INTRODUCTION:
Recent several studies have demonstrated the limited accuracy of conventional culture method for diagnosing peri-prosthetic infection, especially in cases with low-grade infection. We have applied real-time Polymerase Chain Reaction (PCR) assay for a rapid bacterial identification around implant, and reported its utility especially in “intraoperative” identification. Thus, real-time PCR has been recognized as one of the alternative tools because of its extreme rapidity. On the other hand, a capability of quantification is also useful feature of this assay, although there have been no previous study that evaluated its quantifiability compared with other diagnostic tests.

The aim of this study was therefore to validate the usefulness of quantitative analysis using real-time PCR in clinical cases with peri-prosthetic infection, comparing with other tests such as serological evaluation by C-reactive protein (CRP), microbiologic culture, and histopathological findings.

METHODS:
This study was approved by our institutional review board. A total of 51 operations including hip or knee arthroplasty, revision arthroplasty and debridement that applied intraoperative real-time PCR assay were reviewed retrospectively. CRP was measured before operation for all cases. And we evaluated intraoperatively collected tissue samples by microbiologic culture, histopathology and real-time PCR. Method of intraoperative PCR assay was reported previously. Universal PCR assay by LightCycler® system (Roche diagnostics) that targeted 16S-rRNA gene was used for quantitative analysis. The difference of threshold cycles between clinical samples and negative control (ΔCt) in each case was calculated.

< C-reactive protein (CRP) > As a serological marker of inflammation, CRP value (mg/dl) in each case was reviewed and divided into three groups; CRP <0.2 mg/dl, 0.2 mg/dl ≤ CRP <1 mg/dl, and 1 mg/dl≤CRP.
<Microbiologic culture> Results of microbiologic culture were reviewed and divided into three groups; negative, positive (by enrichment culture condition), and strong positive (by normal culture condition)
<Pathological evaluation> Pathological findings were reviewed and divided into three groups based on level of neutrofil infiltration; negative, positive (1–10/HPF), and strong positive (more than 10/HPF).
<Statistics> The differences of ΔCt in each test were evaluated by Kruskal-Wallis’s analysis and one-way factorial analysis of variance followed by post-hoc test by Fisher’s PSLD.

RESULTS:
In evaluation with CRP, there were 20 cases of CRP<0.2, 14 cases of 0.2≤CRP U 1.0, and 17 cases of 1.0<CRP. In culture results, there were 15 cases of negative, 8 cases of positive and 18 cases of strong positive. In pathological evaluations, there were 14 cases of negative, 19 cases of positive and 18 cases of strong positive. There was a significant correlation between CRP and ΔCt (r=0.53, P=0.0007). There was significant differences of ΔCt among each CRP levels, and the group of highest CRP level showed the highest value of ΔCt. Similarly, there were significant differences of ΔCt among culture result, and also among pathological evaluation. Among the groups divided by CRP, there was significant difference of ΔCt between CRP<0.2 and 1.0<CRP group (P=0.0048) (Figure 1). Among those by culture result, there was significant difference between the groups of negative and strong positive (P<0.0001), and between positive and strong positive group (P=0.0007) (Figure 2). Among those by pathological result, there was significant difference between the group of negative and strong positive (P=0.0029), and between positive and strong positive (P=0.0081) (Figure 3).

DISCUSSION:
This is the first study that validated quantitative evaluation of real-time PCR for diagnosing peri-prosthetic infection in clinical samples comparing with other tests. We confirmed that quantification using ΔCt by universal PCR was correlated with level of preoperative CRP, and associated also with microbiologic culture or pathological severity.

Practically we sometimes had occasional cases which showed border line of preoperative CRP and intraoperative pathological findings. In such cases, quantitative evaluation by intraoperative real-time PCR should be useful as an information for making decision, i.e. one or two-stage revision. Previously, we have reported usefulness of real-time PCR for detection of peri-prosthetic infection during revision surgery with 0.87 sensitivity and 0.8 specificity in clinical use. Some other studies have also reported the usefulness of PCR, however, there were no study that validated the quantifiability of PCR for peri-prosthetic infection.

One issue of PCR assay is that there are “PCR positive but culture negative” cases, which means it is impossible to confirm the viability of bacteria in PCR positive cases, or the possibility of contamination. In such situation, quantification by PCR would provide reasonable interpretation. For instance, higher ΔCt suggests the existence of infection more clearly regardless negative culture result, while lower ΔCt suggests the possibility of false positive due to contamination. In conclusion, this study demonstrated that quantitative evaluation by real-time PCR associated with other tests in peri-prosthetic infection cases. Further prospective studies are needed with more clinical cases.

REFERENCES: