiographical classification system established by the working group of the Specific Disease Investigation Committee under the auspices of the Japanese Ministry of Health, Labour, and Welfare.
[b]D'Aubigne scale[6].

To determine the location of necrotic lesions, we used the radiographic classification established by the working group of the Specific Disease Investigation Committee under the auspices of the Japanese Ministry of Health, Labour, and Welfare (Figure 1). Two hips were type A, 1 hip was type B, 5 hips were type C-1, and 9 hips were type C-2 at first examination. Radiological staging was performed according to Ficat's classification[4]; 9 hips were stage I and 8 hips were stage II. Each patient underwent repeated radiographic examination, such as plain radiograph, computed tomography (CT), magnetic resonance imaging (MRI), and bone scanning. To evaluate the progression of collapse, we followed the methods for measuring osteonecrosis progression reported by Aaron et al[5] and Nishii et al[2] (Figure 2). Each patient was asked to indicate the level of hip pain according to the scoring system of the D'Aubigne scale.[6] Patients with a pain score from 1 (intense and permanent pain) to 2 (severe pain when walking) were regarded as having severe pain. Patients failing to achieve a grade of 2 on the pain scale were advised to undergo a hip arthroplasty.

Written informed consent was obtained from the patients included in this study.

Results

Seven of 17 hips (41%) did not exhibit progression to collapse. One hip was type A, 1 hip was type B, 3 hips were type C-1, and 2 hips were type C-2. Radiographic signs of femoral head collapse were observed in the remaining10 hips (59%). Eight hips collapsed within 1 year of first examination, and 2 hips (12%) collapsed after 1 to 3 years. Cessation of collapse occurred within 3 years from first examination in 4 hips, and the amount of collapse of the femoral head was <3 mm in all 4 hips. Two of these 4 hips were type C-1 and 2 hips were type C-2. In 6 hips with >3 mm of collapse, collapse progressed to stage IV. Total hip arthroplasty was performed on 4 of these 6 hips. One hip was type A and the remaining 5 hips were type C-2 (Table). There was no correlation between pain and type of AVN. Total hip arthroplasty was performed on 4 hips having severe pain (pain score 1 or 2).

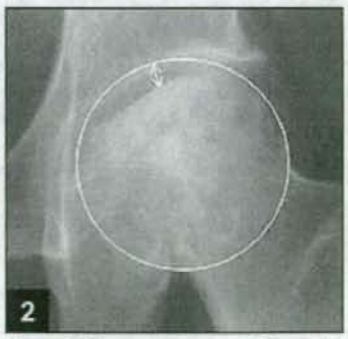

Figure 2: The measurement of femoral head collapse is shown. A circle (white line) encompassing the femoral head was estimated to represent the entire femoral head on AP or lateral radiographs. The distance between the maximum incursion into the femoral head and the estimated outline was determined (arrows).

In 4 stage III hips at final evaluation, the amount of collapse of the femoral head was 1.5 mm in the first 3 years (range, 1-2 mm). In contrast, in the 6 stage IV hips at final evaluation, the amount of collapse of the femoral head was 4 mm in the first 3 years (range, 1-7 mm) and had progressed an average of 0.7 mm a year (0.2-1.7 mm a year) until final evaluation.

Discussion

If the course could be predicted in a given case, it would easier to prescribe appropriate treatment, and it would also allow for accurate comparisons of results obtained with various treatment methods. However, comprehensive studies into the course of AVN are lacking. The most important turning point in the course of AVN is the occurrence of subchondral plate fracture of the femoral head, which leads to collapse of the necrotic segment of the epiphysis. The rate of femoral head collapse reportedly varies from 44% to 79%,[2,7,8] probably because of differences in study populations; the overall average was approximately 50%.[1] In our study, the overall rate of femoral head collapse was 59%.

Collapse depends chiefly on the initial size and location of the necrotic segment. Multiple logistic regression analyses revealed a significant relationship between lesion volume and radiological collapse.[9] In this study, of the 3 hips in which lesion volume was small (type A or B), 1 hip collapsed. Of the 14 hips in which lesion volume was large (type C-1 or C-2), 9 hips (64%) collapsed. If lesion size is extensive in the weight-bearing area, the femoral head will typically collapse.

There is an opinion that the time to femoral head collapse is usually <2 years after diagnosis of AVN.[1,2] Beyond 3 years, the risk of radiographic deterioration is virtually nonexistent. Lafforgue[1] reported that the time to femoral head collapse is within 2 years after diagnosis of AVN. Ohzono et al[10] reported that the interval from entry into the study to the date of collapse was usually <3 years, and within 6 years, 69% had developed osteoarthritis. Bradway and Morrey[11] found that 13 of 15 hips that were negative for any conclusive sign of necrosis on plane radiographs collapsed <3 years after entry. The remaining 2 hips showed collapse of the femoral head at 50 and 66 months after entry, respectively. In our study, all collapses were observed within 3 years after first examination, and thus we believe that if collapse of the femoral head does not occur within 3 years of onset, femoral head collapse will not occur. Moreover, cessation of collapse was also observed within 3 years of onset. The first 3 years are an important period in predicting the history of AVN.

Our analysis indicated that collapse of the femoral head does not necessarily indicate a poor prognosis, and even after collapse occurs, subsequent cessation of collapse can be expected in a certain percentage of hips. In 4 of 10 collapsed hips (40%) in this study, cessation of collapse was observed. In all 4 hips with cessation of collapse, the amount of collapse was <2 mm, while all 6 hips with >3 mm of collapse progressed without cessation of collapse. Therefore, hips with >3 mm of collapse should not be expected to exhibit cessation of collapse, thus indicating poor prognosis. This suggests that patients with collapse of <2 mm may show cessation of collapse and may not require joint-preserving surgery. Nishii et al[2] showed that 28 of 54 hips (52%) with early-stage AVN (without collapse or only a crescent sign) collapsed during a 5-year follow-up; subsequently, 15 of 28 hips (54%) showed cessation of collapse and 10 of 15 hips (67%) showed improvement in pain, even after collapse occurred. In terms of the confirmation period of femoral head collapse cessation, all cases of cessation were observed within 3 years from first presentation. After cessation of collapse was observed, recollapse did not occur in any cases.

Our study has 2 major limitations. First, we were only able to evaluate 17 hips, as surgical treatments were required within short periods from the onset of hip pain in many cases, and thus evaluating many cases is difficult. However, we believe that further study is required to clarify the course of AVN. The second limitation is bias at the time of case selection. This study used a selected sample of patients who could be followed for at least 8 years, and it is thus possible that the results do not reflect the true course of AVN.

