What Is the Optimal Timing for Reading the Leukocyte Esterase Strip for the Diagnosis of Periprosthetic Joint Infection?

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Abstract

Background  The leucocyte esterase (LE) strip test often is used to diagnose periprosthetic joint infection (PJI). In accordance with the manufacturer’s directions, the LE strip test result is read 3 minutes after exposing it to joint fluid, but this has not been supported by robust research. Moreover, we have noted that the results of the LE strip test might change over time, and our previous studies have found that centrifugation causes the results of the LE strip test to degrade. Still, there is no evidence-based recommendation as to when to read the LE strip test to maximize diagnostic accuracy, in general, and the best reading times for the LE strip test before and after centrifugation need to be determined separately, in particular.

Questions/purposes (1) What is the optimal timing for reading LE strip test results before centrifugation to diagnose PJI? (2) What is the optimal timing for reading LE strip test results after centrifugation to diagnose PJI?

Methods  This study was a prospective diagnostic trial. In all, 120 patients who were scheduled for revision arthroplasty and had signs of infection underwent joint aspiration in the outpatient operating room between July 2018 and July 2019 and were enrolled in this single-center study. For inclusion, patients must have had a diagnosis of PJI or non-PJI,
valid synovial fluid samples, and must not have received antibiotics within 2 weeks before arthrocentesis. As such, 36 patients were excluded; 84 patients were included for analysis, and all 84 patients agreed to participate. The 2018 International Consensus Meeting Criteria (ICM 2018) was used for the classification of 49 patients with PJI (score ≥ 6) and 35 without PJI (score ≤ 2). The classification was used as the standard against which the different timings for reading LE strips were compared. All patients without PJI were followed for more than 1 year, during which they did not report the occurrence of PJI. All patients were graded against the International Consensus Meeting Criteria (ICM 2018) was described as the major criteria [8], so the diagnosis of PJI still predominantly relies on a combination of minor criteria. Analysis of joint synovial fluid is a substantial part of the 2018 International Consensus Meeting (ICM 2018) criteria [3]. The leukocyte esterase (LE) strip test, initially used in the diagnosis of urinary tract infections, is an important method in analyzing synovial fluid that is extensively used in clinical practice because it is simple and yields a quick result [6]. The early strip test for diagnosing urinary tract infection required 15 to 30 minutes, but now it takes only 60 to 90 seconds to obtain a result [5]. Given that the viscosity of synovial fluid is higher than that of urine, it is appropriate to extend the reading time correspondingly [7]. In accordance with the manufacturer’s directions, the LE strip test with a 3-minute reading time has been used for the diagnosis of PJI [14].

However, the 3-minute reading time for LE is controversial [15]. We have found no relevant clinical research that supported the best timing for reading these strip tests. In clinical practice, we found that the color of the LE strip gradually deepened over time (more than 3 minutes) after application, changing from nonpositive (0, ±, and 1+) to positive (2+) (Fig. 1). Therefore, the timing for reading the LE strip may affect the final diagnosis because the LE strip test plays an important role in the ICM 2018 criteria. It is imperative to determine the most appropriate time for reading results [3]. The definite reading time will further standardize the clinical application of LE strip tests for diagnosing PJI and will provide a basis for further research to improve the accuracy of LE strip testing (such as the development of quantitative tests).

Introduction

Periprosthetic joint infection (PJI) remains a catastrophic complication after total joint arthroplasty, but diagnosing PJI remains a challenge. Few patients meet what have been
A previous study confirmed that centrifugation will lead to the degradation of LE test paper [10]. We believe that the reading time before and after centrifugation should be studied separately.

We therefore asked: (1) What is the optimal timing for reading LE strip test results before centrifugation to diagnose PJI? (2) What is the optimal timing for reading LE strip test results after centrifugation to diagnose PJI?

**Patients and Methods**

**Study Cohort**

From July 2018 to July 2019, surgeons at our outpatient clinic recommended joint aspiration if after total joint arthroplasty, a patient had symptoms suspicious of infection, such as persistent fever, swelling, unexplained pain, unexplained early prosthetic loosening, and subsidence; an elevated erythrocyte sedimentation rate or C-reactive protein level; or was scheduled to undergo hip or knee revision surgery.

