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Synovial Fluid Biomarkers for Periprosthetic Infection

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Abstract

Background We have previously described a unique gene expression signature exhibited by synovial fluid leukocytes in response to bacterial infection, identifying a number of potential biomarkers for infection. However, the diagnostic performance of these potential biomarkers in an immunoassay format is unknown.

Questions/purposes We therefore evaluated the sensitivity, specificity, and accuracy of several potential synovial fluid biomarkers for infection, and compared them to current standards of testing for periprosthetic infection.

Methods We prospectively collected synovial fluid from 14 patients classified as having a periprosthetic infection and 37 patients classified as having an aseptic failure. The synovial fluid samples were tested for 23 potential biomarkers for periprosthetic infection. We then determined differences in biomarker levels between infected and aseptic groups, then computed the sensitivity, specificity, positive predictive value, negative predictive value and accuracy for select biomarkers, and finally compared those to current standard tests for infection.

Results Twelve synovial fluid biomarkers had substantially higher average levels in the synovial fluid of infected versus aseptic patients. Synovial fluid levels of IL-1 were a mean of 258 times higher in patients with a periprosthetic infection compared to patients having revision for aseptic diagnoses. Synovial fluid IL-1 and IL-6 levels correctly classified all patients in this study with a sensitivity, specificity, positive predictive value, negative predictive value and accuracy equal to 1. Several markers tested in this study outperformed the ESR and CRP tests.

Conclusions Patients with a periprosthetic infection have elevated levels of numerous synovial fluid biomarkers, when compared to patients with aseptic diagnoses. Several of these biomarkers exhibited nearly ideal sensitivity, specificity, and accuracy in this study, suggesting that synovial fluid biomarkers could be a valuable tool for diagnosing periprosthetic infection.

Level of Evidence Level III, prognostic study. See Guidelines for Authors for a complete description of levels of evidence.

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Introduction

The ability to accurately diagnose infection is critical to the management and treatment of patients with a painful joint arthroplasty. In fact, it is the presence or absence of periprosthetic infection that guides the surgeon's decision making and treatment plan when considering revision arthroplasty. Although the recent literature has drawn attention to improved methods with higher diagnostic accuracy [3, 4, 9, 13, 15, 16], there remains a proportion of patients who present with ambiguous laboratory results, leaving the surgeon with a diagnostic dilemma.

The currently available tests for infection fall into two broad categories: bacterial detection techniques and host response measures. The bacterial detection techniques include tissue culture [2] and PCR [11, 18]. Both tests can identify bacteria but are susceptible to contamination in the surgical field, during sample handling, and in the laboratory setting. Therefore, confidence in treating a patient based solely on these tests is mitigated by the concern for a false-positive result. Techniques that assess the host response include systemic measures such as the erythrocyte sedimentation rate (ESR), the C-reactive protein (CRP) level, and the IL-6 level [10], and local measures such as the synovial fluid white blood cell (SF WBC) count and the tissue WBC count as seen by histopathology. Although these tests of the host response can be highly sensitive and specific in the ideal setting, they can be confounded by other causes of inflammation such as concomitant infections, systemic inflammatory diseases, and local diseases such as gout [1].

We previously described a specific genetic signature expressed by synovial fluid WBCs in response to bacterial infection [7]. When neutrophils in the synovial fluid are isolated in the setting of a periprosthetic infection, they exhibit an activated genetic program that is unique, even when compared to neutrophils involved in the acute response from gout. Because the synovial fluid WBCs express specific genes when the etiology for inflammation is bacterial, a more specific measure of the host response to infection could potentially be developed. It is highly likely synovial fluid from joints with a periprosthetic infection have elevated levels of certain proteins that are very specific to infection. This would allow for the development of an inexpensive and rapid test for periprosthetic infection based on well-known technology such as that developed for the pregnancy test.

The purpose of our study was to (1) define the sensitivity, specificity, and accuracy of various synovial fluid biomarkers in patients with periprosthetic infection versus aseptic disease; and (2) compare these results to current standards of diagnostic testing, including the ESR, CRP, synovial fluid WBC count, and percentage of segmented neutrophils in the synovial fluid.

Patients and Methods

We prospectively collected synovial fluid from 51 patients undergoing revision total joint arthroplasty. All samples were aspirated from patients having surgery for a painful joint arthroplasty. Fourteen of these 51 patients had surgery, including irrigation and débridement or the first stage of a two-stage exchange, for presumed infection; 37 patients had surgery for presumed aseptic loosening or a mechanical complication of a hip or knee arthroplasty. Patients were not excluded for a history of inflammatory disease or antibiotic treatment. Patients having revision surgery or surgery for infection had perioperative laboratory testing including ESR, CRP, synovial fluid WBC count with a differential cell count, tissue or fluid cultures, and a frozen section performed of periprosthetic tissues. Synovial fluid cell counts, ESR, and CRP were tested upon admission to the hospital, or in the case of planned revision surgery, at a preoperative office visit. Relevant clinical observations such as a draining sinus were noted. The mean patient age was 65 years, with a range of 37 to 92 years. Twenty-eight patients were female and 23 were male. Twenty-nine of the samples from periprosthetic joints were from a hip, while 22 were from the knee. Fourteen patients had surgery directed at treating a presumed infection, and 37 patients had a surgical procedure aimed at treating a presumed aseptic failure. We noted some differences between the infected and aseptic groups, most notably related to having had preoperative antibiotics and inflammatory markers (Table 1). We had prior approval of the Institutional Review Board.

Patients classified with a periprosthetic joint infection had to demonstrate either (1) one or more positive cultures on solid medium; or (2) a chronic draining sinus communicating with the synovial fluid. We did not use laboratory

Table 1. Comparison of patient characteristics in the infected and aseptic groups

Variable	Infected	Aseptic	Total	p Value
Patients	14	37	51	–
Females	6	22	28	0.35
Knee	9	13	22	0.11
Inflammatory disease	2	3	5	0.6
Patients receiving preoperative antibiotic treatment	5	5	10	0.05*
ESR (mm/hr)	57	22	–	.0002*
CRP (mg/l)	50	3.7	–	< .0001*
SF WBCs (cells/mm^3)	62600	1993	–	< .0001*
% segs	91	40	–	< .0001*

ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; SF WBC = synovial fluid white blood cell count; % segs = percent segmented neutrophils.

*Statistically significant.

tests to classify patients. Fourteen patients were classified into the infection group and had positive culture results on solid medium. Thirteen of these patients had greater than 10 WBCs per high powered field by histopathology, and the only patient with a negative frozen section had multiple positive cultures revealing the same bacterial organism.

In order to be classified into the aseptic inflammation group, a patient had to have negative culture results (excluding broth cultures) and the absence of sinuses. Of 37 patients classified into the aseptic group, all but two had negative histopathology at the time of surgery. Among patients classified as aseptic, 15 had a revision for a mechanical complication, 12 had a revision for aseptic loosening of an implant, nine had a reimplantation after treatment for infection, and one had an irrigation and débridement for presumed acute infection. The patient having a débridement had several negative preoperative and intraoperative cultures. His preoperative ESR and CRP were 34 mm/sec and 3 mg/l.

Samples were collected over an eight month period and represented a random sample of revision arthroplasties performed by five attending surgeons during that time period. Synovial fluid samples were collected on days when a research fellow was on-site and available to collect, process, and freeze the sample. The research fellow was notified when revision surgery was taking place, and was required to process and freeze the sample within 1 hour to prevent enzymatic degradation of proteins. For some patients with a planned revision for presumed aseptic disease, a sample was collected at the time of the preoperative visit.

All synovial fluid was collected into a syringe and centrifuged within 1 hour to remove all cellular and particulate content. The resulting cell-free synovial fluid was frozen on dry ice and stored in a −80°C freezer until final immunoassay testing. All samples were sent to the Cytokine Reference Laboratory at the University of Minnesota (Minneapolis, MN) for immunoassay analysis on the multiplex bead-based Luminex (Luminex Corp, Austin, TX) platform. From our previous study, we identified a list of genes that appeared to be specifically expressed during the response to infection [7]. Based on this list of potential protein targets, we chose to evaluate 23 proteins in the synovial fluid of patients having revision joint arthroplasty. The multiplex immunoassay was utilized to measure the synovial fluid levels of proteins IL-1α, IL-1β, IL-1Rα, IL-4, IL-6, IL-8, IL-10, IL-17, ENA-78, FGF-basic, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP-1α, MIP-1β, Rantes, TNF-α, Tpo, and VEGF. Additionally, synovial fluid levels of the proteins SKALP (Hycult Biotech, Uden, The Netherlands) and SLPI (R & D Systems, Minneapolis, MN) were measured by standard ELISA according to the manufacturer's protocol. In total, the levels of 23 different proteins were quantified in the synovial fluid samples of each patient (Table 2).

