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ORIGINAL STUDY

Intra-articular versus serum C-reactive protein analysis in suspected periprosthetic knee joint infection

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The purpose of this study was to investigate the comparative diagnostic strength of C-reactive protein (CRP) values in synovial fluid and serum in patients with presumed periprosthetic joint infection.

We collected 44 synovial fluid samples and 24 serum samples from 43 patients. Patients were judged to be uninfected or infected based on standardized criteria. Synovial and serum samples were obtained simultaneously. We determined the diagnostic strength of our assay by using receiver operating characteristic curve analysis, calculated the cutoff value and calculated the difference in diagnostic strength for periprosthetic joint infection between synovial fluid and blood serum analysis.

The area under the curve was 0.977 for intra-articular CRP analysis and 0.979 for serum CRP analysis. The cutoff points for intra-articular CRP analysis where 1.8 mg/L and 2.8 mg/L. Both tests showed a very high diagnostic strength ; the difference in diagnostic strength between synovial fluid and blood serum analysis was not significant (p = 0.66).

Keywords : periprosthetic joint infection ; C-reactive protein ; intra-articular fluid analysis ; receiver operating characteristic curve analysis.

INTRODUCTION

Periprosthetic joint infection (PJI) remains a devastating and challenging complication after total joint arthroplasty (*6*). The diagnosis of periprosthetic joint infection is based (*9*) on a combination of clinical suspicion, serological tests, culture results, histology and molecular techniques. No single diagnostic method is highly accurate (*6*). Therefore, researchers focus on improving the numerous diagnostic tests available as well as developing new methods for diagnosing such as polymerase chain reaction (*4*), synovial leukocyte esterase (*7*), sonication of explanted prosthetics (*12*), molecular markers including interleukin-6 (*2*) and synovial CRP analysis (*8*). The standard serum C-reactive protein (CRP) assay and erythrocyte sedimentation rate (ESR) are routine tests in the diagnostic workup for infection. CRP is an acute phase protein produced in the liver and is elevated in case of inflammation or infection. However, both CRP and ESR are highly sensitive but have a low specificity and can be elevated for a number of reasons other than joint infection. It is therefore clear that periprosthetic

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joint infection should not be ruled out on the basis of ESR and CRP results alone (*3*).

In a recent paper, Parvizi *et al* described the analysis of synovial C-reactive protein as possible molecular marker for periprosthetic knee joint infection (*8*), hypothesizing that the CRP level in synovial fluid would be a more specific and/or sensitive predictor than the serum level. Furthermore, the authors expected CRP levels to be higher at the source of an inflammatory process (i.e., the joint) than systemically because the physiological role of CRP is to activate the complement system for disposal of dying cells. Zamani *et al* (*13*) had examined CRP levels in synovial fluid as a method for differentiating inflammatory and non-inflammatory primary arthritis, and Martinot *et al* (*5*) had investigated the concept of improving the accuracy of a biomarker assay by performing the same assay on a localized fluid such as synovial fluid and serum samples. Parvizi *et al* (*8*) determined whether CRP levels in either synovial fluid or in serum would differentiate between infected and uninfected revision knee arthroplasties ; and whether measuring CRP in synovial fluid instead of in serum would improve the accuracy of the diagnosis of PJI. In their study, synovial fluid and serum samples were collected from patients who underwent revision total knee arthroplasty and were analyzed by three different assay methods. The authors mentioned that their positive findings might reflect the strength of the assay itself rather than the source of the fluid tested. They suggested using the same laboratory parameters on blood serum and synovial fluid to compare CRP measurements.

Therefore, we searched the hospital database for synovial fluid aspiration samples obtained in patients with persistent postoperative pain or functional loss after total knee arthroplasty and specifically looked for those that were submitted to a standard hospital CRP assay as part of the diagnostic workup to investigate possible PJI. The method was identical to the serum assay and in several cases a serum sample was examined simultaneously.

We determined whether the diagnostic strength of our assay for intra-articular CRP and serum CRP analysis was comparable with the assays of Parvizi *et al* ; second, whether there was a difference in cut-off value ; and third, if the analysis of blood serum and synovial fluid by the same assay showed a different diagnostic strength for PJI.

MATERIALS AND METHODS

After approval by the clinical trial center and ethics committee, we searched the hospital database and collected a total of 95 synovial fluid samples from 94 patients who underwent an intra-articular aspiration between December 2011 and October 2012 as part of the diagnostic workup to confirm or rule out infection after total knee arthroplasty. All samples were collected in the operating room and analyzed by the same clinical laboratory. We reviewed medical charts of all patients to collect information on patient demographics, clinical presentation and surgical details. On 44 of 95 synovial fluid samples a CRP analysis was feasible. Fifty-one synovial fluid samples were excluded from the study because the fluid was too viscous (43) for detection through standard hospital serum CRP assay or the amount too small (8) for analysis.