Conclusion

The course of AVN without collapse of the femoral head can be classified into 3 patterns: (1) collapse of femoral head does not occur, (2) early progression with subsequent cessation after >2 mm of collapse, and (3) slow progression of collapse and patients tolerate hip pain. In groups 1 and 2, good clinical results are expected. We believe that there are 3 prognostic stages for a poor outcome in AVN: the first stage is the extent of necrotic lesions, the second stage is the onset of collapse, and the third stage is the amount of collapse. In this study, we particularly focused on the second and third stages. Thus, whether collapse and cessation occur within the first 3 years, the amount of femoral head collapse is important in predicting the course of AVN.

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KNEE

Staged bilateral mobile-bearing and fixed-bearing total knee arthroplasty in the same patients: a prospective comparison of a posterior-stabilized prosthesis

Masahiro Hasegawa · Akihiro Sudo · Atsumasa Uchida

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Abstract Mobile-bearing total knee arthroplasty (TKA) has several theoretical advantages over fixed-bearing TKA. We conducted a prospective randomized trial to compare the results of mobile-bearing and fixed-bearing posterior-stabilized TKA in the same patients using the same femoral component design of a mobile-bearing prosthesis in one knee and a fixed-bearing prosthesis in the other knee in 25 patients with osteoarthritis. The mean follow-up was 40 months. No significant differences were found in the mobile-bearing and fixed-bearing knees in terms of clinical and radiographic results. No osteolysis, loosening, or revision occurred. One knee with a mobile-bearing prosthesis had a dislocation of the rotating bearing; however, spontaneous reduction occurred and the dislocation did not recur. Satisfactory early results can be achieved in both mobile-bearing and fixed-bearing knees. We could not demonstrate an advantage of a mobile-bearing TKA.

Keywords Total knee arthroplasty · Mobile bearing · Fixed bearing

Introduction

The long-term results of total knee arthroplasty (TKA) with a fixed-bearing design have shown a high degree of success. There is concern, however, with regard to problems of polyethylene wear and implant loosening. Debris-induced osteolysis due to polyethylene wear is a potential mechanism of long-term TKA failure [17, 21]. Mobile-bearing total knee prostheses were designed to provide dual-surface articulation at both the superior and inferior surfaces of the polyethylene insert. Highly congruent articulating surfaces result in reduced polyethylene contact stresses. In a simulator study, mobile-bearing prostheses exhibited reduced wear rates in comparison to fixed-bearing components [6, 19]. Mobile-bearing prostheses are also postulated to minimize bone prosthesis stress at the fixation surface of the tibial component [4, 6, 7, 19, 23]. Additionally, the self-aligning nature of the implants has been promoted as simplifying the surgical procedure, although the surgery involved requires perfect soft tissue balancing [25]. However, these advantages over fixed-bearing total knee prostheses are theoretical.

We conducted a single-blind, prospective, randomized, trial using the same femoral component design to compare the results of mobile-bearing and fixed-bearing TKAs in the same patients. To our knowledge, the present study is the first trial comparing the same design of a femoral component of a mobile-bearing prosthesis in one knee and a fixed-bearing prosthesis in the other with use of a posterior-stabilized TKA. We hypothesized that early clinical and radiographic results may demonstrate no differences between mobile-bearing and fixed-bearing TKAs.

Patients and methods

Between April 2003 and July 2006, 25 consecutive patients underwent a staged bilateral TKA, with a mobile-bearing TKA on one side and a fixed-bearing TKA on the other (average interval 8.7 months; range 2–28 months). Randomization regarding the use of either a mobile-bearing or a fixed-bearing prosthesis was determined using sealed envelopes. The patients were kept in ignorance as to which

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side had been chosen. The study was approved by the institutional review board, and all patients gave informed consent. Participants included 22 women and 3 men with a mean age at the time of surgery of 73 years (55–81). All patients had osteoarthritis. The mean height of patients was 150.1 cm (137–167) and their mean weight was 56.8 kg (45–75). The mean follow-up was 40 months (18–63). No patient was lost to follow-up.

All operations were performed by a single surgeon (MH) through a midline skin incision with a midvastus approach. Both the anterior cruciate ligament and posterior cruciate ligament were excised. Tibial preparation was done first followed by femoral preparation. The ligaments were balanced. A press-fit condylar Sigma mobile-bearing or fixed-bearing prosthesis (PFC Sigma, DePuy, Warsaw, IN) was used. All implants used a posterior-stabilized design with a post-cam mechanism. All components were fixed with cement. The femoral component in both groups was the same and was made of cobalt–chrome. The cobalt–chrome tibial tray for the mobile-bearing prosthesis was modular and keel shaped with the design of a rotating platform, as was the titanium tibial tray for the fixed-bearing prosthesis. The mobile-bearing tibial component was a highly polished baseplate with nearly full conformity in the coronal and sagittal planes. The polyethylene insert included a central cone that engaged a matching conical cavity in the tibial tray (Fig. 1) [23]. All patellae were routinely resurfaced using an all-polyethylene prosthesis. No cases required lateral retinaculum release. On the second day after surgery, the knee was placed on a continuous passive motion machine and the settings were advanced incrementally until the knee reached 120° of flexion. All patients were allowed full weight bearing 5 days postoperatively.

Preoperative and postoperative ratings according to the system of the Knee Society were obtained for all patients. These ratings included a knee score and a function score [13]. The range of movement (ROM) was measured. In addition, we asked for subjective preference of one knee over the other. We obtained radiographs, including anteroposterior views of both long leg standing and supine, supine lateral, and skyline patellar views before and after surgery. Radiographs were assessed by a single observer (AS) who was blinded to the type of prosthesis for alignment of the limb, the position of the component, and the presence of radiolucent lines at the bone–cement interface, according to the methods of the Knee Society [8]. Any detectable osteolysis around the three components was recorded.

Statistical analyses were carried out using the Wilcoxon signed rank test, Mann–Whitney's U test, and Fisher's exact test. $P < 0.05$ was considered statistically significant.

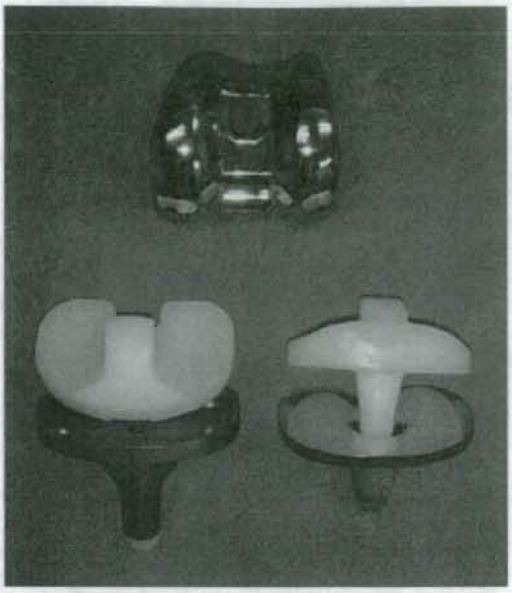

Fig. 1 Photographs of the mobile-bearing prosthesis in the right and fixed-bearing prosthesis in the left

Results

Clinical results

The preoperative knee score and function score were not statistically different between the mobile-bearing and fixed-bearing knees (Table 1). The scores at the time of the last follow-up were also not statistically different between the groups (Table 1). Both knee scores and function scores significantly improved postoperatively in the mobile-bearing and fixed-bearing TKAs ($P < 0.01$). The ROM in mobile-bearing and fixed-bearing knees was not statistically different either preoperative or postoperative evaluation (Table 2). Nine patients preferred the mobile-bearing side, 11 patients preferred the fixed-bearing side, and 5 patients indicated no difference between the two knees.

Radiographic results

The preoperative femorotibial alignment (anatomic axis) averaged 8.7° of varus (0–20) in 24 knees with a mobile-bearing prosthesis. In one knee with a mobile-bearing prosthesis, the preoperative femorotibial alignment was 11° of valgus. The preoperative femorotibial alignment averaged 8.9° of varus (3–20) in all knees with a fixed-bearing prosthesis. The postoperative femorotibial alignment

Table 1 Clinical scores according to the Knee Society

Patient number	Knee score				Function score			
	Preoperative		Postoperative		Preoperative		Postoperative	
	Mobile	Fixed	Mobile	Fixed	Mobile	Fixed	Mobile	Fixed
1	43	33	100	100	35	55	80	80
2	33	38	100	100	20	40	80	80
3	36	23	100	100	45	75	80	80
4	35	26	95	95	60	65	100	100
5	30	27	94	94	55	55	55	55
6	0	0	100	100	40	40	100	100
7	57	37	100	100	60	70	100	80
8	1	14	95	95	0	0	50	50
9	50	0	100	100	15	40	80	80
10	8	0	79	88	20	20	100	100
11	7	12	100	100	45	45	80	80
12	35	20	100	100	35	35	100	100
13	23	52	100	94	30	70	70	70
14	16	28	98	98	45	45	70	65
15	21	44	100	100	45	55	80	80
16	6	40	100	100	35	50	70	100
17	22	29	88	95	45	35	90	90
18	47	33	100	100	35	40	80	80
19	10	0	100	100	70	45	80	80
20	27	35	100	100	35	40	80	80
21	46	26	88	98	65	35	90	90
22	23	31	94	97	70	50	100	100
23	33	0	95	95	55	35	75	90
24	29	5	100	95	35	35	80	80
25	15	35	100	100	65	65	100	100
Mean	26	24	97	98	42	46	83	84
P	0.61		0.86		0.63		0.72	

averaged 5.3° of valgus (1–8) in the mobile-bearing knees and 5.4° of valgus (2–8) in the fixed-bearing knees. In both groups, there were no significant differences in the position of the femoral and tibial components in the coronal and sagittal planes (Fig. 2; Table 3). Three (12%) of the 25 knees with a mobile-bearing prosthesis and 5 (20%) of the 25 knees with a fixed-bearing prosthesis had radiolucent lines around the tibial prostheses. All of the radiolucent lines were <1 mm and were nonprogressive. We found no significant difference between the mobile-bearing and fixed-bearing knees in the occurrence of radiolucent lines ($P = 0.70$).

Complications

One knee in the mobile-bearing group had a dislocation of the rotating bearing 4 days postoperatively. However, spontaneous reduction occurred 6 days after the dislocation, and the dislocation did not recur. This patient has muscle weakness in both legs due to cervical spondylotic myelopathy with severe varus deformity of the knee preoperatively. Both quadriceps deficiency and ligament laxity may contribute to the risk of dislocation [9].

No loosening, revision, or infection occurred in any patient.

Discussion

Several authors have compared the results of different types and designs of mobile-bearing and fixed-bearing TKAs. Most studies showed no difference between the mobile-bearing and fixed-bearing TKAs in terms of clinical score, ROM, and radiographic results as shown in the present study [1, 4, 5, 11, 14–16, 18, 22, 28]. For example, Kim et al. [14] compared the results of a mobile-bearing prosthesis (LCS meniscal-bearing, DePuy) and fixed-bearing prosthesis (AMK, DePuy) in 116 patients who had

Table 2 Range of movement

Patient number	Flexion				Flexion contracture			
	Preoperative		Postoperative		Preoperative		Postoperative	
	Mobile	Fixed	Mobile	Fixed	Mobile	Fixed	Mobile	Fixed
1	130	135	135	135	15	10	0	0
2	130	130	140	140	5	5	0	0
3	120	100	145	130	5	10	0	0
4	115	120	125	130	15	5	0	0
5	125	125	120	120	0	5	0	0
6	120	115	140	135	20	20	0	0
7	130	125	130	130	10	15	0	0
8	95	120	130	125	5	15	0	0
9	130	105	135	130	10	15	0	0
10	110	110	115	115	45	20	10	0
11	100	125	125	135	15	15	0	0
12	120	120	130	135	10	20	0	0
13	105	120	130	120	5	0	0	0
14	110	135	135	130	30	5	5	5
15	100	110	130	130	10	5	0	0
16	120	135	135	130	15	0	0	0
17	100	110	110	130	5	5	5	0
18	110	105	135	125	0	5	0	0
19	90	100	130	125	15	25	0	0
20	110	120	125	140	0	10	0	0
21	120	120	115	115	5	5	0	0
22	90	120	100	110	0	5	5	0
23	130	95	135	140	15	30	0	0
24	125	125	145	140	20	15	0	0
25	120	135	140	135	20	0	0	0
Mean	114	118	129	129	12	11	1	0
P	0.16		0.69		0.71		0.24	