Data were analyzed to evaluate the performance of each potential biomarker for the diagnosis of infection, and comparisons were made with other standard laboratory tests for infection. When the measured biomarker level was below the detectable limit of the assay, the value was set to the lowest detectable value to facilitate data analysis. Biomarker concentrations in the infected and aseptic samples were analyzed to characterize the average and range of values in infected and aseptic groups (Table 3).

The Fisher exact test was used to identify differences between patient characteristics (Table 1). The SAS Version 9.2 Mann-Whitney test was used to evaluate differences between markers in the infected and aseptic groups (Tables 2, 3). The Bonferroni adjustment for multiplicity was also applied. For biomarkers that appeared to discriminate between infected and aseptic groups, cutoff values were chosen to maximize accuracy. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated for these biomarkers, as well as for the CRP level, ESR, synovial fluid WBC count, and percentage of segmented neutrophils (Table 4). Diagnostic cutoff values are listed for these tests (Table 4). The Fisher exact test was used to calculate the differences between the accuracy of tests (Table 5).

Results

We found a difference in concentration for 12 of the 24 synovial fluid proteins tested comparing patients with periprosthetic infection to those with aseptic diagnoses (Table 3). Synovial fluid IL-1α, IL-1β, IL-6, IL-17, G-CSF, and SKALP levels were each further evaluated for sensitivity, specificity, accuracy, NPV, and PPV. Synovial fluid IL-1β and IL-6 demonstrated a sensitivity, specificity, PPV, NPV, and accuracy of 1 (Table 4).

Several biomarkers had superior diagnostic accuracy for infection when compared to current standard tests for infection (Table 5).

Discussion

The diagnosis of periprosthetic infection is a serious clinical challenge, requiring the use of multiple clinical signs and laboratory tests [8]. The recent literature has highlighted the need for improved diagnostics as evidenced by the number of studies attempting to identify the best combination of laboratory tests predicting periprosthetic infection [3, 4, 9, 14–16]. Additionally, new methods of diagnosis such as PCR [12, 18], biofilm detection [17, 20],

Table 2. Biomarkers listed and their fold-elevation in the infection group versus the aseptic group

Biomarker		Fold-elevation	p
IL-1b	Interleukin 1-beta	257.8	< 0.0001**
G-CSF	Granulocyte colony-stimulating factor	119.9	< 0.0001**
IL-17	Interleukin 17	112.5	< 0.0001**
IL-6	Interleukin 6	27.3	< 0.0001**
IL-1a	Interleukin 1-alpha	24.5	< 0.0001**
MIP-1B	Macrophage inflammatory protein 1-beta	18.0	< 0.0001**
IL-10	Interleukin 10	8.0	< 0.0001**
IL-8	Interleukin 8	6.2	< 0.0001**
GM-CSF	Granulocyte monocyte colony-stimulating factor	5.1	0.0678
MIP-1a	Macrophage inflammatory protein 1-alpha	4.9	< 0.0001**
TNF-a	Tumor necrosis factor alpha	4.1	0.0048
IL-5	Interleukin 5	2.5	0.001*
ENA-78	Epithelial cell-derived neutrophil-activating peptide 78	2.2	0.0048
SKALP	Skin derived antileukoproteinase	2.1	< 0.0001**
IFN-g	Interferon gamma	2.0	0.168
FGF-Basic	Fibroblast growth factor basic	1.9	0.0012*
IL-1ra	Interleukin 1 receptor antagonist	1.6	0.0054
IL-4	Interleukin 4	1.3	0.336
VEGF	Vascular endothelial growth factor	1.3	0.0302
Tpo	Thrombopoietin	1.2	0.1019
MCP-1	Monocyte chemotactic protein 1	0.8	0.3185
Rantes	Regulated upon Activation, Normal T-cell Expressed, and Secreted	0.7	0.4706
SLPI	Secretory leukocyte peptidase inhibitor	0.6	N/A

