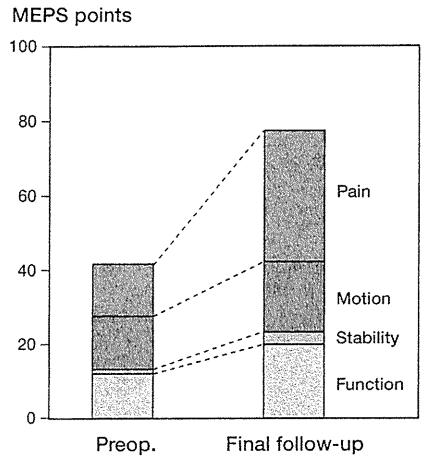

Figure 1. The total Mayo elbow performance score (MEPS) preoperatively and at the final follow-up.

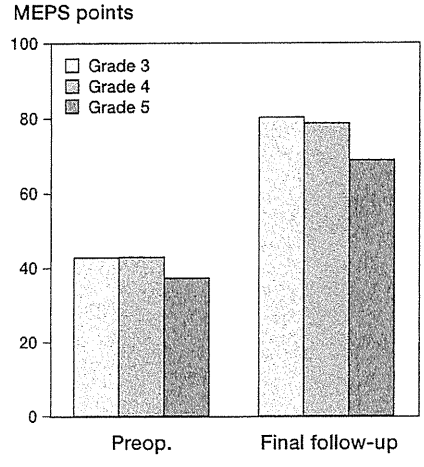

Figure 2. MEPS preoperatively and at the final follow-up examination. The mean scores improved statistically significantly for all grades.

had an average follow-up period of 15 (10–23) years. Surgical indications of synovectomy were continuous pain that did not respond to nonoperative treatment or serious disability due to progressive loss of range of motion.

Functional outcome was assessed using the Mayo elbow performance score (MEPS) (Morrey 1991). Conventional anteroposterior and lateral radiographs were taken before surgery and at the final follow-up examination. The grades of Larsen's classification and the elbow valgus angle before surgery and at the final follow-up examination were evaluated (Larsen et al. 1977).

Surgical procedure and postoperative care

A laterally curved 10-cm skin incision was made in the posterolateral area of the elbow. Dissection was done between the brachioradialis and the triceps muscles in the upper arm and between the extensor carpi radialis and the anconeus in the forearm. The anterior capsule and the lateral ligament were exposed entirely. After resection of the radial head, the anterior joint capsule with the synovium was completely detached from the lateral to the medial condyle of the humerus. The synovium remaining around the capsular attachment to the humerus and around the radioulnar joint was resected with a small rongeur. After the posterolateral joint cavity was exposed, the synovectomy was performed by resecting the capsule adhering to the posterior side of the humerus and resecting the synovium in the posterolateral capsule and around the olecranon fossa. Because we used a lateral approach, most of the synovium in the medial capsule could not be resected. Osteophytes around the olecranon and the coronoid process, if present, were resected until full range of extension and flexion was achieved. A suction drain tube was inserted into the joint space for 24 h. No external fixation was used. Active exercises of the elbow were started on the day after surgery. To avoid flexion contracture, the elbow was gradually extended while a 0.5- to 1.0-kg weight was held in the hand. Home exercises were continued until enough range of motion was obtained.

Statistics

We used Wilcoxon's signed-rank test and chi-square test to compare the clinical results before surgery and at the final follow-up examination. Values of p less than 0.05 were considered significant. Survival analysis was performed by the Kaplan-Meier method, and the endpoint was defined as conversion to TEA.

Results

Clinical and radiographic outcome

According to the MEPS, 48 elbows showed excellent or good results, 12 elbows showed fair results, and 4 elbows had poor results at the final follow-up examination. The total MEPS improved from an average of 42 (15–75) points before surgery to 78 (45–100) points at the final follow-up examination ($p < 0.01$) (Figure 1). There were no cases that had lower MEPS at the final follow-up examination than preoperatively. For all elbows, MEPS was statistically significantly improved at the final follow-up examination (Figure 2).

The mean MEPS for pain improved from 14 (0–30) points before surgery to 35 (15–45) points after surgery. Complete pain relief or only mild pain (scores of 30–45) after the elbow synovectomy was observed in 62 cases (Figure 3). 2 elbows showed moderate pain at the final follow-up examination.

The mean arc of flexion-extension motion increased from 67° (0–125) preoperatively to 101° (65–140) postoperatively ($p < 0.01$) and from 102° (20–70) to 137° (80–180) in pronation and supination ($p < 0.05$). In 2 elbows, the arc of flexion-extension motion worsened postoperatively. In pronation and supina-

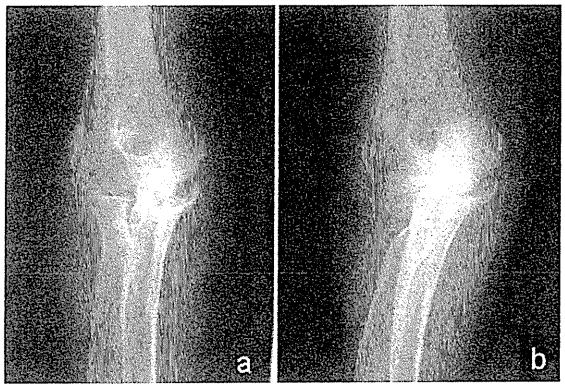

Figure 3. a. A right elbow 6 years after the onset of rheumatoid arthritis; Larsen grade 4. This patient complained of severe pain, swelling, and disability in activities of daily living (ADL), and motion was from 30° to 105°. The preoperative MEPS was 50 points. b. 11 years after surgery; Larsen grade 4. The patient had experienced relief of the pain with no swelling and no disability in ADL. The MEPS was 95 points.

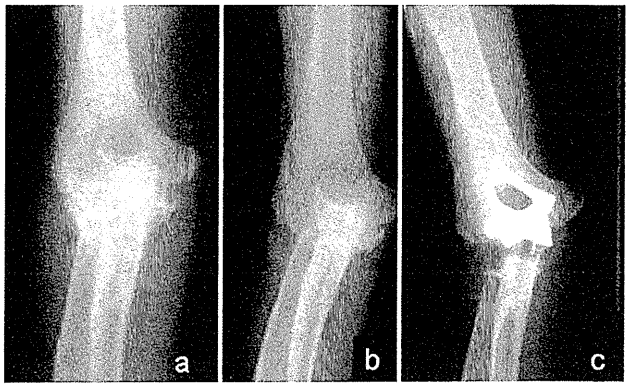

Figure 4. a. Rheumatoid elbow of a 65-year-old woman before synovectomy. b. 13 years after the synovectomy. ADL scores were worse 10 years after the synovectomy. TEA was performed due to the recurrence of arthritis 13 years after the synovectomy. The MEPS was 50 points just before the TEA. c. After TEA, the patient experienced relief of the pain with no swelling and disability in ADL. The MEPS was 80 points at the final follow-up examination, 10 years after TEA.

tion, 52 elbows had an improved arc of motion preoperatively, 10 elbows had a reduced arc of motion, and 2 elbows (3.1%) had the same arc of motion preoperatively. Regarding the arc of motion, the preoperative MEPS increased from 14(5–20) points to 19 (15–25) points at the final follow-up examination. The function score was 12 (0–20) points preoperatively and 20 (15–45) points at final follow-up. The changes in the total score at the final follow-up examination mainly depended on the relief of pain in 12 elbows that had been preoperatively graded as Larsen grade 3; of these, 4 elbows remained at grade 3, and 7 elbows and 1 elbow progressed to Larsen grade 4 and 5, respectively. In 45 elbows that had been graded as Larsen grade 4 preoperatively, 32 elbows remained at grade 4 and 13 elbows progressed to Larsen grade 5. Assessment of clinical mediolateral joint stability showed minimal instability (0–5°) in 59 elbows, moderate instability (6–10°) in 3 elbows, and marked instability (> 10°) in 2 elbows.

Survival analysis

12 elbows had seen converted to TEA at the final follow-up (Figure 4). Of these cases, 1 elbow had been graded as Larsen grade 3, 7 as grade 4, and 4 as grade 5 preoperatively. The mean MEPS in the TEA conversion cases before synovectomy was 40 (20–60) points, which was not significantly different from that in the cases who did not undergo TEA (43 points). The survival rates for synovectomy were 97% at the 10-year point, 75% at the 15-year point, and 70% at the 20-year point (Figure 5).

Postoperative complications

There were no cases of postoperative infection. None of the cases needed an additional synovectomy. Ulnar nerve palsy was observed in 4 cases, which all recovered spontaneously and completely. No cases had any loss of motor function. The

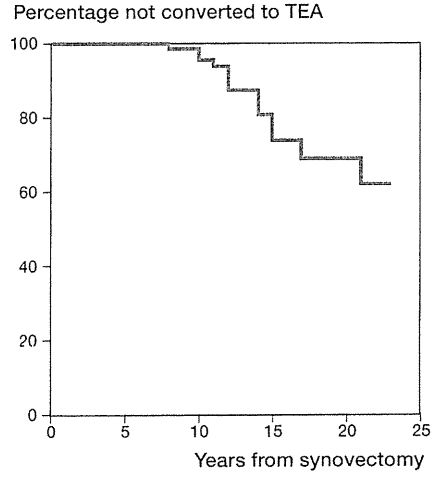

Figure 5. Kaplan-Meier survival curve of synovectomy for rheumatoid elbow with elbows converted to TEA as endpoint. The 10-, 15-, and 20-year survival rates were 96.8%, 74.9%, and 69.9%, respectively.

mean valgus angle of the elbow was 16 (4–28) degrees before surgery and it progressed slightly to 17 (6–33) degrees at the final follow-up examination.

