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Minimally invasive total knee arthroplasty: comparison of jig-based technique versus computer navigation for clinical and alignment outcome

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Abstract
Purpose Correct alignment of the leg and positioning of the components are important factors in good long-term outcome of total knee arthroplasty (TKA). Computer-assisted navigation systems were introduced to improve component alignment accuracies. The three main hypotheses of this study were that the navigated compared to jig-based patient will show the following: (1) No difference in clinical outcomes. (2) Better alignment in the frontal and sagittal plane. (3) Better rotational positioning of components.
Methods The authors evaluated 100 patients who had minimally invasive TKA using either an image-free computer-assisted navigation system ($n = 50$) or a jig-based technique ($n = 50$). Six months postoperatively, clinical and radiological evaluations were performed using full-length standing anteroposterior and lateral radiographs and CT scans of the knee.
Results Knee Society knee score, function score, and range of motion were comparable in the two groups after surgery. The percentage of patients with a frontal tibiofemoral angle within ±3° of the ideal was significantly higher in the navigated group than in the jig-based group (94% vs. 78%, respectively; $P = 0.041$). No significant differences were found between groups in terms of the frontal and sagittal planes as well as rotational alignment of the femoral or tibial components.
Conclusion Computer-assisted TKA gives a better correction of alignment of the leg compared with jig-based TKA when combined with a minimally invasive surgical approach.

Keywords Total knee arthroplasty · Navigation · Minimally invasive surgery · Image-free

Introduction

Minimally invasive (MIS) total knee arthroplasty (TKA) has gained popularity over the past several years [6, 14, 15, 24, 46]. MIS TKA is associated with rapid functional recovery and improvement in range of motion due to minimal soft-tissue damage [6, 14, 15, 23, 24, 46]. Several studies comparing MIS and conventional TKA showed no differences in radiographic findings between patients who underwent these two techniques [6, 20, 24]. However, the MIS approach has potential issues with component malpositioning arising from the limited surgical view [10]. Correct alignment of the leg and positioning of the components are important factors in good long-term outcome of TKA [16, 18, 25, 35]. A previous study reported that 4 of 30 patients who underwent MIS TKA had tibial components placed in 3° or more of varus malalignment, which did not occur in the conventional surgery group [10].

Computer-assisted navigation systems were introduced to improve component alignment accuracies. The navigation system and technique have been described precisely in the previous report by Stulberg et al. [44]. Numerous cohort studies have shown improved prosthetic alignment in association with the use of computer-assisted navigation compared with standard instrumentation [2, 13, 42]. The use of computer-assisted surgery may reduce the risk of malalignment associated with MIS. Biasca et al. [4] compared the mechanical accuracy of implants positioning after

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computer-navigated TKA with MIS versus conventional technique, and there were no outliers in the mechanical axis postoperatively in both groups. The authors compared the accuracy of navigation systems with MIS. The three main hypotheses of this study were that the navigated compared to jig-based patient will show the following: (1) No difference in clinical outcomes. (2) Better alignment in the frontal and sagittal plane. (3) Better rotational positioning of components.

Materials and methods

In this prospective study, 100 consecutive patients were divided into two groups (MIS computer-navigated group or MIS jig-based group) according to the day of the week when the surgery was done. Between 2007 and 2009, 50 patients each underwent primary MIS TKA using either an image-free computer-assisted navigation system (Orthopilot, Aesculap, Tuttlingen, Germany) or a jig-based technique without navigation. No exclusion criteria were defined in terms of age, gender, or severity of the deformity. Preoperative mechanical axis deviation in degrees of valgus was measured. There were no significant differences in demographic characteristics between groups (Table 1). Our institutional review board approved the study. Informed consent was provided by all patients.

All operations were performed by one experienced surgeon (MH) through a midline skin incision of 8–12 cm in length using a mini-midvastus approach without patellar eversion. Posterior stabilized (PS) designs were used for all cases, and all components (Columbus, Aesculap) were fixed with cement. In the navigated group, a balanced gap technique was used. Rotation of the femoral component was determined according to the navigation system after soft-tissue balancing; however, the surgeon checked to ensure that they were parallel to the surgical epicondylar axis [3]. In the MIS jig-based group, extramedullary instrumentation was used for the tibial component, and intramedullary instrumentation was used for the femoral side. The proximal tibia was resected perpendicular to the shaft of the tibia in the frontal plane with a posterior slope of 0° in the sagittal plane. For the distal femur, the intramedullary alignment guide was inserted slightly medial to the midpoint of the femoral condyles. This entry point was determined as the position where the intramedullary line of the femoral canal intersects the femoral condyles on the full-length anteroposterior radiographs [32]. The distal femoral cutting block was set to 6° from the alignment guide. After cutting the distal femur, the cutting block was set to 3° of external rotation from the posterior condylar line. In both groups, the rotational alignment of the tibial component was adjusted to the anteroposterior axis between the center of the cut surface and the border of the medial third of the tibial tuberosity [29, 31].

Table 1 Patient demographic data

	MIS computer-navigated group	MIS jig-based group
Male:female	13:37	7:43
Age (mean ± SD years)	73 ± 8	74 ± 7
BMI (mean ± SD kg/m^2)	25.8 ± 3.5	27.6 ± 5.2
Mechanical axis deviation (mean ± SD)[a]	−2.2 ± 12.4	−2.6 ± 8.2
Diagnosis		
Osteoarthritis	46	44
Rheumatoid arthritis	4	6

MIS minimally invasive surgery, *SD* standard deviation, *BMI* body mass index

[a] Negative values indicate varus alignment

The operative time and amount of blood loss were recorded. Clinical evaluations were performed using range of motion preoperatively and at 1 week, 3 weeks, 6 weeks, 3 months, and 6 months postoperatively as well as ratings according to the system of the Knee Society preoperatively and at 6 months postoperatively [17]. These ratings included a knee score and a function score [17].

Full-length standing anteroposterior and lateral radiographs and CT scans of the knee were carried out 3 weeks after surgery to determine the alignment of the components after surgery. The frontal mechanical axis of the leg was measured (tibiofemoral angle between a line connecting the center of the hip with the center of the knee and the line connecting the center of the knee to the center of the ankle). Zero degree was considered a straight mechanical axis; varus deviation was listed in negative values and valgus deviation in positive values. The authors assessed the frontal femoral component angle; lateral distal femur angle (LDFA), the angle between the mechanical axis of the femur and the transcondylar line of the femoral component as measured on the lateral side of the midline, neutral = 90° (Fig. 1); the frontal tibial component angle; medial proximal tibial angle (MPTA), the angle between the mechanical axis of the tibia and the tibial base plate as measured on the medial side of the midline, neutral = 90° (Fig. 1) [34]; the sagittal femoral component angle, the angle between the sagittal mechanical axis of the femur and the distal femoral cut line of the femoral component as measured on the posterior side of the midline, neutral = 90°; and the sagittal tibial component angle, the posterior slope angle of the tibial component as defined the angle between the sagittal mechanical axis of the tibia and the tibial base plate as measured on the posterior side of the midline, neutral = 90° (Fig. 2) [12]. The rotational alignment of the femoral and tibial components was evaluated