The 44 synovial fluid samples were divided into two groups : infected and uninfected, according to criteria set by the Musculoskeletal Infection Society (*9*). PJI was diagnosed when : there was a sinus tract communicating with the prosthesis ; or a pathogen was isolated by culture from 2 or more separate tissue or fluid samples obtained from the affected prosthetic joint ; or when four of the following six criteria existed : (a) elevated serum erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) concentration, (b) elevated synovial white blood cell count, (c) elevated synovial polymorphonuclear percentage (PMN%), (d) presence of purulence in the affected joint, (e) isolation of a microorganism in one culture of periprosthetic tissue or fluid, or (f) greater than 5 neutrophils per high-power field in 5 high-power fields observed from histologic analysis of periprosthetic tissue at ×400 magnification.

Venipuncture was performed simultaneously with the intra-articular aspiration on 24 of 43 patients (7 infected and 17 uninfected). The same laboratory performed the blood serum analysis, using the same standard hospital serum CRP assay as used to analyze the synovial fluid samples. Results from venipuncture not performed simultaneously with the intra-articular aspiration were not included in this study.

Particle enhanced immunoturbidimetric assay (*11*) (CRPL3 ; Roche Diagnostics) was used for the CRP analysis, with a detection limit of 0.3 mg/L and an extended measuring range of 0.3-350 mg/L, and was

performed on a Roche/Hitachi cobas c 702 analyzer (Roche Diagnostics).

Like Parvizi *et al*, we used receiver operating characteristic (ROC) curve analyses to determine the diagnostic strength of intra-articular CRP and serum CRP analysis in the diagnosis of PJI. The ROC curve can be used to evaluate the effectiveness of a certain biomarker in the differentiation between an infected and uninfected specimen (*10*). As the area under the curve (AUC) increases (to a maximum of 1), the diagnostic strength improves. An AUC of 0.5 indicates a test with no diagnostic strength, an AUC greater than 0.9 is considered an excellent test (*8*). The Youden Index was used to determine the optimal cutoff point, because this is the cutoff point that optimizes the biomarker's differentiating ability when equal weight is given to sensitivity and specificity (*10*). AUC values were compared between intra-articular and serum CRP analysis using an approach proposed by DeLong *et al* (*1*).

RESULTS

Our final cohort contained 44 synovial fluid samples from 43 patients. We classified 11 samples as infected and 33 samples as uninfected. The average CRP concentration of the infected group was 11.98 mg/L (range : 2.00-30.20 mg/L). The average CRP concentration of the uninfected group was 1.08 mg/L (range : 0.30-5.00 mg/L) (Table I). There was a minimal overlap in CRP concentration between the infected and uninfected group.

One patient had a bilateral joint aspiration because of a suspicion for bilateral PJI. Two different CRP values were obtained : 30.2 mg/L on the left side and 16.3 mg/L on the right side.

The ROC analysis of our assay for intra-articular CRP yielded an AUC of 0.977 (95% confidence interval (CI) 0.941-1.000), which indicates a near excellent diagnostic test. In this specific database, there were two optimal cutoff points with the same Youden Index : 1.8 mg/L and 2.8 mg/L.

The cutoff point of 1.8 mg/L showed a sensitivity of 100% (95% CI 71.5-100), a specificity of 84.9% (95% CI : 68.1-94.9), a positive predictive value (PPV) of 68.8% (95% CI : 41.3-89), a negative predictive value (NPV) of 100% (95% CI : 87.7-100) and an accuracy of 88.6% (95% CI : 75.4-96.2).

The cutoff point of 2.8 mg/L showed a sensitivity of 90.9% (95% CI : 58.7-99.8), a specificity of 93.9% (95% CI : 79.8-99.3), a positive predictive value (PPV) of 83.3% (95% CI : 51.6-97.9), a negative predictive value (NPV) of 96.9% (95% CI : 83.8-99.9) and an accuracy of 93.2% (95% CI : 81.3-98.6).

A serum CRP analysis was performed on 24 of 43 patients. We classified 7 samples as infected and 17 samples as uninfected. The average CRP concentration of the infected group was 32.70 mg/L (range : 6.70-145.1 mg/L). The average CRP concentration of the uninfected group was 3.38 mg/L (range : 0.50-10.10 mg/L) (Table I). Again, there was a minimal overlap in CRP concentration between the infected and uninfected group.

The ROC analysis of our assay for serum CRP yielded an AUC of 0.979 (95% CI : 0.935-1.000), which also indicates a nearly excellent diagnostic test.

