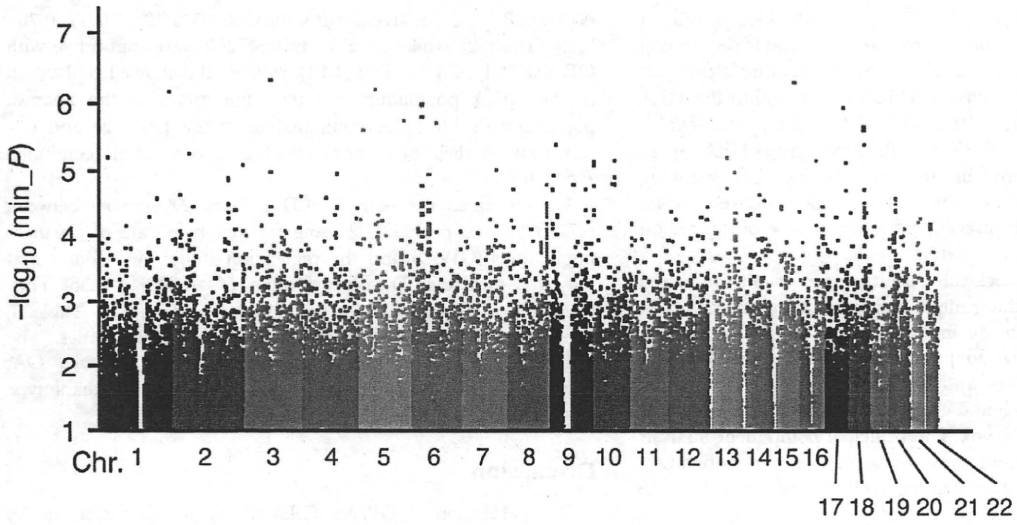

Figure 1. Results of a genome-wide association study ($-\log_{10}$ *P* value plot). Each *P* value is the minimum of Fisher's exact tests for three models: dominant, recessive and allele frequency model.
doi:10.1371/journal.pone.0009723.g001

SNPs selected for the replication study that had the smallest *P* values (minimum $P<1\times10^{-5}$) were next genotyped in an independent set of 167 Japanese knee OA individuals and 347 Japanese controls from a resident cohort study. Through these studies, only two SNPs, rs7775228 (combined $P=2.43\times10^{-8}$; OR = 1.34; 95% CI = 1.21–1.49) and rs10947262 (combined

Table 2. Association of rs7775228 and rs10947262 with knee osteoarthritis.

SNP (Nearest gene)	Allele	Population	Minor allele Frequency				
			Case	Control	OR (95% CI)[a]	*P*[b]	P_{het}[c]
rs7775228	C/T	Japanese					
(*HLA-DQB1*)		GWAS	0.318	0.379	1.31 (1.18–1.47)	1.38 E-06	
		Replication	0.290	0.385	1.53 (1.15–2.02)	3.07 E-03	
		Combined[d]			1.34 (1.21–1.49)	2.43 E-08	0.33
		European Caucasian					
		Spanish	0.194	0.209	1.10 (0.83–1.45)	0.521	
		Greek	0.094	0.075	0.78 (0.58–1.03)	0.084	
		European combined[e]			0.93 (0.76–1.13)	0.178	0.09
		All combined[f]			–	–	0.003
rs10947262	C/T	Japanese					
(*BTNL2*)		GWAS	0.358	0.419	1.30 (1.16–1.44)	2.45 E-06	
		Replication	0.332	0.422	1.47 (1.12–1.93)	5.74 E-03	
		Combined[d]			1.32 (1.19–1.46)	6.73 E-08	0.40
		European Caucasian					
		Spanish	0.122	0.136	1.13 (0.81–1.59)	0.465	
		Greek	0.068	0.094	1.43 (1.06–1.92)	0.019	
		European combined[e]			1.29 (1.03–1.61)	0.025	0.32
		All combined[f]			1.31 (1.20–1.44)	5.10 E-09	0.63

Odds ratios (ORs) and *P* values for the independence test were calculated by the Mantel-Haenszel method.
[a]OR of the risk allele from the two-by-two allele frequency table.
[b]P values of the Pearson's χ^2 test for the allele model.
[c]Results of the Breslow-Day test.
[d]Meta-analysis of Japanese studies.
[e]Meta-analysis of European studies.
[f]Meta-analysis of all studies.
doi:10.1371/journal.pone.0009723.t002

$P = 6.73 \times 10^{-8}$; OR = 1.32; 95% CI = 1.19–1.46) were significant even after the Bonferroni correction for multiple testing ($P = 1.09 \times 10^{-7}$) (Table 2). The two SNPs showing significant associations are located within a 340-kb region within the HLA locus, including *BTNL2*, *HLA-DRA*, *HLA-DRB5*, *HLA-DRB1*, *HLA-DQA1* and *HLA-DQB1* (Figure 2). Although the HLA region is known to show extensive linkage disequilibrium (LD) spanning over 7 Mb, only SNPs in the 340-kb region showed strong associations with OA (Figure 2), and SNPs outside of this region did not have significant association.

Application of the Cochrane-Armitage test to all the tested SNPs indicated that the genetic inflation factor lambda was 1.08 for GWAS (Figure 3), implying a low possibility of false positive associations due to population stratification. We also carried out age, gender- and BMI-adjusted analysis using a logistic regression model, and confirmed similar association after adjustment (data not shown). The principal component analysis [13] revealed that there was no evidence for population stratification between the two control groups used for the GWAS (Figure S1).

To check the association of rs7775228 and rs10947262 in different ethnic populations, we examined the association of the SNPs with knee OA in two European Caucasian populations from Greece and Spain. We genotyped a total of 813 OA and 1,071 control subjects (Table 1). We conducted the meta-analysis using the Mantel-Haenszel method. The combined European results for rs7775228 were not significant with OR (95%CI) of 0.93 (0.76–1.13) (Table 2), while those for rs10947262 were supportive with OR (95%CI) of 1.29 (1.03–1.61). rs10947262 showed replication in the Greek population and the same trend in the Spanish population (Table 2). A meta-analysis of the Japanese and two European studies gave more significant association (combined $P = 5.10 \times 10^{-9}$).

We estimated the pairwise LD indexes (D' and r^2) between rs7775228 and rs10947262 using the genotype data of Japanese populations (GWAS and the replication study), and found that they were in strong LD with each other ($D' = 0.82$, $r^2 = 0.56$). They formed two frequent haplotypes (Haplotype I and II; Table 3) accounting for about 90% of all observed chromosomes. The haplotypes were also significantly associated with knee OA; Haplotype I, the most frequent haplotype was a risk haplotype ($P = 1.48 \times 10^{-8}$; OR = 1.33; 95% CI = 1.20–1.46).

Discussion

We performed a GWAS followed by a replication in an independent population using a total of ~4,800 Japanese subjects, and identified two SNPs (rs7775228 and rs10947262) in the HLA class II/III locus associated with susceptibility to knee OA. To our knowledge, this study represents the first GWAS of OA with extensive coverage (~550,000 markers) and definite genome-wide significance even after Bonferroni's correction, which is very

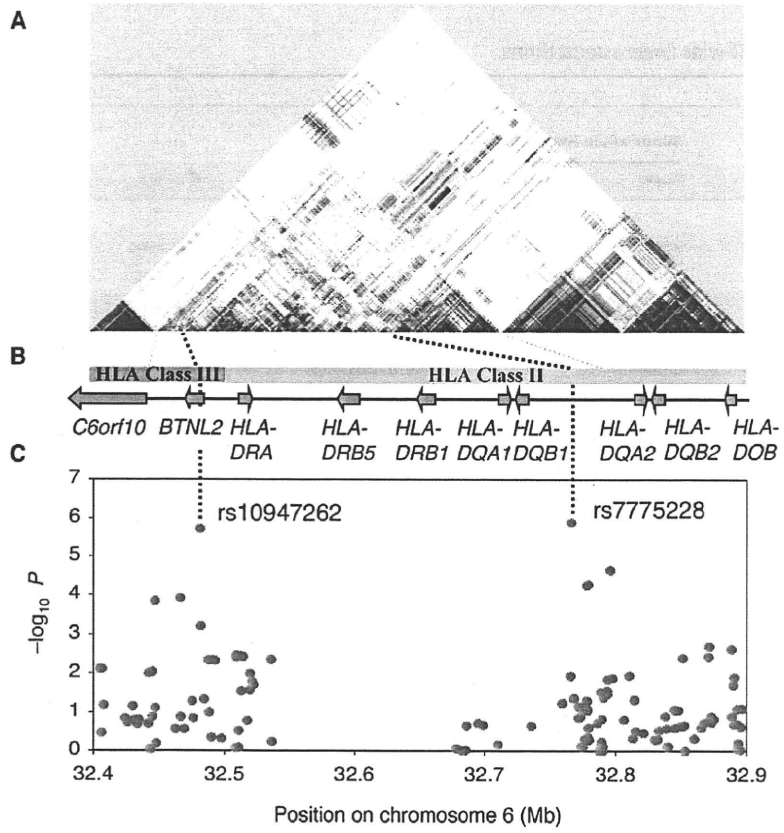

Figure 2. Case-control association analysis and linkage disequilibrium (LD) map of the HLA class II/III region of chromosome 6. (A) The LD map based on D' was drawn using HapMap data release 24 for the JPT population. (B) Genomic structure within the extended HLA-II/III region. (C) Results of GWAS for osteoarthritis in Japanese population. The $\log_{10}$-transformed P values are plotted on the y axis.
doi:10.1371/journal.pone.0009723.g002

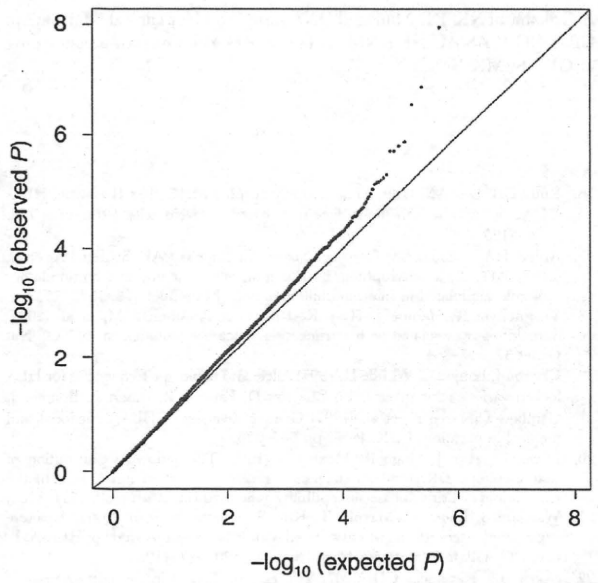

Figure 3. GWAS of knee osteoarthritis. Q-Q plot with Cochrane-Armitage trend P in the GWAS. Horizontal and vertical lines represent expected P values under a null distribution and observed P values, respectively. The genetic inflation factor lambda is 1.08.
doi:10.1371/journal.pone.0009723.g003

conservative. There were no effects of population stratification and confounding factors. Since two groups of controls were used in the GWAS, we evaluated the possibility of genetic heterogeneity between the two groups by a principal component analysis and found it unlikely (Figure S1). Although there was large age difference between the case and control groups of GWAS (Table 1), significant association was observed after the age adjustment.

In the NCBI genome database, rs7775228 and rs10947262 located between upstream region of *HLA-DQA2* and *HLA-DQB1*, and within the intron 1 of *BTNL2*, respectively (Figure 2). *HLA-DQA2* and *HLA-DQB1* encode HLA-DQ α and β chains, which belong to the HLA class II molecules. HLA class II molecules are expressed in antigen presenting cells (B lymphocytes, dendritic cells and macrophages) and play a central role in the immune system by presenting peptides derived from extracellular proteins [14]. The HLA-DQA2 protein is expressed, but at a very low level in comparison with the HLA-DQA1 protein [15,16]. Moreover, the HLA-DQA2 α chain does not dimerize with class II β chains [16]. *BTNL2* encodes butyrophilin-like 2, a member of butyrophilin family that shares sequence homology with the B7 co-stimulatory molecules. BTNL2 regulates T-cell activation through unknown receptor, distinct from CD28 and CTLA-4 [17]. In Japanese population, the haplotype association was more significant than those of respective SNPs (Tables 2 and 3). Therefore, there may be hidden SNP(s) with a lower P value than rs7775228 and rs10947262, or the haplotype may be implicated in the OA susceptibility. An association of sarcoidosis with rs2076530, a coding SNP on exon 5 of the *BTNL2* gene has been reported [18], but the SNP was not in LD with rs10947262 ($D' = 0.11$, $r^2 = 0$).

The 340-kb region of HLA locus, where the two SNPs are located also includes *HLA-DRA*, *HLA-DRB1*, *HLA-DRB3*, *HLA-DRB4*, *HLA-DRB5* and *HLA-DQA1*. *HLA-DRA*, *HLA-DRB1/3/4/5* and *HLA-DQA1* encode HLA-DR α, β and HLA-DQ α chains, which could also belong to the HLA class II molecules. *HLA-DRB1* is present in all individuals. Allelic variants of *HLA-DRB1* are linked with either none or one of the genes *HLA-DRB3*, *HLA-DRB4* and *HLA-DRB5* [19]. Among these genes, *HLA-DRB1* is strongly associated with RA. Some subtypes of HLA-DRB1 alleles, such as *0101, *0401, and *0405, is associated with RA [20], but not with generalized OA [21].

Although OA has generally been considered a non-inflammatory disease, accumulating evidences suggest that this is not the case. Inflammation involving activated T cells in the synovial membrane of OA patients is well documented [22]. Recently, we identified a genetic variant of *EDG2* gene encoding lysophosphatidic acid receptor associated with knee OA [23]. A GWAS has identified a genetic variant of the *PTGS2* gene encoding cyclooxygenase-2 involved in risk for knee OA [6]. These genetic associations of genes such as *EDG2* and *PTGS2* underscore the potential role of inflammatory pathways in the pathogenesis of knee OA.

Several studies have suggested associations of OA with HLA class I and class II alleles. Study on generalized OA revealed association with HLA A1-B8 in Caucasian [24] and with HLA-Cw4 in Japanese [21]. An association of the HLA-DRB1*02 alleles with knee and hip OA was identified in a cohort of 106 patients [25]. Interestingly, chondrocyte, which are normally HLA-DP, DQ and DR-negative, become positive for them in OA [26,27], suggesting their function as antigen-presenting cells. Cartilage fragments are mechanically shaved from the joint surface and frequently found in the synovial membrane of OA patients [28]. So, physical interaction between chondrocytes and T cells is conceivable. Peripheral blood T cells from OA patients show significantly higher proliferative responses to autologous chondrocytes [27]. Our results further support the concept that OA is an immunologic disorder.

Table 3. Haplotype association analysis for knee osteoarthritis susceptibility SNPs, rs7775228 and rs10947262.

		Frequency		Haplotype effect[b]	
Haplotype[a]		Case	Control	OR (95% CI)	P value
I	TC	0.610	0.541	1.33 (1.20–1.46)	1.48 E-8
II	CT	0.277	0.340	0.74 (0.67–0.83)	5.07 E-8
III	CC	0.077	0.080	0.96 (0.80–1.15)	0.653
IV	TT	0.037	0.040	0.91 (0.71–1.18)	0.475

OR: odds ratio, CI: confidence interval.
[a]Alleles of rs7775228 (C/T) and rs10947262 (C/T) with this order.
[b]A haplotype vs. all others.
doi:10.1371/journal.pone.0009723.t003

Supporting Information

Figure S1 Principal component analysis of GWAS samples. Samples in the GWAS and in HapMap database are analyzed by a program of Smartpca [12], and plotted for the first (X axis) and the second (Y axis) principal component (PC), respectively.
Found at: doi:10.1371/journal.pone.0009723.s001 (0.16 MB TIF)

Acknowledgments

We thank all participating patients, doctors and staffs of collaborating institutes. We also thank Ms. Yoshie Takanashi, Carmen Pena-Pena and technical staffs of Laboratories for Genotyping Development and for Bone and Joint Diseases of CGM, RIKEN, and Laboratorio Investigacion 10 and Rheumatology Unit, Hospital Clinico Universitario de Santiago for genotyping.

Author Contributions

Wrote the paper: MN SI. Planned and supervised the whole project: SI. Performed the Japanese association study: MN IK TF JD. Managed the European association study: A. Tsezou AG. Helped with statistic analysis: A. Takahashi NK TT. Managed DNA sample and/or clinical information: CR-F JJG-R AS AU NF KNM A. Tsezou AG YN. Oversaw a genotyping of GWAS: MK YN.

References

1. Ikegawa S (2007) New gene associations in osteoarthritis: what do they provide, and where are we going? Curr Opin Rheumatol 19: 429–434.
2. Dai J, Ikegawa S (2010) Recent advances in association studies of osteoarthritis susceptibility genes. J Hum Genet 55: 77–80.
3. Mototani H, Mabuchi A, Saito S, Fujioka M, Iida A, et al. (2005) A functional single nucleotide polymorphism in the core promoter region of CALM1 is associated with hip osteoarthritis in Japanese. Hum Mol Genet 14: 1009–1017.
4. Spector TD, Reneland RH, Mah S, Valdes AM, Hart DJ, et al. (2006) Association between a variation in LRCH1 and knee osteoarthritis: a genome-wide single-nucleotide polymorphism association study using DNA pooling. Arthritis Rheum 54: 524–532.
5. Miyamoto Y, Shi D, Nakajima M, Ozaki K, Sudo A, et al. (2008) Common variants in DVWA on chromosome 3p24.3 are associated with susceptibility to knee osteoarthritis. Nat Genet 40: 994–998.
6. Valdes AM, Loughlin J, Timms KM, van Meurs JJ, Southam L, et al. (2008) Genome-wide association scan identifies a prostaglandin-endoperoxide synthase 2 variant involved in risk of knee osteoarthritis. Am J Hum Genet 82: 1231–1240.
7. Miyamoto Y, Mabuchi A, Shi D, Kubo T, Takatori Y, et al. (2007) A functional polymorphism in the 5' UTR of GDF5 is associated with susceptibility to osteoarthritis. Nat Genet 39: 529–533.
8. Nakamura Y (2007) The BioBank Japan Project. Clin Adv Hematol Oncol 5: 696–697.
9. Kizawa H, Kou I, Iida A, Sudo A, Miyamoto Y, et al. (2005) An aspartic acid repeat polymorphism in asporin inhibits chondrogenesis and increases susceptibility to osteoarthritis. Nat Genet 37: 138–144.
10. Rodriguez-Lopez J, Pombo-Suarez M, Loughlin J, Tsezou A, Blanco FJ, et al. (2009) Association of a nsSNP in ADAMTS14 to some osteoarthritis phenotypes. Osteoarthritis Cartilage 17: 321–327.
11. Andrieu N, Goldstein AM (1998) Epidemiologic and genetic approaches in the study of gene-environment interaction: an overview of available methods. Epidemiol Rev 20: 137–147.
12. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21: 263–265.
13. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, et al. (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 38: 904–909.
14. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, et al. (2007) Molecular biology of the cell - Fifth edition. New York: Garland Science. pp 1577–1579.
15. Yu LP, Sheehy MJ (1991) The cryptic HLA-DQA2 ("DX alpha") gene is expressed in human B cell lines. J Immunol 147: 4393–4397.
16. Rudy GB, Lew AM (1997) The nonpolymorphic MHC class II isotype, HLA-DQA2, is expressed on the surface of B lymphoblastoid cells. J Immunol 158: 2116–2125.
17. Arnett HA, Escobar SS, Gonzalez-Suarez E, Budelsky AL, Steffen LA, et al. (2007) BTNL2, a butyrophilin/B7-like molecule, is a negative costimulatory molecule modulated in intestinal inflammation. J Immunol 178: 1523–1533.
18. Valentonyte R, Hampe J, Huse K, Rosenstiel P, Albrecht M, et al. (2005) Sarcoidosis is associated with a truncating splice site mutation in BTNL2. Nat Genet 37: 357–364.
19. Clayton J, Lonjou C, Whittle D (1997) Allele and haplotype frequencies for HLA loci in various ethic groups. In: Charron D, Fauchet R, Albert E, Bodmer J, Cambon-Thomsen A, et al. (1997) Genetic diversity of HLA, functional and medical implication. EDK, Paris. pp 665–820.
20. Evans TI, Han J, Singh R, Moxley G (1995) The genotypic distribution of shared-epitope DRB1 alleles suggests a recessive mode of inheritance of the rheumatoid arthritis disease-susceptibility gene. Arthritis Rheum 38: 1754–1761.
21. Wakitani S, Imoto K, Mazuka T, Kim S, Murata N, et al. (2001) Japanese generalised osteoarthritis was associated with HLA class I–A study of HLA-A, B, Cw, DQ, DR in 72 patients. Clin Rheumatol 20: 417–419.
22. Sakkas LI, Platsoucas CD (2007) The role of T cells in the pathogenesis of osteoarthritis. Arthritis Rheum 56: 409–424.
23. Mototani H, Iida A, Nakajima M, Furuichi T, Miyamoto Y, et al. (2008) A functional SNP in EDG2 increases susceptibility to knee osteoarthritis in Japanese. Hum Mol Genet 17: 1790–1797.
24. Pattrick M, Manhire A, Ward AM, Doherty M (1989) HLA-A, B antigens and alpha 1-antitrypsin phenotypes in nodal generalised osteoarthritis and erosive osteoarthritis. Ann Rheum Dis 48: 470–475.
25. Moos V, Menard J, Sieper J, Sparmann M, Muller B (2002) Association of HLA-DRB1*02 with osteoarthritis in a cohort of 106 patients. Rheumatology (Oxford) 41: 666–669.
26. Lance EM, Kimura LH, Manibog CN (1993) The expression of major histocompatibility antigens on human articular chondrocytes. Clin Orthop Relat Res. pp 266–282.
27. Sakata M, Masuko-Hongo K, Nakamura H, Onuma H, Tsuruha JI, et al. (2003) Osteoarthritic articular chondrocytes stimulate autologous T cell responses in vitro. Clin Exp Rheumatol 21: 704–710.
28. Revell PA, Mayston V, Lalor P, Mapp P (1988) The synovial membrane in osteoarthritis: a histological study including the characterisation of the cellular infiltrate present in inflammatory osteoarthritis using monoclonal antibodies. Ann Rheum Dis 47: 300–307.

Clin Rheumatol (2010) 29:671–675
DOI 10.1007/s10067-010-1406-6

BRIEF REPORT

Retrospective clinical study of the efficacy of lower-dose methotrexate and infliximab therapy in patients with rheumatoid arthritis

Hiroki Wakabayashi · Akihiro Sudo · Masahiro Hasegawa · Hiroshi Oka · Atsumasa Uchida · Kusuki Nishioka

Received: 19 October 2009 / Revised: 4 February 2010 / Accepted: 10 February 2010 / Published online: 4 March 2010

Abstract The objective of this study is to compare the long-term outcomes of infliximab therapy with lower-dose methotrexate (MTX; ≤4 mg per week) and with standard-dose MTX (≥6 mg per week) in Japanese rheumatoid arthritis (RA) patients. One hundred thirty-eight patients with refractory RA were treated with intravenous infliximab; 106 patients underwent lower-dose MTX therapy, and 32 patients underwent standard-dose MTX therapy. Treatment responses at 54 weeks or last observation carried forward (LOCF) assessed using the European League Against Rheumatism (EULAR) response criteria were compared between the two groups. Eighty-eight patients (81.1%) in the lower-dose MTX group and 27 patients (84.3%) in the standard-dose MTX therapy completed 54 weeks of infliximab treatment. A EULAR response criteria good and moderate response was seen in 70.9% in the lower-dose group and 74.1% in the standard-dose group. Good and moderate treatment responses at 54 weeks or LOCF were seen in 66.0% in the lower-dose group and 68.7% in the standard-dose group. The outcome in the lower-dose MTX group was not significantly different from that in the standard-dose group. Therapy with MTX and infliximab was effective in Japanese RA patients, regardless of MTX dosage.

Keywords Infliximab · Lower-dose · Methotrexate · Rheumatoid arthritis

H. Wakabayashi (✉) · A. Sudo · M. Hasegawa · A. Uchida
Department of Orthopaedic Surgery,
Mie University Graduate School of Medicine,
2-174 Edobashi,
Tsu 514-8507, Japan
e-mail: whiroki@clin.medic.mie-u.ac.jp

H. Oka · K. Nishioka
Institute of Medical Science,
St. Marianna University School of Medicine,
2-16-1 Sugao Miyamae-ku,
Kawasaki 216-8512, Japan

Introduction

Biologics targeting tumor necrosis factor (TNF) have brought about a paradigm shift in the treatment of rheumatoid arthritis (RA). The aim of treatment for RA is remission, but treatment success can also be assessed based on the induction of low disease activity [1–3]. Infliximab, anti-TNF-alpha chimeric monoclonal antibody, was marketed in Japan in 2003. Methotrexate (MTX) is necessary when treating RA patients with infliximab. Infusions of infliximab in combination with MTX have been reported to be effective not only in reducing signs and symptoms but also in inhibiting the progression of structural damage and improving physical function in patients with RA that remains active despite MTX therapy [4–6]. In Japan, MTX is given at a lower dose (≤8 mg per week) than that used in the USA and many European countries (15 to 20 mg per week). Compared with Europe and America, infliximab therapy in Japan involves less than half the dose of MTX and less than one third the dose of infliximab. Nevertheless, MTX may need to be discontinued because of adverse effects or toxicity. Therefore, we compared the long-term outcomes of a lower-dose MTX group (≤4 mg per week) plus infliximab and a standard-dose MTX group (≥6 mg per week) plus infliximab, and investigated the

efficacy of lower-dose MTX plus infliximab therapy in Japanese RA patients.

Methods

Inclusion and exclusion criteria

Patients with a history of treatment failure with at least one disease-modifying antirheumatic drug (DMARD) and active RA despite the use of MTX could be included. RA was classified according to the American Rheumatism Association 1987 revised criteria [7]. Exclusion criteria were compliant with the contraindications and precautions given by the manufacturer of infliximab and consisted of pregnancy, breast-feeding, ongoing infections, multiple sclerosis, current symptoms and signs of severe, progressive, or uncontrolled disease other than RA, active pathology on chest radiographs or laboratory pathology, and ongoing malignant or premalignant disease. Patients with anti-double-stranded DNA antibodies or autoimmune disease other than RA were not included.

Study design

All patients started treatment with infliximab (Remicade®) in doses close to 3 mg/kg body weight. Infusions were given at weeks 0, 2, 6, and then every eighth week.

Overall, 138 patients with refractory RA were treated with intravenous infliximab; 106 patients underwent lower-dose MTX therapy (≤ 4 mg per week), and 32 patients underwent standard-dose MTX therapy (≥6 mg per week).

Treatment responses at baseline, 22 weeks, and 54 weeks were assessed by the European League Against Rheumatism (EULAR) response criteria and compared between the two groups (lower-dose group and standard-dose group). The last observation carried forward (LOCF) was used in discontinued patients with missing data.

The disease activity score (DAS28) is a validated, continuous index measuring disease activity in RA patients [8]. In recent reports, DAS28-C-reactive protein (CRP), using CRP instead of erythrocyte sedimentation rate (ESR), and DAS28-ESR were well correlated [9–11], but the threshold values should be reconsidered. DAS28-CRP threshold values corresponding to remission, low disease activity, and high disease activity were 2.3, 2.7, and 4.1, respectively [9].

Statistical analysis

Changes from baseline in individual DAS28 values between treatment groups were analyzed using analysis of variance.

Results

Baseline patient characteristics and disease activity

The average age at the start of infliximab therapy was 55.4 years in the lower-dose group and 52.3 years in the standard-dose group. Disease activity state, evaluated using DAS28-CRP, was almost the same in the two groups. About 60% of patients exhibited high disease activity before infliximab therapy in both groups. The mean DAS28-CRP value (±SD) was 4.51 (±1.32) in the lower-dose group and 4.50 (±1.19) in the standard-dose group. DAS28-CRP was not different between the two groups. The mean MTX dose received was 3.4 mg/week in the lower-dose group and 6.6 mg/week in the standard-dose group (Fig. 1).

Continuation rate

By 54 weeks, 25 patients had discontinued infliximab therapy, 20 patients had undergone lower-dose MTX therapy (≤4 mg per week), and 5 patients had undergone standard-dose MTX therapy (≥6 mg per week).

In the 113 patients who completed infliximab therapy for a total period of 54 weeks, 86 had undergone lower-dose MTX therapy, and 27 patients had undergone standard-dose MTX therapy. The continuation rates for infliximab therapy were 81.1% (86/106) in the lower-dose MTX group and 84.3% (27/32) in the standard-dose MTX group (Table 1).

Serious adverse events

Among the 25 patients that discontinued infliximab by 54 weeks, 14 experienced adverse events, 10 showed a lack of effectiveness, and 1 desired to become pregnant (Table 1). Adverse events were infectious events in three, general malaise in three, infusion reactions in two, renal dysfunction

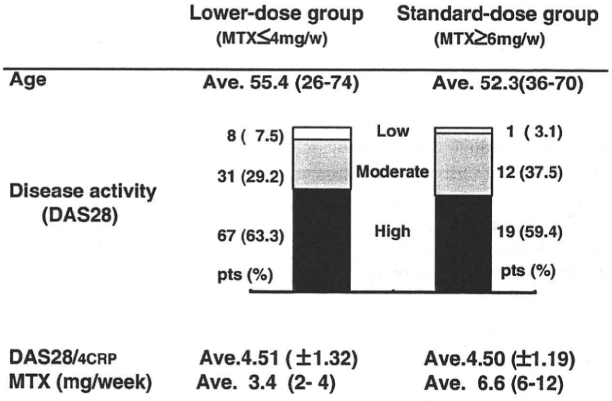

Fig. 1 Baseline clinical characteristics and disease activity

Table 1 Outcome of methotrexate and infliximab therapy

	Lower-dose MTX≤4mg/w	Standard-dose MTX≥6mg/w	Total
Continued at 54 weeks	86 patients (81.1%)	27 patients (84.3%)	113 patients
Discontinued			
Adverse effect	11 patients (10.4%)	3 patients (9.4%)	14 patients
Inefficiency	9 patients (8.5%)	1 patient (3.1%)	10 patients
Pregnant hope	1 patient	1 patient	
Total	106 patients	32 patients	

in one, liver dysfunction in one, anemia in one, eczema in one, increased serum KL-6 levels, which is a serum marker for interstitial pneumonia, in one, and deep vein thrombosis in one.

Response status

The mean DAS28-CRP (±SD) value decreased significantly from 4.51 (±1.32) at baseline to 2.90 (±1.39) at week 54 in the lower-dose group, and from 4.50 (±1.19) at baseline to 2.91 (±1.52) at week 54 in the standard-dose group. The difference was not significant.

Using the EULAR response criteria, the long-term outcomes of infliximab therapy are shown in Fig. 2a, b. At week 54, using EULAR response criteria, good, moderate, and no response were 38.3%, 32.6%, or 29.1%, respectively, in the lower-dose group, and 44.5%, 29.6%, and 25.9%, respectively, in the standard-dose group. A good or moderate response was achieved in 70.9% of patients in the lower-dose group and 74.1% in the standard-dose group using EULAR response criteria. The response rate was not statistically different between the two groups.

With respect to the proportions of disease activity, about 60% of patients had high disease activity before infliximab therapy in both groups. After 54 weeks of infliximab therapy, the proportions of patients exhibiting high activity, moderate activity, low activity, or in clinical remission were 22.1%, 25.6%, 10.5%, and 41.8%, respectively, in the lower-dose group, and 18.6%, 26.9%, 11.1%, and 44.5%, respectively, in the standard-dose group (Fig. 2c, d).

The outcomes of all patients with infliximab therapy at 54 weeks or LOCF are shown in Fig. 2. Using the EULAR response criteria, good, moderate, and no response were 33.0%, 33.0%, and 34.0%, respectively, in the lower-dose group, and 40.6%, 28.1%, and 31.3%, respectively, in the standard-dose group. A good or moderate response was achieved in 66.0% of patients in the lower-dose group and 68.7% in the standard-dose group. The proportions of patients with high activity, moderate activity, low activity, and in clinical remission were 26.4%, 28.3%, 9.4%, and 35.9%, respectively, in the lower-dose group, and 21.9%, 28.1%, 9.4%, and 40.6%, respectively, in the standard-dose group, thereby indicating good overall efficacy of infliximab therapy.

Discussion

MTX is widely used in the USA and Europe as a first-line DMARD. In Japan, MTX was approved in 1999 for RA patients with inadequate response to more than one other DMARD. However, it is difficult to control disease activity in a substantial number of RA patients.

Infliximab, a chimeric monoclonal antibody to TNF-alpha, has been used worldwide for RA patients concomitantly with MTX. MTX virtually abolishes the human antichimeric antibody response. Maini et al. reported that low-dose MTX may be useful in reducing the immunogenicity of other therapeutic monoclonal antibodies [12].

An important problem associated with the use of infliximab in therapeutic drug regimens is that its efficacy often decreases during prolonged treatment [13, 14].

Although it efficacy in Japanese RA patients was demonstrated in the RECONFIRM study [15], the results of this study also indicated that the clinical response to infliximab may decline after 30 weeks of drug therapy. Our results showed the best response with infliximab therapy at 22 weeks. The clinical response to infliximab at 54 weeks was reduced compared with the response at 22 weeks in both groups (Fig. 2a, b).

In Japan, the approved dose of infliximab is up to 3 mg/kg, or 200 mg/body, and that of MTX is up to 8 mg/week. Insufficient doses of these drugs may contribute to a decrease in the clinical efficacy of infliximab. However, the adverse events associated with MTX, such as liver dysfunction, have limited its use or required use at less than the optimal dose. In our study, good response using EULAR criteria was higher in the standard-dose group than in the lower-dose group, but good and moderate response was almost the same in both groups.

Interestingly, the clinical response to infliximab was reduced, but clinical remission was increased, at 54 weeks compared to 22 weeks in both groups (Fig. 2). Thus, there appear to be patients with greater efficacy of infliximab, as

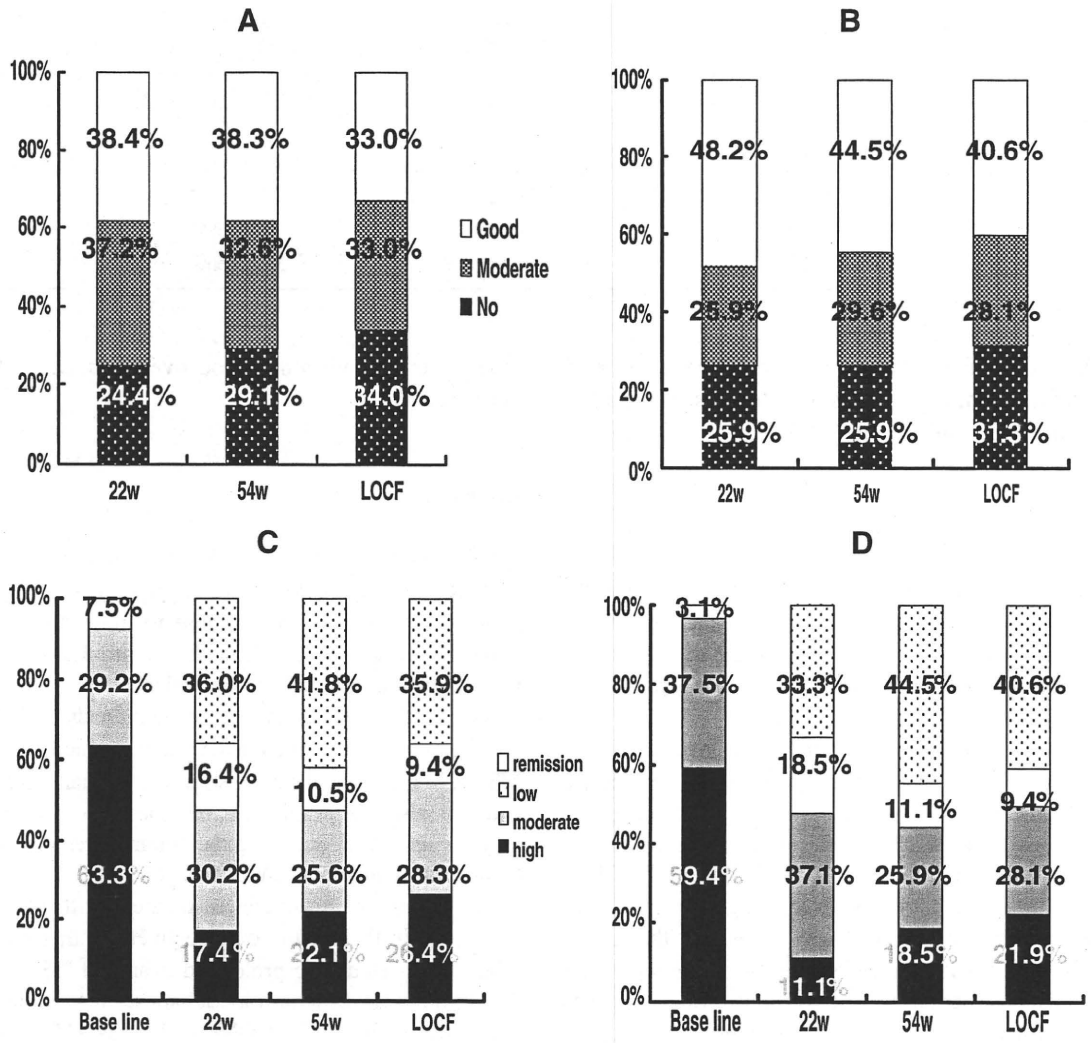

Fig. 2 Outcome of methotrexate (MTX) and infliximab therapy. **a** Clinical response in the lower-dose MTX (≤4 mg/week) group. **b** Clinical response in the standard-dose MTX (≥6 mg per week) group. **c** Proportion of disease activity in the lower-dose MTX (≤4 mg/week) group. **d** Proportion of disease activity in the standard-dose MTX (≥6 mg per week) group

well as patients with a reduced response to infliximab, in both groups, regardless of MTX dosage.

In 25 patients who discontinued infliximab, the incidence of adverse events was similar between the two groups, but lack of effectiveness was more frequent in the lower-dose group. In our opinion, MTX doses greater than 6 mg are good if patients can tolerate them. However, we recommend that the patients who cannot tolerate the optimal MTX dose can be treated with infliximab plus lower-dose MTX therapy.

Some methods have been suggested for cases in which infliximab is ineffective. Horikoshi suggested that in cases where infliximab is ineffective but MTX cannot be increased, mizoribine pulse therapy should be attempted [16]. An increase in the dose of infliximab beyond 3 mg/kg (e.g., 5 or 10 mg/kg) or shortening of the interval between infliximab infusions (e.g., every 6 weeks) has proven to be effective in such cases [17–19]. Recently, the Japanese Ministry of Health, Labor, and Welfare approved these approaches. If we use these methods, infliximab therapy may become more effective, regardless of MTX dosage.

Conclusion

We compared long-term outcomes of infliximab therapy combined with lower-dose MTX (≤4 mg per week) and standard-dose MTX (≥6 mg per week) in Japanese RA patients. Outcomes using the DAS28 value and the response rate using the EULAR response criteria were not

significantly different between the two groups. Infliximab plus MTX therapy was effective in Japanese RA patients, regardless of MTX dosage.

Disclosures None.

References

1. Bansback NJ, Young A, Brennan A (2005) The NICE reappraisal of biologics in 2005: what rheumatologists need to know. Rheumatology (Oxford) 44:3–4
2. Feltelius N, Fored CM, Blomqvist P, Bertilsson L, Geborek P, Jacobsson LT, Lindblad S, Lysholm J, Rantapää-Dahlqvist S, Saxne T, Klareskog L; ARTIS Group (2005) Results from a nationwide postmarketing cohort study of patients in Sweden treated with etanercept. Ann Rheum Dis 64:246–252
3. Vrijhoef HJ, Diederiks JP, Spreeuwenberg C, Van der Linden S (2003) Applying low disease activity criteria using the DAS28 to assess stability in patients with rheumatoid arthritis. Ann Rheum Dis 62:419–422
4. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, Lipsky P (1999) Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. Lancet 354:1932–1939
5. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Furst D, Weisman MH, St Clair EW, Keenan GF, van der Heijde D, Marsters PA, Lipsky PE, Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group (2004) Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. Arthritis Rheum 50:1051–1065
6. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, Smolen JS, Weisman M, Emery P, Feldmann M, Harriman GR, Maini RN, Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group (2000) Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. N Engl J Med 343:1594–1602
7. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31:315–324
8. Van Riel PL, Van Gestel AM, Scott DL, on behalf of the EULAR Standing Committee for International Clinical Studies Including Therapeutic Trials e ESCISIT (2000) The EULAR handbook of clinical assessments in rheumatoid arthritis. Nijmegen, Alpen aan den Rijn van Zuiden Communications BV, The Netherlands
9. Inoue E, Yamanaka H, Hara M, Tomatsu T, Kamatani N (2007) Comparison of Disease Activity Score (DAS)28- erythrocyte sedimentation rate and DAS28- C-reactive protein threshold values. Ann Rheum Dis 66:407–409
10. Matsui T, Kuga Y, Kaneko A, Nishino J, Eto Y, Chiba N, Yasuda M, Saisho K, Shimada K, Tohma S (2007) Disease Activity Score 28 (DAS28) using C-reactive protein underestimates disease activity and overestimates EULAR response criteria compared with DAS28 using erythrocyte sedimentation rate in a large observational cohort of rheumatoid arthritis patients in Japan. Ann Rheum Dis 66:1221–1226
11. Soubrier M, Zerkak D, Gossec L, Ayral X, Roux C, Dougados M (2006) Which variables best predict change in rheumatoid arthritis therapy in daily clinical practice? J Rheumatol 33:1243–1246
12. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD (1998) Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum 41:1552–1563
13. Wolbink GJ, Vis M, Lems W, Voskuyl AE, de Groot E, Nurmohamed MT, Stapel S, Tak PP, Aarden L, Dijkmans B (2006) Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arithritis. Arthritis Rheum 54:711–715
14. van der Bijl AE, Breedveld FC, Antoni CE, Kalden JR, Kary S, Burmester GR, Beckmann C, Unnebrink K, Kupper H (2008) An open-label pilot study of the effectiveness of adalimumab in patients with rheumatoid arthritis and previous infliximab treatment: relationship to reasons for failure and anti-infliximab antibody status. Clin Rheumatol 27:1021–1028
15. Yamanaka H, Tanaka Y, Sekiguchi N, Inoue E, Saito K, Kameda H, Iikuni N, Nawata M, Amano K, Shinozaki M, Takeuchi T (2007) Retrospective clinical study on the notable efficacy and related factors of infliximab therapy in a rheumatoid arthritis management group in Japan (RECONFIRM). Mod Rheumatol 17:178
16. Horikoshi M, Ito S, Ishikawa M, Umeda N, Kondo Y, Tsuboi H, Hayashi T, Goto D, Matsumoto I, Sumida T (2009) Efficacy of mizoribine pulse therapy in patients with rheumatoid arthritis who show a reduced or insufficient response to infliximab. Mod Rheumatol 19:229–234
17. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, Smolen JS, Weisman M, Emery P, Feldmann M, Harriman GR, Maini RN, Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group (2000) Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. N Eng J Med 343:1594–1602
18. St Clair EW, Wagner CL, Fasanmade AA, Wang B, Schaible T, Kavanaugh A, Keystone EC (2002) The relationship of serum infliximab concentrations to clinical improvement in rheumatoid arthritis: results from ATTRACT, a multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 46:1451–1459
19. Hjardem E, Østergaard M, Pødenphant J, Tarp U, Andersen LS, Bing J, Peen E, Lindegaard HM, Ringsdal VS, Rødgaard A, Skøt J, Hansen A, Mogensen HH, Unkerskov J, Hetland ML (2007) Do rheumatoid arthritis patients in clinical practice benefit from switching from infliximab to a second tumor necrosis factor alpha inhibitor? Ann Rheum Dis 66:1184–1189

Arch Orthop Trauma Surg (2010) 130:1447–1450
DOI 10.1007/s00402-010-1056-9

ORTHOPAEDIC SURGERY

Serum metal ion levels after second-generation metal-on-metal total hip arthroplasty

Takao Imanishi · Masahiro Hasegawa · Akihiro Sudo

Received: 13 November 2009 / Published online: 29 January 2010

Abstract
Introduction Metal-on-metal bearings for total hip arthroplasty are increasing in popularity. However, metal ion toxicity, metal hypersensitivity, and metal carcinogenicity are the causes concern for patients with metal-on-metal hip replacement. We investigated serum levels of cobalt and chromium ions in patients with successfully implanted second-generation metal-on-metal total hip arthroplasty (THA) using PINNACLE-A (DePuy, Warsaw, IN, USA).
Materials and methods Thirty-three patients underwent primary cementless THA with the use of a 36-mm femoral head PINNACLE-A with a metal-on-metal articulation. Blood samples were taken preoperatively, at 3 months, and at 1 year, and levels of cobalt and chromium were determined.
Results At 3 months, levels of both cobalt and chromium had increased significantly compared with preoperative levels. There were no significant differences between levels of either metal at 3 months and 1 year.
Conclusion Patients with metal-on-metal THA had higher circulating levels of metal ions than before arthroplasty at 3 months, with no additional significant increases at 1 year in this study.

Keywords Total hip arthroplasty · Metal-on-metal · Serum metal ion level · Cobalt · Chromium

T. Imanishi · M. Hasegawa (✉) · A. Sudo
Department of Orthopaedic Surgery, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan
e-mail: masahase@clin.medic.mie-u.ac.jp

Introduction

Metal-on-metal bearings are being increasingly used in total hip arthroplasty (THA) and hip resurfacing with the understanding that reduced wear and osteolysis will improve long-term survival [1]. However, the possible complications resulting from the dissemination of metal particles and ions throughout the body are likely cause of patient anxiety. The particles are nanometers in size and high in number, and their dissolution results in measurable increases in cobalt and chromium ions in serum, urine, and red blood cells of patients with a metal-on-metal bearing [2]. We investigated serum levels of cobalt and chromium ions in patients with successfully implanted second-generation metal-on-metal THA using PINNACLE-A (DePuy, Warsaw, IN, USA) [3, 4].