bilateral simultaneous TKA. At a mean follow-up of 7.4 years, no difference in the clinical outcome was identified in the two groups. The authors observed the same results in extended follow-up periods [16]. Woolson and Northrop [28] compared the results of 57 mobile-bearing prostheses (LCS rotating-platform, DePuy) and 45 fixed-bearing prostheses (NexGen PS, Zimmer, Warsaw, IN) for a mean follow-up of 41 months and found no differences clinically or radiographically. However, more patients with a mobile-bearing prosthesis required early revision for failure of rotating patellar components in two knees and tibial polyethylene spinout in one knee. Bhan et al. [4] compared the results of a mobile-bearing prosthesis (LCS rotating-platform, DePuy) and fixed-bearing prosthesis (Insall Burstein-II, Zimmer) in 32 patients who had bilateral simultaneous TKA. At a mean follow-up of 6 years, clinical and radiographic results showed no differences between groups. Two knees with a mobile-bearing prosthesis required a second operation: one had an early revision because of recurrent dislocation of the rotating bearing and another required conversion to an arthrodesis to treat a deep infection. Price et al. [22] compared the results of TMK mobile-bearing (Biomet Merck, Bridgend, UK) and AGC fixed-bearing (Biomet Merck) TKAs in 40 patients who had bilateral simultaneous TKA. At 1 year, the authors demonstrated a significant clinical advantage for the mobile-bearing knee. However, at 3 years, there were no significant differences in clinical outcome between the two prostheses [1]. Biau et al. [5] reported the 5-year results of two cohorts of 108 posterior-stabilized HLS prostheses (Hospital Lyon Sud, Tornier SA, Montbonnot, France), one cohort receiving the mobile-bearing design and the other receiving the fixed-bearing design. In all of these previous studies, different types and designs of prostheses were compared. This may have affected the clinical outcome. In a recent prospective randomized trial, Lädermann et al. [18] reported the 7-year results of two groups of 52 knees that were replaced using either a mobile-bearing or a fixed-

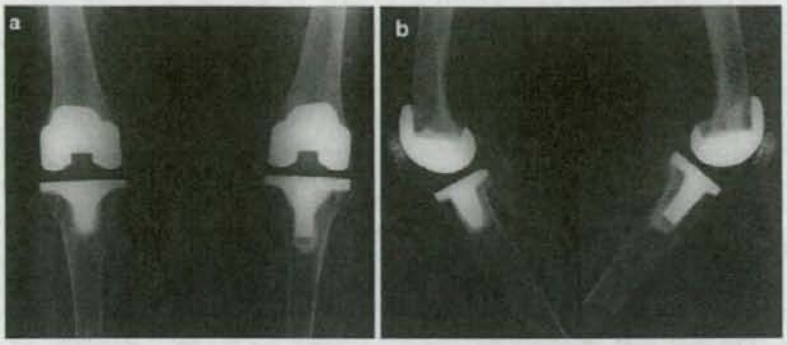

Fig. 2 Radiograph of an 80-year-old woman with osteoarthritis of both knees. **a** Anteroposterior and **b** lateral views with a mobile-bearing prosthesis in the right knee and a fixed-bearing prosthesis in the left knee, taken 4 years postoperatively. The components are well fixed and there are no radiolucent lines or osteolysis

Table 3 Component alignment in mobile-bearing and fixed-bearing TKAs

Patient number	Femoral component				Tibial component			
	Coronal		Sagittal		Coronal		Sagittal	
	Mobile	Fixed	Mobile	Fixed	Mobile	Fixed	Mobile	Fixed
1	96	96	3	0	90	90	84	87
2	96	96	2	−3	90	90	85	87
3	93	96	−2	4	88	90	86	87
4	97	96	4	0	92	90	87	87
5	95	97	0	2	89	90	87	85
6	96	96	3	0	90	88	85	86
7	97	97	−4	0	90	88	87	85
8	97	96	0	0	90	88	83	87
9	95	95	0	0	90	90	87	87
10	96	96	0	0	90	90	87	86
11	96	96	3	4	90	90	86	86
12	96	97	0	0	90	88	88	84
13	95	97	0	0	89	88	87	84
14	96	98	−1	1	90	90	85	87
15	96	96	3	1	91	90	87	84
16	96	94	0	0	90	89	87	86
17	98	96	0	0	90	88	84	86
18	97	96	0	−3	91	90	84	87
19	95	96	0	−2	90	90	87	87
20	97	96	0	0	90	90	85	87
21	97	96	2	0	87	90	84	87
22	96	96	−1	2	88	90	86	87
23	97	96	1	1	90	90	87	87
24	94	96	4	0	90	90	85	84
25	98	96	0	0	92	88	86	87
Mean	96.1	96.1	0.7	0.3	89.9	89.4	85.8	86.2
P	0.88		0.59		0.09		0.4	

bearing variant of the same posterior-stabilized total knee prostheses (PFC Sigma) as was used in this study. Two knees with a mobile-bearing design required reoperation: one for persistent joint stiffness and another to treat septic loosening, however, no significant differences were demonstrated with respect to the clinical and radiographic results between groups. To our knowledge, only two studies have compared the same femoral component design of a mobile-bearing prosthesis in one knee with a fixed-bearing prosthesis in the other [15, 23]. In a case–control

study, Ranawat et al. [23] compared the mobile-bearing rotating-platform TKA (PFC Sigma RP) to the fixed-bearing version of the same PFC Sigma design previously implanted in the opposite knee in 26 patients. All implants were posterior stabilized. At an average follow-up time of 16 months for the mobile-bearing side and 46 months for the fixed-bearing side, no significant differences were found in terms of clinical and radiographic results. Kim et al. [15] compared the results of a mobile-bearing PFC Sigma RP and a fixed-bearing PFC Sigma in 174 patients who had bilateral simultaneous TKA. All implants used a posterior cruciate-retaining design. At an average follow-up time of 5.6 years, the authors could not demonstrate any significant clinical advantage for the mobile-bearing TKA.

In terms of preference for the type of prosthesis, Price et al. [22] demonstrated that two of three patients who expressed a preference favored the mobile-bearing knee. Kim et al. [16] reported that 85% of patients expressed no preference for either knee. Our questionnaire also revealed no tendency for a preference between a mobile-bearing or fixed-bearing TKA.

No osteolysis was found in either group in the present study, and Callaghan et al. [7] reported no knee was revised because of loosening, osteolysis, or polyethylene wear at a minimum follow-up of 15 years for a mobile-bearing TKA. However, the prevalence of osteolysis in failed TKA was reported to be significantly higher in the mobile-bearing TKA (47%) than in the fixed-bearing TKA (13%) [11]. Huang et al. [12] showed that the mobile-bearing knees (LCS, DePuy) produced smaller particulate debris and more granular debris. Minoda et al. [20] compared the size, shape, and number of polyethylene wear particles found in synovial fluids of patients 1 year after implantation of well-functioning mobile-bearing and fixed-bearing total knee prostheses with a posterior-stabilized design and found no differences in these parameters between groups. These in vivo studies did not confirm the theoretical advantages of a mobile-bearing TKA. Recently, Ho et al. [10] examined worn tibial inserts, including mobile-bearing rotating-platform posterior cruciate-sacrificing dished prostheses (LCS, DePuy) and fixed-bearing posterior cruciate-retaining flat prostheses (Miller-Galante I, Zimmer), which were retrieved at revision surgery with an average implantation time of 115 months. Low-grade wear was more common in mobile-bearing knees, whereas high-grade wear was more common in fixed-bearing knees. The authors stated that mobile-bearing designs reduced the incidence of rotational asymmetric wear because of facilitation of movement of the insert relative to the tray when the knee rotates. The follow-up period in our study was not sufficient to evaluate the reduction of polyethylene wear as well as implant loosening. The other limitations of the study were the small sample size and particular type of population, including light mean weight (56.8 kg) and good preoperative mean range of movement (114°–118° of flexion).