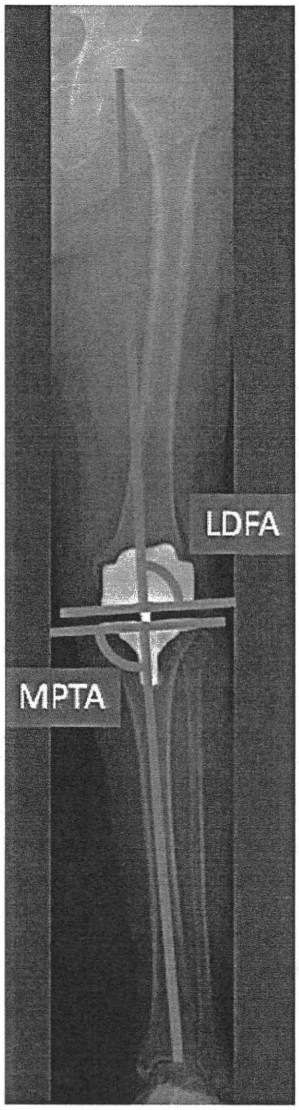

Fig. 1 Full-length standing anteroposterior radiograph. The parameters assessed in the frontal plane are *LDFA* lateral distal femur angle and *MPTA* medial proximal tibial angle

on CT scans. The rotational femoral component angle was the angle between the surgical epicondylar axis [3] and the posterior condylar line of the femoral component. The rotational tibial component angle was defined as the angle between a line connecting the center of the stem of the tibial component and the medial third of the tibial tubercle and a line perpendicular to the posterior border of the tibial keel. The rotational alignments of the femoral and tibial components were analyzed in 1° steps and three types of deviation: within normal (±3°), minor deviation (4–6°), and severe deviation (>6°). A resulting frontal tibiofemoral angle of within ±3° from neutral is the maximum acceptable deviation to minimize early failure [8, 11, 44].

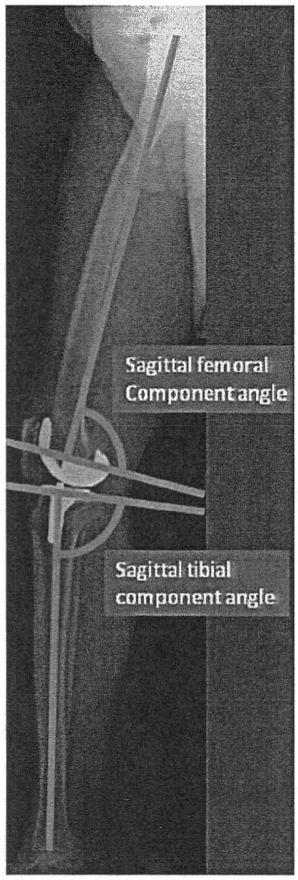

Fig. 2 Full-length standing lateral radiograph. The parameters assessed in the sagittal plane are sagittal femoral component angle and sagittal tibial component angle

Radiographs were assessed by one observer (AS) for twice measurements of angles; the observer was blinded to the surgical technique used. Intraobserver reliability was within 1° on all radiographs.

Statistical analysis

Mann–Whitney's *U* test was used for continuous variables. Fisher's exact test and chi-squared test were used for categorical data. $P < 0.05$ was considered statistically significant.

Results

Postoperative recovery of the patients was uneventful, and there were no infections or wound-healing disorders in both groups. There were no postoperative fractures at the sites of tracker pin insertions. Clinical outcomes are shown in Tables 2 and 3.

Table 2 Mean (±SD) clinical data

	MIS computer-navigated group	MIS jig-based group	*P* value
Operative time (min)	168 (18)	122 (16)	<0.001
Blood loss (ml)	211 (122)	208 (99)	n.s.
Range of motion			
Preoperative	111 (21)	109 (23)	n.s.
After 1 week	87 (18)	82 (21)	n.s.
After 3 weeks	106 (17)	109 (18)	n.s.
After 6 weeks	115 (14)	116 (14)	n.s.
After 3 months	119 (14)	119 (13)	n.s.
After 6 months	121 (13)	120 (13)	n.s.

MIS minimally invasive surgery, *n.s.* not significant ($P > 0.05$)

Table 3 Mean (±SD) clinical scores according to the Knee Society [17]

	MIS computer-navigated group	MIS jig-based group	*P* value
Knee score			
Preoperative	32 (18)	31 (17)	n.s.
Postoperative	95 (7)	92 (9)	n.s.
Function score			
Preoperative	43 (24)	37 (22)	n.s.
Postoperative	76 (16)	78 (14)	n.s.

MIS minimally invasive surgery, *n.s.* not significant ($P > 0.05$)

The percentage of patients with a frontal tibiofemoral angle within ±3° of the ideal in the MIS computer-navigated group was significantly higher than in the MIS jig-based group (Fig. 3. 47 [94%] vs. 39 [78%], respectively; $P = 0.041$). No other significant differences were noted between groups in terms of implant alignment (Tables 4, 5).

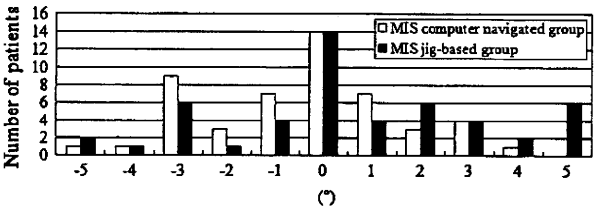

Fig. 3 Distribution of the postoperative mechanical leg axis in both surgical techniques

Discussion

This study showed no difference in clinical outcome between MIS computer-navigated and MIS jig-based TKA. MIS computer-navigated TKA resulted in significantly more accurate limb alignment than that achieved by MIS jig-based TKA; however, no significant differences were found between groups in terms of the frontal and sagittal planes as well as rotational alignment of the femoral or tibial components.

Many studies have demonstrated that TKA with computer navigation leads to more accurate implantation [2, 9, 12, 13, 27, 37, 42], but other reports have found no differences [21, 22, 45]. Recent meta-analyses noted fewer patients outside the critical ranges of 3° varus or valgus malalignment with navigated surgery [1, 28]. However, the authors are not aware of any studies that have established whether these improvements in alignment accuracy with navigated surgery are associated with superior Knee Society knee scores and function scores or range of motion [9, 12, 22, 45]. There were no differences in Knee Society knee scores and function scores between MIS computer-navigated and MIS jig-based TKA soon after surgery. There was limitation of the power of the scores because of ceiling effect. MIS TKA and computer-assisted navigation are both new techniques that have recently been used in combination. However, both represent new technologies that still have unproven benefits over conventional methods [6].

Several studies have compared MIS TKA using navigation with jig-based TKA (Table 6) [2, 5, 9, 12, 26, 38]. In this study of 100 patients, the frontal tibiofemoral alignment was reestablished to within ±3° of neutral in 94% of the patients in the MIS computer-navigated group compared with 78% of those in the MIS jig-based group. The accuracy in achieving a neutral frontal tibiofemoral alignment with MIS in the jig-based group was comparable with the results of other reports, including conventional computer-navigated TKA (range 68–98%) [2, 12, 22, 26, 38]. In the sagittal alignment, Dutton et al. [12] reported the improved accuracy with regard to the sagittal tibial alignment in the computer-navigated group. However, there was no significant difference between the computer-navigated group and jig-based group with regard to the sagittal femoral alignment. Other studies [8, 41] showed the improved accuracy with regard to sagittal femoral alignment; however, there was no difference in sagittal tibial alignment. This study showed no differences in the

Table 4 Number (%) of patients with implant alignment within ±3° from neutral

	MIS computer-navigated group	MIS jig-based group	*P* value
Frontal femoral component angle	50 (100)	46 (92)	n.s.
Frontal tibial component angle	49 (98)	46 (92)	n.s.
Sagittal femoral component angle	46 (92)	38 (76)	n.s.
Sagittal tibial component angle	47 (94)	41 (82)	n.s.
Frontal tibiofemoral angle	47 (94)	39 (78)	0.041