The comparison between the AUC of the intra-articular CRP and serum CRP analysis was made by selecting the 24 patients with both intra-articular and blood serum samples obtained simultaneously. This showed no statistically significant difference ($p = 0.66$). Both tests show a very high diagnostic strength, but according to these results, measuring CRP in synovial fluid does not improve the accuracy of the diagnosis of PJI.

Table I. — Descriptive information regarding intra-articular CRP and serum CRP analysis

		N	Mean	SD	Median	Min	Max	Q1	Q3
IA CRP (mg/L)	Not infected	33	1.08	1.14	0.60	0.30	5.00	0.30	1.30
	Infected	11	11.98	9.65	7.40	2.00	30.20	4.70	16.30
Serum CRP (mg/L)	Not infected	17	3.38	2.61	3.10	0.50	10.10	1.30	4.50
	Infected	7	32.70	50.02	11.80	6.70	145.10	10.10	27.70

To our surprise, CRP values in synovial fluid were substantially lower than CRP values measured in blood serum.

In comparison, we found that our assay showed a higher AUC for both synovial fluid en serum CRP analysis (0.977 and 0.979 versus 0.91 and 0.88) and a higher diagnostic accuracy than the assays used by Parvizi *et al*. Using the Youden Index, we also found different cutoff points for intra-articular CRP analysis (1.8 and 2.8 mg/L versus 0.06 and 3.7 mg/L).

Parvizi *et al* also found suggestive (although not definitive) support for the superiority of a multiplex assay (used for synovial CRP analysis) over a clinical hospital laboratory assay (used for serum CRP analysis), and therefore diagnostic superiority of synovial fluid CRP analysis over serum CRP analysis (*8*). Considering our results, we could not confirm this support.

DISCUSSION

In a recent paper, Parvizi *et al* speculated that measuring CRP in synovial fluid instead of in serum could improve the accuracy of the diagnosis of PJI (*8*). This study aimed to investigate this hypothesis.

We acknowledge the limitations of our study. First, this was a retrospective study and certain values were missing owing to the absence of a standardized prospective study protocol. Therefore, the comparison between the AUC of intra-articular and serum CRP analysis could only be made on 24 patients with both intra-articular and blood serum samples obtained simultaneously, thus allowing an unbiased comparison.

Second, although we report significant findings through an adequate number of samples, there were also samples too viscous for detection through standard hospital serum CRP assay and others did not deliver the appropriate amount of fluid needed for analysis. Further improvement in the test methodology is therefore required in order to allow CRP analysis regardless of the viscosity of the sample.

Our first purpose was to determine the diagnostic strength of our assay for synovial fluid CRP analysis as well as serum CRP analysis. Our assay showed for both intra-articular and serum CRP analysis a very high diagnostic strength in differentiation between infected and uninfected cases.

Our second purpose was to compare the cutoff points, as calculated by Youden J statistic. In this particular database, there were two cutoff points with the same Youden index : 1.8 mg/L and 2.8 mg/L. The first cutoff point showed greater sensitivity, the second showed greater specificity. Both cutoff points differed from the cutoff points found by Parvizi *et al*. This could suggest that the cutoff point of intra-articular CRP analysis depends on the assay itself rather than being an absolute value. Further research is needed to confirm these findings. Nevertheless, because there is still a minimal overlap in CRP values between infected and uninfected cases, periprosthetic infection should not be ruled out on the basis of intra-articular or serum CRP results alone (*3*).

Our third purpose was to determine if measuring CRP in synovial fluid instead of in serum could improve the accuracy of the diagnosis of PJI. The comparison between the AUC of the intra-articular CRP and serum CRP analysis by the same assay showed no statistically significant difference. Both tests showed a very high diagnostic strength, but according to these results, measuring CRP in synovial fluid does not improve the accuracy of the diagnosis of PJI. However, these findings have to be confirmed in a similar larger prospective study.

Similar to Parvizi *et al*, we observed markedly different absolute concentrations for CRP. The CRP values in synovial fluid were substantially lower that the CRP values measured in blood serum. This is in conflict with the assumption that CRP levels have to be higher at the source of an inflammatory process (i.e., the joint) than systemically.

Although we found no evidence in this study for the superiority of intra-articular CRP analysis over serum CRP analysis in the diagnostic accuracy of PJI, there was still one remarkable item : our study included one patient who underwent a bilateral intra-articular aspiration because of a suspicion for bilateral PJI. Two different CRP values were obtained : 30.2 mg/L on the left side and 16.3 mg/L on the right side. There was of course only one (elevated) serum CRP value. According to the medical

charts, the patient's complaints were primarily located in the left knee. This observation may suggest that the intra-articular CRP level could reflect the severity of the PJI. Because this is only a case report, further research with larger cohorts is needed to evaluate these findings.

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