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Case Report

Treatment of Infected Hip Arthroplasty With Antibiotic-Impregnated Calcium Hydroxyapatite

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Abstract: We reviewed the treatment of infected hip arthroplasty with antibiotic-impregnated calcium hydroxyapatite (CHA) ceramic blocks. Seven consecutive patients consisting of 2 men and 5 women with an average age of 65 years were followed up for an average of 5.0 years. All patients received resection arthroplasty and thorough debridement, followed by implantation of antibiotic-impregnated CHA ceramic. Two-stage revision was performed in all patients. There was no evidence of a recurrent infection in 6 patients. The remaining one patient underwent an additional debridement 2 years after the second stage. This patient was free of infection at the time of the latest follow-up. Antibiotic-impregnated CHA ceramic is thus considered to be an excellent drug delivery system for the infected hip arthroplasty. **Key words:** infected hip arthroplasty, antibiotic-impregnated hydroxyapatite, calcium hydroxyapatite ceramic block, drug delivery system, 2-stage revision.

Despite recent advances in surgical techniques, operating room discipline, and prophylactic administration of antibiotic therapy, deep infections remain a devastating complication after total hip arthroplasty. The optimal treatment of an infected hip arthroplasty remains controversial with many protocols having been described [1-10]. The selection of a suitable treatment method requires a careful assessment of patient-related variables and the expected treatment goals. The 6 basic treatment options include antibiotic suppression [1,2], open debridement [3,4], resection arthroplasty [5,6], arthrodesis [7], reimplantation of another prosthesis [8,9], and amputation [10]. Obviously, it is ideal to retain a well-functioning total hip arthroplasty after infection. Unfortunately, in most cases, this is not possible. Accordingly, the removal of the implant is mandatory for the treatment and cure of implant sepsis in most cases. After removing implants, it has been the current trend to insert antibiotic-impregnated bone cement in the form of beads or spacers. However, antibiotic-impregnated bone cement is also associated with certain disadvantages. The potential drawbacks may include a short duration of drug release [11-16], a very low release rate [11,14,17], thermal damage to some antibiotics, reduced biocompatibility with the bone for which additional surgery to remove the bone cement is necessary, and possible bone loss adjacent to loose spacers [18].

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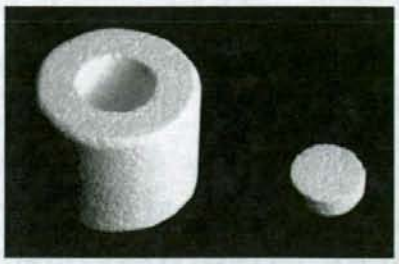

Fig. 1. This photograph is a CHA ceramic block.

To solve these problems, we developed the antibiotic-impregnated calcium hydroxyapatite (CHA) ceramic as a new drug delivery system (Fig. 1) [19,20]. This system is superior to acrylic bone cement. All implanted antibiotics are thus released over a long period, with none being trapped in the composite [19]. The antibiotics studied showed a high release rate [19]. Shinto et al [19] reported that gentamicin sulfate in CHA block was still effective at 12 weeks, when 70% of the antibiotic had been released. At this time, the antibiotic concentration from a 75-mg dose was 5 times the minimum inhibitory concentration for staphylococci. Any antibiotics can be placed in CHA because there is no thermal damage to the drug. Calcium hydroxyapatite ceramics are fully biocompatible, thus avoiding the need for a second operation [19,21-23]. We have already treated the patients with chronic osteomyelitis by this CHA ceramic block [24,25]. The aim of this study is to evaluate the clinical results of the antibiotic-impregnated CHA used for the treatment of infected hip arthroplasty.

Materials and Methods

Between April 1998 and July 2000, 7 consecutive patients who had a deep infection at the site of a hip prosthesis were treated using an antibiotic-impregnated CHA. The diagnosis of infection was based on the clinical criteria, including the presence of a discharging sinus, frank purulent fluid or pus found on preoperative hip aspiration, or positive findings on laboratory and histopathological tests. The study group consisted of 2 men and 5 women, with an average age of 65 years (range, 45-81 years). The initial diagnoses were osteoarthritis in 3 patients, rheumatoid arthritis in 2, and neck fracture in 2.

Protocol of Treatment

First Stage. This consisted of resection arthroplasty and thorough debridement followed by the implantation of antibiotic-impregnated CHA (Fig. 2A). A posterolateral incision was used to approach the hip joint. Three sets of deep cultures were taken from the joint fluid, inflamed synovial tissue, and membrane from the bone-prosthesis interface at the time of debridement.

Calcium hydroxyapatite ceramic was sintered at 1200°C for 2 hours, and it had a porosity of 30% to 40% with the diameter of the micropores between 40 to 150 μm. There was an interconnecting pore structure open to the external surface of the block. The size of this cylindrical block was 15 mm in diameter and 12 mm in height. These blocks were commercially available (Sumitomo Cement, Tokyo, Japan). At operation, the chosen antibiotic powder was packed into a central cylindrical cavity (7 mm in diameter and 8 mm deep) in each porous block, and then, the cavity was sealed with a CHA plug (7 mm in diameter and 3mm in height). The volume of antibiotic powder they were able to accommodate depended on the types of antibiotics. The usual dose of antibiotics in each ceramic block ranged from 100 to 400 mg. The choice of antibiotics in the CHA was determined according to the results of bacterial cultures from the draining

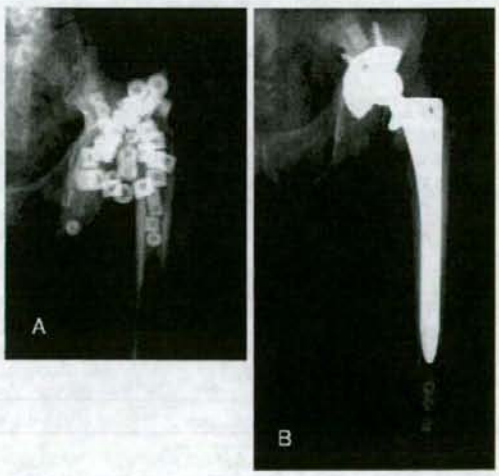

Fig. 2. Radiographs of left hip of a 71-year-old man. A, Thirty-eight CHA blocks were placed in dead space. B, Anteroposterior radiograph after revision total hip arthroplasty with CHA blocks placed into the femoral canal and acetabulum is shown.

Table 1. Patient Demographics

Case	Age	Sex	Previous Surgery	Organism	Antibiotics in the CHA Blocks	IV Antibiotics at Interim Period	PO Antibiotics at Interim Period	Follow-Up
1	71	M	Total hip arthroplasty	*Pseudomonas aeruginosa*	CFS 0.9 g, MEPM 1.5 g, PIPC 0.1 g	CPR, MEPM, PAPM/BP	TFLX	Died
2	74	F	Hemiarthroplasty	MRSA	DKB 0.5 g, FOM 4 g	ABK, CMZ, DOXY	MINO	Died
3	65	F	Total hip arthroplasty	Alpha streptococcus	CTM 0.75 g, CZOP 1.5 g, FMOX 1.5 g	CEZ, CTM, FMOX	CFPN-PI	Reimplantation
4	55	F	Total hip arthroplasty	MRSA	SM 2 g, VCM 1.5 g	ABK, MINO	MINO	Reimplantation
5	81	F	Total hip arthroplasty	MRSA	TEIC 1 g, VCM 1.6 g	ABK, GM	–	Reimplantation
6	63	M	Hemiarthroplasty	MRSA	IPM/CS 0.5 g, VCM 0.5 g	CLDM, MINO	CFPN-PI	Reimplantation
7	45	F	Total hip arthroplasty	*Staphylococcus aureus*	AMK 0.2 g, CEZ 3 g	CTM	CFPN-PI	Reimplantation

ABK, CFPN-PI, CFS, CMZ, CTM, CZOP, DKB, FMOX, PAPM/BP, and TFLX are currently not available in the United States.