MIS minimally invasive surgery, *n.s.* not significant ($P > 0.05$)

Table 5 The differences of absolute value from the target angle of rotational femoral and tibial components

	MIS computer-navigated group	MIS jig-based group	*P* value
Rotational femoral component angle			
Mean ± SD in 1° steps (°)	1.1 ± 1.3	0.9 ± 1.2	n.s.
Number (%) within normal (±3°)	48 (96)	48 (96)	
Number (%) with minor deviation (4°–6°)	2 (4)	2 (4)	n.s.
Number (%) with severe deviation (>6°)	0 (0)	0 (0)	
Rotational tibial component angle			
Mean ± SD in 1° steps (°)	2.3 ± 2.5	3.2 ± 3.0	n.s.
Number (%) within normal (±3°)	33 (66)	30 (60)	
Number (%) with minor deviation (4°–6°)	13 (26)	14 (28)	n.s.
Number (%) with severe deviation (>6°)	4 (8)	6 (12)	

MIS minimally invasive surgery, *SD* standard deviation, *n.s.* not significant ($P > 0.05$)

Table 6 Comparison studies of MIS computer-navigated versus jig-based total knee arthroplasty

Author	Year	MIS computer-navigated group[a] (%)	Jig-based group[a]		*P* value
			Conventional (%)	MIS (%)	
Bäthis et al. [2]	2004	96	78		0.001
Seon et al. [38]	2007	95	81		0.043
Dutton et al. [12]	2008	92	68		0.003
Lüring et al. [26]	2008	100	93	90	n.s.
Confalonieri et al. [9]	2007	100		84	0.025
Bonutti et al. [5]	2008	94		98	n.s.
Present study		94		78	0.041

MIS minimally invasive surgery, *n.s.* not significant ($P > 0.05$)

[a] Percentage of patients with coronal mechanical axis of the leg within ±3° from neutral

sagittal femoral and tibial alignments between the two groups. There are limited data available on rotational alignment after computer-navigated TKA. Stöckl et al. [43] and Chauhan et al. [7] found that the image-free navigation systems achieved significantly better rotational alignment of the femoral component than did conventional surgery. However, other studies [30, 39, 40] showed that the rotational alignments of femoral and tibial components were not improved through navigation. The authors are not aware of any published study comparing the rotational alignment of MIS computer-navigated TKA and MIS jig-based TKA. This study failed to show that using the navigation systems lead to an improvement in femoral and tibial rotational alignments in MIS TKA.

Navigation systems have been associated with increased operation times [6, 8, 12, 22, 26, 36, 45], higher expenses [33], and higher complication rates, especially femur fractures [6, 19]. The mean operative time was significantly longer in computer-navigated surgery in the present study. This was associated with time-consuming base requirements of image-free computer-assisted surgery including positioning of reference arrays, registration of the instruments, and digitization of the knee. However, the intraoperative feedback with regard to resection, implant, and limb alignment provided by computer-navigated surgery offers surgeons an opportunity to improve their judgment with regard to the accuracy with which they perform and evaluate each step of the TKA procedure [45].

There are several limitations to this study. First, the authors did not use the same landmarks for femoral rotation in the navigated and conventional study arms, and this study included the use of 6° of frontal intramedullary

correction angle at the femur and 3° of femur external rotation for all cases in the jig-based group. Another potential limitation is that the surgeon did not have extensive experience using computer-assisted navigation, which may have affected outcomes. Finally, the follow-up periods were short. However, the aim of this study was to assess the rate of functional recovery rather than the longevity of the prosthesis. As there were no functional differences between the two groups by 6 months, and as the patients demonstrated improvement in comparison with their preoperative clinical parameters by 6 months, the authors decided that the data collected at this time were sufficient for an analysis of this endpoint [12]. One of the strength of this study was that the authors used long-standing radiographs pre- and postoperatively, and used postoperative CT for measurements.

Conclusion

There were no differences between MIS computer-navigated and MIS jig-based TKA concerning clinical outcomes, and the first hypothesis can be disproved. MIS computer-navigated TKA resulted in significantly more accurate limb alignment than that achieved by MIS jig-based TKA, and the second hypothesis was partially proved. However, both frontal and sagittal alignments of each component were similar between groups. In addition, rotational positioning of components was similar, and therefore the third hypothesis cannot be verified. Both the MIS and computer-navigated techniques have now been refined and can be used safely in combination. Additional experience with these techniques and longer follow-up periods may reveal further information about the usefulness of these procedures [5].

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Mortality after vertebral fractures in a Japanese population

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Mortality after vertebral fractures in a Japanese population

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ABSTRACT

Purpose. To assess the association between vertebral fractures and mortality.

Methods. 419 women and 210 men aged 60 to 98 (mean, 73) years participated in an osteoporosis screening exercise. Patient age, gender, comorbidity, lumbar pain, smoking, and alcohol consumption were recorded, as were the number of vertebral fractures and bone mineral density. Vertebral fractures were evaluated using lateral radiographs and quantitative morphometry. Anterior, central, and posterior vertebral heights were measured; vertebral fractures were defined as a decrease of ≥20% in any of these heights.

Results. 131 (21%) of the participants had vertebral fractures. At the 10-year follow-up, 121 (19%) of the participants (55 men and 66 women) had died; 43 of them had vertebral fractures and 78 did not. The respective 10-year survival rates for participants with and without vertebral fractures were 69% and 86% ($p<0.0001$). The survival rate was lower in those with greater number of vertebral fractures (76% for those with one or 2 fractures and 50% for those with ≥3 fractures). Multiple regression analysis showed that advanced age ($p<0.0001$), male gender ($p=0.003$), and presence of vertebral fractures ($p=0.013$) correlated significantly with survival.

Conclusion. The presence and number of vertebral fractures were associated with mortality. Prevention of vertebral fractures may be important for improving the prognosis of patients with osteoporosis.

Key words: *epidemiology; mortality; osteoporosis; spinal fractures*

INTRODUCTION

Vertebral fractures are often asymptomatic, and therefore many patients with this condition do not seek medical attention. The incidences of vertebral fractures in the Wakayama and Hiroshima areas in Japan are 40 and 84 per 1000 person-years for women in their 70s and 80s, respectively.[1-3] Such

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incidence is higher among Japanese than European subjects.[3,4] Vertebral fractures[5–13] and hip fractures[14,15] affect mortality. We therefore assessed the association between vertebral fractures and mortality in 633 subjects.

MATERIALS AND METHODS

In December 1997, 419 women and 210 men aged 60 to 98 (mean, 73) years from the mountain village of Miyagawa in central Mie Prefecture in Japan participated in an osteoporosis screening exercise. The ethics committee of our university approved the study protocol, and informed consent was obtained from each participant.

Patient age, gender, comorbidity, lumbar pain, smoking, and alcohol consumption were recorded (Table 1), as were the number of vertebral fractures and bone mineral density. Alcohol consumption was categorised as frequent (nearly every day), sometimes (one to 4 times per week), or seldom (less than once per week). Bone mineral density of the non-dominant, distal forearm was measured using dual energy X-ray absorptiometry.

Fractures of the thoracic and lumbar vertebrae were evaluated using lateral radiographs and quantitative morphometry. Anterior, central, and posterior vertebral heights were measured; vertebral fractures were defined as a decrease of ≥20% in any of these heights.

The 10-year survival/mortality rate of 629 participants (4 dropped out) was assessed by reviewing medical histories, telephone interviews, and death certificates.