Materials and methods

From December 2006 to April 2008, 85 consecutive primary THA procedures using PINNACLE-A were performed in our department. Study exclusion criteria included the presence of other metallic implants, metal allergy, pregnancy, and renal insufficiency. This resulted in 33 patients being included in the study, including 4 men and 29 women. The mean age of participants was 60 years (range, 41–83 years), and the mean body mass index was 25 (range, 18–34). The preoperative diagnosis was osteoarthritis in 30 patients and rheumatoid arthritis in 3 patients. Demographic data are shown in Table 1.

All patients underwent primary cementless THA with the use of a 36-mm femoral head PINNACLE-A with a metal-on-metal articulation [3, 4]. S-ROM-A (DePuy) was used in the femoral stem. The acetabular component was

Table 1 Demographic data of the study population (range)

Number of patients	33
Male:female	4:29
Mean age (years)	60 (41–83)
Mean body mass index (kg/m^2)	25 (18–34)
Pre-operative diagnosis	
Osteoarthritis	30
Rheumatoid arthritis	3

made of wrought-forged, high-carbon content cobalt–chromium alloy (0.35% C) with a diametric bearing clearance of 80–120 μm. The mean diameter of the acetabular component was 54 mm (range, 52–58 mm). The acetabular component inclination angle was measured on anteroposterior pelvic radiographs. The inclination angle was defined as the angle between the line joining the inferior teardrop points and the axis of opening of the acetabular component. The mean inclination angle was 49° (range, 32°–70°). Clinical evaluation was performed using the Japanese Orthopaedic Association (JOA) score [5]. The JOA score carries a maximum score of 100, with scales to evaluate pain (0–40 points), range of movement (0–20 points), walking ability (0–20 points), and activities of daily living (0–20 points) [5]. This study was approved by our institution's Ethics Committee, and all patients gave informed consent.

Twelve milliliters of blood samples was taken preoperatively, at 3 months, and at 1 year after surgery using cobalt-free needles and glass tubes for trace metal analysis without additives for blood collection to avoid metal contamination. Blood samples were submitted for analysis by Mitsubishi Chemistry Medience Co., Ltd. (Tokyo, Japan), and serum samples were stored at −20°C in inert polystylene tubes until assayed. Levels of cobalt were determined by Inductively Coupled Plasma Mass Spectrometry (Perkin-Elmer SCIEX Elan 6100 DRC ICP-MS system; Perkin-Elmer Instruments, Norwalk, CT, USA) at Mayo Medical Laboratories. The detection limit of the method was estimated to be 0.2 μg/L. Levels of chromium were determined using a graphite furnace atomic absorption spectrometer (Z-5700; Hitachi Ltd., Tokyo, Japan) with polarization-Zeeman absorption. The detection limit of the method was estimated to be 0.2 μg/L. All concentrations below that limit were defined as 0.2 μg/L for both cobalt and chromium to allow for statistical calculation.

Statistical analysis was performed using the Wilcoxon signed rank test to compare preoperative and postoperative JOA scores. For each time-point, the median as well as the 25th and 75th percentiles of cobalt and chromium concentrations was calculated. We used the median concentrations of cobalt and chromium, and applied the Wilcoxon signed rank test for comparisons over time. The acetabular component inclination, the cup diameter, the age of the patient, body mass index, and JOA score were correlated with serum concentrations of cobalt and chromium using the Spearman correlation coefficient. Statistical significance was set at $P < 0.05$.

Results

The JOA score improved significantly from 45 points (range, 22–73 points) preoperatively to 76 points (range, 56–97 points) at 3 months ($P < 0.001$) and 80 points (57–100 points) at 1 year ($P < 0.001$).

Pre- and postoperative serum concentrations of cobalt and chromium are summarized in Fig. 1. The median preoperative serum cobalt concentration was 0.3 μg/L. At 3 months, cobalt levels (0.7 μg/L) had increased

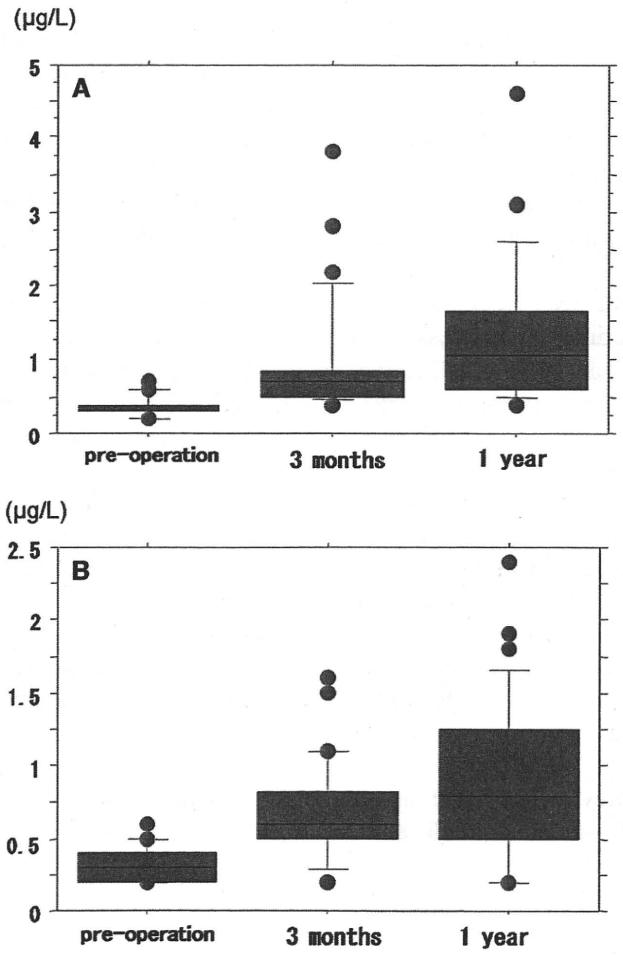

Fig. 1 Serum concentrations of cobalt (**a**) and chromium (**b**). *Top*, *bottom*, and *middle lines* of the graph correspond to the 75th percentile, 25th percentile, and median, respectively. *Bars* indicate the range of the 10th and 90th percentiles. Each *circle* represents an outlier

significantly compared with preoperative levels ($P < 0.001$). There was no significant difference between cobalt levels at 3 months and 1 year (1.1 μg/L; $P = 0.1301$). The median preoperative serum chromium concentration was 0.3 μg/L. At 3 months, chromium levels had increased significantly (0.6 μg/L) compared with preoperative levels ($P < 0.001$). There was no significant difference between the chromium levels at 3 months and 1 year (0.8 μg/L; $P = 0.0854$).

Neither the acetabular component inclination, cup diameter, nor the age of the patient, body mass index, or JOA score showed a significant correlation with cobalt and chromium serum concentrations in the present study.

Discussion

There are limited data available on systemic metal concentrations in patients implanted with PINNACLE-A [6–8]. Antoniou et al. [6] reported a comparison of the serum metal ion levels between the 28- and 36-mm head metal-on metal-prostheses. At 6 months, serum metal levels for the 36-mm femoral head with a metal-on-metal articulation PINNACLE-A was 1.8 μg/L for cobalt and 0.25 μg/L for chromium. At 1 year, the serum metal level of cobalt was 2.3 μg/L and that of chromium was 0.4 μg/L. Isaac et al. [8] measured the whole blood metal ion levels with 28-mm head metal-on-metal prostheses. The median cobalt and chromium levels were 0.83 and 0.66 μg/L at 1 year.

The levels of both cobalt and chromium in our study were lower than levels reported in previous studies of different types of metal-on-metal bearings [9–12]. In contrast, serum metal levels of cobalt and chromium for the 28-mm Metasul bearing (Zimmer, Warsaw, IN) were similar to those found in previously reported investigations for modern, second-generation metal-on-metal bearings [13, 14].

Recent study evaluated the release of metal ions after metal-on-metal total disc arthroplasty [15]. The average concentrations in the serum were 4.8 μg/L for cobalt and 1.9 μg/L for chromium after average follow-up of 15 months [15]. The levels were similar or higher than the levels shown in the literature following implantation of total hip endoprosthetics with metal-on-metal bearings [6–14].

In terms of acetabular component inclination, we found no significant correlation between cobalt and chromium serum concentrations. Vendittoli et al. [16] was also unable to draw strong conclusions regarding the acetabular inclination and levels of cobalt. On the other hand, some authors [17, 18] reported significantly higher levels of metal ions in patients with steeply inclined components. In our study, there was no significant correlation between patient activity, as assessed using activities of daily living score of the JOA score [5] and serum metal levels. This is in agreement with recent results showing that metal ion levels are not acutely affected by patient activity [16, 17, 19]. However, another study showed a mild increase in levels of cobalt but not chromium following exercise [20].

Despite concerns regarding chromosome aberrations and translocations, the International Agency for Research on Cancer concluded that there is inadequate evidence in humans regarding the carcinogenicity of orthopaedic implants [6]. Metal-on-metal bearings often are not recommended in women of child-bearing age because of the theoretical risk of metal ion exposure to the fetus [21]. Data from Nordic registries are used to estimate adverse effects on a large scale, based mostly on metal-on-polyethylene bearings, and cancer incidence was in line with the general population [2]. Elevated ion levels have been associated with an increased prevalence of chromosomal changes, suggesting a possible carcinogenic effect [21, 22].

Our results showed that patients with metal-on-metal total hip prostheses had higher circulating levels of metal ions than before arthroplasty at 3 months, with no additional significant increases at 1 year; however, future, follow-up studies will investigate the long-term concentration of metal ions.

References

1. Cobb AG, Schmalzreid TP (2006) The clinical significance of metal ion release from cobalt–chromium metal-on-metal hip joint arthroplasty. Proc Inst Mech Eng 220:385–398
2. Visuri TI, Pukkala E, Pulkkinen P, Paavolainen P (2006) Cancer incidence and causes of death among total hip replacement patients: a review based on Nordic cohorts with a special emphasis on metal-on-metal bearings. Proc Inst Mech Eng 220:399–407
3. Engh CA, Hopper RH Jr, Engh CA Jr (2004) Long-term porous-coated cup survivorship using spikes, screws, and press-fitting for initial fixation. J Arthroplasty 19(7 Suppl 2):54–60
4. Powers CC, Ho H, Beykirch SE, Huynh C, Hopper RH Jr, Engh CA Jr, Engh CA (2009) A comparison of a second- and a third-generation modular cup design is new improved? J Arthroplasty. April 8. [Epub ahead of print]
5. Ohashi H, Hirohashi K, Yamano Y (2000) Factors influencing the outcome of Chiari pelvic osteotomy: a long-term follow-up. J Bone Joint Surg Br B 82-B:517–525
6. Antoniou J, Zukor DJ, Mwale F, Minarik W, Petit A, Huk OL (2008) Metal ion levels in the blood of patients after hip resurfacing: A comparison between twenty-eight and thirty-six-millimeter-head metal-on-metal prostheses. J Bone Joint Surg Am A 90(Suppl 3):142–148
7. Williams S, Schepers A, Isaac G, Hardaker C, Ingham E, van der Jagt D, Breckon A, Fisher J (2007) Ceramic-on-metal hip arthroplasties: a comparative in vitro and in vivo study. Clin Orthop Relat Res 465:23–32
8. Isaac GH, Brockett C, Breckon A, van der Jagt D, Williams S, Hardaker C, Fisher J, Schepers A (2009) Ceramic-on-metal

bearings in total hip replacement: whole blood metal ion levels and analysis of retrieved components. J Bone Joint Surg Br 91(9):1134–1141
9. Savarino L, Granchi D, Ciapetti G, Cenni E, Nardi Pantoli A, Rotini R, Veronesi CA, Baldini N, Giunti A (2002) Ion release in patients with metal-on-metal hip bearings in total joint replacement:a comparison with metal-on-polyethylene bearings. J Biomed Mater Res 63:467–474
10. Clarke MT, Lee PTH, Arora A, Villar RN (2003) Levels of metal ions after small- and large-diameter metal-on-metal hip arthroplasty. J Bone Joint Surg Br B 85:913–917
11. Witzleb W, Ziegler J, Krummenauer F, Neumeister V, Guenther K (2006) Exposure to chromium, cobalt and molybdenum from metal-on-metal total hip replacement and hip resurfacing arthroplasty. Acta Orthop 77:697–705
12. Moroni A, Savarino L, Cadossi M, Baldini N, Giannini S (2008) Does ion release differ between hip resurfacing and metal-on-metal THA? Clin Orthop Relat Res 466:700–707
13. Brodner W, Bitzan P, Meisinger V, Kaider A, Gottsauner-Wolf F, Kotz R (2003) Serum cobalt levels after metal-on-metal total hip arthroplasty. J Bone Joint Surg Am A 85:2168–2173
14. Hur CI, Yoon TR, Cho SG, Song EK, Seon JK (2008) Serum ion level after metal-on-metal THA in patients with renal failure. Clin Orthop Relat Res 466:696–699
15. Zeh A, Becker C, Planert M, Lattke P, Wohlrab D (2009) Time-dependent release of cobalt and chromium ions into the serum following implantation of the metal-on-metal Maverick type artificial lumbar disc (Medtronic Sofamor Danek). Arch Orthop Trauma Surg 129(6):741–746
16. Vendittoli PA, Mottard S, Roy AG, Dupont C, Lavigne M (2007) Chromium and cobalt ion release following the Durom high carbon content, forged metal-on-metal surface replacement of the hip. J Bone Joint Surg Br B 89:441–448
17. Haan RD, Pattyn C, Gill HS, Murray DW, Campbell PA, Smet KD (2008) Correlation between inclination of the acetabular component and metal ion levels in metal-on-metal hip resurfacing replacement. J Bone Joint Surg Br B 90:1291–1297
18. Langton DJ, Jameson SS, Joyce TJ, Webb J, Nargol AV (2008) The effect of component size and orientation on the concentrations of metal ions after resurfacing arthroplasty of the hip. J Bone Joint Surg Br B 90:1143–1151
19. Heisel C, Silva M, Skipor AK, Jacobs JJ, Schmalzried TP (2005) The relationship between activity and ions in patients with metal-on-metal bearing hip prostheses. J Bone Joint Surg Am A 87:781–787
20. Kahn M, Kuiper JH, Richardson JB (2008) The exercise-related rise in plasma cobalt levels after metal-on-metal hip resurfacing arthroplasty. J Bone Joint Surg Br B 90:1152–1157
21. Ladon D, Doherty A, Newson R, Turner J, Bhamra M, Case CP (2004) Changes in metal levels and chromosome aberrations in the peripheral blood of patients after metal-on-metal hip arthroplasty. J Arthroplasty 19(8 Suppl 3):78–83
22. Dunstan E, Ladon D, Whittingham-Jones P, Carrington R, Briggs TW (2008) Chromosomal aberrations in the peripheral blood of patients with metal-on-metal hip bearings. J Bone Joint Surg Am A 90:517–522

Biomarkers, 2010; 15(2): 149–157

informa
healthcare

ORIGINAL ARTICLE

Evaluation of soluble fibrin and D-dimer in the diagnosis of postoperative deep vein thrombosis

Rui Niimi[1], Masahiro Hasegawa[1], Akihiro Sudo[1], Dequan Shi[1], Tomomi Yamada[2], and Atsumasa Uchida[1]

[1]*Department of Orthopaedic Surgery, Mie University Graduate School of Medicine, Mie, Japan, and* [2]*Translational Medical Science, Social and Environmental Medicine, Graduate School of Medicine, Clinical Research Support Center, Mie University Hospital, Mie, Japan*

Abstract

Soluble fibrin (SF) and D-dimer are useful for making the diagnosis of deep vein thrombosis (DVT). However, the evidence for using such markers and optimal timing to diagnose postoperative DVT are unclear. We evaluate the usefulness of SF and D-dimer testing for the diagnosis of postoperative DVT. A total of 207 patients who had total hip arthroplasty or knee arthroplasty were evaluated. SF and D-dimer were tested on postoperative days 1 and 7. DVT was confirmed with ultrasonography. SF level on postoperative day 1 was the most useful, although D-dimer evaluation on postoperative days 1 and 7 was also useful. Using a SF cut-off of more than 4.00 μg ml^{-1}, the sensitivity was 90%, the specificity was 33%. Although the SF and D-dimer tests cannot be used as stand-alone tests, SF and D-dimer are valuable screening tools. We recommend two-stage screening including first with the SF or D-dimer test, followed by ultrasonography or venography.

Keywords: *D-dimer; deep vein thrombosis; postoperative condition; screening; soluble fibrin*

Introduction

Venous thromboembolism (VTE) is a common and serious complication after surgery, especially after total hip arthroplasty (THA) or total knee arthroplasty (TKA), and it is a leading cause of morbidity and mortality during the postoperative recovery period (Douketis et al. 1997, Geerts et al. 2001, Stringer et al. 1989). Without prophylaxis after THA and TKA, the overall incidence of DVT is 40–70% (Geerts et al. 2001) and 40–84% (Geerts et al. 2001, Stringer et al. 1989, Rabinov & Paulin 1972), respectively. Physical immobility and blood hypercoagulability are important factors related to the development of postoperative deep vein thrombosis (DVT). The clinical signs and symptoms of DVT are unreliable, and both venography and ultrasonography remain the 'gold standard' methods, despite their many limitations: venography is invasive, costly and not easily repeatable (Kamikura et al. 2005), while ultrasonography requires skill and manpower. As these methods are time-consuming and expensive, to perform a DVT check on all patients by venography or ultrasonography repeatedly is impossible in institutions where several thousand orthopaedic operations are performed per year. A plasma marker reflecting the presence of DVT would be a very attractive diagnostic tool that would overcome these weak points.

Coagulation and fibrinolysis occur in a complex manner in the blood, and many markers are available for detecting the products of thrombin and plasmin action in patients with thrombotic and fibrinolytic disorders (Suzuki et al. 2007). Of these markers, soluble fibrin (SF) reflects thrombin activation and the cleavage of fibrinogen in the early stage of disease, and it is used as an indicator of coagulation (Soe et al. 1996, Wada et al. 2006, Ota et al. 2005); SF detection could be a useful tool for diagnosing thrombotic diseases (Bounameaux et al. 1991). On the other hand, D-dimer is a specific

Address for Correspondence: Masahiro Hasegawa, Department of Orthopaedic Surgery, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu City, Mie, 514-8507, Japan. Tel: +81 59 231 5022. Fax: +81 59 231 5211. E-mail: masahase@clin.medic.mie-u.ac.jp

(Received 22 July 2009; revised 26 September 2009; accepted 27 September 2009)

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/13547500903367276

http://www.informahealthcare.com/bmk

degradation product of plasmin-cleaved, cross-linked fibrin. Therefore, it is considered to be a sensitive indicator of coagulation activation or secondary fibrinolysis, and it has been shown to be helpful in the diagnosis of DVT (Tan et al. 2009, Michiels et al. 2006, Rectenwald et al. 2005, Ota et al. 2005, Friera-Reyes et al. 2005, Ginsberg et al. 1998, Kearon et al. 2006, Kelly et al. 2002, Stein et al. 2004, Wells et al. 2001, 2003, Wijns et al. 1998, Bounameaux et al. 1994).

Several studies on D-dimer have indicated that the D-dimer assay could be used as a simple, inexpensive, screening test for VTE. Bounameaux et al. 1994. showed that the plasma D-dimer level has an overall diagnostic sensitivity of 97% and a specificity of 47% in various patient populations suspected of having DVT who were referred for venography (Bounameaux et al. 1994). However, the roles of the SF and D-dimer assays with respect to DVT screening in postoperative patients remain controversial, because the diagnostic SF and D-dimer levels are affected by many variables (primary disease, thrombus size, anticoagulant therapy and timing).

Recently, we reported the usefulness of SF and D-dimer tests after orthopaedic surgery by examination of 99 patients. In this report, we have increased the investigated number of patients (Sudo et al. 2009). The primary aim of the present study was to compare the diagnostic efficacy of the SF and D-dimer assays used as a screening test to detect postoperative DVT in patients undergoing THA and TKA. The secondary aim was to determine the optimal timing for the evaluation of SF and D-dimer levels for the diagnosis of DVT.

Materials and methods

Patients

From April 2003 to December 2007, 207 patients undergoing THA or TKA at Mie University Hospital were eligible for the study ($n=207$: THA $n=145$, TKA $n=62$). In this study, we excluded patients with DVT before surgery ($n=40$: THA $n=24$, TKA $n=16$). The operative diagnosis was degenerative osteoarthritis in 161 patients (THA $n=112$, TKA $n=49$), rheumatoid arthritis in 20 patients (THA $n=9$, TKA $n=11$), failure of a previous implant in 23 patients (THA $n=21$, TKA $n=2$) and 'other' in three patients (THA $n=3$). None of the patients received anticoagulant therapy. The patients' sex, height, weight, body mass index (BMI, weight in kilograms divided by the square of the height in meters), operation time, blood loss during surgery and blood loss during drainage were recorded. The institutional review boards approved the study protocol, and written informed consent was obtained from all participants.

Study design

The study was designed as a prospective management trial with 7-day follow-up. All patients underwent ultrasonography, and SF and D-dimer assays before surgery. Postoperative ultrasonography was performed 4 days after surgery in consideration of the rehabilitation schedule by very skilled physicians who were blinded to the SF and D-dimer results. The popliteal and calf vein were examined in the sitting position and to perform ultrasonography in the sitting position just after the surgery was difficult. Therefore we performed ultrasonography 4 days after surgery. Postoperative SF and D-dimer measurements were performed on postoperative days 1 and 7.

Diagnosis of VTE

B-mode ultrasonography with compression and colour Doppler imaging were performed for bilateral common femoral veins, the superficial veins, the popliteal veins and the calf veins. Augmentation by calf squeezing or Valsalva's manoeuvre were included as needed. The criteria for the diagnosis of DVT were: loss of compressibility of the vein; presence of intraluminal echogenicity; and absence of venous flow using an Aplio (Toshiba Medical Systems Corp., Tokyo, Japan) sonographic scanner with a linear transducer (frequency 6 MHz).

DVT was classified as proximal if the thrombus involved the iliac, femoral or popliteal veins, and distal if the thrombus was limited to the calf veins. Patients with a proximal DVT proceeded to a pulmonary thromboembolism (PTE) survey. PTE was confirmed by helical computer tomography (CT) scan, pulmonary angiogram or ventilation-perfusion lung scan.

Laboratory studies

Citrated blood samples were taken from each patient the day before the operation and on postoperative days 1 and 7 between 06:00 a.m. and 07:30 a.m., before and after venous occlusion, in order to evaluate plasma SF and D-dimer levels. Citrated blood samples were centrifuged for 20 min at 1500g. The supernatants (plasma) were analysed within 4 h. Plasma SF and D-dimer levels were measured.

Measurement of plasma SF and D-dimer levels

SF was measured using the latex agglutination method (IATRO SF; Mitsubishi Kagaku Iatron Inc., Tokyo, Japan), which is based on monoclonal antibody (mAb) IF-43. mAb IF-43 recognizes a segment of the fibrin Aα chain (Aα17–78 residue segment) exposed in the E region of the fibrin monomer (FM) when the FM molecule binds

the D region of another fibrin monomer or fibrinogen. The antibody is coated for the SF assay. The normal range is <7.0 μg ml^{-1}. Plasma D-dimer levels were also measured using LPIA-ACE D-dimer (Mitsubishi Kagaku Iatron Inc.) with JIF23 mAb. The JIF23 mAb, which recognizes the plasmin-digested N-terminus of the γ-chain on the D region, was used for latex agglutination. The normal range is <1.0 μg ml^{-1}.

