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The Leukocyte Esterase Test Strip Is a Poor Rule-Out Test for Periprosthetic Joint Infection

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ABSTRACT

Background: The urinary leukocyte esterase (LE) test strip has been suggested as a good screening test for periprosthetic joint infection (PJI). The purpose of this study is to compare the diagnostic profile of LE assays from different manufacturers and determine whether the LE test strip is a good rule-out test.
Methods: Synovial fluid samples (N = 344), sent to 1 laboratory for PJI testing, were used in this prospective study. Four different tests for synovial fluid LE were simultaneously evaluated for their performance in detecting white blood cell (WBC) positive samples (>3000 cells/μL).
Results: Both neutrophil elastase immunoassays demonstrated greater sensitivity than urinary LE test strips (92.0% and 90.8% vs 72.4% and 80.3%; all $P < 0.011$). Fifty-three percent of false-negative urinary LE test strip results clearly missed the presence of elevated levels of synovial fluid LE. Invalid urinary LE test strip results were 4-fold more likely among WBC (+) compared with WBC (−) samples (27.0% vs 6.8%; $P < 0.0001$). The combined failure to detect an elevated WBC count, because of either false-negative or invalid results, was 47.1% and 41.4% for the Roche and Siemens test strips, respectively.
Conclusions: This study agrees with the existing literature demonstrating that the LE test strips are among the lowest sensitivity tests for PJI. The urinary LE tests strips should not be used to rule-out PJI, as they often fail to detect abundant levels of LE in synovial fluid. Instead, it is more appropriate to use the (++) LE test strip result as a secondary confirmatory rule-in test for PJI because of its high specificity.

The urinary leukocyte esterase (LE) test strip was first developed to provide for a rapid estimate of urinary white blood cells (WBCs) as a screening test for pyuria. One of the major proteases that is detected by the LE test strip is neutrophil elastase (NE), which catalyzes the esterase reaction on the LE test strip pad. The test optimization and regulatory approvals related to the urinary LE test strip were achieved with the assumption that the test strip would be used for urinary screening.

The arthroplasty literature has recently suggested considering the use of the urinary LE test strip for the purposes of screening synovial fluid for periprosthetic joint infection (PJI) [1]. However, the performance of the LE test strip in synovial fluid has demonstrated suboptimal diagnostic characteristics for PJI. In fact, research on the use of the urinary LE test strip to screen synovial fluid has revealed 3 serious concerns: (1) several institutions have demonstrated a low LE test strip sensitivity for PJI [1–6], (2) several institutions have reported on a high rate of uninterpretable results because of the presence of blood [1,5–7], and (3) the optimal cutoff for LE test strip positivity in synovial fluid has varied between institutions [6,7]. These are the type of issues that need to be accounted for when validating an off-label use of a diagnostic test.

The Synovasure brand of diagnostic tests (Zimmer Biomet, CD Diagnostics) offers several different tests for the differential diagnosis of a painful joint. These include the Synovasure Alpha-Defensin Test for PJI, the Synovasure Alpha-Defensin Test for

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native septic arthritis, the Synovasure Microbial Identification Test, and the Synovasure NE Test. The Synovasure NE Test is an immunoassay for the major protein in synovial fluid contributing to LE activity. Therefore, while the LE test strip is a test of a protein's enzyme activity, the Synovasure NE test is a test of the actual protein's concentration.

Although the Musculoskeletal Infection Society (MSIS) has included the LE test strip as a proxy to the WBC count [8], very few large studies have tested the agreement between LE assays of different technologies, or their accuracy as a proxy for the WBC count. The purpose of this study is to compare the diagnostic profile of LE assays of differing technologies, and determine whether the LE test strip is a good rule-out test.

Methods

A prospective diagnostic study using remnant synovial fluid samples was conducted to evaluate the results of LE testing in synovial fluid. Institutional review board's approval was attained for this remnant sample study.

Patient and Sample Population

All studies were completed at the laboratories of CD Diagnostics (Zimmer Biomet, Towson, MD). The laboratory receives clinical synovial fluid samples from 49 states in the United States, for the purposes of diagnosing PJI. In February and March of 2017, our group selected 13 days when all remnant synovial fluid samples received at CD Diagnostics for clinical testing were considered for inclusion in this study. During these 13 days, a total of 1574 synovial fluid samples were evaluated for testing at our laboratory. Several inclusion criteria were necessary to allow for this study to be conducted. Availability of a WBC count for the samples was required, as this test was used in data analysis. This inclusion resulted in 671 samples from the total of 1574 samples received. Furthermore, the samples qualifying for inclusion must have at least 1 cc of remaining remnant fluid for the purposes of this study, resulting in a 344 synovial fluid sample cohort for this study.