After mobile-bearing TKA, dislocation or spinout can occur as a result of excessive rotation of the polyethylene bearing accompanied by translation of the femur on the tibia [2, 3, 9, 24, 25]. Although bearing dislocation is an unusual complication, it is the most important potential early complication. The reported incidence of polyethylene dislocation ranges from 0 to 9.3% [4, 12, 23, 26]. The causes of dislocation after TKA are multifactorial, including component malposition, prosthesis design, extensor mechanism dysfunction, hamstring spasm, extensive posterolateral release, and increased flexion laxity [3, 9, 24–26]. Many surgeons feel that the use of an unconstrained mobile-bearing TKA may be contraindicated in cases of severe varus and valgus deformity because of the difficulty in ligament balancing and the requirement for extensive soft tissue release; however, the degree of deformity that can be treated with mobile-bearing knees is unclear [5, 27].

Conclusion

Although it is difficult to draw valid conclusions from our small study and long-term results from our patients are required to provide useful information, early results indicate no significant differences in the clinical and radiographic findings between mobile-bearing and fixed-bearing posterior-stabilized TKAs using the same design of femoral component in the same patients. Satisfactory early results can be achieved in both prostheses. We could not demonstrate an early advantage for a mobile-bearing knee and our hypothesis was verified.

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Thrombin-Cleaved Osteopontin in Synovial Fluid of Subjects with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Osteopontin (OPN) is an extracellular matrix glycoprotein that has been recognized as a potential inflammatory cytokine. The function of OPN is modulated by protease digestion, and a thrombin-cleaved form of OPN is involved in the pathogenesis of various inflammatory disorders. We examined thrombin-cleaved OPN products in synovial fluid from patients with rheumatoid arthritis (RA) and osteoarthritis (OA).

Methods. Synovial fluid samples were obtained from knees of 20 patients with RA and 111 patients with OA. Thrombin-cleaved OPN product was determined using Western blotting. Levels of thrombin-cleaved and full-length OPN in synovial fluid were determined by ELISA. Synovia were analyzed by immunohistochemistry using an antibody specific to the thrombin-cleaved form.

Results. Immunoblotting showed the presence of thrombin-cleaved OPN in synovial fluid from patients with RA and OA. ELISA results showed no difference between concentrations of full-length OPN in the synovial fluid of RA and OA patients; however, thrombin-cleaved OPN concentrations in RA synovial fluid samples were roughly 30-fold higher compared with OA samples ($p < 0.001$). Synovial fluid concentrations of thrombin-cleaved OPN in RA did not correlate with C-reactive protein levels. Immunohistochemistry of the synovium showed stronger reactivity in RA than in OA samples.

Conclusion. Local generation of thrombin-cleaved OPN was increased in RA joints. Thrombin-cleaved OPN may be a useful biochemical marker of RA. (First Release Jan 15 2009; J Rheumatol 2009;36:240–5; doi:10.3899/jrheum.080753)

Key Indexing Terms:
OSTEOPONTIN SYNOVIAL FLUID BIOMARKER
RHEUMATOID ARTHRITIS OSTEOARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by pathologic changes in joints, including synovitis, cartilage degradation, subchondral bone erosion, and alterations in cellular immune responses. Proinflammatory cytokines, including interleukin 1ß (IL-1ß), IL-6, and tumor necrosis factor-α (TNF-α), which are produced mainly by macrophages, are involved in the development of synovitis[1-3].

Recent studies have shown that osteopontin (OPN), an extracellular matrix glycoprotein, is a potential inflammatory cytokine[4-6] and modulates a variety of pathological conditions[7,8]. OPN is also expressed in synovial tissue and cartilage from patients with RA and osteoarthritis (OA)[9,10], suggesting an involvement in the pathogenesis of inflammatory arthritis[11].

OPN is a highly phosphorylated and sulfated glycoprotein, with a molecular weight of roughly 32 kDa. Variable sizes up to 75 kDa have been reported[7]. It is expressed in bone-forming cells, as well as in mesenchymal cells from the uterus, placenta, kidney, and nervous system[12]. During inflammation and wound-healing, OPN is also expressed by cells related to both innate and adaptive immunity, such as activated T lymphocytes, macrophages, and resident fibroblasts[5,13]. OPN contains several cell adhesive domains, including an arginine-glycine-aspartate (RGD)-containing domain that interacts with cell-surface integrins $\alpha_v\beta_3$, $\alpha_v\beta_1$, and $\alpha_v\beta_5$. Proteolytic modification of OPN by thrombin cleavage reveals cryptic binding sites for $\alpha_9\beta_1$ and $\alpha_4\beta_1$ integrins, preferentially expressed by neutrophils and by monocytes and lymphocytes, respectively[4,7,14,15]. The newly exposed binding sites within OPN, SVVYGLR in humans and SLAYGLR in mice and rats, promote adhesion and migration of leukocytes and neutrophils through these alternative sites in an RGD-independent manner[7,15] (Figure 1). The presence of the thrombin-cleaved form of OPN is well correlated with various inflammatory disease activities[16].

In terms of RA pathogenesis, inflammatory cells such as

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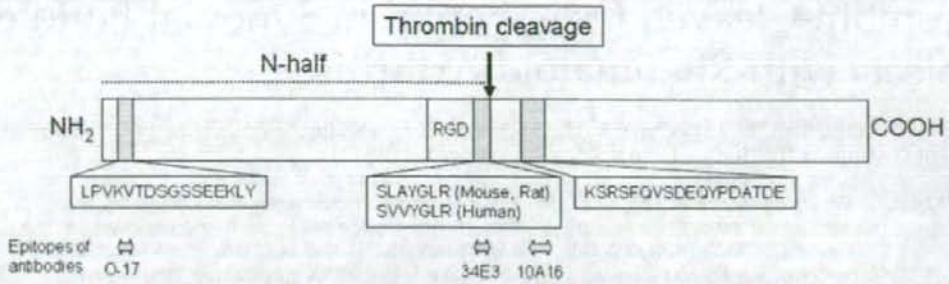

Figure 1. Structure of mouse OPN as described. The position of the RGD domain and the thrombin cleavage site at position 168R/S169 are indicated[7,15]. Epitopes for antibodies used in this study are also indicated.

macrophages, neutrophils, and lymphocytes are infiltrated into the diseased synovium. Moreover, activation of the coagulation cascade in RA synovial fluid, resulting in increased thrombin activity, has been found[17]. It is conceivable that the thrombin-cleaved form of OPN participates in the development of arthritis[18]. The purpose of our cross-sectional study was to quantify this form of OPN in synovial fluid from patients with RA and OA and to compare these levels between diseases.