A total of 120 patients accepted the recommendation and received arthrocentesis in our outpatient operating room. These patients signed an informed consent form to allow researchers to use their synovial fluid samples and clinical data. Patients were excluded if they had no valid synovial fluid samples (11 patients; six patients with grossly bloody taps and five patients with dry taps), scored 3 to 5 against the ICM 2018 diagnostic criteria, their data were insufficient to determine whether an infection was present (18 patients), or they received antibiotics within 2 weeks before arthrocentesis (seven patients). After excluding these 36 patients, 84 were eventually included in the cohort.

Of 84 patients, 49 were classified as having PJI (score $\geq 6$) and the other 35 were classified as not having PJI (score $\leq 2$) according to the ICM 2018 diagnostic criteria. All patients without PJI were followed up for more than 1 year, during which they did not report the occurrence of PJI. The serum C-reactive protein level, erythrocyte sedimentation rate, and ICM 2018 scores were higher in the PJI group than in the nonPJI group ($p < 0.05$) (Table 1).

**Gold Standard for Diagnosis of PJI: ICM Criteria**

The definition of PJI was based on the ICM 2018 diagnostic criteria [3], which consist of major criteria and minor criteria. The minor criteria include the C-reactive protein or D-dimer level (2 points); erythrocyte sedimentation rate (1 point); elevated synovial white blood cell count, LE, or alpha-defensin (3 points); synovial polymorphonuclear percentage (2 points); culture (2 points); histologic analysis (3 points); and intraoperative purulence (3 points).

A total score of 6 or more indicated infection, a score between 3 and 5 was inconclusive, and a score of 2 or less indicated no infection. Forty-nine patients were classified as having PJI (score $\geq 6$) and the other 35 were classified as having no PJI (score $\leq 2$) according to the definition. All patients were followed regularly for more than 1 year, and 35 patients without PJI did not report an occurrence of PJI during the period. For this study, the ICM 2018 diagnostic criteria turned out to be robust, serving as the gold standard for PJI diagnosis. We scored all patients included in this study against the ICM 2018 diagnostic criteria, regardless of their LE strip test results. We used white blood cell count instead of LE strip test results in the gold standard test because they are equivalent in the diagnostic criteria.

**Acquisition and Processing of Synovial Fluid**

All diagnostic arthrocentesis procedures were performed in the outpatient operating room of our hospital, and all

| Characteristic                  | PJI (n = 49) | NonPJI (n = 35) | p value |
|--------------------------------|-------------|----------------|---------|
| Female sex, % (n)              | 69 (34)     | 63 (22)        | 0.53    |
| Age in years, mean (range)     | 63 (21-82)  | 64 (28-78)     | 0.73    |
| BMI in kg/m², mean (range)     | 26 (18-44)  | 26 (21-32)     | 0.98    |
| Joint, % (n)                   | 20 (10)     | 11 (4)         | 0.43    |
| Hip                            | 20 (10)     | 11 (4)         |         |
| Knee                           | 80 (39)     | 89 (31)        |         |
| With immune system disease,% (n) | 24 (12)     | 20 (7)         | 0.63    |
| Serum CRP in mg/L, mean (range)| 3.86 (0.1-14.7) | 0.68 (0.05-4.83) | <0.01    |
| Serum ESR in mm/h, mean (range)| 67 (8-120)  | 25 (2-90)      | <0.01    |
| ICM 2018 score, mean (range)   | 9 (6-16)    | 1 (0-2)        | <0.01    |

*Includes a history of rheumatoid arthritis (10 patients), ankylosing spondylitis (six patients), sicca syndrome (one patient), systemic lupus erythematosus (one patient), and psoriatic arthritis (one patient).
operating steps followed the standardized anatomic positioning procedure. After arthrocentesis, we divided the harvested joint fluid into two portions, one of which was put into a normal centrifuge tube by using a syringe and centrifuged as recommended by Aggarwal et al. [2] (6600 rpm, 180 seconds; D3024, SCILLOXGEN, Pittsburgh, PA, USA). For one patient, the sample was not centrifuged because the collected synovial fluid was less than 1.5 mL. Of the 84 samples before centrifugation and the 83 samples after centrifugation, most of the LE strips gradually deepened in color over time after sample application.