Univariate analysis for factors that could influence prognosis (lumbar pain and alcohol consumption) was conducted using the Kaplan-Meier graphs. Intergroup comparisons were made using the log rank test. Multiple regression analysis was performed using the stepwise Cox proportional hazards model. Time to event was computed as the number of years that elapsed between each participant's respective final wave of testing and the date of death (for decedents) or the end of follow-up in March 2007 (for surviving participants). A p value of <0.05 was considered statistically significant.

RESULTS

131 (21%) of the participants had vertebral fractures. At the 10-year follow-up, 121 (19%) of the participants (55 men and 66 women) had died; 43 of them had vertebral fractures and 78 did not. The causes of death in 91 participants are shown in Table 2.

The respective 10-year survival rates for participants with and without vertebral fractures were 69% and 86% ($p<0.0001$, Fig. a). The survival rate was lower in those with greater number of vertebral fractures (76% for those with one or 2 fractures and 50% for those with ≥3 fractures), with the mean number of vertebral fractures being 2.3. The difference between those with ≥3 fractures and those with one or 2 fractures ($p=0.004$) or those without

Table 1
Patient characteristics at baseline

Characteristic	No. of patients
No. of female:male	419:210
Mean (range) age (years)	73 (60–98)
Mean (range) height (cm)	150 (105–181)
Mean (range) weight (kg)	52 (30–76)
Mean (range) body mass index (kg/m²)	23 (16–33)
Bone mineral density (% of young adult mean value)	
≥80	317
70–79	121
<70	191
Comorbidity	
Diabetes mellitus	57
Gout	9
Lumbar pain	327
Smoking	
Smoker	116
Non-smoker	513
Alcohol consumption	
Frequent	87
Sometimes	34
Seldom	504

Table 2
Cause of death of participants

Cause of death	No. of participants	
	With vertebral fracture	Without vertebral fracture
Pneumonia	7	9
Myocardial infarction	5	8
Lung cancer	3	4
Acute renal failure	3	3
Brain infarction	0	5
Congestive heart failure	2	1
Other causes	14	27
Unknown	9	21
Total	**43**	**78**

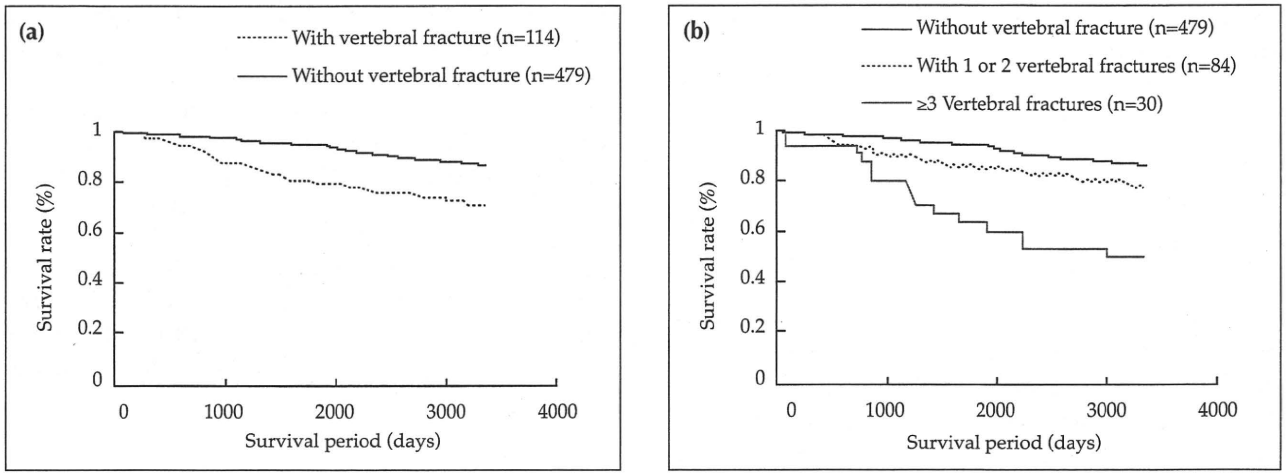

Figure The 10-year survival rates were significantly higher in participants without vertebral fractures than **(a)** those with vertebral fractures (p<0.0001) or **(b)** those with ≥3 vertebral fractures (p<0.0001) or one to 2 fractures (p=0.004).

fractures (p<0.0001) was significant after Bonferroni correction (Fig. b).

The respective survival rates for women and men were 86% and 77% (p=0.006). The survival rates of participants in their 60s and 70s were both significantly higher than those in their 80s (p<0.0001).

The respective survival rates for participants with ≥80%, 70–79%, and <70% of the mean bone mineral density expected in young adults were 86%, 82%, and 77%. The difference between those with <70% and ≥80% of that bone mineral density was significant (p=0.011).

The respective survival rates for participants with and without lumbar pain were 83% and 82% (p=0.868). The corresponding rates were 74% and 84% for smokers and non-smokers (p=0.012) and were 82%, 86%, and 82% for those who seldom, sometimes, and frequently drank alcohol (p=0.829).

The survival rate was significantly lower in participants of male gender, advanced age, with ≥3 vertebral fractures, low bone mineral density, and smoking habit. Neither lumbar pain nor alcohol consumption was associated with survival.

Multiple regression analysis was performed using the Cox proportional hazards model. The stepwise method showed that advanced age (p<0.0001), male gender (p=0.003), and presence of a vertebral fracture (p=0.013) correlated significantly with survival (Table

Table 3
Risk of death in association with vertebral fractures*

Variable	Hazards ratio	95% CI	p Value
Vertebral fracture (yes vs. no)	1.72	1.12–2.65	0.013
Age (years)			
60s	0.07	0.04–0.14	<0.0001
70s	0.18	0.12–0.27	<0.0001
80s	1.00		
Gender (men vs. women)	1.82	1.22–2.72	0.003
Vertebral fracture (≥3 vs. none)	3.28	1.77–6.10	<0.0001
Vertebral fracture (1 or 2 vs. none)	1.33	0.80–2.22	0.271
Age (years)			
60s	0.08	0.04–0.15	<0.0001
70s	0.18	0.12–0.28	<0.0001
80s	1.00		
Gender (men vs. women)	2.04	1.34–3.10	0.001

* Bone mineral density, smoking, alcohol consumption, lumbar pain are not selected

3). Participants with vertebral fractures was 1.7 fold more likely to die than those without vertebral fractures (p=0.013, Table 3), whereas participants with ≥3 vertebral fractures was 3.3 fold more likely to die than those without fractures (p<0.0001, Table 3).

DISCUSSION

The 5-year survival rates for individuals with vertebral fracture were 28% in Sweden,[5] and 61% in the United States.[6] Both rates were lower than the 10-year survival rate of our participants.

The number of vertebral fractures is associated with mortality.[7,8] Women with one, 2, and ≥3 vertebral deformities had 1.3, 2.5, and 3.9 fold greater risks of mortality, respectively, than those without deformities.[9] In our study, the stepwise Cox proportional hazards model indicated that both the presence and the number of vertebral fractures were associated with mortality.