MATERIALS AND METHODS

Patients and samples. All patients with RA had knee joint arthritis and fulfilled the revised criteria for RA of the American College of Rheumatology (ACR)[19]. All patients with OA fulfilled the ACR clinical and radiological diagnostic criteria[20]. Synovial fluid samples were obtained at the time of surgery or before intraarticular injection of hyaluronic acid from 20 patients with RA and 111 patients with OA. Synovial fluid was centrifuged at 15,000 × g for 15 min, and the supernatants were stored at –80°C until analyzed.

The group with RA consisted of 16 women and 4 men with a mean age of 66.9 years and a mean body mass index (BMI) of 22.5 kg/m^2. The group with OA consisted of 78 women and 33 men with a mean age of 73.9 years and mean BMI of 23.6 kg/m^2. Synovial membrane samples were obtained at the time of surgery from 7 patients with RA and 23 with OA of the knee. C-reactive protein (CRP) and matrix metalloproteinase-3 (MMP-3) were measured in sera as a marker of inflammation. All patients gave informed consent, and the study was approved by the local ethics committee.

Western blot analysis. Immunoblotting of synovial fluid was done to examine the presence of the N-terminal half of thrombin-cleaved osteopontin (OPN N-half). Synovial fluid was examined from 3 OA patients, 2 RA patients, and one control patient with a meniscus tear. From each synovial fluid sample, 0.15 ml was diluted twice with phosphate buffered saline (PBS), added to 20 µl of DEAE Sepharose Fast Flow (GE Healthcare UK Ltd., Buckinghamshire, UK), mixed for 30 min at room temperature, washed 5 times with PBS, then eluted with 1.0 ml of 0.7 M NaCl in PBS. The eluates were diluted twice with 2× sodium dodecyl sulfate (SDS) buffer [4% SDS, 20% glycerol, 125 mM Tris HCl (pH 6.8), 10% 2-mercaptoethanol], boiled, and applied on Western blotting with horseradish peroxidase-labeled anti-human OPN (O-17) rabbit IgG Fab' (IBL, Gunma, Japan). Antibody (O-17) is raised against synthetic peptides corresponding to the internal sequence of mouse OPN (^{17}LPVKVTDSGSSEEKLY^{32}) and can bind both non-thrombin-cleaved OPN (full-length) and OPN N-half[21] (Figure 1).

Enzyme linked immunosorbent assay. An ELISA (IBL) was applied to quantify the levels of non-thrombin-cleaved OPN (OPN full-length) and OPN N-half. For the OPN N-half ELISA, Immuno Module Plates (Nalge Nunc, Rochester, NY, USA) were coated with anti-OPN N-half (34E3) mouse monoclonal antibody (in 0.1 M carbonate buffer, pH 9.5) at 4°C overnight, then blocked with 1% bovine serum albumin in PBS containing 0.05% NaN_3 at 4°C overnight. The mouse monoclonal antibody (34E3) specifically reacts to SLAYGLR and SVVYGLR, exposed by thrombin cleavage of mouse and human OPN, respectively. The OPN N-half ELISA system does not recognize full-length OPN and detects OPN after thrombin cleavage[21] (Figure 1). Sample and standard proteins were diluted with 1% BSA, 0.05% Tween 20 in PBS, added to each well, and incubated at 37°C for 1 h. After 7 washes with washing buffer (0.05% Tween 20 in phosphate buffer), 100 µl of horseradish peroxidase (HRP)-labeled anti-human OPN (O-17) rabbit polyclonal antibody was added to each well and incubated for 30 min at 4°C. After 9 washes with washing buffer, 100 µl of tetramethyl benzidine buffer as a substrate was added to each well and incubated for 30 min at room temperature in the dark. Color development was stopped by addition of 100 µl of stop solution (1 N H_2SO_4). Optic density of each sample was measured at 450 nm.

To quantify the levels of non-thrombin-cleaved OPN (OPN full-length), the human osteopontin assay kit (IBL) was used with 2 antibodies (O-17 and 10A16). A mouse monoclonal antibody (10A16) is raised against synthetic peptides corresponding to the internal sequence of human OPN (^{155}KSRSFQVSDEQYPDATDE^{172}) and can bind to OPN full-length but not OPN N-half (Figure 1).

The percentage of N-half was expressed as a percentage (OPN N-half divided by all OPN (OPN N-half plus OPN full-length).

Immunohistochemistry. Expression of thrombin-cleaved OPN in synovial membranes was determined by immunohistochemistry using the avidin-biotin complex method. Synovial membrane samples were fixed in 10% buffered formalin, embedded in paraffin, and cut into 4-µm thick sections. After deparaffinization with xylene and rehydration through a series of graded ethanol solutions, the sections were pretreated in 10 mM citrate buffer, pH 6.0, in a microwave oven for 5 min for antigen retrieval. Sections were treated with superblock solution (Scytek Laboratories, Logan, UT, USA). After washing, sections were incubated in 0.3% H_2O_2 in methanol for 15 min to block endogenous peroxidase activity, blocked with 2% bovine serum albumin, and incubated with the primary antibody [anti-OPN N-half (34E3) mouse monoclonal antibody] overnight at 4°C. Sections were washed with PBS and reacted with the secondary antibody (biotinylated goat anti-mouse IgG antibody) for 1 h at room temperature. After washing, they were incubated with avidin-peroxidase complex for 30 min. Sections were then developed with diaminobenzidine tetrahydrochloride substrate solution and counterstained with hematoxylin.

The results of immunoreactivity for synoviocytes and subintimal tissues were identified using the point system of Salter[22], as follows: For synoviocytes, no staining = 0 points; staining of < 25% of synoviocytes = 1 point; staining of 25%–75% of synoviocytes = 2 points; and staining of > 75% of synoviocytes = 3 points. For subintimal tissues: no staining = 0

points; focal weak staining of subintimal tissues = 1 point; focal strong staining of subintimal tissues = 2 points; and extensive strong staining of subintimal connective tissue = 3 points. The protocol was tested with 3 observers (MH, YN, AS) to standardize the scoring system.

Statistical analysis. The Mann-Whitney U-test was used to determine the differences between values in RA and OA patients. Correlation analyses were performed for serum biomarker levels of RA, including CRP and MMP-3, and synovial fluid levels of OPN N-half in patients with RA using Spearman's rank correlation test. Correlation between levels of OPN N-half and age and BMI were also estimated. P values < 0.05 were considered significant.