Data Collection

Two independent observers (PR, JYS) and one assistant (JCL) who were blinded to the patients’ data were intensively trained to conduct all procedures. The two observers independently collected 50 µL of synovial fluid before and after centrifugation with a pipette and applied the synovial fluid to the LE strip (AUTION Sticks, 10PA, ARKRAY, Kyoto, Japan). One minute after the application of synovial fluid, the two observers separately put the LE strip into a fully automatic semiquantitative urine analysis system (PocketChem UA PU-4010, ARKRAY, Kyoto, Japan), which reads the color change of strips by semiquantitatively measuring reflected light via a spherical integrator that receives dual wavelengths of light [18]. Afterward, the two observers took an automatic reading every 1 minute, and the assistant used a stopwatch throughout the process to check the time to ensure that the procedures of the two independent observers were synchronized and that automatic readings of the machine were 1 minute apart.

The two observers separately recorded 15 sets of readings within 16 minutes after application of the synovial fluid. The readings were at four different levels (negative, ±, 1+, and 2+). According to the ICM 2018 diagnostic criteria, we classified 2+ as a positive result, and deemed negative, ±, and 1+ as nonpositive.

Ethical Approval

Ethical approval for this study was obtained from the ethics committee of Chinese PLA General Hospital (QNC19013).

Statistical Analysis

Parametric data were assessed using t tests, and categorical variables were evaluated by using chi-square tests or the Fisher exact probability test. Receiver operating characteristic (ROC) curves were generated to determine the diagnostic value of each reading time point for the assessment of PJI. The area under the curve (AUC) and the 95% confidence interval was calculated. The discriminatory value of the curves was rated as excellent (0.9-1), good (0.8-0.89), fair (0.7-0.79), poor (0.6-0.69), or failing to have or having no discriminatory value (0.5-0.59). Before plotting the ROC curve, we converted the scale points of the LE strip test results (negative, ±, 1+, and 2+ to 0, 1, 2, and 3) and frequency-weighted the resultant data because the results of the LE strip test were ordinal categorical variables. We determined the optimal timing for reading the LE strip by looking for peaks in the AUC. The aforementioned statistical analyses were performed by using the SPSS software package, version 26.0.0.0 (IBM Corp, Armonk, NY, USA).

The sensitivity, specificity, positive predictive value, and negative predictive value of the LE strip test of synovial fluid before and after centrifugation and the related 95% CI were calculated for the timepoint of max AUC. Statistical analyses were conducted by using the EmpowerStats, version 3.0 (X&Y Solutions, Shanghai, China) and the open source statistical program R version 3.3.1 (R Development Core Team, Vienna, Austria). A p value < 0.05 was considered statistically significant.

Results

Best Time to Read LE Strip Test: Before Centrifugation

The ROC curve revealed that LE strips test results before centrifugation had the highest AUC 5 minutes after application (0.90 [95% CI 0.83 to 0.98]), indicating that this timepoint is optimal for the reading of the LE strip before centrifugation for the diagnosis of PJI. At this timepoint, the sensitivity, specificity, positive predictive value, and negative predictive value were 0.88 [95% CI 0.75 to 0.95], 0.89 [95% CI 0.72 to 0.96], 0.92 [95% CI 0.79 to 0.97], and 0.84 [95% CI 0.67 to 0.93], respectively. The AUC of LE strip test results before centrifugation at the other 14 timepoints were between 0.80 and 0.89, indicating that they were all good timing for the diagnosis of PJI (Table 2). For the LE strip test before centrifugation, the results remained unchanged from 13 minutes after application to the end of the detection period, or 16 minutes after application. During this plateau period, the sensitivity, specificity, positive predictive value, and negative predictive value were 0.90 [95% CI 0.77 to 0.96], 0.71 [95% CI 0.54 to 0.85], 0.82 [95% CI 0.68 to 0.90], and 0.83 [95% CI 0.65 to 0.94], respectively. Over the 16 minutes of observation, in specimens that did not undergo centrifugation, 24% (20 of 84) showed an increase in LE grade, with negative tests decreasing to zero by 8 minutes (Fig. 2).
The ROC curve showed that the LE strip test results after centrifugation had the highest AUC 10 minutes after application (0.92 [95% CI 0.86 to 0.98]), which indicated that this timepoint is best for reading the LE strip after centrifugation for the diagnosis of PJI. At this timepoint, the sensitivity, specificity, positive predictive value, and negative predictive value were 0.65 (95% CI 0.50 to 0.78), 0.97 (95% CI 0.83 to 1.00), 0.97 (95% CI 0.83 to 1.00), and 0.66 (95% CI 0.51 to 0.78), respectively. The AUC of LE strip test results after centrifugation 6, 7, 9, and 11 minutes after sample application were also greater than 0.9, indicating that they were all timepoints for attaining good results for PJI diagnosis. The AUC of LE strip test results after centrifugation at the other 10 timepoints were between 0.80 and 0.89, indicating that these timepoints were good timing for PJI diagnosis (Table 3). After the samples were centrifuged, the results of the LE strip test remained unchanged from 14 minutes after application to the end of the detection period, which was 16 minutes after application. During this plateau period, the sensitivity, specificity, positive predictive value, and negative predictive value were 0.74 (95% CI 0.59 to 0.85), 0.88 (95% CI 0.72 to 0.96), 0.90 (95% CI 0.75 to 0.97), and 0.70 (95% CI 0.54 to 0.82), respectively. In specimens that underwent centrifugation, 34% (28 of 83) exhibited an increase in LE grade (Fig. 3).