Statistical analysis

We compared the patients' baseline characteristics using the Mann–Whitney *U*-test and the χ^2 test. Because their distributions were skewed, the SF and D-dimer levels were log-transformed for the statistical analysis.

To determine the clinical performance of the SF and D-dimer assays, diagnostic sensitivity, specificity, best-fit value (combination of sensitivity + specificity), positive and negative predictive values, positive likelihood ratio (sensitivity / [1-specificity]) were calculated, and receiver operating characteristic (ROC) curves were constructed. The ROC curves and the area under the curve (AUC) were calculated. Analyses were performed using SAS software, version 9.1 (SAS Institute, Inc., Cary, NC, USA); $p < 0.05$ was considered statistically significant.

Results

Figure 1 shows the study flow diagram and Table 1 shows the patients' general characteristics. The series included 207 patients (32 male, 175 female). DVT tended to occur more commonly in females than in males. The patients' median age (± SD) was 64.0 ± 12.1 years. Age had a clinically relevant effect: patients with DVT were older (67.1 years) than patients without DVT (60.9 years). BMI, operation time, blood loss during surgery and blood loss during drainage did not appear to have a clinically relevant effect.

Postoperative DVT was diagnosed in 104 of 207 patients (50.2%). Of these, 92 patients (44.4%) had distal DVT and 12 patients (5.8%) had proximal DVT. Postoperative PTE was diagnosed in five of 207 patients (2.4%). We consulted cardiology with all PTE patients, and three of five patients did not need special treatment for the PTE because the PTE size was very small. None of the patients developed signs or symptoms of PTE, and there were no deaths from PTE.

Table 2 shows the SF and D-dimer results for DVT. After surgery, the SF level increased both in patients with DVT and in those without DVT, with a significant increase in the SF level on the day after surgery in the DVT group (25.7 vs 12.9 μg ml^{-1}). The SF level increased rapidly after surgery and was high 1 day after surgery. However, the SF level declined to a low level 7 days after surgery (6.6 vs 4.0 μg ml^{-1}) (Figure 1). Therefore, there was no significant difference in the SF levels 7 days after surgery between patients with and without DVT.

The D-dimer level was elevated both in patients with DVT and in those without DVT after surgery. D-dimer levels were significantly higher in patients with DVT than in patients without DVT on postoperative days 1 and 7 (day 1, 17.1 vs 10.8 μg ml^{-1}; day 7, 11.8 vs 8.9 μg ml^{-1}). No significant differences between the operative techniques were found (Table 2).

The ROC curves obtained for various cut-off values of SF on postoperative day 1 confirmed that 4.00 μg ml^{-1} is the most reasonable cut-off value for screening for DVT when using the luminescence immunoassay (LIA) assay, yielding a high sensitivity (90.4%), a fair specificity (33.0%), a positive predictive value of 57.7% and a negative predictive value of 77.3% (Figure 3). On the other hand, on postoperative day 1, for the D-dimer test using the LIA, 4.88 μg ml^{-1} is the most reasonable cut-off value for DVT screening, yielding a sensitivity of 91.4%, a specificity of 28.2%, a positive predictive value of 56.5% and a negative predictive value of 78.4%. With respect to the D-dimer test on postoperative day 7, 5.35 μg ml^{-1} is the most reasonable cut-off value for screening for DVT when using the LIA assay, yielding a sensitivity of 90.4%, a specificity of 23.3%, a positive predictive value of 54.9%, and a negative predictive value of 75.0% (Table 3).

The AUC for the SF was 0.7296 on postoperative day 1, while for D-dimer, the AUC was 0.6834 on postoperative day 1 and 0.6513 on postoperative day 7. There were no significant differences in the SF and D-dimer levels between the operative techniques. The best-fit values (sensitivity + specificity) were: 1.4002 (cut-off value, 7.61 μg ml^{-1}) for SF on postoperative day 1; 1.2654 (cut-off value, 9.78 μg ml^{-1}) for D-dimer on postoperative day 1; and 1.2842 (cut-off value, 8.26 μg ml^{-1}) for D-dimer on postoperative day 7.

Discussion

The present study demonstrates that the patients with DVT had a significantly higher SF on the postoperative day 1 and D-dimer on postoperative days 1 and 7 in Mann–Whitney *U*-tests. The incidence of VTE after THA and TKA continues to be high despite current prophylactic regimens. Fatal PTE is a catastrophic complication, with a reported incidence after THA of from 0.19% to 2.3% (Johnson et al. 1977, Khaw et al. 1993, Salzman & Davies.1980). Prevention of fatal PTE depends on appropriate use of prophylaxis and early detection and treatment of DVT. The traditional 'gold standard' test, venography, is a good test for the detection of DVT, but it is too invasive and expensive to use routinely and repeatedly

Table 1. Patient characteristics.

Characteristic	All patients ($n=207$)	Patients with DVT ($n=104$)	Patients without DVT ($n=103$)	p-Value[a]	Patients after THA ($n=145$)	Patients with DVT after THA ($n=60$)	Patients without DVT after THA ($n=85$)	p-Value[a]	Patients after TKA ($n=62$)	Patients with DVT after TKA ($n=44$)	Patients without DVT after TKA ($n=18$)	p-Value[a]
Age (years), median (range)	64.0 (12.1)	67.1 (9.5)	60.9 (13.6)	0.0005	61.0 (12.3)	64.1 (9.4)	58.9 (13.7)	0.0005	70.9 (8.1)	71.2 (8.1)	70.2 (8.2)	0.4748
Women, n (%)	175 (84.5)	98 (94.2)	77 (74.8%)	0.001	122 (84.1)	57 (95.0)	65 (76.5)	0.0026	53 (85.5)	41 (93.2)	12 (66.7)	0.0071
Body weight (kg), mean (SD)	55.7 (10.8)	55.7 (10.0)	55.8 (11.5)	0.0675	55.3 (11.1)	55.4 (10.8)	55.2 (11.3)	0.691	56.8 (10.0)	56.1 (8.9)	58.7 (12.2)	0.2414
Height (cm), mean (SD)	151.9 (8.6)	150.7 (7.2)	153.0 (9.7)	0.7815	152.8 (9.2)	151.1 (7.7)	150.0 (9.9)	0.0462	149.6 (6.7)	150.1 (6.4)	148.5 (7.2)	0.187
Body mass index ($kg\,m^{-2}$), mean (SD)	24.1 (3.8)	24.5 (3.3)	23.7 (4.2)	0.0872	23.6 (3.8)	24.1 (3.3)	23.2 (4.0)	0.0693	25.2 (3.6)	24.9 (3.2)	25.8 (4.5)	0.1514
Operation time (min), mean (SD)	112.3 (40.0)	113.8 (36.8)	110.8 (42.8)	0.1126	106.0 (41.5)	105.3 (40.3)	106.6 (42.5)	0.7480	127.0 (31.7)	125.4 (27.8)	131.1 (38.9)	0.8767
Intraoperative bleeding (g), mean (SD)	336.6 (315.0)	341.4 (368.8)	331.8 (251.0)	0.2366	350.5 (313.5)	357.3 (383.2)	345.7 (255.6)	0.3643	304.1 (40.5)	319.7 (351.4)	266.1 (222.6)	0.9197
Drain bleeding (g), mean (SD)	543.2 (324.6)	552.3 (318.0)	534.1 (332.4)	0.505	561.5 (342.2)	569.6 (359.4)	555.8 (331.5)	0.8946	500.4 (276.9)	528.6 (252.9)	431.5 (326.1)	0.1227
Total bleeding (g), mean (SD)	880.0 (504.9)	893.7 (545.5)	865.8 (462.5)	0.8573	912.0 (528.6)	926.9 (598.3)	901.5 (476.7)	0.592	804.6 (439.4)	848.3 (466.8)	697.6 (352.9)	0.2035

DVT, deep vein thrombosis; THA, total hip arthroplasty; TKA, total knee arthroplasty. [a]Mann–Whitney U-test; χ^2 test.

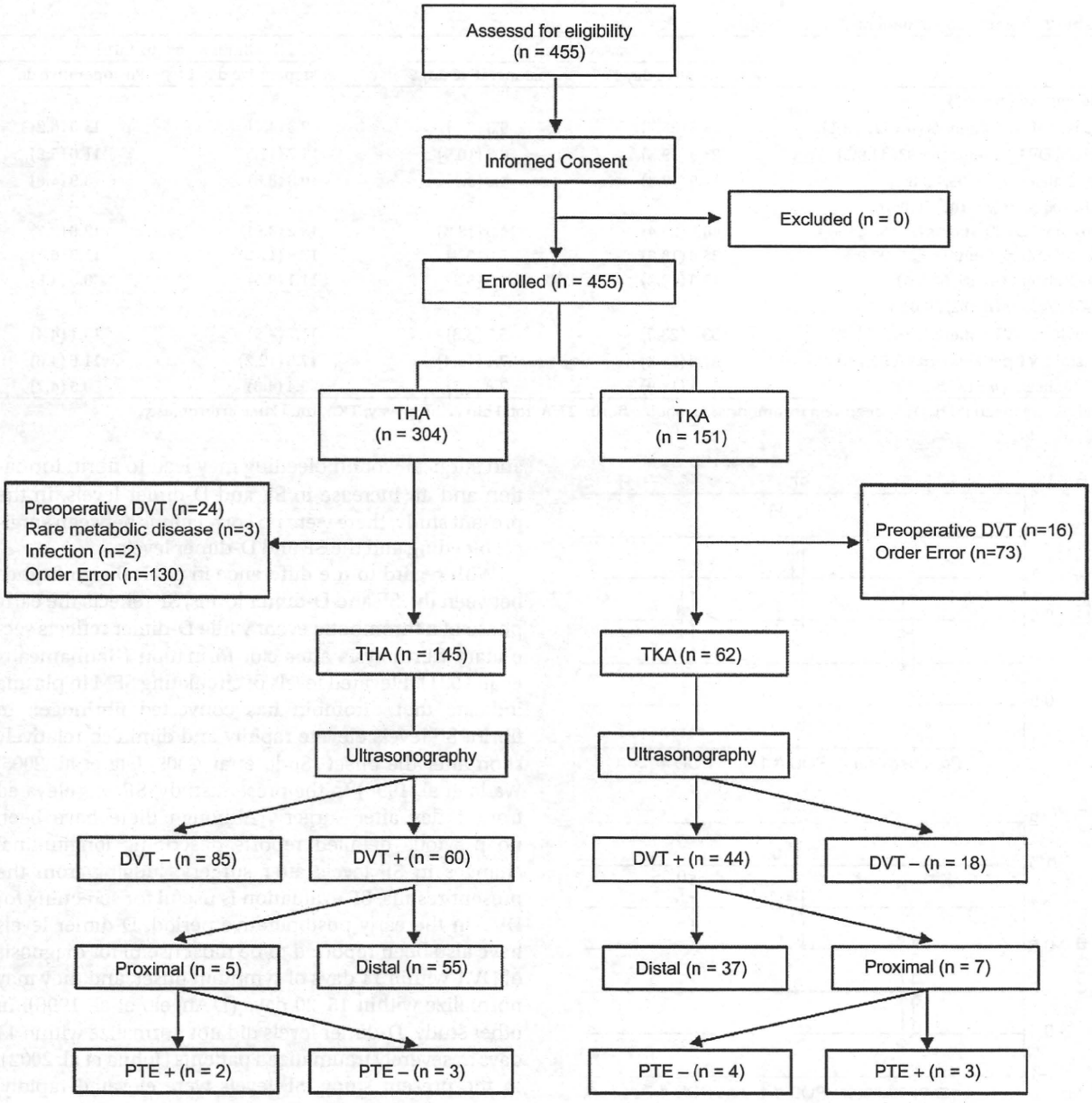

Figure 1. Study flow diagram. THA, total hip arthroplasty; TKA, total knee arthroplasty; DVT, deep vein thrombosis; PTE, thromboembolism.

for screening (Kamikura et al. 2005). Ultrasonography is also a good alternative test that is quite non-invasive compared with venography, but ultrasonography is too complicated for screening to check all operative patients repeatedly because it is too time-consuming. The use of SF and D-dimer testing has the potential to make the screening of DVT more convenient, reproducible everywhere and economical.