RESULTS

Expression of thrombin-cleaved OPN protein. 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 OA (Figure 2, lanes 1–3) and RA (Figure 2, lanes 5, 6). A control sample showed no band (Figure 2, lane 4). These results showed that all samples of OA and RA contained full-length OPN, and that the levels of N-half OPN were considerably elevated in patients with RA (Figure 2, lanes 5, 6), although faint bands were rarely detected in OA samples (Figure 2, lane 1).

Concentrations of full-length and thrombin-cleaved OPN in synovial fluid. ELISA results showed a tendency for higher levels of full-length OPN in synovial fluid from RA over OA samples, but differences were not significant. In contrast, N-half OPN levels in synovial fluid of RA samples (median 0.376 nM) were roughly 30-fold higher ($p < 0.001$) compared with OA samples (median 0.013 nM). In addition, percentage of N-half was significantly higher in RA samples than in OA samples ($p < 0.001$; Figure 3).

All OA patients had serum CRP levels within the normal range, whereas patients with RA showed elevated serum CRP, with mean level of 2.6 μg/ml. However, no significant correlation was found between serum CRP levels and syn-

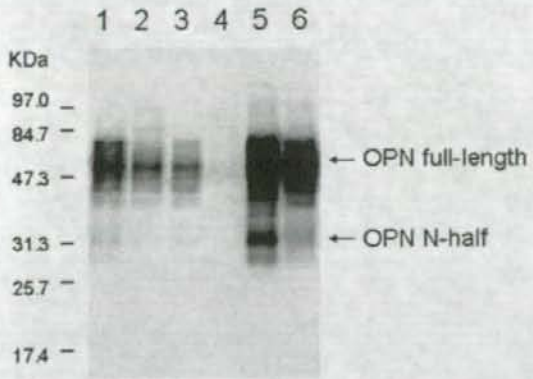

Figure 2. Western blot analyses by HRP-labeled anti-human OPN (O-17) Fab'. Synovial fluid results from patients with OA (lanes 1-3), a control patient (lane 4), and patients with RA (lanes 5, 6) are shown. Synovial fluid from patients with RA contained a considerable amount of thrombin-cleaved OPN.

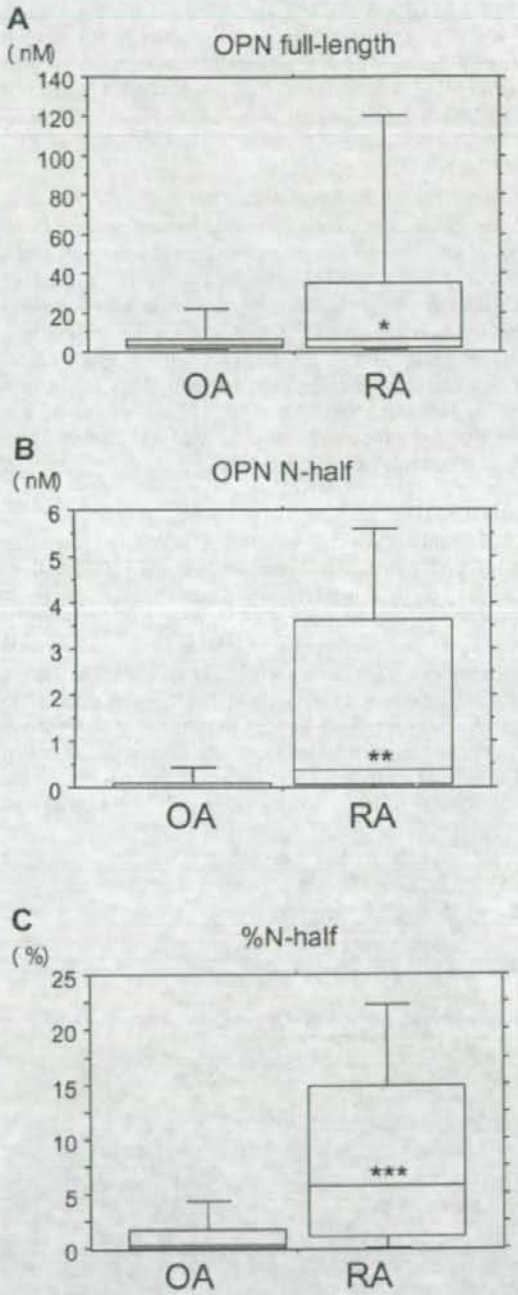

Figure 3. Synovial fluid concentrations of OPN full-length (A) and OPN N-half (B), and percentage of N-half (C). Lower and upper lines in the box represent 25th and 75th percentiles, with the median marked in the box. Bars show range of 10th and 90th percentiles. *p = 0.174, **p < 0.001, ***p < 0.001.

ovial fluid OPN N-half levels in patients with RA ($r = 0.343$, $p = 0.137$). The mean serum MMP-3 level in RA patients was 239 ng/ml. We found no significant correlation between serum MMP-3 and synovial fluid levels of OPN N-half ($r = 0.497$, $p = 0.116$). Similarly, synovial fluid levels of OPN N-half did not correlate with patient age ($r = -0.029$, $p = 0.457$) or BMI ($r = 0.018$, $p = 0.858$).

Localization of thrombin-cleaved OPN in synovial membrane. Synovial membrane samples showed positive labeling of OPN N-half in synovial lining cells and subintimal tissues in both RA and OA samples (Figure 4). The mean (± standard deviation) scores for synoviocytes in RA samples and OA samples were 2.0 ± 1.0 and 1.7 ± 0.7, respectively, and immunostaining for OPN N-half in synoviocytes showed no difference between RA and OA samples ($p = 0.539$). However, expression of OPN N-half was stronger in RA subintimal tissues (2.0 ± 0.6) compared with OA subintimal tissues (1.4 ± 0.5; $p = 0.019$).

DISCUSSION

OPN could be involved in arthritis, which is induced by many proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, and this protein is produced by macrophages in response to the activation by inflammatory stimuli[12]. Among its multiple properties, OPN, an extracellular matrix glycoprotein, has been shown to act as a T-helper type 1 immunoregulatory cytokine, and has been implicated in inflammatory responses through recruitment of inflammatory cells and augmentation of cytokine expression, including TNF-α and integrins[18]. OPN plays a pivotal role in the pathogenesis of RA in the mouse model[23]. Compared with arthritic wild-type mice, OPN-deficient mice are resistant to type II collagen-induced arthritis, showing attenuated joint swelling and cartilage destruction, chondrocyte apoptosis, and synovial angiogenesis[11]. Administration of an antibody directed against the SLAYGLR sequence, exposed by thrombin cleavage of murine OPN, has been shown to inhibit synovitis, bone erosion, and inflammatory cell infiltration in arthritic joints of animal models[18].