The p values listed are the raw p values based on the Mann-Whitney test; * = p value significant after Bonferroni adjustment for multiplicity; ** = p value highly significant after Bonferroni adjustment for multiplicity.

and cultures augmented by implant ultrasound [19] have been described and evaluated for diagnostic purposes. The patient, physician, and economic burdens of infected arthroplasties [5, 6] are expected to grow rapidly with the increase in joint arthroplasties performed each year. For these reasons, the ability to accurately diagnose infection is critical. Currently there are no commercially available synovial fluid immunoassays for the purpose of diagnosing infection, and the only commonly used systemic immunoassay for periprosthetic infection is the CRP. Because the CRP and any other systemic test for infection would be confounded by concomitant infection at another anatomic site or systemic inflammatory disease, a synovial fluid test for infection is intuitively more appealing. We previously reported the genome-wide synovial fluid WBC response to periprosthetic infection [7], attempting to identify potential biomarkers for infection. The purpose of the current study is to evaluate several potential synovial fluid biomarkers for infection, and compare their diagnostic characteristics to current standard laboratory tests for infection.

We acknowledge several weaknesses and limitations in this study. First, there is no gold standard test or clinical evaluation for infection in the literature. There have been a variety of laboratory tests and combinations of clinical criteria and laboratory tests used as the diagnostic criteria to classify patients previously in the literature. We used the solid medium culture results and/or draining sinus tracts to define the groups as infected or aseptic. Because we compared the markers in the current study to the ESR, CRP level, synovial fluid WBC count, and percent segmented neutrophils, we did not want to include them in the classification of patients. One could argue that weaknesses in the classification of patients could lead to alteration of the primary results of this study; however this is an inherent weakness of any study evaluating a diagnostic test for infection. Second is the relatively small number of patients. As this was a preliminary study to identify potentially useful diagnostic biomarkers for infection, we wanted to eliminate the biomarkers that showed poor diagnostic accuracy before pursuing a larger prospective multicenter study. Despite the small number of patients though, we were able to show differences in the diagnostic potential of many of the biomarkers studied.

Table 3. Minimum, maximum, and mean values for all biomarkers included in the study, listed by patient groups

Biomarker	Aseptic			Infected		
	Min	Max	Mean	Min	Max	Mean
IL-1a	0.1	34.1	1.0	0.1	110.8	24.9
IL-1b	0.2	110.4	8.0	114.7	10937.2	2067.2
IL-1ra	1871.4	149958.7	66784.6	34197.1	185112.9	104945.2
IL-4	4.0	93.4	10.9	4.0	65.4	14.7
IL-5	0.2	23.1	1.7	0.4	14.7	4.3
IL-6	16.4	13328.3	2171.7	13355.8	125494.1	59324.8
IL-8	124.2	23516.3	3402.7	2607.3	56246.9	21238.8
IL-10	0.1	14.1	4.1	7.5	84.0	32.6
IL-17	1.5	25.6	2.8	2.0	1692.2	314.6
ENA-78	4.0	78833.0	7386.0	53.3	49250.1	16038.0
FGF-Basic	40.0	625.3	83.9	40.0	572.9	163.4
G-CSF	0.2	54.9	10.7	37.1	8533.9	1283.8
GM-CSF	2.2	25.1	3.8	3.2	201.9	19.5
IFN-g	0.4	40.1	5.3	0.4	57.2	10.4
MCP-1	521.3	31696.6	7461.8	143.2	18602.8	5890.5
MIP-1a	30.0	905.5	224.1	161.7	3205.6	1101.8
MIP-1B	9.1	779.3	171.9	187.6	15927.7	3085.7
Rantes	0.8	7584.8	824.5	0.8	2746.5	615.6
TNF-a	0.4	54.7	6.5	1.2	102.7	26.6
Tpo	0.4	243.5	78.2	8.0	207.4	97.0
VEGF	258.2	9079.5	2374.5	1159.6	7062.7	3100.6
SKALP	443.4	5399.9	1241.1	1198.0	5883.9	2650.7
SLPI	0.2	6.0	0.9	0.1	1.3	0.6

All values are in pg/ml.