The pathogenesis of DVT is multifactorial and only partly understood. Several factors are thought to be involved, such as increased blood coagulability, endothelial damage and impaired fibrinolytic activity (Trotti et al. 1997). Thrombin-catalysed cleavage of fibrinogen yields several species of SF monomer (SFM) (Wada et al. 2006). When they are produced in the presence of an excess of fibrinogen, they form complexes with fibrinogen and exist as soluble monomer complexes (SMC), called SF. Under pathological conditions when blood coagulation is activated and thrombin is eventually generated, SF is known to be present in the circulating blood. Therefore, detection and quantification of SF in plasma derived from patients with thrombotic disease has been expected to provide useful information on the state and degree of intravascular

Table 2. Comparison of patients.

	SF assay (μg ml^{-1})		D-dimer assay (μg ml^{-1})	
	Postoperative day 1	Postoperative day 7	Postoperative day 1	Postoperative day 7
All patients (n=207)				
Proximal DVT patients (n=12, 5.3%)	39.6 (25.7)	9.3 (9.6)	12.3 (4.4)	13.0 (6.2)
Distal DVT patients (n=93, 44.9%)	23.9 (19.3)	6.2 (10.9)	17.7 (11.5)	11.6 (5.9)
DVT absent (n=103, 49.8%)	12.9 (17.3)	4.0 (3.1)	10.8 (8.8)	8.9 (4.8)
THA patients (n=145, 70.0%)				
Proximal DVT patients (n=5, 3.4%)	48.1 (29.4)	14.3 (13.5)	13.2 (3.9)	12.8 (8.8)
Distal DVT patients (n=55, 37.9%)	25.8 (18.6)	5.6 (6.3)	17.9 (11.2)	11.6 (6.7)
DVT absent (n=85, 58.6%)	13.3 (18.2)	4.2 (3.2)	11.1 (9.5)	8.8 (4.8)
TKA patients (n=62, 30.0%)				
Proximal DVT patients (n=7, 11.3%)	33.6 (23.0)	5.7 (3.3)	11.7 (4.9)	13.1 (4.4)
Distal DVT patients (n=37, 59.7%)	21.0 (20.3)	7.1 (15.4)	17.5 (12.2)	11.6 (4.6)
DVT absent (n=18, 29.0%)	11.1 (12.9)	2.8 (1.7)	9.4 (4.6)	9.5 (4.7)

Values are mean (SD). DVT, deep vein thrombosis; SF, souble fibrin; THA, total hip arthroplasty; TKA, total knee arthroplasty.

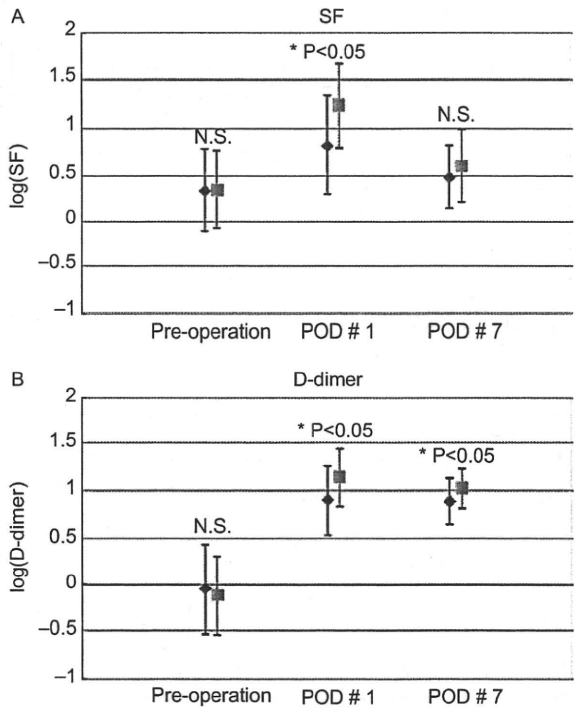

Figure 2. Plasma soluble fibrin (SF) (A) and D-dimer (B) levels. N.S., not significant; POD, postoperative day.

coagulation (Suzuki et al. 2007, Bounameaux et al. 1991, Vogel et al. 1996).

D-dimer is a fibrin-derived fragment that is released into the circulation when cross-linked fibrin is broken down by the fibrinolytic system (Stein et al. 2004, Wells et al. 2003). Elevated D-dimer levels generally can be seen with intravascular activation of the coagulation system (Tan et al. 2009, Michiels et al. 2006, Rectenwald et al. 2005, Kelly et al. 2002, Wells et al. 2001, Le Gal et al. 2006) and secondary fibrinolysis. Although both haemostasis and surgical wound bleeding may lead to fibrin formation and an increase in SF and D-dimer levels, in the present study, there were no correlations between surgical bleeding and the SF and D-dimer levels.

With regard to the difference in clinical significance between the SF and D-dimer levels, SF reflects the early phase of a thrombotic event while D-dimer reflects secondary fibrinolysis after clot formation (Bounameaux et al. 1991). Elevated levels of circulating SFM in plasma indicate that thrombin has converted fibrinogen to fibrin. SF levels elevate rapidly and diminish relatively soon after the onset (Sudo et al. 2009, Ota et al. 2005, Wada et al. 1996) In the present study, SF was elevated from 1 day after surgery. Although there have been no previous detailed reports describing longitudinal changes in SF levels after surgery, judging from the present results, SF evaluation is useful for screening for DVT in the early postoperative period. D-dimer levels have also been reported to be most useful for diagnosis of DVT within 11 days of symptom onset, and they may normalize within 15–20 days (D'Angelo et al. 1996). In other study, D-dimer levels did not normalize within 14 days in severely traumatized patients (Johna et al. 2002). In the present study, SF levels were elevated rapidly, while D-dimer levels were elevated on postoperative day 1 and remained high 7 days after surgery.

The diagnostic capability of these methods was tested using AUC analysis, and the SF assay on postoperative day 1 was the most reliable assay based on the AUC (0.72) and the best-fit values. Thus, SF evaluation for screening should be emphasized, because SF evaluation offers early detection of DVT and has an excellent best-fit value. Thus, treatment and walking exercises can be started early. D-dimer is useful when the onset of DVT is unclear in cases of idiopathic DVT because D-dimer levels remain higher for a longer period than for SF.

Our results confirmed the findings from recent studies that reported persistently elevated D-dimer levels 7

days following major orthopaedic surgery; in contrast SF levels rapidly decreased within a few days after surgery (D'Angelo et al. 1996, Johna et al. 2002, Ota et al. 2005, Sudo et al. 2009, Wada et al. 1996). As orthopaedic surgery is associated with haemostasis and surgical wound bleeding, both of which lead to fibrin formation, both SF and D-dimer may increase without any relationship to DVT. However the patients who developed DVT showed a more marked increase due to DVT. We think the longitudinal difference between SF levels and D-dimer levels is due to the difference of the coagulation system and fibrinolytic system. Our findings imply that the activation of the coagulation system induced by an operation settles within a few days; in contrast, the activation of the fibrinolytic system induced by an operation remains persistently elevated at least 7days following the operation.

Whether the cut-off value is different according to the operative procedure is not known at present. In the present study, the optimal SF and D-dimer level cut-off values for determining the DVT status of patients were examined separately after THA and TKA. The SF and D-dimer levels were similar for patients following THA and TKA. It may be necessary to study SF and D-dimer levels following other surgical procedures to determine whether the cut-off value is independent of surgery. In the present study, ROC curve analysis showed that a cut-off value of 4.00 μg ml^{-1} was the most reasonable to screen for DVT in postoperative patients. In many patients with DVT, the levels of these markers were lower than the cut-off values in the present study. This reason for this may be due to the presence of some occult thrombosis in these patients.

The sensitivity and the negative predictive values of the D-dimer test for DVT and PTE have been reported to be high (Michiels et al. 2006, Douketis et al. 1996, Friera-Reyeset al. 2005, Ginsberg et al. 1998, Kearon et al. 2006, Kelly et al. 2002, Stein et al. 2004, Wells et al. 2001, 2003, Perrier et al. 1999, van Belle et al. 2006). Pooled data from 20 studies of outpatients with clinically suspected VTE have shown a diagnostic sensitivity of 97%, with false negatives presumably explained on the basis of a small thrombus mass (sensitivity may be lower in patients with isolated, below-the-knee DVT) (Stein et al. 2004). Reber et al. showed that, in hospitalized patients, high concentrations of D-dimer due to reasons other than VTE decrease the utility of the test, and in surgical patients the situation is even more complicated, which leads to lower accuracy (Reber et al. 2000).

Vogel et al. followed 129 patients who underwent abdominal surgery. They found that evaluation of SF levels using enzyme-linked immunosorbent assay (ELISA) leads to a high sensitivity (91.7%), with a specificity of 73.2% (Vogel et al. 1996). Bongard et al. (1994) evaluated 173 patients undergoing hip surgery using the D-dimer assay (ELISA). The sensitivity and specificity for proximal DVT were 79% and 36%, respectively. Our results revealed SF and D-dimer evaluation could not be used as a stand-alone test for all postoperative patients suspected of having DVT. Our results suggest that a two-step screening process, involving an initial SF and D-dimer estimation, might significantly decrease the number of patients requiring imaging. Further studies are required to evaluate the utility of such an approach in improving clinical end points. Under postoperative condition, we put emphasis on SF evaluation for screening, because SF evaluation offers early detection of DVT, and thereby we can start early treatment and early start of walking exercises without a break. D-dimer is useful if DVT is suspected and the time of onset is unclear, because high

Table 3. Performance of the luminescence immunoassay (LIA) and pretest probability.

	Total patients (n=207)	DVT present (n=104, 50.2%)	DVT absent (n=103, 49.8%)	Sensitivity, % (n/n)	Specificity, % (n/n)	Positive predictive value % (n/n)	Negative predictive value % (n/n)	Positive likelihood ratio (95% CI)	Accuracy
SF assay result									
Postoperative day 1 (cut-off value 4.00 μg ml^{-1})									
Positive	163	94	69	90.4 (94/104)	33.0 (34/103)	57.7 (94/163)	77.3 (34/44)	1.35 (0.77–2.36)	
Negative	44	10	34						
Best fit value (cut-off value 7.61 μg ml^{-1})				0.7788	0.6214				1.4002
D-dimer assay result									
Postoperative day 1 (cut-off value 4.88 μg ml^{-1})									
Positive	170	96	74	91.4 (96/104)	28.2 (29/103)	56.5 (96/170)	78.4 (29/37)	1.28 (0.69–2.41)	
Negative	37	8	29						
Best fit value (cut-off value 9.78 μg ml^{-1})				0.6635	0.6019				1.2654
Postoperative day 7 (cut-off value 5.35 μg ml^{-1})									
Positive	173	94	79	90.4 (94/104)	23.3 (24/103)	54.9 (96/175)	75.0 (24/32)	1.18 (0.69–2.02)	
Negative	34	10	24						
Best fit value (cut-off value 8.26 μg ml^{-1})				0.7308	0.5534				1.2842

DVT, deep vein thrombosis; SF, soluble fibrin; THA, total hip arthroplasty; TKA, total knee arthroplasty; CI, confidence interval.