The microvascular network of the RA synovium has an increased permeability to plasma proteins, and morphological abnormalities of the microvasculature are well described[24,25]. Inflammatory changes in vessel permeability and bleeding from fragile capillaries in the hyperplastic synovium may allow plasma thrombin to enter the joint space. Vascular endothelial growth factor (VEGF) induces mRNA expression encoding $\alpha_v\beta_3$ integrin subunits and OPN, an $\alpha_v\beta_3$ ligand, in dermal microvascular endothelial cells[26]. Concomitant induction of both the receptor and ligand promotes endothelial cell migration. OPN N-half may be involved in functional changes of microvasculature and inflammatory cell infiltration. Gattorno, *et al*[27] reported that a positive correlation between OPN and VEGF concentrations was found in synovial fluid in juvenile idiopathic arthritis. In addition, OPN expression in the lining layer correlated with the number of vessels present in the areas underlying the sublining layer[27]. We need to study morphometric analysis of vessel walls and their expression of VEGF and cellular infiltration around the vessels. In addition, OPN is an autocrine/paracrine migratory and adhesive factor for tissue macrophages[28]. Macrophages in the synovium show expression of OPN[9]. OPN and VEGF are often expressed in

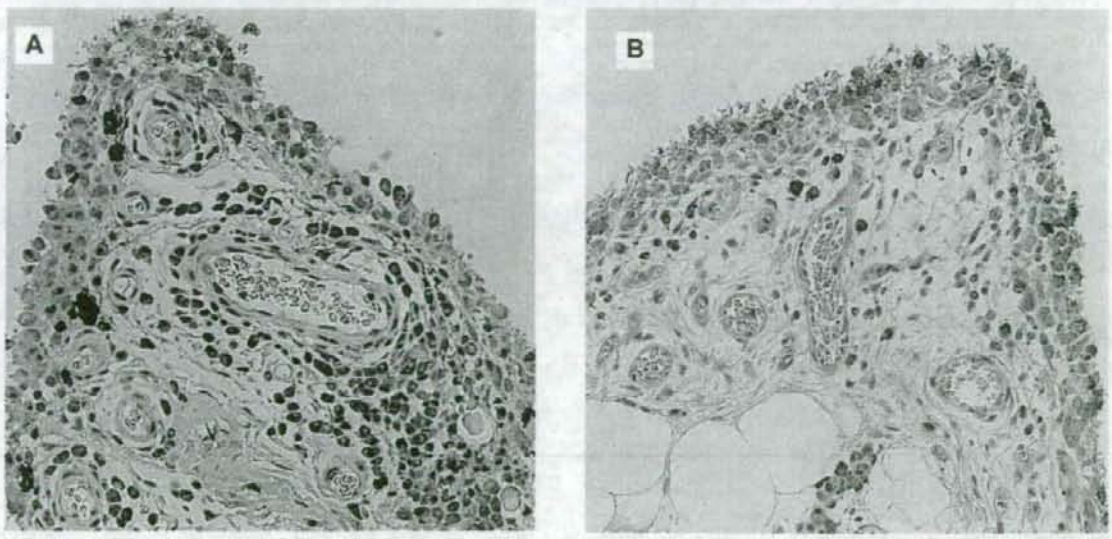

Figure 4. Expression of thrombin-cleaved OPN in synovium. Synovial lining and sublining cells stained for OPN in RA (A) and OA (B). Strong OPN staining was found in RA synovium (original magnification ×200).

close proximity to each other during angiogenesis[26,29]. Synovial macrophage-like cells produce thrombin as well as the components of extrinsic coagulation cascade in situ, promoting activation of coagulation pathways at biologically meaningful rates. Through its mitogenic effects, thrombin may elicit a cellular healing response inappropriate to the chronic inflammatory condition of the rheumatoid joints. It is capable of both causing the proliferation of synoviocytes and stimulating angiogenesis[17,30]. A study has revealed that thrombin concentration was higher in synovial fluid of patients with RA than in patients with OA[31]. Thrombin receptor-positive cells were present in rheumatoid synovia, but osteoarthritic and normal synovia contained few cells expressing thrombin receptors[32].

Results of our present immunohistochemical study revealed that thrombin-cleaved OPN is highly expressed in the rheumatoid synovium at both synovial lining cells and subintimal tissues. Petrow, *et al*[9] reported a similar expression pattern of intact OPN and mRNA by immunohistochemistry using monospecific affinity-purified rabbit antibody against human OPN and in situ hybridization, whereas Ohshima, *et al*[33] found that the expression was predominantly in synovial lining cells using a mouse monoclonal IgG anti-human OPN antibody, 4C1. Petrow, *et al*[9] showed no significant differences of OPN levels in synovial fluid between patients with RA and those with OA using a human OPN enzyme immunometric assay kit (Assay Designs, Ann Arbor, MI, USA). In contrast, Ohshima, *et al*[33] reported that non-thrombin-cleaved synovial fluid OPN levels of RA patients were significantly higher than those of OA patients. They used 2 distinct sandwich ELISA systems, which can detect only non-thrombin-cleaved OPN and both non-thrombin-cleaved and thrombin-cleaved OPN. For detecting only non-thrombin-cleaved OPN, a rabbit polyclonal anti-human OPN antibody, OPN 1, was used for a coating antibody and a mouse monoclonal anti-human OPN antibody, OPN 3, was used for a detecting antibody. For detecting both non-thrombin-cleaved and thrombin-cleaved OPN, another rabbit polyclonal anti-human OPN antibody, OPN 5, was used for a coating antibody, and OPN 1 was used for a detecting antibody generated by their laboratory[34]. One possible reason for the difference in these results may be the molecular fragility of OPN. OPN can easily be cleaved into 2 fragments by thrombin, because the thrombin cleavage site is present in the OPN molecule. We used a novel ELISA system that we developed[21]; the system can measure only thrombin-cleaved OPN directly.

Limitations of our study include the cross-sectional design of the trial and the small RA patient sample, and lack of analysis of serum levels of thrombin-cleaved OPN. However, to our knowledge, this is the first report to measure the synovial fluid levels of thrombin-cleaved OPN in RA directly, and we have demonstrated that levels of thrombin-cleaved OPN are elevated in RA compared with OA. A recent study demonstrated that neutralizing antibody against the cryptic epitope of OPN, which is exposed by thrombin cleavage, could be a future therapeutic choice for patients with RA[16].

Our study showed that local generation of thrombin-cleaved OPN was increased during rheumatic disease, indicating that the cleaved form may be a useful biochemical marker of RA. Further investigations are needed regarding the relationship between synovial fluid levels of thrombin-cleaved OPN levels and serum levels of thrombin-cleaved OPN, as well as progression of RA.

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