Of the 344 samples included, there were 329 samples from an arthroplasty and 15 samples from a native knee. Of the 329 samples from an arthroplasty, there were 256 from a knee arthroplasty, 27 from a hip arthroplasty, 1 from a shoulder arthroplasty, and 45 from unspecified arthroplasties.

Study Design

Each of the 344 synovial fluid samples had a WBC count. With the remaining fresh remnant synovial fluid, several tests were performed in duplicate by 2 trained laboratory staff members: (1) the urinary LE test strip (Roche), (2) the urinary LE test strip (Siemens), and (3) the NE lateral flow immunoassay (Synovasure NE lateral flow test; Zimmer Biomet). In addition, the NE laboratory-based immunoassay (Synovasure NE Laboratory-Based test; Zimmer Biomet) was completed. Therefore, all included synovial fluid samples had a WBC count from the laboratory, in addition to 4 different LE tests simultaneously run in duplicate in our laboratory.

Given the utilization of the urinary LE test strip as a rapid test estimate of the synovial fluid WBC count, and also given the fact that the MSIS chose to include the urinary LE test strip result as an equivalent minor criteria to the synovial fluid WBC count [8], we chose to use the synovial fluid WBC count as the gold standard in this study. Therefore, all synovial fluid samples with a WBC count >3000 cells/μL were considered positive in this study.

The synovial fluid WBC counts in this study were first completed on a clinical laboratory Sysmex 2000 automated cell counter, as standard in the clinical laboratory. In addition, as a quality control measure, whenever a WBC count was found to be >3000 cells/μL on the automated cell counter, a reflex manual cell count was completed to confirm the automated cell counter results. Of the 344 samples in this study, 87 (25%) were positive and 257 were negative.

For the 2 urinary LE test strips, fresh synovial fluid was placed on the reagent pad and the test was performed and interpreted based on the manufacturer's directions. In cases where blood interfered with the interpretation of the colorimetric reagent pad, the result was considered invalid. For the urinary LE test strips, a reading of (++) was considered positive, as recommended by several previous studies [6,9] and the MSIS consensus [8].

For the lateral-flow LE test (Synovasure NE lateral flow test; Zimmer Biomet), synovial fluid was added to the testing well and the fluid was allowed to traverse the lateral flow cartridge. The appearance of a test line was considered a positive result. For the laboratory-based LE test (Synovasure NE Laboratory-Based Test; Zimmer Biomet), a signal to cutoff value $\geq$1 was considered positive.

Data Analysis

The urinary LE test strips and the lateral-flow LE immunoassay were read by 2 trained technicians. The totals of both technicians' results were used to calculate diagnostic data and percentages. The results of each LE test were compared with the WBC count. Each test's invalid result percentage was calculated, as was each test's sensitivity, specificity, and relevant confidence intervals. The Fisher exact test was used to compare the tests in a 2 $\times$ 2 fashion, evaluating the statistical significance of differences of proportion.

Results

Both Synovasure NE immunoassays demonstrated significantly greater sensitivity that the urinary LE test strips (92.0% and 90.8% vs 72.4% and 80.3%; all $P < .011$; Table 1). The lower sensitivities exhibited by the Roche and Siemens urinary LE test strips translated to synovial fluid false-negative rates of 19.7% and 27.6%, respectively. Of 60 total false-negative LE test strip reads (including

Table 1
Diagnostic Profile of Various LE Tests in Detecting WBC >3000 cells/μL.

	Sensitivity	Specificity
Roche LE test strip	72.44% (95% CI: 63.81%-79.99%)	97.29% ^ (95% CI: 95.40%-98.55%)
Siemens LE test strip	80.31% (95% CI: 72.33%-86.84%)	97.08% ^ (95% CI: 95.14%-98.39%)
Synovasure Neutrophil Elastase Lateral Flow immunoassay	91.95% * (95% CI: 86.87%-95.53%)	90.47% (95% CI: 87.59%-92.86%)
Synovasure Neutrophil Elastase Laboratory-Based immunoassay	90.80% * (95% CI: 85.50%-94.65%)	94.94% (95% CI: 92.68%-96.67%)

CI, confidence interval; LE, leukocyte esterase; WBC, white blood cell.
* Equals significantly higher sensitivity ($P < .011$).
^ Equals significantly higher specificity ($P < .0001$).