**Discussion**

The LE strip test is routinely used as an early screening test for patients with suspected PJI in outpatient and intraoperative settings since it is simple, quick, and inexpensive [18]. This test for diagnosing PJI is believed to be effective and useful and has an important place in the ICM 2018 diagnostic criteria [1]. We usually read the LE strips 3 minutes after sample application as per the manufacturer’s recommendation. This timepoint applies to urine, but no relevant clinical studies have confirmed that it also pertains to synovial fluid. Our study demonstrated that the color of LE strips deepened over time, leading to a higher grade. The cause of this phenomenon is unclear, but the LE strip test is biochemically based on diazoreaction, in which LE secreted by activated neutrophils causes hydrolysis of

**Table 2. Diagnostic accuracy at different timepoints before centrifugation (n = 84)**

| Time   | AUC (95%CI) |
|--------|-------------|
| 2 minutes | 0.84 (0.76-0.93) |
| 3 minutes | 0.87 (0.79-0.95) |
| 4 minutes | 0.89 (0.82-0.97) |
| 5 minutes | 0.90 (0.83-0.98) |
| 6 minutes | 0.88 (0.80-0.96) |
| 7 minutes | 0.86 (0.78-0.95) |
| 8 minutes | 0.841 (0.75-0.94) |
| 9 minutes | 0.836 (0.74-0.93) |
| 10 minutes | 0.815 (0.71-0.92) |
| 11 minutes | 0.821 (0.72-0.92) |
| 12 minutes | 0.822 (0.72-0.92) |
| 13 minutes | 0.81 (0.71-0.91) |
| 14 minutes | 0.81 (0.71-0.91) |
| 15 minutes | 0.81 (0.71-0.91) |
| 16 minutes | 0.81 (0.71-0.91) |

*The AUC before centrifugation reaches its maximum at 5 minutes.

Two decimal places to the right of the decimal point are insufficient to indicate the difference in AUC of these timepoints; AUC = area under the curve

**Fig. 2.** Change of results before centrifugation (n = 84). A color image accompanies the online version of this article.
indophenol ester to produce free phenol, which, via phenol oxidation and coupling reaction, combines with diazonium salt in the paper to develop color. We suggested that as a strip with synovial fluid is exposed to air for a protracted time, oxidation and coupling reactions will be more complete and the strip will be darker [4]. In addition, LE synovial fluid may take longer to fully react with substrate on the strips because the synovial fluid is more viscous than urine [7]. This assumption is also premised on the aforementioned principles. Therefore, we began to read the result 1 minute after sample application to allow the synovial fluid to react with the substrate. Although the timepoint at which reaction between the synovial fluid and LE substrate ends may vary, we found that the results of the LE strip test eventually stabilized. The stabilization could be seen as the end of the reaction. Reading the strip at the time of termination (13 or 14 minutes after application) is inappropriate because the prolonged reaction resulted in more false positives (10 false positives before centrifugation and four false positives after centrifugation). Reading the test 3 minutes after application as previously recommended resulted in too many false negatives due to short reaction time (eight false negatives before centrifugation and 27 false negatives after centrifugation). Therefore, it is necessary to determine the optimal timing for reading the strip to improve the accuracy of the LE strip test for diagnosing PJI.

