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

(人工関節周囲感染に対するリアルタイム PCR の定量評価：他検査との比較)

Yushi Miyamae

宮前 祐之

Orthopaedic Surgery

Yokohama City University Graduate School of Medicine

（Doctoral Supervisor: Tomoyuki Saito, Professor）

（指導教員：齋藤知行 教授）
Quantitative evaluation of periprosthetic infection by real-time polymerase chain reaction: a comparison with conventional methods

Introduction

Total joint arthroplasty is commonly recognized as the most effective treatment for osteoarthritis and rheumatoid arthritis, however, periprosthetic joint infection (PJI) remains a serious potential complication of joint arthroplasty. Several recent studies have demonstrated the limited accuracy of conventional culture methods for diagnosing PJI, particularly low-grade infections that often cause false-negative results (Kobayashi et al., 2008, Tunney et al., 1999). Since no particular method has been able to provide definitive information, a more comprehensive approach is needed. We have applied real-time polymerase chain reaction (PCR) assays for the rapid identification of bacteria around implants and reported its utility (Kobayashi et al., 2009). PCR has some main advantages: one is the capability of quantification, another is its high sensitivity in diagnosis of PJI.

The first aim of our study was to validate the usefulness of quantitative analyses using real-time PCR of cases with clinical PJI in comparison with more established tests, such as C-reactive protein (CRP) levels, microbiologic cultures, and histopathology (study 1).

The second aim of this study is to compare the properties of each diagnostic test— including the sensitivity and specificity of preoperative serum CRP, real-time PCR, and histopathology of frozen and paraffin tissue sections—in clinical cases with PJI, using the culture result as the definitive diagnostic test. There are several diagnostic tests for PJI. Because the premises of these diagnostic tests are basically different (quantification of a protein induced by inflammatory responses (CRP), bacterial DNA in PCR, and neutrophil infiltration in histopathology), their sensitivity and specificity might also be different (study 2).

Study 1

Methods 1

56 cases of suspected infection were reviewed retrospectively. The CRP levels were measured prior to each operation in all cases, and intraoperatively collected tissue samples were evaluated by PCR, microbiologic culture, and histopathology. Universal PCR assay that targets the 16S rRNA gene was used for quantitative analysis. The
differences in the threshold cycles between clinical samples and a negative control (ΔCt) in each case were calculated.

**Serum CRP**

CRP levels were reviewed in each case, and the patients were accordingly divided into the following 3 groups: CRP b 0.2 mg/dL, 0.2 mg/dL ≤ CRP ≤ 1 mg/dL, and CRP N 1 mg/dL.

**Microbiological culture**

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).

**Histopathology**

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 highpower 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). The results of the quantitative PCR assay were compared with CRP levels, microbiologic cultures, and histopathology.

**Results**

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.

**Study 2**

Methods

63 joints involving 86 operations were enrolled in this study. In all cases, serum CRP was measured just before each operation, and tissue samples were collected intraoperatively for real-time PCR, histopathology (using frozen and paraffin tissue sections), and for microbiological culture.

**Serum CRP**

We set the cutoff to 1.0 mg/dL for the diagnosis of infection, based on the
results of receiver operating characteristic analysis (data not shown).

**Real-time PCR**

A universal PCR assay was performed for the broad detection of microbes and for quantitative analysis (same as described above in method 1), while an MRS-PCR assay that targeted the mecA gene was performed for specific detection of methicillin-resistant staphylococcus (MRS). Using a universal PCR, ΔCt was calculated in each case. We regarded cases as infected with over 1.9 cycles of ΔCt or with the detection of MRS.

**Histopathology**

Postoperative histopathological analysis of the intraoperative frozen and paraffin tissue sections was performed. We regarded cases with more than 10 neutrophils per high-power field (HPF) to be infected (5 fields counted).

**Microbiological culture**

The culture was scored as positive if the same bacterial organism was identified in at least 2 different tissue or joint fluid samples.

We calculated the sensitivity, specificity, likelihood ratio of positive test results (PLR), and likelihood ratio of negative test results (NLR) for each test in relation to positive microbiological culture results as the gold standard.

**Results**

The sensitivity and specificity of diagnosis with serum CRP were 90% and 85%, respectively. The corresponding values for real-time PCR and histopathology of frozen and paraffin tissue sections were 90% and 45%, 71% and 89%, and 90% and 87%, respectively. Serum CRP had a PLR of 5.8 and an NLR of 0.12, and real-time PCR had a PLR of 1.6 and an NLR of 0.18. The corresponding figures for frozen tissue sections were 6.6 and 0.32, and those for paraffin sections were 7.1 and 0.11, respectively.

**Discussion**

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. 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. In contrast, we can suspect infection more strongly in cases with a ΔCt higher than 1.9, and it would assist with decision-making, i.e. whether to conduct a one or two-stage revision surgery.

In addition, as for intraoperative evaluation of PJI, we found that real-time PCR is useful for screening of infections, with its high sensitivity and good negative likelihood ratio, while histopathological evaluation is suitable for definitive diagnosis of infection, with its excellent specificity and good positive likelihood ratio. These 2 different characteristics make them a reasonable combination for accurate intraoperative diagnosis. As we face cases that are PCR-positive and histopathology-negative, or vice versa, the properties of each diagnostic test should be considered. In some cases, we could not diagnose infection confidently with serum CRP value and a negative histopathology result; however, the positive result from real-time PCR gave suspicion of infection, with its high sensitivity. In these instances, the possibility of low-grade infection should be kept in mind. On the other hand, in cases with strong suspicion of infection from preoperative evaluation, the positive histopathological result was important for a definitive diagnosis, with its high specificity.

References


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

Ⅰ 主論文
Quantitative evaluation of periprosthetic infection by real-time polymerase chain reaction: a comparison with conventional methods.

Ⅱ 副論文
1. Different diagnostic properties of C-reactive protein, real-time PCR, and histopathology of frozen and permanent sections in diagnosis of periprosthetic joint infection.

2. インプラント周囲感染におけるリアルタイムPCRによる定量評価の有用性
宮前祐之, 稲葉裕, 小林直実, 崔賢民, 池裕之, 百瀬たか子, 藤原秀輔, 斎藤知行：
日本骨・関節感染症学会雑誌 25 巻 5 頁～9 頁 平成 24 年 1 月発行