Discussion

The efficacy of synovectomy for joints at radiographically early stages and TEA for joints at radiographically advanced stages has been confirmed repeatedly (Brumfield and Resnick 1985, Stein et al. 1975, Saito 1986, Ferlic et al. 1987, Figgie et al 1989, Tulp and Winia 1989, Koshino 1991, Gendi et al. 1997, Connor and Morrey 1998, Fuerst et al. 2006, Nakagawa et al. 2007). Our cases underwent open elbow synovectomy,

but several reports suggest the efficacy of arthroscopic synovectomy for rheumatoid elbow at radiographically early stages (Lee and Morrey 1997, Horiuchi et al. 2002, Tanaka et al. 2006). However, there have been few reports on long-term outcome of arthroscopic synovectomy (Tanaka et al. 2006), and several authors have reported a relatively high incidence of complications such as local nerve impairment (Lee and Morrey 1997). Thus, we performed open synovectomy to resect the synovium sufficiently and safely.

Medium-term results of elbow synovectomy with an average follow-up period of up to 10 years have been published by several authors. Ferlic et al. (1987) reported excellent results with an average follow-up of 7 years and a maximum follow-up of 20 years. In their report, 44/57 elbows had excellent results, and they had better clinical results in patients who underwent surgery at earlier stages of the disease. Brumfield and Resnick (1985) stated that synovectomy was not contraindicated even for joints at radiographically advanced stages, such as Larsen grade 3 or 4, because an improvement in the range of motion can be expected. We also performed elbow synovectomy at advanced stages and this was not followed by substantial joint instability. In the present study, 12/16 elbows required conversion to TEA after a minimum of 10 years and an average of 15 years of follow-up. We consider this result to be more favorable than those in previous reports that included synovectomies for elbows at radiographically advanced stages (Ferlic et al. 1987, Fuerst et al. 2006). Recurrence of severe pain from progressive joint destruction was the most common cause of the conversion to TEA: 4 of 7 elbows of Larsen grade 5 underwent conversion.

During an elbow synovectomy, the radial head is often resected. Resection of the radial head enhances the operative visual field and the performance of an adequate resection of the joint synovium. Additionally, resection of the radial head improves flexion, especially in cases with anterior subluxation of the radial head. However, resection of the radial head during an elbow synovectomy is controversial. Ferlic et al. (1987) reported that there was no difference in clinical results between cases that had resection of the radial head and those with resection and radial head replacement. Lehtinen et al. (2001) reported that the elbow seemed to turn into valgus during rheumatoid destruction and resection of the radial head. Rymaszewski et al. (1984) suggested that resection of the radial head caused joint instability in the long term, and recommended radial head replacement rather than radial head resection. In addition, Taylor et al. (1976) and Copeland and Taylor (1979) reported that resection of the radial head caused progression of valgus deformities of the elbow in more than half of their cases. They also found that excessive axial pressure to the ulna by resection of the radial head caused pain on the ulnar side of the wrist. We resected the radial head in all our cases with only a slight increase in the valgus angle at the final follow-up examination (mean 2 degrees). At the final examination, only 2 elbows showed marked valgus deformities with severe instability of the elbow joint. Thus, we believe that resection of the radial head is appropriate.

One of the limitations of our study was the evaluation of joint instability, which was assessed clinically, not radiographically. Radiographic examination is a more precise method.

In conclusion, synovectomy for the treatment of rheumatoid elbow gives a good long-term outcome, even for radiologically advanced joints of Larsen grades 3 or 4, but not for those of grade 5.

KI: design of the study, data collection, literature search, and manuscript preparation. YI, YM, TS: design of the study, surgery, manuscript preparation, and supervision.

We thank YutakaInaba, Yuichi Mochida, and Tomoyuki Saito-all the orthopedic surgeons who participated at Yokohama City University.

No competing interests declared.

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Diagnostic Microbiology and Infectious Disease 74 (2012) 125–130

Contents lists available at SciVerse ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Quantitative evaluation of periprosthetic infection by real-time polymerase chain reaction: a comparison with conventional methods☆,☆☆

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ARTICLE INFO

Article history:
Received 9 March 2012
Accepted 18 June 2012
Available online 24 July 2012

Keywords:
Real-time PCR
Quantification
Periprosthetic infection

ABSTRACT

Several recent studies have demonstrated the limited accuracy of conventional culture methods for diagnosing periprosthetic infections. We have applied real-time polymerase chain reaction (PCR) assays for the rapid identification of bacteria around implants and reported its utility. However, the capability of quantification is also a useful feature of this type of assay. The aim of our study was to validate the usefulness of quantitative analyses using real-time PCR of cases with clinical periprosthetic infections in comparison with more established tests, such as C-reactive protein (CRP) levels, microbiologic cultures, and histopathology. Fifty-six joints with suspected infections were reviewed retrospectively. A universal PCR assay was used to perform the quantitative analyses. The differences in the threshold cycles between clinical samples and a negative control (ΔCt) in each case were calculated. The results of the quantitative PCR assay were compared with CRP levels, microbiologic cultures, and histopathology. There was a significant correlation found between the CRP and ΔCt values. There were also significant differences found in the ΔCt values according to CRP levels, with higher CRP levels showing higher ΔCt values. Similarly, there were significant differences in the ΔCt measurements in our culture results and among our pathologic evaluations. We confirmed that quantification by universal PCR based on the ΔCt correlated with preoperative CRP levels and was associated with the microbiologic culture results and pathologic severity. This quantification method may be valuable for assessing infection severity.

1. Introduction

Total joint arthroplasty is commonly recognized as the most effective treatment for osteoarthritis and rheumatoid arthritis, and this procedure has been improved over the years in terms of the implant design, more stable long-term clinical results, and a reduced frequency of complications. Despite these improvements, however, periprosthetic infection remains a serious potential complication of joint arthroplasty. Difficulties have occurred in the detection of such infections with conventional culture methods, and several recent studies have demonstrated the limited accuracy of this approach for diagnosing periprosthetic infections, particularly low-grade infections that often cause false-negative results (Kobayashi et al., 2008; Tunney et al., 1999). No single laboratory test has sufficient sensitivity or specificity for diagnosing such low-grade infections (Bare et al., 2006). Histopathologic evaluations are thought to be the most reliable tests for diagnosing bacterial infections because of their high specificity, but previous reports have indicated that intraoperative analyses using frozen sections have poor sensitivity in this regard (Della Valle et al., 1999; Kanner et al., 2008). Hence, the diagnosis of periprosthetic infections remains a challenge for clinicians and requires an evaluation with several different tests in parallel.

A number of molecular diagnostic tests have recently been developed for detecting microbial infections. Most are polymerase chain reaction (PCR) based and have been reported to be effective in cases of respiratory (Peters et al., 2009; Yang et al., 2005), neurologic (Darton et al., 2009; Hackett et al., 2002; Kearns et al., 1999), and pediatric (Chen et al., 2004; Perkins et al., 2005) infections, which are of a viral or bacterial nature. In addition, several previous studies have demonstrated the usefulness of PCR-based assays for the detection of periprosthetic bacterial infections in an orthopedic setting (Clarke et al., 2004; Mariani et al., 1996; Tarkin et al., 2003). Notably, the rapid and sensitive real-time PCR technique is increasingly being recognized as an alternative tool for diagnostic testing (Mackay, 2004). In addition, we previously employed and reported the utility of a real-time PCR assay for the detection of periprosthetic infections, particularly as an intraoperative identification tool, due to its extreme rapidity and ability to specifically identify methicillin

☆ Funding: No financial support has been received for this work.
☆☆ Conflicts of interest: None for all authors.
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doi:10.1016/j.diagmicrobio.2012.06.017

resistance (Kobayashi et al., 2009a). However, although the quantification capability of real-time PCR is a useful feature of this detection method, there have been few previous studies that have evaluated its utility in comparison with other diagnostic tests.

The aim of our present study was to validate the usefulness of quantitative analyses using real-time PCR of clinical periprosthetic infection cases in comparison with more established tests, such as serologic evaluations of C-reactive protein (CRP) levels, microbiologic culture, and histopathology.

2. Methods

2.1. Patients

This study was approved by our institutional review board. From January 2007 to April 2010, a total of 38 cases, which involved 56 joints including 41 hip joints (12 one-stage revision arthroplasty, 16 two-stage revision arthroplasty, and 13 implant removal), 11 knee joints (5 two-stage revision arthroplasty, 3 implant removal, and 3 hydroxyapatite block replacement), and 4 debridement joints of suspected infection or aseptic loosening that had undergone surgery were enrolled to this study. Fourteen patients were treated by antibiotics within a week of the surgical procedure. Intraoperative real-time PCR assays were performed for all cases, and quantitative evaluation was reviewed retrospectively. The CRP levels were measured prior to each operation in all cases, and intraoperatively collected tissue samples were evaluated by microbiologic culture and histopathology. As the underlying inflammatory conditions, 1 patient had rheumatoid arthritis and 2 patients had malignant tumor (osteosarcoma and pancreatic carcinoma).

2.2. Real-time PCR

Intraoperative real-time PCR assays were performed as described previously (Kobayashi et al., 2009a). Briefly, for manual DNA extraction from samples, we performed ultrasonication (Bransonic 2510 Ultrasonic cleaner; Branson Ultrasonics, Danbury, CT, USA) at a frequency of 40 kHz for 5 min using a plastic bag and 1 mL of sterile water. The sonicated solutions were collected and then applied to a DNA purification column (QIAamp DNA Mini Kit; QIAGEN, Hilden, Germany). A universal PCR assay with a LightCycler® system (Roche Diagnostics Corporation, Indianapolis, IN, USA) that targets the 16S rRNA gene was used for the quantitative analysis. Differences in the threshold cycles between the clinical samples and a negative control (ΔCt) were calculated in each case based on the LightCycler quantification mode.

2.3. C-Reactive protein

As a serologic marker of inflammation, the CRP levels (mg/dL) were reviewed in each case, and the patients were accordingly divided into the following 3 groups: CRP < 0.2 mg/dL, 0.2 mg/dL $\leq$ CRP $\leq$ 1 mg/dL, and CRP > 1 mg/dL.

2.4. Microbiologic culture

All specimens were analyzed with standard microbiologic cultures with a direct plating method and broth medium, conducted on both blood agar and Gifu anaerobic medium agar simultaneously. The results were reviewed, and the samples were divided into 3 groups based on the following results: negative, positive (under enrichment culture conditions with Gifu anaerobic medium agar), and strongly positive (under normal culture conditions with blood agar). Briefly, Gifu anaerobic medium agar, which has high nutrient condition enough for anaerobes to grow, was developed for anaerobic bacterial growth of a wide range. Some organisms that grew in blood agar sooner were sent for a Gram stain and then, according to the result, added to the appropriate agar, and, finally, the grown-up organisms represented strong positive result. On the other hand, after incubation in anaerobic jars containing hydrogen and carbon dioxide generators at 35 °C for 5 days, some organisms that grew in Gifu anaerobic medium agar represented positive result.