MRSA indicates methicillin-resistant *Staphylococcus aureus*; ABK, arbekacin sulfate; AMK, amikacin sulfate; CEZ, cefazolin sodium; CFPN-PI, cefcapene pivoxil hydrochloride; CFS, cefsulodin sodium; CLDM, clindamycin; CMZ, cefmetazole sodium; CPR, cefpirome sulfate; CTM, cefotiam hydrochloride; CZOP, cefozopran hydrochloride; DKB, dibekacin sulfate; DOXY, doxycycline hydrochloride; FMOX, flomoxef sodium; FOM, fosfomycin sulfate; GM, gentamicin sulfate; IPM/CS, imipenem/cilastatin sodium; MEPM, meropenem trihydrate; MINO, minocycline hydrochloride; PAPM/BP, panipenem/betamipron; PIPC, piperacillin sodium; SM, streptomycin sulfate; TEIC, teicoplanin; TFLX, tosufloxacin tosilate; VCM, vancomycin hydrochloride.

sinuses or preoperative joint aspirations. If no preoperative culture was positive, we use the several antibiotics, including the broad spectrum antibiotics and antistaphylococcal antibiotics. A closed suction drain was placed to diminish the occurrence of any hematoma formation. All wounds were closed primarily.

Interim Period. All patients were given intravenous antibiotics for 3 or 4 weeks (Table 1). The selection was determined according to a microbiological assessment before operation and, thereafter, was modified according to the results of the operative cultures. Oral antibiotics were prescribed for a further 3 or 4 weeks (Table 1). The patients were encouraged to move using non–weight-bearing methods. Several factors were periodically assessed in the interval before reimplantation, including clinical examinations for wound healing and signs of infection, radiographs, and laboratory studies, including the C-reactive protein and the erythrocyte sedimentation rate.

Second Stage. The second-stage procedure was carried out only after there was sufficient clinical, radiographic, and laboratory evidence to support the eradication of an infection. The reimplantation procedure was performed through a posterior approach to the hip joint. The CHA ceramic blocks were removed. Three intraoperative specimens were taken from synovial fluid, synovial tissue, and membrane on the bone. At least 10 polymorphonuclear leukocytes per high-power field was considered positive for infection [26]. A new prosthesis was then inserted only if there was sufficient intraoperative evidence to support the absence of infection. The type of reimplantation was a cementless component, with or without antibiotic-impregnated CHA additionally implanted in the surrounding bone. In addition, we used CHA at the time of the reimplantation for 3 cases that further surgery would be impossible because of medical condition or severe bone loss. All patients received intravenous antibiotics for 3 weeks postoperatively.

Postoperative Care and Radiographic Evaluation

The patients were examined at 1, 3, 6, and 12 months and, thereafter, every half year. The clinical findings including pain at rest, radiographs, and laboratory findings were monitored for any evidence of recurrent infection.

Cup loosening was radiographically defined as a change in the cup angle or radiolucent line in the zones of DeLee and Charnley [27]. A loosening of the stem was evaluated according to the system described by Engh et al [28].

Results

The average duration of follow-up was 5.0 years (range, 2.3-6.1 years). No patients were lost to follow-up. Two patients included in the study have

died of other causes, with an average follow-up of 4.2 years before death. There was no evidence of a recurrent infection in these 2 patients in a lifetime.

Gram-positive microorganisms were responsible for the infection in most hips (Table 1). The types of antibiotics that were used in the CHA are also listed in Table 1. No complications with this antibiotic-impregnated CHA ceramic, such as excessive postoperative drainage, erythema, bone damage from friction, or any particle disease were observed. At the second stage, there was no visible evidence of infection and the cultures were all negative. There was no evidence of a recurrent infection in 6 patients. The remaining 1 patient had an additional debridement with prosthesis retention after 2 years from the second stage because of recurrent infection. This recurrence had a different organism on the culture specimens at the time of reinfection. This patient was free of infection at the time of the latest follow-up. Radiographically, there was no loosening or migration of the components in all 7 patients.

Discussion

Infection remains one of the most devastating complications of hip arthroplasty. Antibiotic-impregnated bone cement has been reported to be effective for the prophylaxis and treatment of infected prostheses; however, some limitations such as a short duration of drug release, a low release rate, a suitable substrate for bacterial growth [11-16,29-32], and complications such as fracture and dislocation of spacer [8,33] have been reported.

Cement beads usually provide local bactericidal levels of antibiotics for only 2 to 4 weeks. Once the level of antibiotics eluting from the implant has waned, there is an increased propensity for the overgrowth of antibiotic resistant organisms that were not eliminated by the original high concentration of antimicrobials [15]. Moreover, the antibiotics in the cement beads often leech from the outer cortex of implant, thus leaving behind a central core of unused antibiotics. Once the antibiotics have leeched from the implant cortex, the cement beads provide a perfect substrate for additional bacterial colonization [15]. Once colonized, many bacteria are thus able to synthesize a slime layer, termed the *glycocalyx* [15]. This layer prevents the inward diffusion of numerous antimicrobials, thus allowing bacterial escape from the bactericidal and bacteriostatic effects of the antimicrobial therapy. Kendall et al [12] reported the bacterial growth on the surface of antibiotic-loaded acrylic cement was examined in an in vitro model. Therefore, the surface of bone cement is a suitable substrate for bacterial growth, even in the presence of antibiotics.

Calcium hydroxyapatite has been used for the sustained release and long-term delivery of various antibiotics to treat completely the particular orthopedic infection. We have been able to maintain high concentrations of antibiotics for long periods both in vivo and in vitro with antibiotic-impregnated CHA ceramic [19]. We have also reported that high concentrations of antibiotics were detected at the site of infection, and bacteria were eradicated without removal of the metal implant for the treatment of experimentally produced, implant-related osteomyelitis in rats [34]. Moreover, gentamicin-impregnated CHA was reported to produce 2.5 times higher concentrations, for 1.2 times longer, than the acrylic bone-cement drug delivery system [34]. The ability of gentamicin impregnated CHA ceramic to deliver 5 times the minimum inhibitory concentrations for *Staphylococcus* species for at least 12 weeks.

Any antibiotic can be placed in a CHA ceramic cylinder because there is no thermal damage to the drug. It is reported that some antibiotics inactivated by the polymerization process such as chloramphenicol should not be used in bone cement [35]. Brien et al [11] reported that elution of vancomycin from bone cement was suboptimal, and the bioactivity of vancomycin was variable. In another study, vancomycin elution from bone cement was also reported not as good as tobramycin [36]. These data might be related to the thermal damage.

Moreover, this CHA ceramic is fully biocompatible with bone and marrow cells, which grow into the pores [19,21-23]. Therefore, we can use this system not only for the dead space after the removal of the prosthesis but also for the drug delivery system at revision surgery. At revision surgery with the cemented prosthesis, the success rate with antibiotic-impregnated bone cement has been reported to be higher than that without antibiotic-impregnated bone cement [37-40]. However, at revision surgery with the cementless prosthesis, a high concentration of antibiotics could not be obtained at the site of reimplantation. We suggest that this new drug delivery system might be a useful tool for revision surgery using a cementless prosthesis (Fig. 2B). We applied this CHA ceramic in the major trochanter, the acetabulum, and into the femoral canal during revision surgery. All impregnated antibiotics are thereafter released over a long period of time. Thereafter, these CHA

ceramics encourage osteoconduction into their pores [19,21], thus avoiding the need to remove them at the end of the treatment.

Finally, this drug delivery system is very simple and easy to use. Any antibiotics can be selected to correspond to the sensitivities of the wound pathogens. In the operating room, the surgeon just packs the selected antibiotic powder in each porous block, as necessary.

This is the first report regarding the treatment of the infected hip arthroplasty with antibiotic-impregnated CHA. We already successfully treated the patients with chronic osteomyelitis by implanting this CHA ceramic with antibiotics into a cavity produced after through surgical excision of necrotic tissue. These favorable results using the antibiotic CHA should encourage further clinical studies in patients with infected arthroplasty.

The present study has several limitations. Patients in this retrospective study were not treated prospectively under a uniform protocol. As a result, antibiotic selection was not controlled. Another limitation is the small number of patients. Further investigation is needed with more patients for a longer period in a prospective design, although the results are promising.

In conclusion, although thorough surgical debridement and appropriate parenteral antibiotic therapy might be individually successful for eradication of deep periprosthetic infection, this study suggests that a subsequent procedure with antibiotic-impregnated CHA ceramic produce a higher rate of success.

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ORIGINAL ARTICLE

A long-term follow-up study of the cementless THA with anatomic stem/HGPII cup with 22-mm head

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Abstract The anatomic femoral component and Harris-Galante porous II (HGPII) cup were developed to provide more reliable bone ingrowth. We performed 20 cementless total hip arthroplasties (THAs) with anatomic stem/HGPII cup with 22-mm head in 14 consecutive patients, and evaluated the clinical and radiological results for a mean follow-up of 12.8 years. The all-anatomically designed stem provided excellent clinical and radiographic results. Four acetabular components underwent revision: three for fracture of the locking mechanism and wear of the polyethylene liner and one for the locking mechanism failure with dislocation of the HGPII cup. The abduction angles of the four revised acetabular components were apparently higher. The survivorship 13 years after surgery was 78%. Our findings show good long-term results using the anatomic femoral component, while the HGPII cup combined with 22-mm head seems to have poor durability due to locking mechanism failure.

Résumé Le composant fémoral anatomique et la cupule Harris-Galante porous II (HGPII) ont été développés de façon à améliorer la repousse osseuse. Nous avons réalisé 20 prothèses totales de hanche sans ciment avec une queue anatomique et une cupule HGPII et un couple de friction avec une tête de 22 mm chez 14 patients consécutifs. Nous avons réalisé une évaluation clinique et radiologique des résultats après un suivi moyen de 12,8 ans. Toutes les pièces fémorales ont donné d'excellents résultats sur le plan clinique et radiographique. Cependant, 4 composants acétabulaires ont nécessité une révision, 3 pour fracture de mécanisme de verrouillage de l'insert et usure de l'insert, 1 pour défaut du verrouillage du mécanisme de la fixation de l'insert et luxation de la cupule. L'angle de verticalisation des 4 cupules révisées a été plus important. Le taux de survie à 13 ans a été de 78%. Nous pensons que cet implant donne de bons résultats à long terme sur le plan fémoral, il n'en est pas de même en ce qui concerne la cupule HGPII associée à une tête de 22 mm, celle-ci semble avoir une durée de vie diminuée du fait du défaut de mécanisme de blocage de l'insert.

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Introduction

Since the introduction of total hip arthroplasty (THA), implant fixation and polyethylene wear have been the most important problems requiring solutions. There are many system designs available today for cementless THA. The anatomic THA was one of the early cementless designs [15]. A published study of the anatomic hip indicated that the anatomically designed femoral component can provide early, satisfactory pain relief in younger, active patients. Additional experience and follow-up were required to determine if the long-term objectives were realised. Loosening of the acetabular component is recognised as the major long-term problem associated with THA with cement [3, 9]. A number of techniques to improve fixation of the acetabular component have been reported [2, 12, 22]. In the 1980s, cementless porous-coated acetabular components were developed to improve the durability of acetabular fixation. We began to use the cementless hemispheric

porous-coated acetabular component, Harris-Galante porous II (HGPII), and anatomic femoral component with 22-mm head (Zimmer, Warsaw, IN) in November 1991.

The purpose of this study is to provide the clinical and radiographic outcomes, after long-term follow-up, in a consecutive series of patients treated with cementless THA with the anatomic stem/HGPII cup with 22-mm head.

Patients and methods

Between November 1991 and March 1997, 20 primary cementless THA with the HGPII cup/anatomic stem were performed in 14 consecutive patients (6 men and 8 women). The average age of the patients at the time of surgery was 54.4 years (range, 20.0–78.0 years). The average weight was 56.9 kg (range, 45.0–74.0 kg) and the average height was 155.0 cm (range, 145–178.0 cm). The preoperative diagnosis was osteoarthritis in all 20 hips, including developmental dysplasia in 19 hips and post-traumatic arthritis in one hip. All of the operations were performed by the same surgeon via a posterior approach. No patient was lost to follow-up. All 20 hips were evaluated after a mean follow-up of 12.8 years (range, 10.0–15.3 years).

The HGPII acetabular component and anatomic femoral component were used in all patients. The uncemented acetabular component is made from a titanium alloy metal shell and has a sintered titanium fiber-metal porous coating. The metal shell was inserted; the diameter of the implant matched the two-plus diameter of the last reamer used to prepare the acetabular. The outer diameter of the metal shell varied. Multiple screwholes allowed for dome screw fixation. The acetabular component was fixed with titanium 6.5-mm-diameter screws to ensure primary stability. The polyethylene used at this time was machined and sterilised with gamma irradiation in air. The femoral component was the anatomic stem consisting of Ti-6Al-4V alloy, with the surfaces of the proximal aspect coated with titanium fiber-metal mesh. The stem had no collar. The fluted stem composed the distal half of the device. The design of the femoral component has a posterior intertrochanteric bow and an anterior distal bow. This prosthetic design relies on maximum metaphyseal fill to provide initial implant stability. Flexible reamers were used to ream the medullary canal to the diameter of the desired stem, followed by rasping of the metaphysic and canal. If any motion of the broach was detected within the proximal part of the femur, the next larger broach was used until this motion was eliminated and the final component was then implanted. The femoral component varied. The modular femoral head was a chromium-cobalt alloy component mated to the femur by interference fit with a conical taper. A 22-mm head was used in all patients. In nine hips an acetabular autograft was used to fill a bone defect. All patients walked with partial weight-bearing allowed on the seventh postoperative day and then full weight-bearing at 1 month postoperatively.

The clinical results were analysed using the Merle d'Aubigne and Postel scoring system [18]. Serial radiographs were obtained and analysed by an independent observer. The inclination of the acetabular component was measured and the presence of radiolucent lines was evaluated using the zones described by DeLee and Charnley [4] for the acetabular component and those described by Gruen et al. [10] for the femoral component. Radiological loosening of the acetabular component was evaluated using the methods of Hodgkinson et al. [13]; loosening was defined as migration or radiolucency greater than 1 mm in all of the DeLee and Charnley zones [4]. Radiological loosening of the femoral component was evaluated using the method described by Engh et al. [17]. Linear wear of the acetabular component was determined by measuring the change in the shortest distance between the centre of the femoral head and the periphery of the acetabular component and by comparing the immediate postoperative radiograph with that taken at the last follow-up visit, as described by Livermore et al. [22]. The measurements were taken using calipers with an accuracy of 0.5 mm. Peri-prosthetic cystic or scalloped lesions with a diameter greater than 2 mm that were not present on the immediate postoperative radiograph were defined as osteolysis.

Statistical analysis was performed using the Wilcoxon signed rank test or Mann-Whitney U test. A p value of 0.05 was considered to be significant. Kaplan-Meier survivorship analysis was performed using revision for any reason as the end point.

Results

At the final follow-up, four hips (in three patients) had undergone revision with a mean duration between the initial operation and revision of 7.9 years (range, 4.9–11.4 years). Of these, three hips were revised at 8.9 years (range, 5.5–11.4 years) after the initial operation and their radiographs showed probable acetabular liner locking mechanism failure and wear of the polyethylene liner (Fig. 1a). The metallic locking mechanism was broken and the polyethylene liner was worn and loose in the acetabular component (Fig. 1b). Both the metal shell components and the femoral components were well fixed. The well-fixed acetabular component and worn polyethylene liner were changed to a new metal shell and polyethylene liner using the same approach. One patient fell, 4.9 years after the arthroplasty, and sustained a fracture of the metal tines of the locking mechanism with dislocation of the acetabular component.

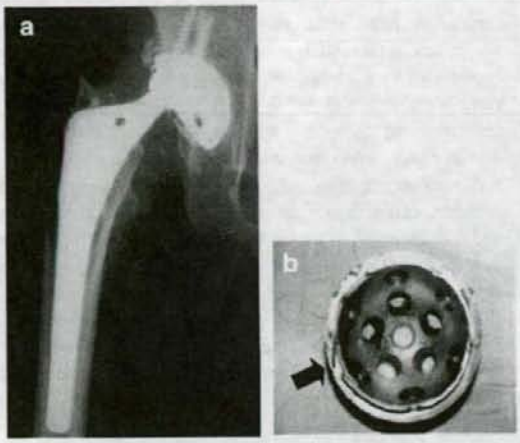

Fig. 1 **a** Anteroposterior radiograph showing acetabular component dissociation. The 22-mm inner head is positioned eccentrically within the acetabular metal shell. The liner is dissociated inferiorly from the shell. **b** Retrieved metal shell showing the broken metallic tine locking mechanism

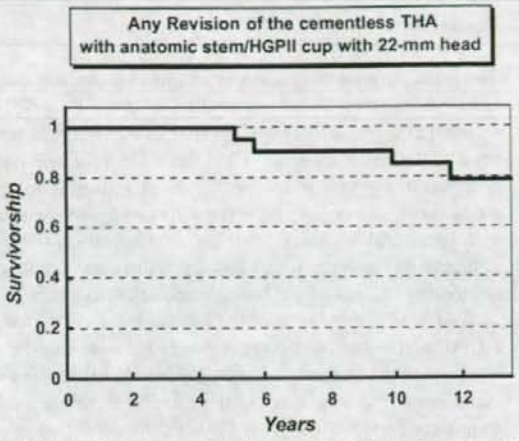

Fig. 2 Survivorship curve for the cementless THA with anatomic stem/HGPII cup combined with 22-mm femoral head with revision for any reason as the end point

The mean abduction angles of four revised hips (average, 43.5°±6.0°) were significantly higher than the angles of the 16 hips that were not revised (average, 35.3°±5.4°; $p<0.05$). The average polyethylene thickness and oscillation angle in the unrevised group were not significantly different from the four revised hips (Table 1). The femoral component was not loosened and the acetabular component required replacement.

The Kaplan-Meier survivorship analysis revealed a 13-year survival rate of 78% (95% CI:, 59–97%) with revision for any cause as the end point (Fig. 2). The clinical results of the 11 patients (16 hips) who did not undergo revision revealed that the mean Merle d'Aubigne and Postel score for this group had improved significantly from 11.1 points (range, 5–15 points) preoperatively to 16.5 (range, 12–18 points) postoperatively ($p<0.01$). The mean score for pain improved from 3.7 preoperatively to 5.7 at the last follow-up, while that of mobility improved from 5.3 to 4.9 and that of walking ability improved from 2.1 to 5.8. None of the patients reported any pain in the thigh.

Table 1 Comparison of results for unrevised and revised hips as mean ± SD

	Unrevised hips	Revised hips
Thickness of polyethylene	10.2±1.1	10.5±1.3
Abduction angle	35.3±5.4	43.5±6.0*
Oscillation angle	106.3±0.9	105.5±0.9

*Significantly higher than those of unrevised hips, $P<0.05$

Radiological findings revealed that three hips had a radiolucent line in zone 1 on the acetabular side according to the classification of DeLee and Charnley [4]: one in zone 2 and three in zone 3. The mean rate of wear of the acetabular component was 0.16 mm/y (range, 0.00–0.54 mm/y). On the femoral side, 12 hips had a radiolucent line in Gruen zone 1, two in zone 2, 13 in zone 4, two in zone 5, and three in zone 6, but none had a radiolucent line in zones 3 and 7 [10]. Some hips had lesions in more than one zone. None of the radiolucent lines was wider than 2 mm. Loosening of the acetabular component was observed in one of the unrevised hips. All of the femoral components exhibited radiological evidence of bone ingrowth at the last follow-up (Fig. 3) [6]. None of the 20 hips exhibited osteolysis.

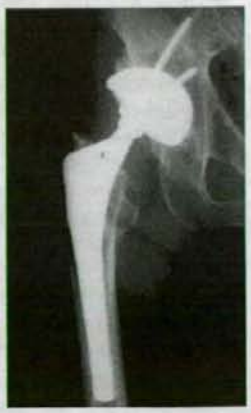

Fig. 3 Anteroposterior radiograph showing the components are well fixed 11 years postoperatively

Discussion

The main purpose of this study was to evaluate the long-term (more than 10 years) results of cementless THA incorporating an anatomic stem/HGPII cup with 22-mm head. After an average of 12.8 years, 78% of the hips demonstrated good or excellent clinical results. Furthermore, there was no aseptic femoral loosening, no femoral osteolysis, and no thigh pain up to 10 years after the arthroplasty. However, it was found that there was failure of the locking mechanism in four acetabular components.

We found that the tines of the locking mechanism in the HGPII acetabular component were broken in four hips. Lachiewicz et al. reported that only three HGPII acetabular components required a revision in 78 hips with a mean follow-up period of 8 years [16]. Archibeck et al. also reported that five patients in 74 underwent exchange of the HGPII cup at middle-term follow up [1]. The diameter of these femoral heads was larger than the 22-mm inner diameter used in our series. There was no significant difference as to mean polyethylene thickness and oscillation angle; however, it was suggested that the failure of the HGPII cup was associated with size of inner head diameter. Dislocation associated with a radiographically visible inner head positioned eccentrically within the acetabular metal shell was the primary indication for revision for locking mechanism failure. Mechanical failure, defined as broken metal tines detected during revision, was responsible for the locking mechanism fractures. An association was generated between radiographically visible dislocation and polyethylene failure with a portion of the locking mechanism showing distorted metal flanges. In our study, the abduction angles of the revised acetabular components were apparently higher. Peters et al. reported that the angle of inclination was changed or the orientation of the polyethylene liner was changed relative to the elevated rim and dislocation occurred because failure of the locking mechanism occurred from wear and shifting of the polyethylene liner [1, 5, 16, 19]. We suggested that failure of the locking mechanism arose from the mobility of the polyethylene liner because of a higher inclination angle of the acetabular component from wear. Gaffey et al. reported that the rate of wear was greater in association with cementless components than in association with cemented components [8]. Rotational force onto the articular surface, which disrupts the normally low-friction articulation and transfers oblique forces to the polyethylene liner through interaction with the femoral head, was augmented and then the polyethylene liner became worn and mobile.