**Limitations**

This study has several limitations. First, given that the optimal time was determined by identifying the peak of the AUC, statistically, the overlap of 95% CI across timepoints might raise some doubts. Direct comparison of the AUC may cause statistical problems due to the presence of errors. However, we believe that direct comparison of the AUC was appropriate in this study, even though 95% CIs overlapped. Errors tend to be caused by differences in diagnostic tools or samples. We tested the same set of samples using the same diagnostic tool, which avoided the impact of errors on the results. As to the overlapping of 95% CIs, two factors need

![Fig. 3. Change of results after centrifugation (n = 83). A color image accompanies the online version of this article.](image-url)
to be considered. First, this is a single-center study and the sample size was not large enough to narrow the 95% CIs. Moreover, the set of consecutive timepoints we compared might increase the overlapping of the 95% CIs. Therefore, the results of this study are reliable; however, large multicenter studies are needed to confirm our findings.

Second, in this study, we used an automated colorimetric reader (PocketChem UA-4010, ARKRAY, Kyoto, Japan) and AUCTION sticks (strips) that went with the reader. Although Koh et al. [9] have confirmed that the reading results of several different brands of automated colorimetric readers have a good consistency, whether differences in equipment and strips affect the final results still needs further study. This study facilitates future research.

Third, the 35 patients without PJI have been followed for less than 2 years, which seems to raise concerns about false negatives. Parvizi et al. [13] validated the 2018 definition of periprosthetic hip and knee infection through a randomly selected sample of 200 aseptic cases that were followed for 1 year. Therefore, the follow-up time for patients without PJI (more than 1 year) should be deemed sufficient.

Finally, one limitation that should be mentioned is that 18 patients who scored 3 to 5 on the ICM 2018 criteria had to be excluded. They did not complete all the diagnostic tests included in the diagnostic criteria, and the absence of intraoperative diagnostic indicators was a major factor. It is practically inevitable since not all patients will receive surgery, even if they are at risk for PJI. However, for the purpose of this study, it was reasonable to exclude this group.

Best Time to Read LE Strip Test: Before Centrifugation

By comparing the AUC of LE strip test results at different timepoints before and after centrifugation, we found that 5 minutes may be the most appropriate time for reading the results of synovial fluid before centrifugation. With 2+ set as the best threshold value, Shafafy et al. [16] demonstrated that the LE strip test had an AUC of 0.914, and the sensitivity and specificity of the LE test strip to diagnose PJI were 0.82 and 0.93, respectively. Our results were consistent with their findings [16]. Nonetheless, those authors did not further stratify their results in terms of the timepoint and centrifugation. We recommend that, for the diagnosis of PJI, clinicians should read LE strips, without centrifugation, 5 minutes after sample application.

Best Time to Read LE Strip Test: After Centrifugation

Our results suggest that after centrifugation, the most accurate time to read the LE strip test would be 10 minutes after application. Li et al. [10] showed that centrifugation could lead to lower grades of the LE strip test, but they routinely read results 3 minutes after application in their study. On the basis of our data, we speculate that synovial fluid after centrifugation may need a longer reaction time to achieve the ideal result. Unlike previous studies, which centrifuged only mixed synovial fluids [2, 16, 19], this study subjected all samples to centrifugation (except the sample of one patient with a minimal sample). Our findings could explain why, if samples were read earlier, the sensitivity of the test after centrifugation was lower than that in previous studies, which reported sensitivities ranging from 70% to 100% [10, 11]. On one hand, centrifuging only obviously mixed synovial fluid may bias the results of the optimal timing after centrifugation. Conversely, the sensitivity of the 10-minute timing we recommend is essentially consistent with the range of previous studies [17]. The results reported by Tischler et al. [17] were similar to our postcentrifugation results. They demonstrated that the sensitivity and specificity of the LE strip test for diagnosing PJI were 66.0% (95% CI 51.7% to 78.5%) and 97.1% (95% CI 92.6% to 99.2%), respectively, when 2+ was used as a positive result and the Musculoskeletal Infection Society infection criteria was used as the gold standard.

Although centrifugation may decrease the sensitivity of the LE strip test, we cannot explicitly reject centrifugation unless a better way is available to solve the sample-mingling problem. For PJI diagnosis, we recommend that clinicians read LE strips, after centrifugation, 10 minutes after sample application.

Conclusion

We recommend that LE strips be read 5 minutes after application and before centrifugation, while 10 minutes after application may be the appropriate time for reading the results of LE strip tests postcentrifugation. We cannot abandon centrifugation since this is an effective way to resolve the sample-mingling problem at present. Centrifugation should only be used in cases where it is necessary, and the LE strips after centrifugation should be read at the timepoint we recommend. Multicenter and large sample size clinical studies are needed to further validate our conclusions.

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