2.5. Histopathologic evaluation

Histopathologic evaluations of all specimens were performed intraoperatively using frozen sections and postoperatively using permanent preparations. Histopathologic findings were reviewed, and the samples were divided into the following 3 groups based on the level of neutrophil infiltration that was determined under a high-power field (HPF, 400×): negative (no neutrophil), positive (minimum of 1 HPF containing 1–10 neutrophils), and strongly positive (minimum of 5 HPF containing 10 or more neutrophils).

2.6. Definition of infection

We defined a result as an infection if strongly positive results were obtained with microbiologic culture, pathologic findings, or both.

2.7. Clinical evaluations

The average follow-up period was 14.2 months (range, 1–36). At the final follow-up examination, the existence of a radiolucent line or any other evidence of implant loosening was evaluated on an anteroposterior and lateral view by plain X-ray, and CRP values were evaluated serologically.

2.8. Statistics

The correlation between ΔCt and CRP value was analyzed by Pearson's correlation. The differences in the ΔCt values in each group were evaluated using the Kruskal–Wallis test and 1-way factorial analysis of variance followed by a post-hoc test using Fisher's protected least significant difference test. Analyses of receiver operating characteristic (ROC) curves for ΔCt for infection were then performed.

3. Results

In the evaluations of the preoperative CRP levels, there were 27 cases with CRP levels of less than 0.2, 14 cases with CRP levels between 0.2 and 1.0, and 15 cases with CRP levels higher than 1.0 among our study cohort. The culture results indicated 41 negative, 8 positive, and 7 strongly positive cases. In our pathologic evaluations, there were 15 negative, 26 positive, and 15 strongly positive findings. There was a significant correlation found between the preoperative CRP levels and ΔCt values (Fig. 1; $r = 0.54$, $P < 0.01$). There were also significant differences found in the ΔCt for each group divided by the preoperative CRP level, with higher CRP levels showing higher ΔCt values (Fig. 2). Similarly, there were significant differences in the ΔCt measurements among the groups divided by our culture results and among those divided by our pathologic evaluations. In the culture tests, there were significant differences detected between the negative and strongly positive ($P < 0.01$) and between the positive and strongly positive groups ($P < 0.01$) (Fig. 3). In terms of the pathologic results, significant differences were found between the negative and strongly positive ($P < 0.01$) and between the positive and strongly positive groups ($P < 0.01$) (Fig. 4). ROC curves revealed that the sensitivity and specificity of PCR with a ΔCt cut-off at 1.9 cycles were 94% and 51%, respectively.

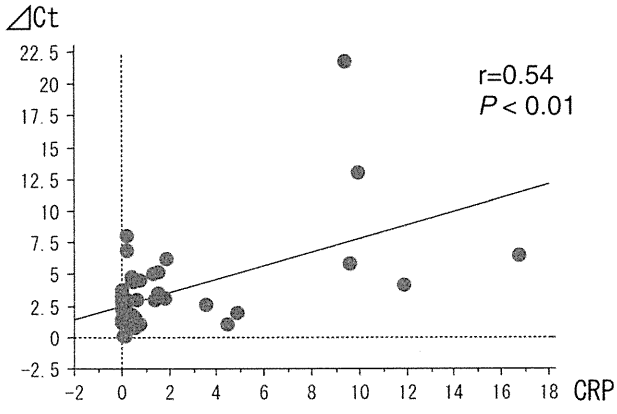

Fig. 1. Correlation between the differences in threshold cycles between the clinical samples and a negative control (ΔCt) value and the C-reactive protein (CRP) levels. There was a significant correlation found between the CRP levels and ΔCt values ($r = 0.54$, $P < 0.01$).

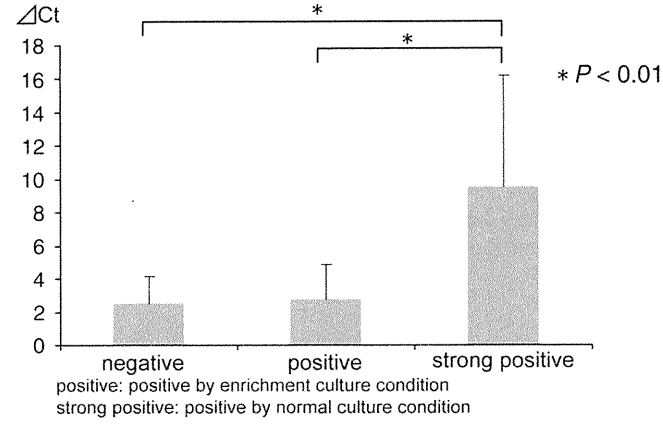

Fig. 3. Differences in ΔCt values among the patients grouped by culture results. Among the groups divided by culture results, there were significant differences between the negative and strongly positive ($P < 0.01$) and between the positive and strongly positive groups ($P < 0.01$).

3.1. Clinical evaluation

We found that all 38 cases had been followed continuously, but that 2 patients were not evaluated in this way as they had died from either a medical complication or an unknown cause. At the last follow-up examination, there were 14 cases with CRP levels between 0.2 and 1.0, and 5 cases with CRP levels higher than 1.0. The remaining 17 cases had CRP levels that were less than 0.2. Radiologically, 4 cases exhibited radiolucent lines around the acetabular components at the last follow-up examination, and 3 had radiolucent lines that were 2 mm wide or less. However, no loosening or further progression was observed in these 3 patients, while the other patient had a radiolucent line that was more than 5 mm wide that had been earmarked for revision surgery on the suspicion of reinfection. No cases with a radiolucent line around the stem were observed. One of the patients without a radiolucent line required a cup revision because of a traumatic dislocation and the implant coming off the acetabulum.

Fig. 5 shows the real-time PCR quantification of a representative case. In this patient, the CRP value before the procedure was 0.41 (mg/dL), and intraoperative histopathologic findings showed several neutrophils. We could not diagnose as infection confidently with the results of these tests. Then we analyzed tissue samples around the implant in this patient using real-time PCR and calculated a ΔCt of 4.81 intraoperatively. Based on the result of real-time PCR, we considered the patient with a periprosthetic infection and performed an implant removal for this case. Several days after implant removal, the tissue culturing consistently revealed a Gram-positive bacillus. After a waiting period of 3 months, a 2-stage revision was performed upon the intraoperative confirmation of a negative result by real-time PCR. Fig. 6 shows the results of another representative case with an infection that was diagnosed by intraoperative real-time PCR. In this patient, the CRP value before the procedure was 16.78 (mg/dL). The PCR analysis revealed an infection with a Gram-positive species and a high ΔCt of 6.43. We performed a joint implant removal during which we applied our intraoperative histopathologic findings that showed an abundance of neutrophils. In addition, cultures of the aspiration sample produced methicillin-sensitive *Staphylococcus aureus* several days after the implant removal.

4. Discussion

Several previous reports have demonstrated the usefulness of PCR-based assays for the detection and quantification of viral or bacterial infections (Darton et al., 2009; Hackett et al., 2002; Kearns et al., 1999; Perkins et al., 2005; Peters et al., 2009; Yang et al., 2005). Although the application of PCR to quantitative assessments seems to be better developed for some infectious diseases and certain types of viral infection, there have been no studies to date that have validated the quantifiability of PCR for periprosthetic infections.

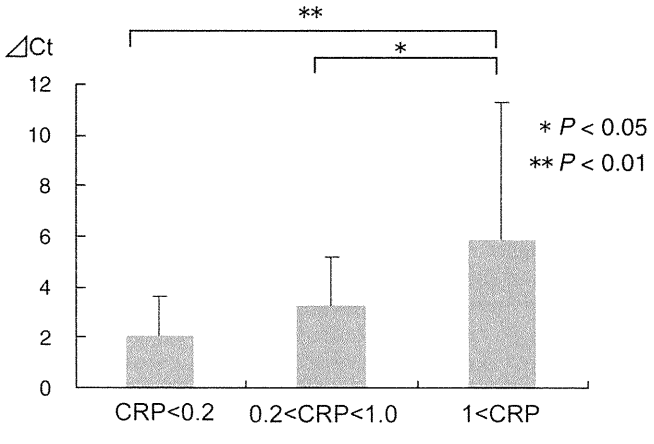

Fig. 2. Differences in ΔCt values among the groups divided according to CRP levels. Among the groups classified according to their preoperative CRP levels, there was a significant difference in the ΔCt values between the CRP <0.2 and CRP >1.0 groups ($P < 0.01$).

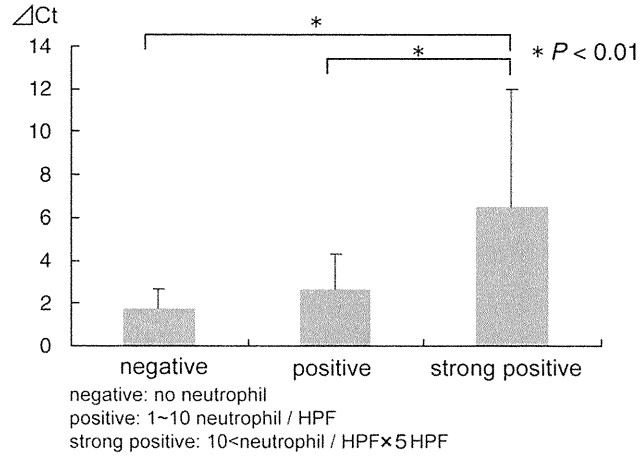

Fig. 4. Differences in ΔCt values among the patients grouped by pathological findings. Among the patient groups divided by pathological results, there were significant differences between the negative and strongly positive ($P < 0.01$) and between the positive and strongly positive ($P < 0.01$) groups.