Table 4. Fold-elevation in the infected group, cutoff values, specificity, sensitivity, positive predictive value, negative predictive value and accuracy are listed for 6 biomarkers

Biomarker or variable	Fold-elevation	Cutoff value	Spec.	Sens.	PPV	NPV	ACC
IL-1b	258	112 pg/ml	1.00	1.00	1.00	1.00	1.00
IL-6	27	13350 pg/ml	1.00	1.00	1.00	1.00	1.00
G-CSF	120	35 pg/ml	0.95	1.00	0.88	1.00	0.96
IL-1a	24	1 pg/ml	0.97	0.86	0.92	0.95	0.94
IL-17	112	7.2 pg/ml	0.97	0.86	0.92	0.95	0.94
SKALP	2	1880 pg/ml	0.89	0.79	0.73	0.92	0.86
SF WBC	31	2000 cells/mm^3	0.84	0.93	0.68	0.97	0.86
CRP	13	10 mg/l	0.86	0.71	0.67	0.89	0.82
% Segs	2	65	0.73	1.00	0.58	1.00	0.80
ESR	3	30 mm/hr	0.73	0.86	0.55	0.93	0.76

Spec = specificity; Sens = sensitivity; PPV = positive predictive value; NPV = negative predictive value; ACC = accuracy; SF WBC = synovial fluid white blood cell count; CRP = C-reactive protein; % segs = percent segmented neutrophils; ESR = erythrocyte sedimentation rate.

We found several synovial fluid biomarkers that were substantially elevated in patients with periprosthetic infection when compared to those patients with aseptic reasons for revision. Several of the synovial fluid biomarkers exhibited fluid levels that were more than 100-fold elevated in patients with periprosthetic infection; a

Table 5. Difference in accuracy between select biomarkers in this study and several current standard tests for periprosthetic infection and percentage of segmented neutrophils

Biomarker	CRP	ESR	SF WBC	%Segs
IL-1a	0.122	***0.023***	0.318	0.072
IL-1B	***0.003***	***< .001***	***0.013***	***0.001***
IL-6	***0.003***	***< .001***	***0.013***	***0.001***
IL-17	0.122	***0.023***	0.318	0.072
G-CSF	0.051	***0.008***	0.16	***0.028***
SKALP	0.786	0.309	1	0.596

P values listed are in bold italics if the synovial fluid biomarker was more accurate than the respective current standard test with statistical significance. CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; SF WBC = synovial fluid WBC count; %Segs = percentage of segmented neutrophils.

conservative estimate given they were undetectable in the majority of patients without overt infection. Synovial fluid IL-1B, IL1-α IL-6, IL-17, and G-CSF, had excellent diagnostic performance, with accuracy above 0.9 in this study, despite the inclusion of patients with confounding characteristics such as systemic inflammatory disease and preoperative antibiotic treatments.

Furthermore, the synovial fluid biomarkers in this study substantially outperformed many currently used laboratory diagnostics for infection. The diagnostic accuracy of the ESR (0.76) and CRP level (0.82) we found was similar to previous reports in the literature [4, 9, 15]. The synovial fluid IL-1B and IL-6 levels were more accurate in diagnosing infection than the ESR, CRP level, synovial fluid WBC count, or percent segmented neutrophils. Several of the other biomarkers also demonstrated improved accuracy when compared to current standards of testing. We emphasize patients treated with antibiotics and patients with systemic inflammatory disease were not excluded. The synovial fluid biomarkers are especially intriguing for diagnostic use in this group of patients, given their historical exclusion from previous studies evaluating the current standards of diagnostic testing [3, 4, 9, 15, 16]. However a larger group of patients with these confounding factors would be necessary to validate these results.

Our data suggest synovial fluid biomarkers could provide an additional valuable resource for the diagnosis of periprosthetic infection. We have identified biomarkers that appear to accurately identify the local host response to infection. They appear to have improved accuracy over current standards of testing, and may be useful even in a population of patients who have confounding systemic variables. Validation of these results is critical to the clinical application of synovial fluid biomarkers, and planning for a larger prospective multicenter study is currently underway.

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