The effects of vertebral fractures on mortality remain unknown. Vertebral fractures may cause kyphosis, and may exacerbate dysfunction of various organs, particularly the thoracic organs. Patients may become more susceptible to lung and heart diseases, thus affecting prognosis. The degree of kyphosis is significantly associated with the risk of subsequent pulmonary death.[8] Women with vertebral deformities have an increased risk of death due to cardiovascular disease and cancer, compared to women without such deformities.[9] Osteoporosis is associated with atherosclerosis[16–19] and cardiovascular mortality.[20,21]

Multiple vertebral fractures may cause esophageal hiatal hernia and esophagitis,[22,23] and restrict physical function, activities of daily living, and quality of life.[24,25] As the size of the elderly population grows, the incidences of osteoporosis and accompanying vertebral fractures are anticipated to increase rapidly. Preventing vertebral fractures may be important for improving patient prognosis.[10]

Our study had several limitations. It is necessary to follow up patients with low numbers of vertebral fractures to evaluate how their prognosis is affected compared to those with more fractures. Although patients with poor health are at increased risk of mortality and tend to become bedridden after vertebral fracture, activities of daily living do not always worsen in such patients. Thus, further investigation into the association between restriction in activities of daily living and health are warranted. Our sample was selected from a limited geographic region, which may not be representative of Japan as a whole.

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Regular Article

Incidence and time course of asymptomatic deep vein thrombosis with fondaparinux in patients undergoing total joint arthroplasty

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ABSTRACT

Introduction: There are many reports concerning fondaparinux prophylaxis of asymptomatic deep vein thrombosis (DVT) after total hip arthroplasty (THA) or total knee arthroplasty (TKA), but little is known about the time course of aymptomatic DVT development during the administration of fondaparinux. The aim of the present study was to define the incidence and time course of aymptomatic DVT development during administration of fondaparinux, and to assess the efficacy of fondaparinux in resolving DVT.

Materials and Methods: We studied consecutive71 patients who underwent THA surgery, and 30 patients who underwent TKA surgery with fondaparinux prophylaxis. Patients received once-daily subcutaneous injections of 2.5 mg of fondaparinux for 14 days after surgery. DVT was diagnosed by ultrasonography, and it was scheduled on the day of surgery on day 1, day 4, and day 14 after surgery.

Results: In patients who received fondaparinux for 14 days after THA surgery, the incidence of DVT was 0% on the day of the surgery, 13.6% at day 1, 27.1% at day 4, and 11.9% at day 14. In patients who received fondaparinux for 14 days after TKA surgery, the incidence of DVT was 4.2% on the day after surgery, 50.0% at day 1, 58.3% at day 4, and 20.8% at day 14. The incidence of DVT after THA or TKA surgery at day 14 was significantly reduced compared to that at day 4.

Conclusion: The incidence of asymptomatic DVT up to day 4 was high, but with 14 days continued treatment of fondaparinux, the incidence of asymptomatic DVT occurring at postoperative day 4 was significantly reduced at day 14.

1. Introduction

Patients undergoing total hip arthroplasty (THA) or total knee arthroplasty (TKA) have a high risk for postoperative venous thromboembolism (VTE). Among Western patients who do not receive any prophylaxis therapy, the prevalence of venographic deep vein thrombosis (DVT) ranges from 40 to 85% after THA or TKA surgery [1]. DVT often begins intraoperatively, and about half resolve spontaneously, without treatment [2] and [3]. However, if asymptomatic DVTs extend to the proximal veins, patients can progress to symptomatic pulmonary thromboembolism (PTE). Some DVTs embolise, resulting in a PTE that is fatal in 0.1-2.0% of unprotected operated patients [1]. Thus, VTE remains an important complication of total joint arthroplasty surgery, and thromboprophylactic treatment needs to be improved in total joint arthroplasty surgery. The 8th American College of Chest Physicians (ACCP) Guidelines recommend use of fondaparinux along with a low molecular weight heparin (LMWH) and a vitamin K antagonist for the prevention of VTE [1].

Fondaparinux is a synthetic pentasaccharide that acts as a specific inhibitor of factor Xa with no direct inhibition of thrombin. The antithrombotic activity of fondaparinux is due to its selective binding to antithrombin III. This inhibition of factor Xa via antithrombin results in effective inhibition of thrombin generation [4], [5] and [6]. In a study of fondaparinux for the prevention of VTE in patients undergoing THA, 0.75 to 8.0 mg fondaparinux once daily, starting 6 hours postoperatively, demonstrated a statistically significant dose response, with higher doses being associated with a lower incidence of VTE [7]. Moreover, recent studies of patients undergoing THA or TKA surgery suggested that a once-daily subcutaneous injection of 2.5 mg fondaparinux reduced the risk of VTE more than dose LMWH [8], [9] and [10]. However, little is known about the time course of asymptomatic DVT development during the administration of fondaparinux. The aim of the present study was to define the incidence and time course of aymptomatic DVT development during administration of fondaparinux, and to assess the efficacy of fondaparinux in resolving DVT.

2. Materials and Methods

2.1. Study Design

This study was a prospective clinical trial, and conducted between December 2007 and December 2008 at Mie University Hospital, Mie,

Abbreviations: DVT, deep vein thrombosis; THA, total hip arthroplasty; TKA, total knee arthroplasty; VTE, venous thromboembolism; PTE, pulmonary thromboembolism; ACCP, American College of Chest Physicians; LMWH, low molecular weight heparin.

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Japan. The study was performed in accordance with the Declaration of Hershinki. The protocol was approved by the Ethics Committee of the University of Mie, and before enrolment in the study, all patients gave written informed consent. Patients had received the thromboprophylaxis with fondaparinux for 14 days after THA or TKA surgery. Ultrasonography, the outcome assessment was performed on the day of surgery, day 1, day 4, and day 14 after surgery.

2.2. Patients

We studied consecutive patients who received prophylaxis with fondaparinux after THA or TKA surgery. There were 71 patients who underwent THA surgery, and 30 patients who underwent TKA surgery between December 2007 and December 2008. We excluded patients who had the following criteria: (1) younger than 18 years age, (2) body weight less than 40 kg, (3) renal failure (serum creatinine concentration >1.5 mg/dl or liver insufficiency, (4) a known hypersensitivity to heparin, (5) chronic or acute DVT within 7 days before surgery as determined by ultrasonography examination, or (6) ultrasonographic examinations were technically inadequate.

2.3. Ultrasonography

Bilateral ultrasonography of the lower extremities (equipment: a 7.5 or 10 MHz linear probe with pulse and color Doppler, ProSound α5SV, Aloka Company, Tokyo, Japan) was performed by highly skilled physicians. Veins were compressed every 1-2 cm, moving from the proximal to the distal end of the venous segment. The following venous segments were visualized: calf muscle veins, anterior and posterior tibial veins, popliteal vein, femoral vein, and common femoral vein. The results of ultrasonography were considered positive if a vein or venous segment was not fully compressible. Pulse and color Doppler modalities could be used for anatomic orientation and venous examination, but not for documentation of the venous findings. Thigh veins were examined with the patient in a supine position; popliteal and calf veins were examined while the patient was sitting. Patients with thrombi were classified by thrombi location: below the popliteal level within the calf veins (distal thrombi) or at the popliteal and femoral veins (proximal thrombi). In patients after total joint arthroplasty, ultrasonography was scheduled on the day of surgery as well as on day 1, day 4, and day 14 after surgery.

2.4. Prophylaxis

All patients received continuous epidural cathetel analgesia from the day of surgery to postoperative 1 day and were removed at least 2 h before the first injection of fondaparinux. Moreover, all patients wore intermittent pneumatic compression stockings from the day of surgery to postoperative day 2, and wore graduated compression stockings continuously during the 3 months after surgery. About the dosage regimen of fondaparinux used in the European and the North American clinical study [8], [9] and [10], patients received a once-daily subcutaneous injection of 2.5 mg fondaparinux for 5-9 days following total joint arthroplasty surgery, and the first postoperative dose was given 6-8 h after surgery. However, in Japanese randomized dose-ranging clinical trial [11], the first postoperative dose was given 24 h after surgery, and the treatment was continued for 10-14 days. Therefore, in our study, all patients received a once-daily subcutaneous injections of 2.5 mg of fondaparinux after surgery, and the first administration was performed 24 hours postoperatively and the second administration was performed more than 12 hours after the first and treatment was scheduled to continue for 14 days. All patients were encouraged to perform exercises in bed immediately after surgery, and patients were allowed to walk in the physical therapy department on day 5 after total joint arthroplasty surgery.

2.5. Outcome measures

Outcome measures included the incidence and time course of asymptomatic DVT and safety end points up to 14 days after surgery. The primary efficacy outcome was assessed by the incidence of asymptomatic DVT occurring at each time point in patients who received fondaparinux for 14 days. The secondary efficacy outcome was the comparison of incidence of asymptomatic DVT between at 4 day and 14 days after surgery in patients who received fondaparinux for 14 days. DVT was determined by ultrasonography of the legs. The primary safety outcome was the incidence of major bleeding. Major bleeding included fatal bleeding; bleeding that was retroperitoneal, intracranial, or intraspinal or that involved any other critical organs; bleeding leading to reoperation; and overt bleeding with a bleeding index of 2 or more. Bleeding index was calculated as the number of units of packed red cells or whole-blood transfused (except autologous blood) plus the hemoglobin value before the bleeding episode minus the hemoglobin value after the episode. Fondaparinux was discontinued if major bleeding, transfusion of more than 2 packed red blood cells, or whole blood transfusion occurred during fondaparinux prophylaxis, or if anemia (a decrease in hemoglobin of >2 g/dl from day 1) occurred.

2.6. Statistical analysis

Our study was designed to demonstrate the efficacy of fondaparinux in resolving DVT with 14 days of treatment using fondaparinux. The primary safety analysis included data on patients who had received at least 1 dose of study medication. A p value of less than 0.05 indicated statistical significance. The chi-square test was used to determine if there was a significant difference about the analysis of efficacy and safety outcomes.

3. Results

Demographic data for both patients groups, divided by type of surgery, are shown in Table 1. The incidence of overall asymptomatic DVT, the location, major bleeding in these groups are listed in Table 2. No patient who underwent THA or TKA surgery had symptomatic or fatal pulmonary thromboembolism up to 14 day after surgery.

3.1. Patients receiving 14 days of fondaparinux

Of the 71 patients undergoing THA surgery, 59 patients received fondaparinux for 14 days, and 12 patients discontinued fondaparinux before 14 days. Reasons for discontinuing treatment with fondaparinux are listed in Table 3. Eighteen of the 59 patients who received fondaparinux for 14 days had asymptomatic DVT within 14 days assessed by ultrasonography examination. No patient had thrombosis on the day of the surgery, but 8 patients had new thrombosis at day 1, 16 patients had thrombosis at day 4 (8 with new thrombosis), and 7 patients had thrombosis at day 14 (11 of 16 thromboses occurring at day 4 resolved, and 2 patients had new thrombosis at day 14). Thus, in patients who received fondaparinux for 14 day after THA surgery, the incidence of aymptomatic DVT was 0% on the day of the surgery, 13.6%

Table 1
Baseline characteristics of patients undergoing THA or TKA surgery.

Characteristics	THA surgery (n=71)	TKA surgery (n=30)
Age (years), median (range)	63.0 34–84	73.3 53–82
Gender, men/women	11/60	5/25
BMI (kg/m²), median (range)	23.8 16.4–35.4	26.0 19.2–34.0
Diagnosis		
Osteoarthritis	69/71	28/30
Rheumatoid arthritis	2/71	2/30

THA, total hip arthroplasty; TKA total knee arthroplasty.

Table 2
Incidence of asymptomatic deep vein thrombosis, the location, and major bleeding.

Characteristics	THA surgery (n=71)	TKA surgery (n=30)
All DVT (%)	20/71 (28.2)	17/30 (56.7)
Proximal+Distal DVT (%)	1/20(5.0)	1/17 (5.9)
Proximal DVT (%)	0/20 (0)	0/17 (0)
Distal DVT (%)	19/20 (95.0)	16/17((94.1)
Symptomatic PTE (%)	0 (0)	0 (0)
Fatal PTE (%)	0 (0)	0 (0)
Major bleeding (%)	1 (1.4)	1 (3.3)

THA, total hip arthroplasty; TKA total knee arthroplasty.

at day 1, 27.1% at day 4, and 11.9% at day 14. The incidence of aymptomatic DVT at day 14 was significantly reduced compared to that at day 4 ($p=0.036$).

Of the 30 patients undergoing TKA surgery, 24 patients received fondaparinux for 14 days, and 6 patients discontinued fondaparinux before 14 days. Reasons for discontinuing treatment with fondaparinux are listed in Table 3.

Fifteen of 24 patients who received fondaparinux for 14 days had asymptomatic DVT within 14 days assessed by ultrasonography examination. No patient had symptomatic or fatal pulmonary thromboembolism. One patient had new thrombosis on the day of the surgery, 12 patients had thrombosis at day 1 (11 with new thrombosis), 14 patients had thrombosis at day 4 (1 of 12 DVT occurring at day 1 resolved at day 4, and 3 patients had new thrombosis at day 4), and 5 patients had thrombosis at day 14 (9 of 14 DVT occurring at day 4 resolved at day 14, and no patient had new thrombosis at day 14). Thus, in patients who received fondaparinux for 14 day after TKA surgery, the incidence of aymptomatic DVT was 4.2% on the day after surgery, 50.0% at day 1, 58.3% at day 4, and 20.8% at day 14. The incidence of aymptomatic DVT at day 14 was significantly reduced compared to that at day 4 ($p=0.008$). The incidence and each time point of asymptomatic DVT over 14 days of fondaparinux therapy are shown in Fig. 1.

3.2. Safety outcomes

There was no fatal bleeding, bleeding in a critical organ, or bleeding leading to reoperation in either treatment group. In this study, major bleeding during the treatment period was the primary safety endpoint in fondaparinux-treated patients. After THA surgery, there was 1 major bleeding event (1.4%) among the 71 fondaparinux-treated patients. After TKA surgery, there was 1 major bleeding event (3.3%) among the 30 fondaparinux-treated patients.

4. Discussion

This study demonstrated that the incidence of asymptomatic DVT after THA or TKA surgery with treatment of fondaparinux was high at day 4, but among patients who received fondaparinux for 14 days, the incidence of asymptomatic DVT was significantly reduced at day 14 compared to that at day 4. To our knowledge, this report is the first to show the time course of the thrombolytic effects of fondaparinux after joint arthroplasty.

Table 3
Reasons for discontinuing fondaparinux prophylaxis.

Characteristics	THA surgery (n=71)	TKA surgery (n=30)
Major Bleeding		
Bleeding index of 2 or more	1	1
Other major bleeding	0	0
Transfusion	0	1
Subcutaneous hematoma at surgical site	1	0
Worsening of anemia without major bleeding	10	4
Total	12	6

THA, total hip arthroplasty; TKA, total knee arthroplasty.

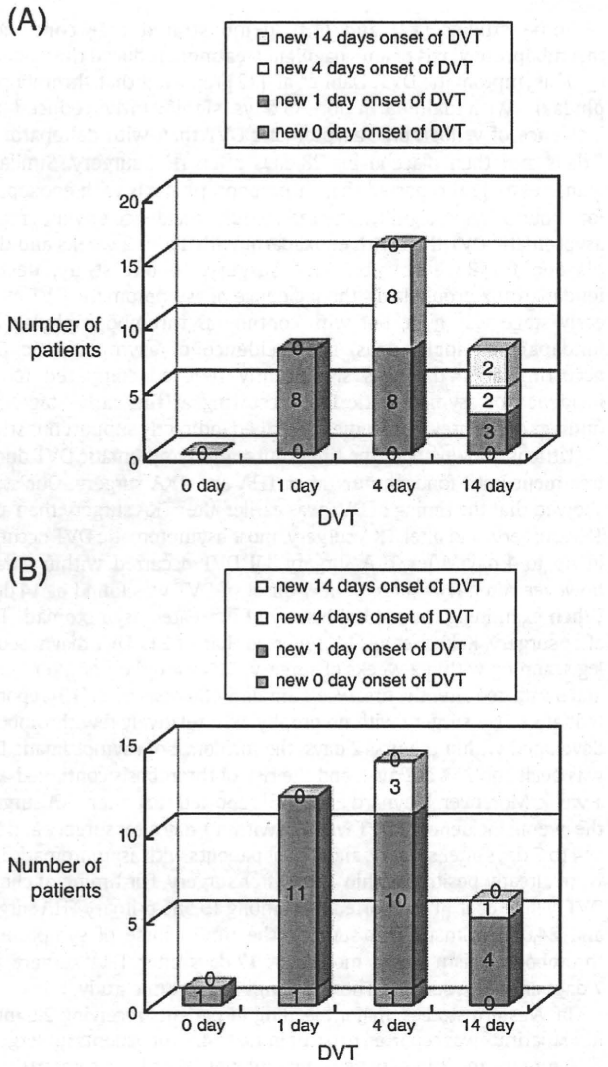

Fig. 1. Time course of aymptomatic DVT with 14 days of fondaparinux treatment after (A) total hip arthroplasty (THA) surgery and (B) total knee arthroplasty (TKA) surgery. The number of newly developed DVT at each time point are shown. For both A and B, please change the text in the key to the following: New onset DVT, day 0; New onset DVT, day 1; New onset DVT, day 4; New onset DVT, day 14. For both A and B, please change the x axis to read Day 0, Day 1, Day 4, and Day 14.

In patients who received fondaparinux for 14 days, the incidence of ultrasonographic asymptomatic DVT occurring at Day 4 was 27.1% after THA surgery and 58.3% after TKA surgery, and it was twice after TKA surgery compared to after THA surgery. In patients who received no prophylaxis, randomized clinical trials have demonstrated venographic DVT rates 7 to 14 days following THA surgery and TKA surgery of 42 to 57% and 41 to 85%, respectively [1]. These findings are similar to our results regarding DVT incidence at day 4 with fondaparinux prophylaxis. However, with 14 days treatment of fondaparinux, the incidence of ultrasonographic asymptomatic DVT occurring at Day 14 was 11.9% after THA surgery and 20.8% after TKA surgery. Randomized, double-blind, large clinical trials examining the efficacy of fondaparinux have shown an incidence of venographic asymptomatic DVT at 5 to 11 days after surgery of 4.0-5.6% after THA surgery and 12.5% after major knee surgery[8], [9] and [10]. In those trials, fondaparinux 2.5 mg was given for 5-9 days starting 6 ± 2 hours after surgery[8], [9] and [10]. The incidence of asymptomatic DVT at Day 14 in our study was similar to that in Western studies.

Some studies [12] and [13], demonstrated that continuous thromboprophylaxis of anticoagulant treatment reduced the frequency of asymptomatic DVT. Dahl et al. [12] reported that thromboprophylaxis with dalteparin for 35 days significantly reduced the incidence of venographic asyptomatic DVT than with dalteparin for 7 days and then placebo for 28 days after THA surgery. Similarly, Planes et al. [13] reported that thromboprophylaxis with enoxaparin for about 5 weeks significantly reduced the incidence of venographic asyptomatic DVT than with enoxaparin with about 2 weeks and then placebo for 3 weeks after THA surgery. In our study, despite fondaparinux prophylaxis, the incidence of asymptomatic DVT at the early stage was high, but with continuous thromboprophylaxis of fondaparinux for14 days, the incidence of asymptomatic DVT occurring at 14 day was significantly reduced compared to the incidence of asymptomatic DVT occurring at the early stage. The findings of the previous studies described indirectly support our study.

Little is known about the time course of asymptomatic DVT during treatment with fondaparinux after THA and TKA surgery. Our study showed that the timing of DVT was earlier after TKA surgery than after THA surgery, and after TKA surgery, most asymptomatic DVT occurred in up to 1 day. After TKA surgery, all DVT occurred within 4 days; however, after THA surgery, new-onset of DVT was found at 14 days. When examining the natural history of untreated asymptomatic DVT after surgery, Kakkar et al. [14] reported that, of 211 DVT diagnosed by leg scanning within 2 weeks of surgery, 61% started on or after 3 days and 9% started after the first week. Similarly, Sikorski et al. [15] reported that after THA surgery with no prophylaxis, relatively few thromboses developed within the first 2 days, the incidence of asymptomatic DVT was peak and 25% at Day 4, and the risk of thrombosis continued after 1 week. Moreover, Maynard et al. [16] reported that after TKA surgery, the overall incidence of DVT was 47% within 1 day after surgery and 54% at 4 to 7 days after surgery, and 87% of patients with asymptomatic DVT were already positive within 1 day after surgery. For timing of clinical DVT, White et al. [17] reported that among 19,586 primary THA surgery and 24,059 primary TKA surgery, the time course of symptomatic thromboembolism was a median of 17 days after THA surgery and 7 days after TKA surgery. These findings support our study.

In Western studies, major bleeding in patients receiving 2.5 mg of fondaparinux was reported to occur in 1.8 to 4.1% of patients undergoing THA surgery and 2.1% of patients undergoing major knee surgery [8], [9] and [10]. The incidence of major bleeding in our study was similar to that in Western studies.

Our study has some limitations. First, the number of patients is small. Secondary, ultrasonograophy is a non-invasive and easily repeatable technique with almost no contraindications [18] and [19], and it can reliably detect thrombi in the proximal veins of symptomatic patients after total joint arthroplasty surgery [20]. However, some studies [21] and [22], demonstrated that ultrasonography has failed to demonstrate sufficient accuracy for the detection of screening for asymptomatic DVT after major orthopaedic surgery. Schellong et al.[21] reported that, sensitivity and specificity of complete compression ultrasound compared venography was 31.1% and 93.0% for any DVT respectively. Whether, contrast venograophy has long been the diagnostic standard in thrmboprophylaxis trials because of its high sensitivity for detecting DVT [23], and practice-changing prophylaxis trials have used contrast venography at the primary outcome measure of efficacy[7], [8], [9] and [10]. Therefore, further studies are needed because of lack of control with contrast venography in our findings.

5. Conclusion

Our study demonstrated that even if fondaparinux was administered starting 24 hours after THA or TKA surgery, the incidence of asymptomatic DVT up to postoperative day 4 was high. However, in patients who had received fondaparinux for 14 days, the incidence of asymptomatic DVT occurring at postoperative day 4 was significantly reduced at postoperative day 14.

Conflict of interest

The authors state that they have no conflict of interest.

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New Sequence Variants in HLA Class II/III Region Associated with Susceptibility to Knee Osteoarthritis Identified by Genome-Wide Association Study

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Abstract

Osteoarthritis (OA) is a common disease that has a definite genetic component. Only a few OA susceptibility genes that have definite functional evidence and replication of association have been reported, however. Through a genome-wide association study and a replication using a total of ~4,800 Japanese subjects, we identified two single nucleotide polymorphisms (SNPs) (rs7775228 and rs10947262) associated with susceptibility to knee OA. The two SNPs were in a region containing HLA class II/III genes and their association reached genome-wide significance (combined $P = 2.43 \times 10^{-8}$ for rs7775228 and 6.73×10^{-8} for rs10947262). Our results suggest that immunologic mechanism is implicated in the etiology of OA.

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Introduction

We are living in the "Bone and Joint Decade" (http://www.boneandjointdecade.org/). As the WHO initiative shows, bone and joint diseases are serious problems all over the world, putting us under severe medical, economical and social burden. Osteoarthritis (OA; MIM 165720) is one of the most common diseases among them. OA affects synovial joints of all over the body, mainly knee, hip, hand and spine. OA is characterized by progressive loss of articular cartilage and, often, proliferation of synovium and bone, which lead to pain, loss of joint function and disability. More than tens of millions patients in the world are suffering from this non-lethal, but intractable disease, and the number is relentlessly increasing; however, its etiological picture remains unclear and we have no fundamental treatment for it.

OA is a polygenic disease. Both environmental and genetic factors contribute to its etiology and pathogenesis [1]. To understand its genetic factor, identification of its susceptibility gene(s) must be the first step. Many OA susceptibility genes identified by candidate-gene association studies have been reported, but only a few have supporting functional evidence and replication of the results in different populations [1,2]. Large-scale association studies including the genome-wide association study (GWAS) using high-density single nucleotide polymorphisms (SNPs) have been reported by a few groups in Asia and Europe [3–6], but only a gene fulfilled genome-wide significance level [2]. The genetic basis of OA susceptibility remains largely uncharacterized. To identify OA susceptibility gene(s), we conducted a GWAS for knee OA and identified two SNPs with genome-wide significance level.

Methods

Samples

Characteristics of each cohort group are shown in Table 1. Case samples of GWAS for the Japanese population were obtained from

Table 1. Basal characteristics of the subjects.

Cohort	Source	Platform	Number of samples	Nationality	Female (%)	Age (mean +/− sd)	BMI (mean +/− sd)	Severity[a] (% severe OA)
GWAS								
knee OA	RIKEN	Illumina HumanHap550	899	Japanese	759 (84.4)	71.6+/−7.6	24.9+/−3.6	76.5
control	ORC+BioBank Japan	Illumina HumanHap550	3,396	Japanese	1,491 (43.9)	52.5+/−15.2	22.5+/−3.7	–
Replication								
Japanese								
knee OA	RIKEN	Invader assay	167	Japanese	124 (74.3)	73.8+/−6.1	24.5+/−3.3	48.5
control	RIKEN	Invader assay	347	Japanese	223 (64.3)	65.9+/−8.7	22.3+/−2.7	–
European Caucasian								
knee OA	Santiago de Compostela	SNaPshot	243	Spanish	197 (81.1)	68.0+/−5.7	32.8+/−4.8	ND[b]
control	Santiago de Compostela	SNaPshot	426	Spanish	165 (38.7)	68.4+/−9.1	28.3+/−3.8	–
knee OA	University of Thessaly	SNaPshot	570	Greek	468 (82.1)	65.8+/−8.7	29.1+/−3.3	77.1
control	University of Thessaly	SNaPshot	645	Greek	417 (64.6)	59.5+/−11.6	25.4+/−3.7	–

OA: osteoarthritis, ORC: Osaka-Midosuji Rotary Club.
[a]Kellegren-Laurence grade ≥3 was considered as severe OA.
[b]All cases underwent TKR (total knee replacement) surgery.
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several medical institutes in Japan, as previously described [5,7]. Knee OA was diagnosed on the basis of clinical and radiographic findings using previously described criteria [5,7]. Rheumatoid arthritis (RA) and polyarthritis associated with autoimmune diseases were excluded, as were secondary OA due to crystal deposition (gout and pseudogout), posttraumatic OA and infection-induced OA. Patients who had clinical and radiographic findings suggestive of skeletal dysplasias, including overt short stature, multiple symmetric involvements of epiphyses and a definitely positive Mendelian family history were also excluded from the study. The control groups consisted of 3,396 individuals that were registered in the Leading Project for Personalized Medicine in the Ministry of Education, Culture, Sports, Science and Technology, Japan as the subjects with diseases unrelated to OA and the volunteers in the Osaka-Midosuji Rotary Club, Osaka, Japan [8]. For replication study, we recruited population-based cohorts from inhabitants of Odai and Minami-ise town (previously Miyagawa village and Nansei town, respectively in the Mie prefecture in Japan) [9]. The Spanish and Greek knee OA and control populations were recruited as described previously from the Hospital Clinico de Santiago, the Departments of Biology and Genetics and of Orthopaedics, University of Thessaly and the Institute of Musculoskeletal Sciences [10]. All the participants provided written informed consent. This research project was approved by the ethical committees at Center for Genomic Medicine (formerly, SNP Research Center), RIKEN and the participating institutions.

SNP genotyping

For the GWAS, we genotyped 906 patients with OA and 3,396 controls using Illumina HumanHap550v3 Genotyping BeadChip. After excluding seven cases with call rate of <0.98, we applied SNP QC (call rate of ≥0.99 in both cases and controls and P value of Hardy-Weinberg equilibrium test of $\geq 1.0\times10^{-6}$ in controls). Finally, 459,393 SNPs on autosomal chromosomes passed the QC filters and were further analyzed. Among the SNPs analyzed in the GWAS, we selected top 15 SNPs showing the smallest P values ($P<1\times10^{-5}$) for the replication study using an independent 514 Japanese subjects from a resident cohort. SNPs with minor allele frequency of ≤0.1 in both case and control samples were excluded from the further analysis. In the replication analysis, we genotyped SNPs using the multiplex PCR-based invader assay (Third Wave Technologies) or by direct sequencing of PCR products using ABI 3700 DNA analyzers (Applied Biosystems), or by SNaPshot Multiplex System (Applied Biosystems) according to manufacturers' protocols.

Statistical analysis

In the GWAS and replication analyses, we applied Fisher's exact test to two-by-two contingency table in three genetic models: an allele frequency model, a dominant-effect model, and a recessive-effect model. We conducted the meta-analysis using the Mantel-Haenszel method. We examined heterogeneity among studies by using the Breslow-Day test. Significance levels after the Bonferroni correction for multiple testing were $P=1.09\times10^{-7}$ (0.05/459,393). Age, gender- and BMI-adjusted odds ratios were obtained by logistic regression analysis [11]. Odds ratios and confidence intervals were calculated using the risk allele as a reference. We analyzed the haplotype association using Haploview software [12]. We conducted a principal component analysis to detect population stratification [13].

Software

For general statistical analysis, we used R statistical environment version 2.6.1 or Microsoft Excel. Drawing the LD map, estimation of haplotype frequencies and analysis of haplotype association were performed by Haploview software.

Results

To identify genetic variants that determine OA susceptibility, we conducted a GWAS in Japanese knee OA. We examined 906 individuals with knee OA and 3,396 control individuals (Table 1) using Illumina HumanHap550v3 Genotyping BeadChip. After confirming the data quality, we compared the results of 459,393 SNPs between cases and controls by Fisher's exact test for three genetic models: allelic, dominant or recessive (Figure 1). Fifteen