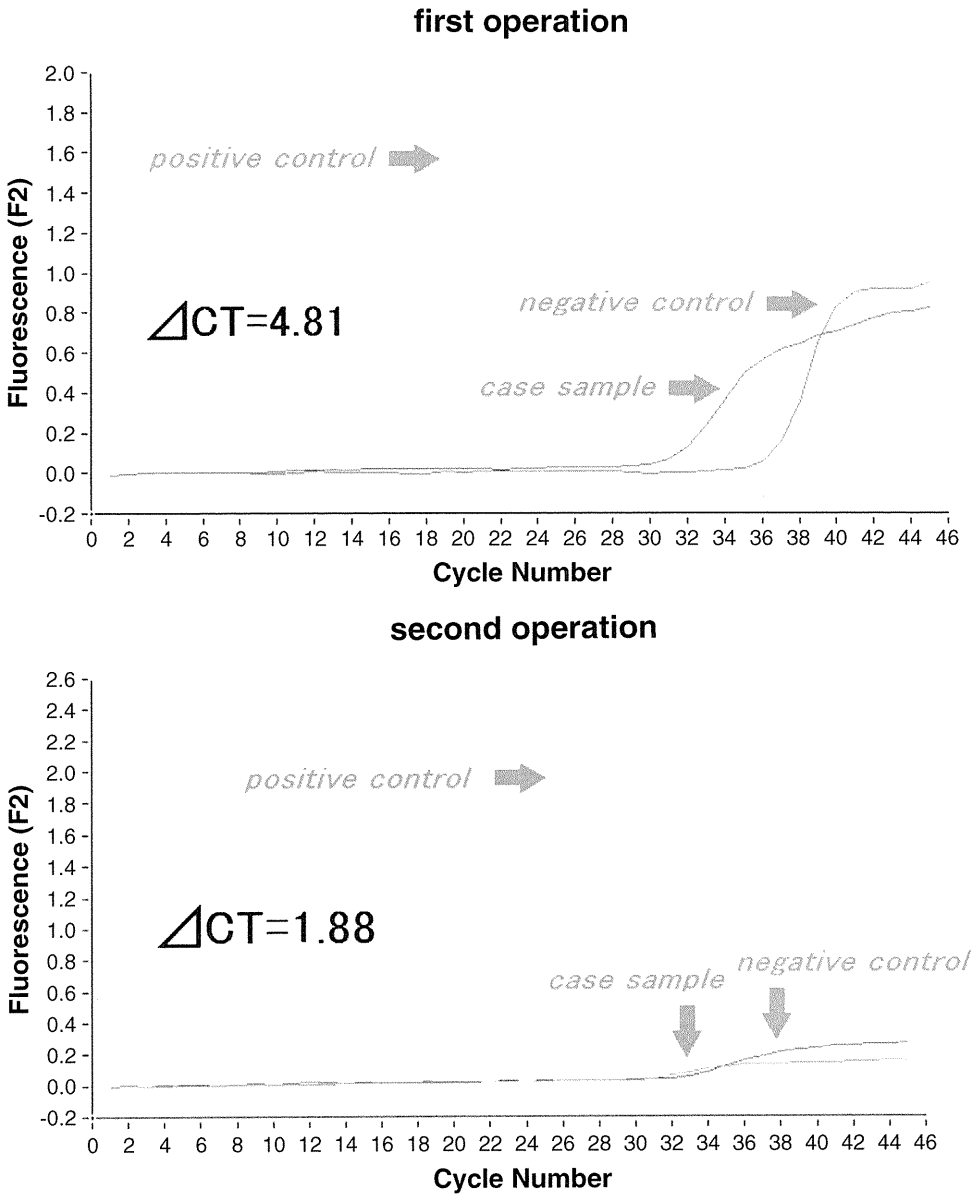

Fig. 5. Real-time polymerase chain reaction (PCR) quantification results from a representative 2-stage revision case. At the first operation, this patient showed a positive result after around 31 cycles of PCR, whilst the positive and negative control were positive after about 14 and 36 cycles, respectively. In this case, the ΔCt was calculated as 4.81. At the second operation, this decreased to 1.88, indicating a reduced level of bacterial DNA.

Hence, our current report is the first study to validate the use of real-time PCR for the quantitative evaluation and diagnosis of periprosthetic infections in clinical samples and compare this method with other tests. We confirmed that quantification by universal PCR based on the ΔCt value correlated with the preoperative CRP values and was associated with the microbiologic culture results and pathologic severity.

In the field of orthopedics, PCR assays have now been compared in a number of settings with conventional microbiologic cultures, which have long been the gold standard for the diagnosis of periprosthetic infections. Although some reports have demonstrated that PCR was not superior to microbiologic culture (Ince et al., 2004), the majority of studies on this issue have emphasized the usefulness of PCR-based techniques for the diagnosis of periprosthetic bacterial infections (Clarke et al., 2004; Mariani et al., 1996; Tarkin et al., 2003; Tunney et al., 1999). For example, Tunney et al. (1999) reported that the prevalence of infection around prosthetic joints might be underestimated by conventional tests. They utilized ultrasonication to dislodge any biofilm around total joint implants and then employed a 16S rRNA universal PCR assay for the detection of bacterial DNA. A much higher detection of occult infection was found with this technique compared with conventional cultures. We have also previously described the usefulness of the intraoperative identification of bacterial DNA by PCR to detect periprosthetic infections and reported a comparatively high specificity of 80% in clinical use (Kobayashi et al., 2009a), even though PCR has been generally recognized in the past to have a poor specificity (Clarke et al., 2004).

Over the past decades, previous studies have suggested that conventional PCR testing is unreliable because of its inability to discriminate between colonization and infection. To overcome this problem, there have been several previous studies that have validated the use of PCR assays for quantitative evaluations (Chen et al., 2004; Darton et al., 2009; Hackett et al., 2002; Perkins et al., 2005; Peters et al., 2009; Yang et al., 2005). Yang et al. (2005) were the first to report the clinical utility of a quantitative PCR assay in diagnosing pneumonia and demonstrated that using this test with sputum samples can detect *Streptococcus pneumoniae* in adult patients with community-acquired pneumonia. Peters et al. (2009), who only

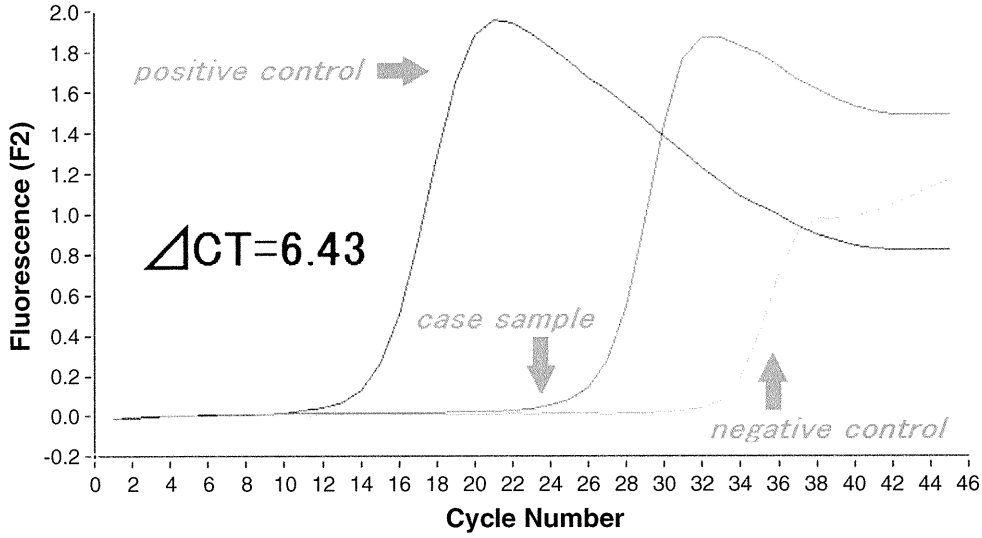

Fig. 6. Quantification results from a representative case of infection diagnosed by intraoperative real-time PCR. This patient showed a positive PCR result after around 26 cycles, while the negative control showed positivity after about 33 cycles. The ΔCt value was thus calculated as 6.43.

validated the usefulness of quantitative analyses using real-time PCR in comparison with more established laboratory tests, reported that the quantification of bacteria using PCR has a higher positive predictive value than the CRP levels or white blood cell (WBC) counts in patients with community-acquired pneumonia and that positive correlations could be observed for the quantification of bacteria based on the WBC counts, CRP levels, and length of stay. These authors evaluated the bacterial DNA load (BDL) as a diagnostic tool and demonstrated that this was related to both clinical and laboratory parameters, including CRP levels and WBC counts. They further confirmed that measuring the BDL not only supported the diagnosis of pneumonia but also provided a marker for the severity of this disease. In our current study, quantification by PCR using the ΔCt, whose method was demonstrated in a previous in vivo study (Corless et al., 2000) and which we use in clinical use, was found to correlate with the preoperative CRP levels.

In a clinical setting, occasional cases arise that show borderline characteristics with both preoperative CRP levels and intraoperative pathologic findings. In such instances, quantitative evaluations by intraoperative real-time PCR should provide useful supplemental information that may suggest suspicious infections. For example, the possibility of infection is regarded as relatively low in cases with a ΔCt under 1.9, and this resulted in a sensitivity of 94% and a specificity of 51%, as determined by universal PCR. In contrast, we can suspect infection more strongly in cases with a ΔCt higher than 1.9 as we have shown in our case example. A noteworthy issue in relation to the use of a PCR assay is the existence of PCR-positive, but culture-negative, cases, which may sometimes be false positives. Over the past decade, PCR has been recognized as a highly sensitive method that can detect low-grade infections that cannot be cultured, and it debuted as a technique with poor specificity at this sensitivity level, raising the issue of possible contamination (Clarke et al., 2004; Corless et al., 2000; Kobayashi et al., 2008). The universal PCR assays we used in our present analyses were based on the 16S rRNA gene and can be subject to contamination through the use of some commercially available Taq polymerase reagents that contain traces of *Escherichia coli* DNA, which is the bacterial system used to produce the enzyme as a recombinant protein (Corless et al., 2000). In PCR-positive but culture-negative cases, however, quantification by PCR can be considered to be a robust way of validating infection. This is because a higher ΔCt, which means a higher amount of bacterial DNA, suggests the clear existence of infection despite a negative culture result. In contrast, a lower ΔCt, which means a lower amount of bacterial DNA, suggests the possibility of a false positive due to contamination. Therefore, in a clinical setting also, we actually selected 2-stage revision surgery after implant removal and a waiting period in the case with PCR-positive (with ΔCt higher than 1.9) but culture-negative result. It is not described in this article, but we also evaluated melting peak analysis in real-time PCR, which enables us to distinguish whether bacterial DNA was from Gram-positive or Gram-negative species as described in a previous study (Kobayashi et al., 2009a).