As a result of the disappointing studies associated with proximally coated cementless femoral components such as the Harris-Galante-I stem (HGPI), the next generation of anatomic stems was developed [23]. The HGPI femoral component had a relatively small porous surface area that was noncircumferential, resulting in a susceptibility to distal osteolysis. As a consequence a proximally coated femoral component with a circumferential porous surface was used and compared favourably with previously published reports on other successful cementless designs. Incomplete sclerotic and radiolucent lines adjacent to the prosthesis do not correlate well with implant stability. Most stems in our series had a radiolucent line next to the noncoated surface of the implant that was not indicative of stem loosening. In none of these hips was there other evidence of aseptic loosening, such as subsidence or complete radiolucency. Ragab et al., in their review, noted that the porous coated anatomic stem used in our series had a partial pedestal at a 28% rate and all of these stems had a radiographically stable implant [20]. In the case of proximally coated anatomic femoral components, no completely radiolucent line as an indicator of loosening was found and all stems were stable, so partial radiolucencies are not considered an important finding at long-term follow-up.

In our series, none of the hips were associated with thigh pain. In other series of proximally coated femoral components, the prevalence of thigh pain has been variable [21, 24]. Xenos et al., in a study of the porous-coated anatomic stem, found a peak prevalence of thigh pain, which decreased to 12% after 10 years [25]. Engh et al. identified 8% thigh pain in a study of extensively coated femoral stems followed for a minimum of 10 years [7]. We generated data over an average of 13 years on the cementless proximally porous-coated anatomic femoral components and our findings of clinically excellent results compare favourably with those available in the literature.

Particulate debris from polyethylene wear and the resultant osteolysis remain the primary factors limiting the longevity of hip prostheses [11, 14]. Femoral osteolysis was not seen in our series.

In conclusion, the outcome of cementless THA depends on many factors, including component design and head size, inclination angle of the acetabular component, and surgical technique. In our long-term (mean 12.8 years) results, the use of the anatomic circumferentially porous-coated femoral stem provides reproducible ingrowth and excellent clinical results, while a tendency for locking mechanism failure and loosening of the polyethylene liner of the HGPII cup combined with 22-mm femoral head introduces a note of caution.

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ORIGINAL ARTICLE

Negative predictive value of D-dimer for diagnosis of venous thromboembolism

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Abstract The D-dimer levels are considered to be useful for the diagnosis of thrombosis, and they can be clinically used as a negative predictive value (NPV). However, evidence for the efficacy of diagnosing thrombosis based on the D-dimer levels is still not well established. The present study was designed to evaluate the cut-off values of D-dimer levels as a negative predictor for thrombosis. The plasma concentrations of D-dimer were measured in inpatients suspected of having thrombosis, and then the findings were evaluated to assess the correlation with the diagnosis of thrombosis. In healthy volunteers, the median value of VIDAS-D-dimer was 0.12 μg/ml, and the 95% confidence interval was from 0.05 to 0.38 μg/ml. However, the plasma D-dimer levels were significantly higher in patients with thrombosis than in those without thrombosis; there was no significant difference in D-dimer levels among various thromboses such as pulmonary embolism (PE), deep vein thrombosis (DVT), and disseminated intravascular coagulation (DIC). The NPV for venous thromboembolism was 100% in patients with 0.5 μg/ml VIDAS-D-dimer and 1.2 μg/ml LPIA-D-dimer levels. Elevated D-dimer levels might indicate a high risk of thrombosis, especially DVT/PE, and they are thus considered to be useful as a negative predictor for thrombosis.

Keywords Hypercoagulable state · DVT · D-dimer · DIC · PE

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1 Introduction

The D-dimer levels are considered to be useful for the diagnosis of thrombosis, and they have been reported to be elevated in deep vein thrombosis (DVT)/pulmonary embolism (PE; 1–4), disseminated intravascular coagulation (DIC; 5–7), acute myocardial infarction (AMI; 8, 9), and thrombotic thrombocytopenic purpura (TTP; 10). The D-dimer levels are widely used to diagnose thrombosis such as DVT/PE, but many of the commercially available D-dimer assay kits contain different monoclonal antibodies, standard substances, and are based on different assay systems. Since these problems regarding the standardization of D-dimer assays remain to be resolved, several studies [11, 12] were designed to generate basic data for the standardization of D-dimer evaluation procedures. PE is a common, frequently undiagnosed, and potentially fatal cause of several symptoms, including dyspnea and chest pain [13–15]. Since PE is often a fatal disease caused by DVT, the early diagnosis of

DVT [16] and PE [17] is important to improve the possible outcomes. In this regard, the D-dimer levels have been reported to be a negative predictor for DVT; levels of less than 0.5 μg/ml of D-dimer are considered to exclude DVT/PE in Europe and North America [16].

DIC [18, 19] is often observed in patients with leukemia, solid cancers, infections, gynecological conditions, and aneurysms, and it is frequently associated with severe bleeding and organ failure. Since DIC is still a fatal condition [20], it is important to diagnose it early through the use of hemostatic molecular markers [21]. The International Society of Thrombosis and Haemostasis (ISTH) established diagnostic criteria for overt-DIC using fibrin-related markers such as D-dimer [22].

The present study was designed to evaluate the cut-off values of D-dimer as a negative predictor for DVT and PE. For this purpose, the plasma D-dimer levels were determined in 381 patients suspected of having thrombosis, and in 100 healthy volunteers.

2 Materials and methods

2.1 Subjects

From January 1, 2005 to December 31, 2005, 381 patients (median age 61.0 years, 25–75% range 50.0–72.0 years, and sex: 245 females and 136 males) were suspected of having some type of thrombosis in several hospitals affiliated with Mie University School of Medicine. The plasma concentrations of D-dimer were examined in these patients, and were then evaluated in order to identify any correlations with the diagnosis of thrombosis. The study protocol was approved by the Human Ethics Review Committees of all participating institutions, and a signed consent form was obtained from each subject. The underlying diseases in these patients included orthopedic conditions in 125 patients, cancer in 65, digestive diseases in 44, cardiovascular diseases in 46, autoimmune diseases in 18, infectious diseases in 15, hematological diseases in 13, diabetes mellitus in 10, trauma and burn in 7, obstetric diseases in 6, thrombophilia in 4, other diseases in 2, and no underlying disease in 26. Of these patients, 184 were diagnosed with thrombosis while 169 were not. Twenty-one of the patients were examined after undergoing liver transplantation, and seven were examined within 3 days after the operation, and these patients were excluded from analysis of the cut-off value. In the thrombotic patients, 76 patients had DVT, 37 had PE, 43 had DIC, 14 had cerebral vascular accident due to thrombosis (CVA), 8 had portal vein thrombosis (PVT), and 6 had AMI or arteriosclerosis obliterans (ASO). DVT was diagnosed with echo or venography. PE was diagnosed either by ventilation–perfusion lung scanning, computed tomography (CT), or pulmonary angiography. DIC was diagnosed by ISTH overt-DIC diagnostic criteria [10]. CVA was diagnosed by CT or magnetic resonance imaging (MRI) and AMI was diagnosed based on the electrocardiogram findings and laboratory data. ASO was diagnosed using the ankle brachial index. For receiver operating characteristic (ROC) analysis, the subjects consisted of DVT, other thrombotic diseases, and non-thrombotic diseases with high D-dimer levels.

The plasma concentrations of D-dimer were measured in patients with thrombosis at onset and those without thrombosis at the first consultation. The same parameters were also measured in 100 healthy volunteers (HV; mean age 41.5 years, range 20–58 years; 47 males and 53 females).

2.2 Measurement of plasma concentrations of D-dimer

The new D-dimer assay (VIDAS D-dimer EXCLUSION, bioMerieux, Marcy l'Etoile, France) is a quantitative ELISA method automated via a VIDAS immunoanalyzer. The new D-dimer assay combines the two-step sandwich immunoenzymatic method followed by final fluorescence detection. All values higher than 10,000 ng/ml were obtained after manual predilution (1/10) of the sample. All values are expressed in ng/ml of fibrinogen equivalent units (FEU).

The plasma D-dimer level was also measured by LPIA-D-dimer (Mitsubishi Chemical Medience, Tokyo, Japan) using a JIF23 monoclonal antibody, which recognizes the plasmin-digested N-terminus of the γ chain on the D region, was used for latex agglutination [23].

2.3 Statistical analysis

The data are expressed as the median (25–75% tile). Differences between groups were examined for significance using Mann–Whitney's U test, while correlations between two variables were evaluated by Pearson's correlation analysis. A P-value of less than 0.05 indicated a significant difference. The usefulness of the D-dimer levels in the diagnosis of thrombosis and DVT or PE was examined by ROC analysis [24]. The cut-off values were determined by ROC analysis. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo).

3 Results

In healthy volunteers, the plasma D-dimer level was not normally distributed with a median value of 0.12 μg/ml using VIDAS-D-dimer and 0.28 μg/ml using LPIA-D-dimer. The 95% confidence interval (CI) of VIDAS-D-dimer and

LPIA-D-dimer ranged from 0.05 to 0.38 μg/ml and from 0.10 to 1.27 μg/ml, respectively. Plasma D-dimer levels as assessed by VIDAS-D-dimer (median 25–75% tile) were significantly higher in patients with thrombosis (5.04 μg/ml; 3.12–6.85 μg/ml), liver transplantation (3.02 μg/ml; 1.75–5.03 μg/ml), and after surgery (2.89 μg/ml; 2.45–5.30 μg/ml) than in those without thrombosis (0.95 μg/ml; 0.40–1.87 μg/ml; $P < 0.01$, each) (Fig. 1a). The plasma D-dimer level as assessed by LPIA-D-dimer was significantly higher in patients with thrombosis (14.42 μg/ml; 8.13–26.75 μg/ml), after liver transplantation (11.56 μg/ml; 6.45–21.88 μg/ml), and surgery (8.05 μg/ml; 4.86–15.98 μg/ml) than in those without thrombosis (1.27 μg/ml; 0.71–2.82 μg/ml) ($P < 0.01$, each) (Fig. 1b). The 95% CI in patients with thrombosis was 0.98–26.89 μg/ml for VIDAS-D-dimer and 3.02–44.59 μg/ml for LPIA-D-dimer.

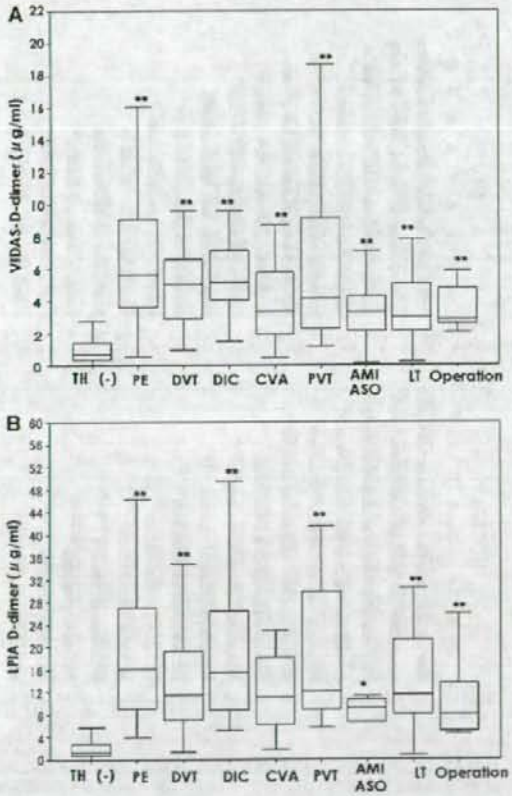

Fig. 1 The plasma level of D-dimer by VIDAS (a) and LPIA (b) in patients without thrombosis, in those with various thromboses, and in those after liver transplantation and surgery. *PE* pulmonary embolism, *DVT* deep vein thrombosis, *DIC* disseminated intravascular coagulation, *CVA* cerebral vascular accident due to thrombosis, *PVT* portal vein thrombosis, *AMI* acute myocardial infarction, *ASO* arteriosclerosis obliterans. ** $P < 0.01$; * $P < 0.05$

In each case of thrombosis, no significant difference was observed in the VIDAS-D-dimer levels among DVT (5.05 μg/ml; 2.92–6.63 μg/ml), PE (5.65 μg/ml; 3.63–9.08 μg/ml), DIC (5.18 μg/ml; 4.07–7.22 μg/ml), CVA (3.36 μg/ml; 1.93–5.79 μg/ml), PVT (4.12 μg/ml; 2.24–9.15 μg/ml), and AMI/ASO (3.27 μg/ml; 2.12–4.27 μg/ml)(Fig. 1a).

No significant difference was observed in the LPIA-D-dimer levels among DVT (11.55 μg/ml; 7.10–19.40 μg/ml), PE (16.13 μg/ml; 9.15–27.00 μg/ml), DIC (15.35 μg/ml; 8.81–26.40 μg/ml), CVA (11.05 μg/ml; 6.06–17.97 μg/ml), PVT (12.07 μg/ml; 8.95–29.73 μg/ml), and AMI/ASO (9.18 μg/ml; 4.91–10.70 μg/ml) (Fig. 1b).

ROC analysis showed the VIDAS-D-dimer levels to be useful in the diagnosis of all thromboses (Fig. 2a) and DVT/PE/PVT (Fig. 2b). The areas under the curves (AUC) in all thromboses (VIDAS-D-dimer: 0.957, and LPIA-D-dimer: 0.968) and DVT/PE/PVT (VIDAS-D-dimer: 0.958, and LPIA-D-dimer: 0.971) were significantly higher. ROC analysis provided adequate cut-off values of the D-dimer levels in the diagnosis of all thromboses and DVT/PE.

VIDAS-D-dimer levels were closely correlated with LPIA-D-dimer levels [$Y = 0.491736 + 3.006X$, $r = 0.8204$ ($P < 0.001$)] (Fig. 3).

The negative predictive value (NPV) for both VIDAS-D-dimer and LPIA-D-dimer for all thrombosis was not 100% at any plasma D-dimer level (Fig. 4a). In contrast, the NPV for DVT/PE/PVT was 100% at levels of less than 0.5 μg/ml using VIDAS-D-dimer and less than 1.2 μg/ml using LPIA-D-dimer (Fig. 4b).

4 Discussion

According to the current findings, the median value of the plasma D-dimer level by VIDAS-D-dimer in healthy volunteers was 0.12 μg/ml, and the 95% CI was from 0.05 to 1.27 μg/ml. In a European report [16] that used the same D-dimer kit, the mean D-dimer level was 0.21 μg/ml, and ranged from 0.07 to 0.49 μg/ml. The current findings are similar to those described in the European report.

The plasma D-dimer level was significantly higher in patients with thrombosis and after liver transplantation and surgery, but there was no significant difference in D-dimer levels among patients with thrombosis, liver transplantation, and after surgery. These findings suggest that the high plasma level of D-dimer is not just a marker of thrombosis but also a high risk for thrombosis referred to as a hypercoagulable state [25], as it was reported to be elevated in DVT [26, 27], DIC [4, 28], and hyperlipidemia [29]. However, the plasma LPIA-D-dimer level was not markedly higher in patients with AMI or ASO. AMI- or ASO-related thrombosis occurred either in small vessels or developed slowly.

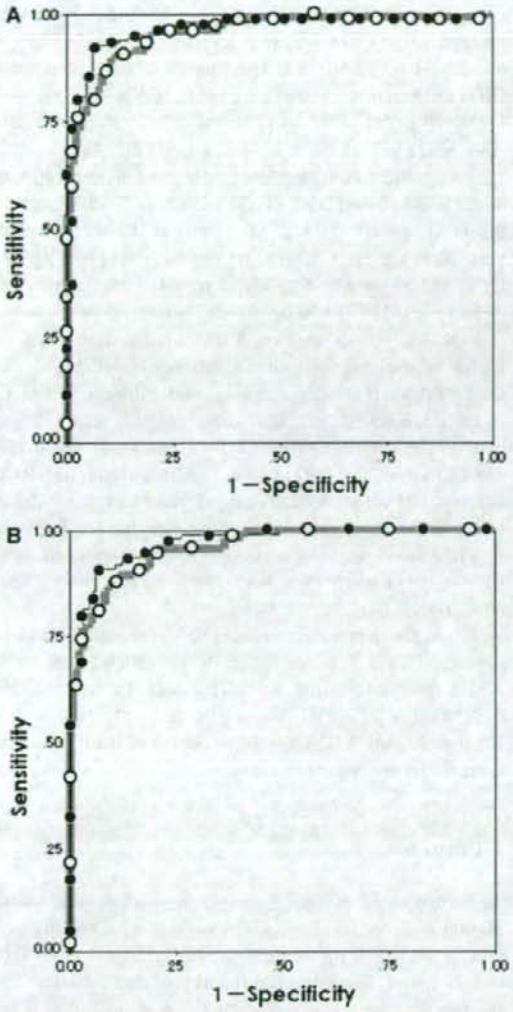

Fig. 2 ROC analysis of D-dimer levels for all thrombosis (**a**) or DVT/PE (**b**). *Open circle* VIDAS-D-dimer, *filled circle* LPIA-D-dimer. **a** AUC was 0.957 in VIDAS-D-dimer and 0.968 in LPIA-D-dimer. **b** AUC was 0.958 in VIDAS-D-dimer and 0.971 in LPIA-D-dimer

VIDAS-D-dimer levels were different from LPIA-D-dimer levels, which is frequently used in Japan; thus, LPIA-D-dimer levels were 2- or 3-fold higher than VIDAS-D-dimer levels. However, both D-dimer levels were significantly higher in patients with thrombosis. ROC analysis including the AUC also showed that both D-dimer results were useful for the diagnosis of various thromboses such as DVT, PE, and PVT. Both the AUC were similar. The VIDAS-D-dimer levels were closely correlated with the LPIA-D-dimer levels, with the latter being about 3× higher than the former.

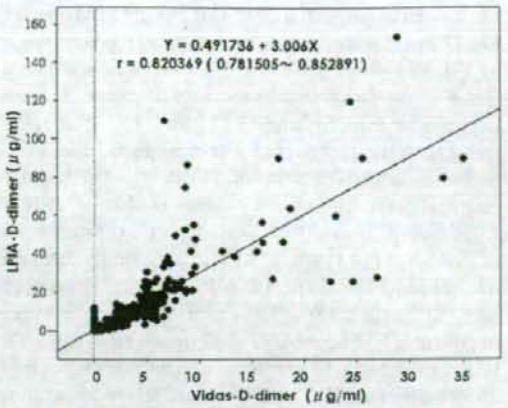

Fig. 3 The relationship between VIDAS-D-dimer and LPIA-D-dimer

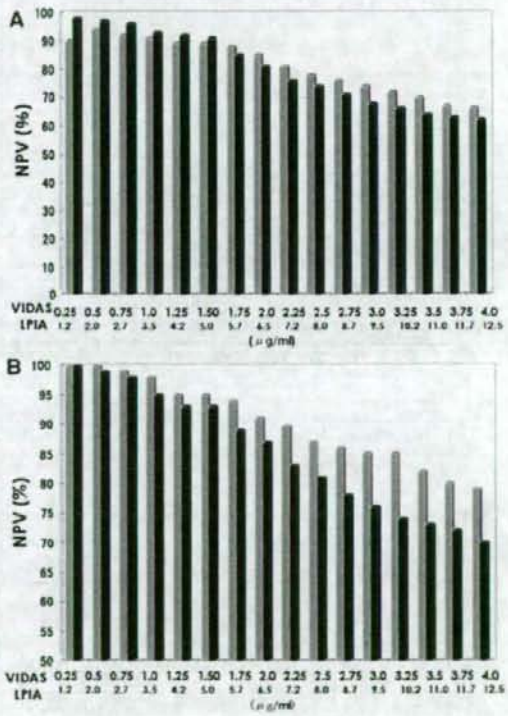

Fig. 4 The NPV in VIDAS-D-dimer and LPIA-D-dimer for all cases of thrombosis (**a**) and DVT/PE/PVT (**b**). *Gray bar* VIDAS-D-dimer, *black bar* LPIA-D-dimer

To maintain the 100% NPV, the cut-off value of VIDAS-D-dimer was 0.5 μg/ml for DVT/PE, and that of LPIA-D-dimer was 1.2 μg/ml. In Europe and North America, plasma D-dimer levels of less than 0.5 μg/ml are

thought to exclude DVT/PE [16]. However, in Japan, the D-dimer level is more than 0.5 μg/ml in many patients, and this cut-off value is not useful as an NPV for DVT/PE in Japan [30], especially because D-dimer kits, which are frequently used in Japan, tend to have a wide normal range (about 0.3–2.5 μg/ml; [4]).

A high false positive rate for D-dimer can potentially result in an increase in pulmonary vascular imaging, increased length of stay in overcrowded emergency departments, and an increased rate of false-positive diagnoses of DVT or PE [31]. Therefore, the cut-off values of LPIA-D-dimer for thrombosis should be more than the 95% CI of healthy volunteers (1.27 μg/ml). However, the cut-off value of VIDAS for NPV was the same as that in European countries (0.5 μg/ml). Although, Japanese physicians trust the 0.5 μg/ml cut-off value as the NPV to exclude DVT or PE, when they are using LPIA-D-dimer, an adequate cut-off value still needs to be established for the D-dimer assay.

In conclusion, these findings suggest that a high plasma level of D-dimer, known as a reliable marker for a hypercoagulable state, indicates a high risk of thrombosis, and is especially useful as an NPV; however, each D-dimer requires a specific cut-off value.

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A functional SNP in *EDG2* increases susceptibility to knee osteoarthritis in Japanese

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Osteoarthritis (OA) is the most common form of arthritis and is characterized by the gradual loss of articular cartilage. Several OA-susceptibility genes have been identified; however, there are few pharmaceutical targets that can be targeted with small-molecule compounds. To investigate whether a susceptibility gene for OA exists among G-protein-coupled receptors (GPCRs), we performed a stepwise association study for 167 single nucleotide polymorphisms (SNPs) in 44 GPCR genes that were present in cartilage. Through the stepwise association study, an SNP located in the promoter region of *EDG2* [endothelial differentiation, lysophosphatidic acid (LPA) GPCR, 2] (−2,820G/A; rs10980705) showed significant association with knee OA in two independent populations (pooled $P = 2.6 \times 10^{-5}$). Luciferase and electrophoretic mobility shift assays indicate that this SNP exerts an allelic difference on transcriptional activity and DNA binding in synovial cells, with the susceptibility allele showing increased activity and binding. *EDG2* encodes an LPA receptor dominantly expressed in the synovium. The LPA receptor increased the expression of inflammatory cytokines and matrix metalloproteases in synovial cells. Our findings suggest that the LPA–EDG2 signal is involved in the pathogenesis of OA via catabolic process.

INTRODUCTION

Osteoarthritis (OA, MIM 165720) is the most common form of arthritis and is characterized by the gradual loss of articular cartilage. It causes pain and dysfunction of joints, and is becoming a major burden on the aging society (1,2). Millions of people are suffering from this disease; however, there is no good medical treatment for it. We have only a few pharmaceutical options such as oral analgesics, topical steroids and hyaluronan (3). Development of innovative drugs has long been awaited. Searching for drug target molecules in the genome is a promising approach.

Epidemiological and genetic studies have shown that OA has a genetic component (4,5). Identification of disease-susceptibility genes brings us not only a better understanding of the pathogenesis of OA, but also new therapeutic targets.

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Several OA-susceptibility genes have been identified (6); however, there are few pharmaceutical targets that can be targeted with small-molecule compounds. Currently, a huge gap exists between the identification of susceptibility genes and the finding of 'druggable' targets.

The seven transmembrane G-protein-coupled receptor (GPCR) families have been considered a good druggable target because >30% of marketed drugs interact with GPCRs (7). To move on to the final goal of drug invention efficiently and swiftly, we planed to search susceptibility genes for OA among GPCRs. In this study, we found that an SNP located on the promoter region of *EDG2* [endothelial differentiation, lysophosphatidic acid (LPA) GPCR, 2] was associated with knee OA by stepwise association study. Functional analysis revealed that the OA susceptibility allele increased its promoter activity. Furthermore, we found LPA–EDG2 signal induced inflammatory cytokine and matrix metalloproteases (MMPs) gene in synovial cells. Therefore, our findings bring a novel therapeutic target for OA treatment.

RESULTS

Identification of landmark SNP that associate with knee OA

We first selected 64 GPCR candidates expressed in the cartilage as determined by micro-array analysis (data not shown). Among these, we genotyped 167 SNPs for 44 genes selected as described in Materials and Methods (Supplementary Material, Table S1). We then examined the association between these SNPs and knee OA using 368 individuals with knee OA and 323 controls. Among 167 SNPs, 160 SNPs were polymorphic, and 155 of these were in Hardy–Weinberg equilibrium. Comparison of genotypic and allelic frequencies for these 155 SNPs (the first screen) showed a significant association ($P < 0.05$) for 13 SNPs. To confirm the association, we genotyped these 13 SNPs in an independent case–control population comprising 276 individuals with knee OA and 654 controls (the second screen). Finally, we found only one SNP (rs3739708) showed a significant association in a recessive model in two independent populations (Supplementary Material, Table S2).

Linkage disequilibrium mapping and identification of OA susceptibility SNP

To localize the susceptibility gene, we evaluated the linkage disequilibrium (LD) extension with the landmark SNP. We genotyped 368 individuals with knee OA for SNPs in the 800 kb region around rs3739708 and examined the LD index (D') between rs3739708 and SNPs that had allele frequencies >10%. D' scores were decreased at rs11794726 ($D' = 0.69$) and rs12353088 ($D' = 0.40$), which are located 35 kb upstream and 10 kb downstream of rs3739708, respectively (Supplementary Material, Fig. S1A), limiting the LD extension with rs3739708 to a 45 kb region.

To identify a disease-causing variation, we first re-sequenced this 45 kb region, except for repetitive genomic sequences, in 48 individuals with knee OA. We identified a total of 27 polymorphisms (Supplementary Material, Fig. S1B, Table S3). We genotyped these 27 polymorphisms in individuals with knee OA used in the first screen, and constructed a pairwise LD structure using polymorphisms having allele frequencies >10% (Supplementary Material, Fig. S1C). These polymorphisms had high D' scores (>0.9), indicating that disease-causing variations could exist among these 27 polymorphisms. We next examined the association of all of 27 polymorphisms using 644 knee OA patients and 640 controls. We identified three SNPs that had an association more significant than rs3739708 (i-*EDG2*-25) in the recessive model (Table 1). Moreover, we examined haplotypes based on the 13 SNPs having allele frequencies >10% within the 45 kb region. Eight haplotypes with frequencies >1% represented >95% of both case and control populations. The haplotype association was much less significant than that of the landmark SNP alone (Supplementary Material, Table S4). Thus, the presence of a hidden ungenotyped sequence variation is unlikely. We then tested the association of the three SNPs and i-*EDG2*-25 in resident-cohort populations. The association of *EDG2* with knee OA was reproduced in the independent populations: three SNPs, i-*EDG2*-9 and -12 located on the 5′-flanking sequence and i-*EDG2*-25 located on the intron 1 of *EDG2*, showed significant association (Table 2).

Because i-*EDG2*-9 showed higher association and odds ratio than i-*EDG2*-12 and 25, we considered i-*EDG2*-9 to be the disease-causing SNP. The homozygotic A allele was overrepresented in the knee OA population. The final *P*-value for the association of the SNP was calculated as 2.6×10^{-5} (odds ratio = 2.3; 95% CI = 1.6–3.3) by using a Mantel–Haenszel analysis on two independent populations (case–control and resident-cohort populations), which was still significant after conservative Bonferroni's correction (using the number of SNPs in first screen, 155 tests).

We checked the effects of confounding factors such as age and body mass index (BMI) to evaluate whether they could make a pseudo-positive association. There was no significant difference in mean age or BMI for the SNP i-*EDG2*-9 (Supplementary Material, Table S5).

To gain insight into the role of *EDG2* in OA, we analyzed its expression in cartilage and synovium from knee OA patients by quantitative real-time PCR (Supplementary Material, Fig. S2). *EDG2* expression was significantly higher in the synovium than in the cartilage. EDG2 is an LPA receptor. There are five identified LPA receptors in mammals (*EDG2*, *EDG4*, *EDG7*, *GPR23* and *GPR92*). LPA receptors are involved in various cellular functions such as cell survival, proliferation and migration thorough binding its intrinsic ligand, LPA (8). *EDG2* expression in the synovium was higher than that of other LPA receptors (Supplementary Material, Fig. S2). These results suggest that *EDG2* is a critical LPA receptor in the synovium.

Functional analysis of susceptibility SNP

i-*EDG2*-9 is located in a putative promoter region (2,820 bp upstream of the transcription start site) and is located immediately after a putative AP-1 binding motif (TGAGCTA). Therefore, we hypothesized that i-*EDG2*-9 may alter *EDG2* transcriptional activity. We transiently transfected a synovial cell line E11 (9) with vectors containing five tandem copies of the sequences surrounding i-*EDG2*-9 coupled to a luciferase