Another issue to consider is the inability to confirm the viability of bacteria in PCR-positive cases. PCR can detect bacterial DNA from both viable and necrotic organisms, such that traces of only a few necrotic bacteria that have been dislodged from an implant surface may yield a false-positive PCR result (Kobayashi et al., 2009b). In an attempt to overcome this problem, Birmingham et al. (2008) reported the possibility of using reverse transcription-PCR to detect only live bacteria as necrotic organisms do not produce mRNA. In addition, Kobayashi et al. (2009b) investigated the use of propidium monoazide (PMA) to differentiate viable from dead bacteria. PMA has been used as a DNA-binding agent for differentiating intact from membrane-compromised bacterial cells and thus has the ability to penetrate dead bacterial cells with compromised membranes. Although we demonstrated the relationship between ΔCt and the other tests in the current study, this issue should be addressed in a future study.

One of the limitations of our present study was related to the method of quantification by real-time PCR. We simply calculated the difference in the crossing point between the samples and a negative control and represented this as the ΔCt. Hence, we did not quantify the actual total amount of amplified DNA in comparison with reference control samples. Nevertheless, we detected a relationship between these ΔCt measurements and the results of other tests that indicated that our simple PCR-based method has utility for routine clinical use. Another limitation was that we have to consider the influence of antibiotics on the results. In our present study, 14 patients were treated by antibiotics within a week of the surgical procedure. The antibiotics treatment possibly affected the results of the microbiologic culture and also the PCR results. In addition, we have to consider the effects of some underlying inflammatory conditions such as rheumatoid arthritis. In the current series, we included 1 case of rheumatoid arthritis and 2 cases of malignant tumor, which might have influenced the results of CRP and histopathology.

In conclusion, we demonstrated that quantitative evaluations by real-time PCR in conjunction with other tests have potential value for

assessing cases with possible periprosthetic infections. The quantification advantages of this PCR method may provide supplemental information for determining the severity of the infection. Additional studies are needed with more clinical cases to further validate this methodology.

Acknowledgment

The authors thank a laboratory technician, Kimi Ishikawa, for performing the real-time PCR procedure and for the management of the LightCycler system.

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J Thromb Thrombolysis
DOI 10.1007/s11239-012-0773-z

Plasma accumulation of fondaparinux 2.5 mg in patients after total hip arthroplasty

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Abstract Fondaparinux (FPX), a selective inhibitor of factor Xa, is widely used for the prophylaxis of venous thromboembolism (VTE) after total joint arthroplasty. However, the association between plasma FPX concentration and adverse events and the occurrence of VTE has not been clarified thus far. We aimed to prospectively evaluate these associations by measuring anti-Xa activity of FPX in patients undergoing total hip arthroplasty (THA) and investigate whether factors such as age, body weight, and renal function influence the anti-Xa levels. We enrolled 85 patients who underwent primary THA. All patients received subcutaneous FPX (2.5 mg/day for 14 days) after surgery. Anti-Xa activity was measured on postoperative days 1, 3, 7, and 14. To assess VTE, multidetector row computed tomography was performed in all patients at 1 week after surgery. The median levels of anti-Xa activity increased as follows (medians with 95 % confidence interval): 0.00 (0.00–0.01) mg/L, 0.13 (0.11–0.14) mg/L, 0.19 (0.17–0.20) mg/L, and 0.24 (0.22–0.25) mg/L on postoperative days 1, 3, 7, and 14, respectively. The plasma accumulation of FPX was more likely in patients with renal impairment than in those with normal renal function. In contrast, a poor correlation was observed between the plasma levels of anti-Xa activity and age or body weight. No differences were observed in the anti-Xa activity in patients with and without postoperative VTE or bleeding. Substantial increase in the levels of anti-Xa activity was observed, especially in patients with renal impairment, after subcutaneous administration of FPX 2.5 mg after THA.

Keywords Venous thromboembolism · Total hip arthroplasty · Fondaparinux · Drug monitoring · Renal dysfunction

Introduction

Fondaparinux (FPX), a selective inhibitor of factor Xa, is indicated for the prophylaxis of venous thromboembolism (VTE) after total joint arthroplasty of the lower extremities [1]. Selective inhibition of factor Xa is effective for prevention of development of a hypercoagulant state because factor Xa is located at the junction of the extrinsic and intrinsic coagulation pathways [2]. The Ephesus [3] and Pentathlon trials [4] showed that compared to enoxaparin, FPX 2.5 mg once daily reduced the risk of postoperative VTE by approximately 50 %. Moreover, FPX is a synthetic product with a high affinity for antithrombin [5] and rarely requires drug monitoring owing to its long half-life and predictable anticoagulant activity [6]. In contrast, although

Electronic supplementary material The online version of this article (doi:10.1007/s11239-012-0773-z) contains supplementary material, which is available to authorized users.

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Published online: 17 July 2012

FPX is supposed to only minimally enhance bleeding and not affect platelet functions [5], higher prophylactic efficacy of FPX is associated with a higher risk of bleeding complications [7, 8].

The American Academy of Orthopaedic Surgeons guidelines recommend that surgeons should evaluate the patient's risks for pulmonary embolism (PE) and serious bleeding and individualize pharmacologic prophylaxis on the basis of a risk–benefit ratio [9, 10]. According to post hoc analysis [11], the rate of major bleeding during FPX administration was the highest in patients with moderate renal impairment, aged 75 years or more, and weighing less than 50 kg. Patients undergoing total joint arthroplasty of the lower extremities are mainly older patients who have got pre-existing renal impairment at a high rate [12]. Moreover, the number of elderly female patients weighing less than 50 kg is high in Japan. Thus, it is suggested that an accumulation of FPX is possibly observed in patients receiving repeated administration of fixed doses after surgery, and the need for monitoring plasma FPX levels will increase with an increase in the use of FPX.

Fondaparinux levels can be monitored by measuring its anti-Xa activity [13]. However, few studies have reported the anti-Xa activity profiles of FPX administered after total hip arthroplasty (THA). The purpose of our prospective study was to measure the anti-Xa activity of FPX in patients undergoing THA, and to evaluate the association between the anti-Xa levels and bleeding complications and occurrence of postoperative VTE. In addition, we determined whether other factors such as age, body weight, and renal function influenced the anti-Xa levels.

Methods

Patients

We prospectively enrolled consecutive patients who received subcutaneous FPX as a prophylactic for VTE after primary THA at Yokohama City University Hospital between December 2007 and June 2010. Patients were excluded if they had any of the following conditions: (a) body weight <40 kg; (b) cerebral or gastrointestinal bleeding within the previous 6 months; (c) severe renal insufficiency (estimated glomerular filtration rate [eGFR] <30 mL/min·1.73 m^{-2}) [14]; (d) hepatic failure; or (e) coagulation or fibrinolysis disorder. The values of eGFR were calculated using the following formula: eGFR (for men) = 194 × serum creatinine concentration$^{-1.094}$ × age$^{-0.287}$ and eGFR (for women) = 143 × serum creatinine concentration$^{-1.094}$ × age$^{-0.287}$. Before enrollment, all patients were given a detailed explanation of the risks and alternatives to participation in this study, and all provided written informed consent.

If patients had taken oral anticoagulant or antiplatelet agents before surgery for any documented diseases, such as atrial fibrillation and cerebral infarction, these medicines were discontinued 3–7 days before surgery. For postoperative thromboprophylaxis, 2.5 mg of FPX was subcutaneously administered from the day after surgery for 14 days. In addition, intermittent pneumatic compression was applied from the time of induction of anesthesia to the day patients started walking exercise. Preoperative anticoagulant or antiplatelet therapy was reintroduced after thromboprophylaxis with FPX was concluded.

Postoperative mobilization was performed according to a set protocol under the supervision of experienced orthopedic physiotherapists. Early walking with a tolerable weight load using crutches or a walker was performed from the day after surgery. Fixed postoperative rehabilitation was scheduled for all patients staying at the hospital; subsequently, the patients were discharged on postoperative day 14, the day on which this protocol was completed.

Data acquisition

Blood samples were obtained from the peripheral veins, before subcutaneous injection of FPX, on postoperative days 1, 3, 7, and 14. The anti-Xa levels of FPX were measured using Rotachrom Heparin® (Roche Diagnostics K.K., Tokyo, Japan) that uses a FPX-based chromogenic substrate, and the results were expressed in mg/L. We noted the clinical incidences of bleeding complications, VTE, and all other adverse events during FPX administration. Clinical and demographic data (age, gender, renal function, body mass index [BMI], duration of operation, and the amount of blood loss) were obtained and subsequently analyzed.

Diagnostic criteria for major bleeding

Major bleeding was diagnosed on the basis of the following criteria: fatal bleeding; bleeding that was retroperitoneal, intracranial, intraspinal, or involving any other critical organ; bleeding leading to reoperation; and overt bleeding with a bleeding index of 2 or more. The bleeding index was calculated as the number of units of packed red blood cells transfused plus a decrease in hemoglobin level ≥2 g/dL from the pre-bleeding value within 48 h of onset [15]. For rapid detection of bleeding complications, the levels of hemoglobin were measured on postoperative days 1, 3, 7, and 14, and the condition of the surgical wound and examination of feces was performed every day during the postoperative period.

Diagnostic methods for VTE

Duplex ultrasonography (US) was performed in all patients 28 days before surgery to detect any evidence of pre-

existing deep vein thrombosis (DVT) of the lower limbs. For the diagnosis of postoperative VTE, including PE and DVT, we performed angiography of the pulmonary artery and the deep veins of the pelvis and lower limbs in all patients on postoperative day 7 using 64-slice multidetector row computed tomography (MDCT). When the presence of VTE was suspected by MDCT, duplex US was additionally performed to confirm the presence of DVT.

Statistical analysis

Statistical analyses were performed using SPSS II software (SPSS Japan Inc., Tokyo, Japan). According to a Kolmogorov–Smirnov analysis, the continuous variables showed a skewed distribution. Thus, these variables are presented as medians with 95 % confidence interval in the text. The medians and interquartile ranges are plotted in the figures as a box-and-whisker plot. The perpendicular bars (whiskers) in the figures represent the 5th and 95th percentiles, and the box plots portray the median and the 25th and 75th percentiles (boxes). Differences in the variables between patients with and without renal dysfunction or VTE were statistically examined using the Kruskal–Wallis test, Student's t test, or a two-tailed Mann–Whitney U-test. Furthermore, the χ^2 test and Fisher exact probabilities were used for the comparison between the observed and expected frequencies. $p < 0.05$ was considered statistically significant.

Results

We enrolled 100 patients who underwent primary THA in our study. Thirteen patients were excluded because of missing data, and 2 patients were excluded because of withdrawal of FPX due to bleeding complications. Thus, 85 patients with a mean age of 59.2 years (range, 32–94 years) completed the protocol and were available for analysis (Table 1). The preoperative levels of eGFR indicated that 6 patients had moderate renal insufficiency (eGFR, 30–59 mL/min·1.73 m^{-2}), and 45 patients had mildly

Table 1 Patient Characteristics

	Moderate RD $n = 6$	Mild RD $n = 45$	Without RD $n = 34$	p
Female, no. (%)	4 (67)	40 (89)	31 (91)	NS
Male, no. (%)	2 (33)	5 (11)	3 (9)	NS
Age, y (95 % CI)	68.5 (55.2–81.8)	60.8 (56.8–64.8)	54.9 (52.1–57.7)	<0.01
Weight, kg (95 % CI)	59.6 (47.0–72.2)	57.9 (54.1–61.6)	57.7 (54.5–60.9)	NS
Body mass index, kg m^{-2} (95 % CI)	24.5 (21.4–27.6)	23.6 (22.4–24.8)	23.9 (22.8–25.0)	NS
eGFR, mL/min·1.73 m^{-2} (95 % CI)	51.4 (46.7–56.1)	76.8 (74.6–79.0)	101.4 (99.1–103.7)	<0.01
Primary hip disease, no. (%)				
OA	5 (83)	37 (82)	32 (94)	NS
RA	0 (0)	5 (11)	1 (3)	NS
ANFH	1 (17)	3 (7)	1 (3)	NS
Operation duration, min (95 % CI)	145 (132–158)	155 (142–168)	149 (138–160)	NS
Blood loss, mL (95 % CI)	466 (341–591)	587 (503–671)	520 (452–588)	NS
Previous illness, no. (%)				
Hypertension	1 (17)	8 (18)	2 (6)	NS
Atrial fibrillation	0 (0)	0 (0)	0 (0)	NS
Old cerebral infarction	1 (17)	2 (2)	0 (0)	NS
VTE	1 (17)	1 (4)	1 (3)	NS
Preoperative anticoagulation, no. (%)	1 (17)	2 (4)	1 (3)	NS
Preoperative antiplatelet therapy, no. (%)	1 (17)	2 (4)	0 (0)	NS
Postoperative events associated with FPX, no (%)				
VTE	0 (0)	6 (13)	2 (6)	NS
Worsen renal function	0 (0)	0 (0)	0 (0)	NS
Skin rash	0 (0)	0 (0)	1 (3)	NS

RD renal dysfunction, *NS* not significant, *eGFR* estimated glomerular filtration rate, *OA* osteoarthritis, *RA* rheumatoid arthritis, *ANFH* avascular necrosis of femoral head, *VTE* venous thromboembolism, *FPX* fondaparinux

reduced renal function (eGFR, 60–89 mL/min·1.73 m^{-2}). Eight patients received chronic anticoagulant or antiplatelet therapy because of a past history of cerebral infarction or VTE.

The overall values of anti-Xa activity of FPX are shown in Fig. 1. The anti-Xa levels showed a gradual increase as follows: 0.00 mg/L (0.00–0.01 mg/L) on postoperative day 1, 0.13 mg/L (0.11–0.14 mg/L) on day 3, 0.19 mg/L (0.17–0.20 mg/L) on day 7, and 0.24 mg/L (0.22–0.25 mg/L) on day 14 ($p < 0.001$).

Univariate analysis indicated no obvious correlation between anti-Xa activity and age or body weight. On the other hand, a negative correlation was observed between anti-Xa activity and eGFR value on postoperative days 7 and 14 ($p < 0.05$).

Anti-Xa activity of FPX in patients categorized according to their renal function is shown in Fig. 2. Patients with moderate ($n = 6$, 7 %) and mild renal insufficiency ($n = 45$, 53 %) had significantly higher plasma levels of anti-Xa activity than patients with normal renal function on postoperative days 3, 7, and 14 ($p < 0.01$). In addition, all groups, including patients with normal renal function showed significant increase in anti-Xa activity of FPX over the course of this study ($p < 0.01$). The demographic characteristics of the patients (Table 1) indicate that patients with renal dysfunction were significantly older than those with normal renal function ($p < 0.01$). The distributions of other characteristics such sex, body weight, BMI, duration of operation, blood loss, and previous illness were similar between patients with and without renal dysfunction.

Postoperative VTE was diagnosed in 8 (9.4 %) of 85 patients, and the incidence of PE and DVT was 3 (3.5 %) and 5 (5.9 %), respectively. No patients in our cohort had symptomatic PE or DVT, and all patients with DVT showed DVT of the calf vein. There were no statistically significant differences in study parameters, including median plasma anti-Xa activity of FPX at different intervals, between patients who developed postoperative VTE and those who did not (Fig. 2).

Overall, 2 (2.3 %) patients had postoperative bleeding complications in the observation period during the hospital stay. The plasma anti-Xa levels of these patients are shown in Fig. 3. The first patient was a 62-year-old woman who was administered FPX after THA for right hip osteoarthritis in Fig. 4. On postoperative day 10, the administration of FPX was discontinued because of bleeding from her surgical wound. Her eGFR level was 106 min^{-1}·1.73 m^{-2}. The second patient was an 85-year-old woman who underwent THA for right hip osteoarthritis. On postoperative day 3, a marked reduction in hemoglobin level (more than 2 g/dL decrease compared to that on the day after surgery) was observed. Although other bleeding symptoms could not be found, we diagnosed major bleeding and discontinued administration of FPX from postoperative day 3. Her eGFR level was 63 min^{-1}·1.73 m^{-2}. However, the

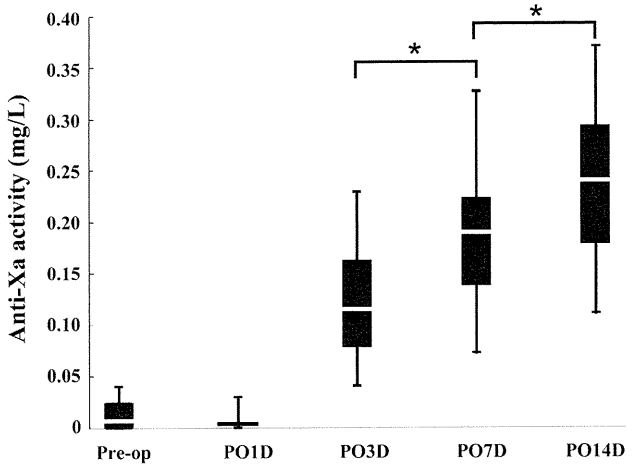

Fig. 1 Levels of anti-Xa activity in patients received repeated administration of FPX 2.5 mg following THA. Plasma levels of anti-Xa activity in patients who received subcutaneous FPX 2.5 mg following THA were measured preoperatively (preope) and on postoperative days 1 (PO1D), 3 (PO3D), 7 (PO7D), and 14 (PO14D). The *boxes* represent the interquartile ranges. The *perpendicular lines* (*whiskers*) represent the 5th and 95th percentiles, and the *box plots* portray the medians and 25th and 75th percentiles (*boxes*). The levels of anti-Xa activity were significantly increased as repeating administration of FPX (**$p < 0.001$)

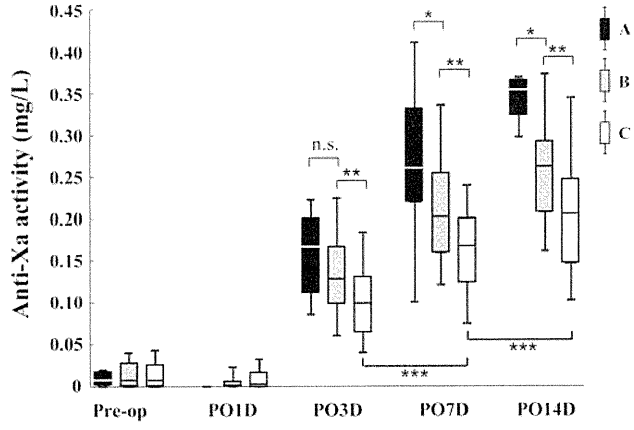

Fig. 2 Levels of anti-Xa activity in patients with different renal function. The *perpendicular lines* (*whiskers*) represent the 5th and 95th percentiles, and the *box plots* portray the medians and 25th and 75th percentiles (*boxes*). Preope, PO1D, PO3D, PO7D, and PO14D denote preoperative day, postoperative days 1, 3, 7, and 14, respectively. In patients with moderate renal insufficiency (*A*, $n = 6$), the plasma levels of anti-Xa activity were significantly higher than patients with mild renal insufficiency (*B*, $n = 45$) on PO7D and PO14D (*$p < 0.05$). Similarly, the higher levels of anti-Xa activity were observed in patients with mild renal insufficiency than those with normal renal function (*C*, $n = 33$) on postoperative days 3, 7, and 14 (**$p < 0.01$)

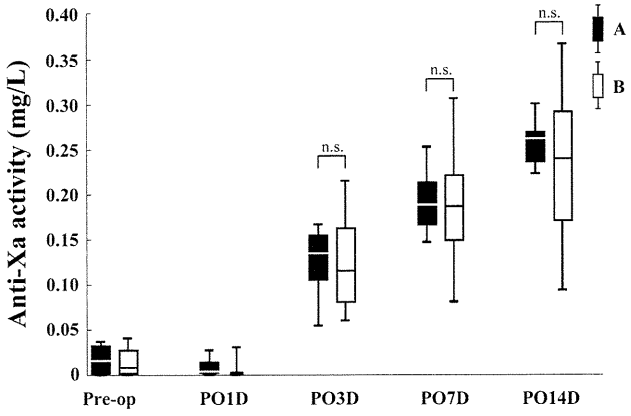

Fig. 3 Different levels of anti-Xa activity in patients with and without postoperative VTE. The *perpendicular lines* (*whiskers*) represent the 5th and 95th percentiles, and the *box plots* portray the medians and 25th and 75th percentiles (*boxes*). Preope, PO1D, PO3D, PO7D, and PO14D denote preoperative day, postoperative days 1, 3, 7, and 14, respectively. Comparing to plasma anti-Xa activity of FPX in patients with and without postoperative VTE (*A*; $n = 8$, and *B*; $n = 78$, respectively), there were no differences in the variables throughout our study

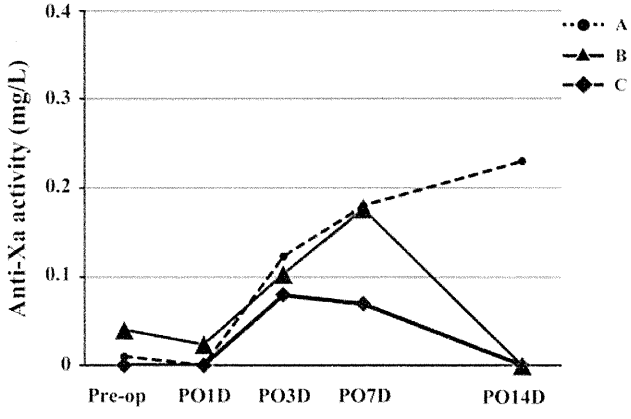

Fig. 4 Levels of anti-Xa activity observed in patients with bleeding complications. The *dashed line* (*A*) represents the medians of plasma anti-Xa activity in the overall patients excluding the patients with bleeding complications. The *solid line* with *triangular markers* (*B*) represents the values observed in the patient of 62-year-old woman who was given FPX following THA for right hip osteoarthritis. On postoperative day 10, administration of FPX was suspended because of bleeding from her surgical wound. Her eGFR level was 106 $min^{-1} \cdot 1.73\ m^{-2}$. The *solid line* with *squared markers* (*C*) represents the anti-Xa activity in the patient of 85-year-old woman who underwent THA for right hip osteoarthritis. On postoperative day 3, a marked reduction in hemoglobin level (more than 2 g/dL decrease compared to that on the day after surgery) was observed. Although other bleeding symptoms could not be found, we diagnosed it major bleeding and discontinued administration of FPX from postoperative day 3. Her eGFR was 63 $min^{-1} \cdot 1.73\ m^{-2}$. However, the plasma anti-Xa levels of FPX in both patients were not higher than the overall median levels, and these levels reduced after the termination of FPX

plasma anti-Xa levels of FPX in both patients were not higher than the overall median levels, and these levels reduced after the termination of FPX.

Discussion

Subcutaneous FPX has critically high bioactivity and is mainly excreted by the kidneys with a half-life of 17–21 h [16]. The anticoagulant level and the plasma concentration of FPX is most likely to be determined by the dose or the patient's ability to excrete FPX. Delavenne et al. [17] estimated the population parameters and inter-individual variability using NONMEM VI software and found that factors such as moderate renal failure and body weight less than 50 kg increased the drug exposure. The estimated levels of anti-Xa activity and the data obtained in our study indicated that anti-Xa activity levels were significantly higher in patients with renal dysfunction. On postoperative day 7, the median values of anti-Xa activity of FPX in patients with mild or moderate renal dysfunction were approximately 18–47 % higher than those in patients with normal renal function. Furthermore, on day 14, the difference in the levels of the anti-Xa activity among these groups increased, that is, anti-Xa activity levels were approximately 30–80 % higher in patients with compromised renal function. On the other hand, the patients with normal renal function also showed an increase in anti-Xa activity with repeated administration of FPX.

The other factor that results in the accumulation of FPX is supposed to be lower body weight of Japanese patients compared to people in the Western countries [18, 19]. In the present study, the patients' physiques were mostly small and the dispersion of body weight seemed to be low for evaluating an association between anti-Xa activity of FPX and body weight. Thus, further investigations of greater numbers of subjects with more heterogeneous body weights should be performed for assaying the pharmacokinetics of FPX in Japanese patients.

The present study showed that the incidence of bleeding complications and VTE in patients who received subcutaneous FPX after THA was low, which was similar to that reported in Europe and United States [15, 20]. Considering that no obvious associations were found between anti-Xa activity and the occurrence of postoperative bleeding and VTE, measurement of plasma anti-Xa activity of FPX will not be useful for predicting whether patients will develop these disorders after THA; further, measurement of this parameter alone cannot be recommended because a small number of patients in our study developed bleeding complications and VTE.

Unlike unfractionated heparin or low-molecular-weight heparin, FPX is a completely synthetic product that targets a single coagulation factor, Xa, and has a predictable dose–response relationship [21]. In addition, FPX inactivates Xa at a different rate and more selectively compared to heparin [5, 7]. Therefore, determining the anti-Xa activity of FPX can indicate plasma FPX concentration [22, 23]. This assay is, however, affected by decreased levels of antithrombin,

and the values of anti-Xa activity decrease when the antithrombin level is less than 60 % [22]. Thus, when plasma levels of FPX are evaluated, it is necessary to consider levels of antithrombin as well. In the present study, none of our patients had antithrombin deficiency.

Our study had some limitations. First, the protocol of this prospective study concluded on postoperative day 14. The Ministry of Health, Labour and Welfare in Japan limits the period of FPX administration for 14 days after surgery. To evaluate an association between anti-Xa activity of FPX and its adverse events, it is necessary to perform further investigation under prolonged period of FPX administration. Second, to our knowledge, this is the first study in which FPX levels have been monitored using patient-related data; however, the sample size was small. Third, the incidence of VTE could be affected by the imaging modality. We used a 64-slice MDCT because this technique can simultaneously perform both pulmonary angiography and venography of the lower extremities. Moreover, this technique is less invasive compared to conventional ascending venography, requires considerably less time (approximately 5–8 min), and is easy to perform [24]. Several studies have shown that the diagnostic ability of indirect CT venography is comparable to that of US [24–27]. VTE can develop during operation, in the postoperative period without mobilization, or in 1–2 months after surgery. Thus, the anti-Xa activity levels and VTE occurrence may vary depending on when the test is performed.

Conclusion

Monitoring the plasma levels of FPX indicated the accumulation of FPX, particularly in patients with renal impairment, when 2.5 mg of FPX was subcutaneously administered after THA. We found no obvious associations between anti-Xa activity of FPX and postoperative VTE or bleeding complications; however, our results should be confirmed by pharmacokinetic studies in a larger patient population.

Acknowledgments This work was supported by Grant-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Culture, Sports, Science and Technology (No. 22591666). We thank the following people for their contributions: Chie Aoki, Hiroshi Fujimaki, Taro Tezuka, Yasuhide Hirata, Takako Momose, and Yushi Miyamae, all the orthopedic surgeons who participated at Yokohama City University. We also thank Roche Diagnostics K.K. for measuring anti-Xa activity.

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Mod Rheumatol
DOI 10.1007/s10165-012-0711-0

ORIGINAL ARTICLE

Radiologic analysis of the effect of tocilizumab on hands and large joints in children with systemic juvenile idiopathic arthritis

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Received: 14 March 2012 / Accepted: 14 June 2012

Abstract
Objectives To assess the efficacy of tocilizumab for preventing damage to the joints of systemic juvenile idiopathic arthritis (sJIA) patients, we examined serial radiographs of the hands and large weight-bearing joints of these patients before and after treatment with this agent.
Methods Nine patients with sJIA receiving 8 mg/kg of tocilizumab intravenously every 2 weeks were studied. The mean follow-up period was 82 months. The number of active joints and laboratory markers of inflammation were assessed before and after tocilizumab treatment, together with radiologic evaluation of the hips, knees, ankles, shoulders, and elbows. The latter examination included soft tissue swelling, juxta-articular osteoporosis, epiphyseal irregularity, joint-space narrowing, cyst formation, erosion, and localized growth abnormalities. Modified Larsen scores for the large joints and the Poznanski score were also recorded.
Results After tocilizumab treatment, the number of active joints and serum inflammatory markers decreased ($p < 0.01$). There was a decrease in radiologic abnormalities at the final follow-up ($p < 0.01$) with the exception of localized growth abnormalities. Radiologic improvement was observed in 47 joints (52 %), but ten (11 %) worsened. Total Larsen score was decreased from 15.8 to 10.9 at the final follow-up. Although the Poznanski score did not change after tocilizumab treatment, it was closely correlated with the total Larsen score ($r = 0.53$, $p < 0.05$).
Conclusions We describe radiologic improvement of the majority of damaged large joints in sJIA following tocilizumab therapy, but some deteriorated further despite stabilization of systemic inflammatory responses. Further studies with a larger number of patients are needed.

Keywords Juvenile idiopathic arthritis · Larsen score · Pozanaski score · Systemic course · Tocilizumab

Introduction

Systemic juvenile idiopathic arthritis (sJIA), classified as a subtype of JIA, is a chronic inflammatory disease characterized by arthritis and systemic features such as spiking fever, skin rash, hepatosplenomegaly, and serositis [1]. Most patients show progressive involvement of increasing numbers of joints, and, as a result of prolonged, repetitive synovitis, the destruction of articular cartilage develops and leads to long-term disability with striking growth impairment. Moreover, sJIA progresses to macrophage activation syndrome, which is associated with serious morbidity and, sometimes, death.

It is becoming clear that abnormalities in the proinflammatory cytokines interleukin (IL)-6 and/or IL-1β, but not tumor necrosis factor-α (TNF-α), play a major role in the pathogenesis of sJIA [2, 3]. This distinguishes it from other JIA subtypes in that biological response modifiers [4, 5], IL-6 blockade (with tocilizumab [6–8], an anti-IL-6 receptor monoclonal antibody) and IL-1β blockade (with anakinra [9], an IL-1 receptor antagonist or with canakinumab [10], an anti-IL-1β monoclonal antibody), have recently been reported as safe and effective for stabilizing not only the

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Published online: 13 July 2012

arthritic manifestations but also the systemic inflammatory responses.

Our recent phase II [7] and III [8] trials of tocilizumab for patients with sJIA indicate that in addition to systemic improvement, functional recovery of joints could be achieved. A few studies have reported radiologic improvement of damaged joints in patients with JIA following treatment with biological response modifiers. One study reported retardation of radiologic progression in hands (Poznanski score) of patients with polyarticular JIA treated with etanercept for 1 year [11]. There is also a case report of improvement in enthesitis-related arthritis following etanercept treatment [12]. We experienced radiologic improvement of damaged large joints in patients with sJIA following tocilizumab treatment [13]. The primary objective of this study was to analyze clinical and radiologic outcomes of tocilizumab treatment for sJIA patients with >5 years of follow-up.

Patients and methods

Patients and clinical responses to tocilizumab

Patients were eligible if they were 2–19 years of age with disease onset before their 16th birthday and if they met the International League of Associations for Rheumatology (ILAR) classification criteria for sJIA [1]. Eleven children with sJIA were enrolled in a phase II, open-label, dose-escalating trial and 56 children in a phase III, double-blind, placebo-controlled trial. Patients in the phase II [7] and III [8] trials were migrated to the next extension trial. Briefly, after completion of each phase II and III trial, all patients in the extension trial received 8 mg/kg of tocilizumab intravenously every 2 weeks. The efficacy of tocilizumab was assessed by the achievement of American College of Rheumatology Pediatric 30 response criteria (ACR Pedi 30) [14]—i.e., at least three of six ACR Pedi variables improved by at least 30 %. The ACR Pedi variables were physician's and patients'/parents' general assessment on a 10-cm visual analogue scale (VAS), functional ability, number of active joints, number of joints with restriction of movement, and erythrocyte sedimentation rate (ESR). Children were also assessed for ACR Pedi 50 and 70 responses—i.e., at least three of six response variables improved by at least 50 and at least 70 %, respectively. Patients were monitored for safety by routine physical examinations, urinalysis, and blood examinations, including antitocilizumab immunoglobulin (Ig)G and IgE antibodies, serum tocilizumab, IL-6, and soluble IL-6 receptor concentrations. Clinical responses to tocilizumab in phases II and III trials and also in the extension trial have been described in detail previously [7, 8].

For this study, patients were included if they had serial radiographs available for review before, during, and after tocilizumab treatment. The number of active joints and laboratory data [peripheral white blood cell (WBC), serum C-reactive protein (CRP), and ESR] were quantified to confirm tocilizumab activity. The Childhood Health Assessment Questionnaire (CHAQ) [15] (0 = best, 3 = worst) was used to assess functional ability before and after tocilizumab treatment. Protocols and amendments were approved by the Institutional Ethics Committee at this medical school. Each patient's parent or legal guardian gave written informed consent, and patients >15 years personally signed and dated written informed consent forms. Younger patients with sufficient intellectual maturity to understand what was proposed signed and dated a separately designed written informed assent form in addition to that signed by their parent or guardian.

Radiologic assessment

The large joints (shoulders, elbows, hips, knees, ankles) were evaluated for soft tissue swelling, juxta-articular osteoporosis, epiphyseal irregularity, joint-space narrowing, cyst formation, erosion, and localized growth abnormalities. Radiographs were read by two experienced orthopedists and a pediatric rheumatologist who had to reach agreement in all cases. High-intensity light was used to assess soft-tissue swelling, scored as present if any evidence for it was found around a joint. Juxta-articular osteoporosis was defined as being present when a localized decrease of bone density was noticed around a joint. Epiphyseal irregularity was defined as a marginal irregularity or an abnormal ossification of the epiphysis. Subchondral bone cysts were defined as localized areas of bone destruction, and erosions were defined as discrete areas of damage to the cortical surface of the bone. Growth abnormalities were analyzed with regard to bone shape, development, and maturation and were defined as asymmetry in epiphyseal development, premature closure of an epiphysis, or a growth deformity characterized by irregular ossification at an epiphysis resulting in a bony deformity. All these radiologic abnormalities were scored as present or absent, and the frequency of each radiologic finding was serially examined before, during, and after tocilizumab treatment.

The degree of damage to large joints was also evaluated radiologically by a modified Larsen method [16]. The grading scale ranges from 0 to 5 (0 = normal joint, 5 = mutilating changes) in each joint. Thus, the Larsen score of ten large joints at each site (bilateral shoulders, elbows, hips, knees, ankles) ranges from 0 to 50. When the patients could stand by themselves, the standing X-ray was also used to evaluate weight-bearing joints.

To assess joint-space narrowing of the hand, carpal length was measured by the Poznanski method [17]. This is based on measurement of the radiometacarpal length (RM) and length of the second metacarpal bone (M2). The number of standard deviations (SD) between the expected and observed RM for measuring M2 was calculated according to the formula for Japanese children published by Inamo et al. [18].

Statistical analysis

Radiologic abnormalities were summarized for each patient as joint scores before and after treatment. Student's t test was used to assess the significance of differences of results before and after treatment. Correlations were assessed using Spearman's rank correlation coefficient. A p value <0.05 was considered significant.

Results

Patient characteristics and clinical responses

We studied nine patients whose serial radiographs of all large joints before and after tocilizumab treatment were available. Mean duration of tocilizumab treatment and follow-up period was 82 (range 60–116) months. Mean active joint counts before tocilizumab treatment were 3.7 (range 0–12), decreasing to 0 at the final follow-up ($p < 0.01$). WBC, CRP, and ESR decreased significantly at the final follow-up, from 13,267/ml, 8.3 mg/dl, and 46.8 mm/h, respectively, to 6,722/ml, 0 mg/dl, and 2.3 mm/h, respectively ($p < 0.01$). Mean CHAQ value decreased from 2.1 (range 0.4–3) at baseline to 0.4 (range 0–1.6) at the final follow-up ($p < 0.01$) (Table 1).

Radiologic assessment

Radiologic abnormalities had all decreased at the final follow-up, with the exception of growth abnormalities. In total, the rate of juxta-articular osteoporosis and soft-tissue swelling in all radiographs of patients enrolled before treatment was 87.6 and 47.8 %, respectively, which decreased to 15.2 and 5.5 % at the final follow-up ($p < 0.01$). Joint-space narrowing, subchondral bone cysts, and erosion also improved after tocilizumab treatment, from 44.3, 13.1, and 22.5 to 29.7, 1.3, and 0 %, respectively ($p < 0.05$ for joint space narrowing, $p < 0.01$ for subchondral bone cyst and erosion). However, the frequency of localized growth abnormalities increased from 13.4 to 38.9 % after tocilizumab treatment ($p < 0.01$). At the final follow-up, localized growth abnormalities (38.9 %), epiphyseal irregularity (27.1 %), and mild joint-space narrowing (29.7 %) were the most frequent abnormalities. In contrast, no erosion and only a low frequency of subchondral bone cysts (1.3 %) were observed (Fig. 1).

Before tocilizumab treatment, radiologic abnormalities were observed more frequently in weight-bearing joints (53.2 % in knees, 49.2 % in hips, and 46.1 % in ankles) than in joints of the upper extremities (33.3 % in shoulders and 35.2 % in elbows). These decreased in all joints after tocilizumab treatment, being 14.7, 4.1, 21.3, 10.5, and 15.8 % in shoulder, elbow, knee, hip, and ankle joints, respectively, at the final follow-up ($p < 0.01$) (Fig. 2).

Modified Larsen score, Poznanski score, and radiologic changes after tocilizumab treatment are summarized in Table 1. The total Larsen score decreased from 15.8 before tocilizumab treatment to 10.9 at the final follow-up; however, there was no significant difference ($p = 0.09$). Radiologic improvement was observed in 47 joints (52 %),

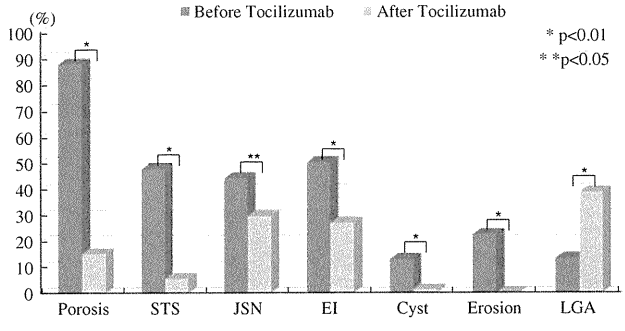

Fig. 1 Frequency of radiologic abnormalities before and after tocilizumab treatment. Frequency of all radiologic findings except LGA decreased after tocilizumab treatment. Only LGA frequency had increased at the final follow-up. *STS* soft-tissue swelling, *JSN* joint-space narrowing, *EI* epiphyseal irregularity, *LGA* localized growth abnormality

Table 1 Clinical and radiologic scores before and after tocilizumab treatment

	Active joints	CHAQ	WBC (/μl)	CRP (mg/dl)	ESR (mm/h)	Larsen score	Pozanaski score
Before TCZ	3.7 (4.8)	2.1 (1.0)	13,267 (5,828)	8.3 (9.7)	46.8 (32.2)	15.8 (10.6)	−2.7 (2.0)
After TCZ	0 (0)	0.4 (0.6)	6,722 (3,959)	0 (0)	3.7 (4.4)	10.9 (8.2)	−2.0 (2.6)

Values in parentheses indicate standard deviation

TCZ tocilizumab, *CHAQ* Childhood Health Assessment Questionnaire, *WBC* white blood cell, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate