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Original Article

Centrifugation May Change the Results of Leukocyte Esterase Strip Testing in the Diagnosis of Periprosthetic Joint Infection

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ABSTRACT

Background: Centrifugation is used to remove the color interference by erythrocytes in blood-synovial fluid samples before leukocyte esterase (LE) strip testing. However, the impact of centrifugation requires further study.

Methods: From April 2016 to October 2017, 133 (53 infected and 80 noninfected) patients were included in this study. One drop of synovial fluid was applied to LE strips before and after centrifugation in 110 cases. The other 23 cases could not be read without centrifugation due to the color disturbance caused by blood contamination. The results were recorded after approximately 3 minutes according to different color grades on a color chart, including grade 3 (++), grade 2 (+), and grade 1 (others).

Results: After centrifugation, almost every sample was lighter in color than before. Although most results changed inconspicuously and remained in the same grade, 18.6% (8/43) and 17.9% (12/67) of cases were downgraded in the periprosthetic joint infection and non-periprosthetic joint infection groups, respectively. Before centrifugation, when grade 3 (++) was used as the positive threshold, the sensitivity and specificity were 97.7% (86.2%-99.9%) and 100% (94.3%-100%), respectively. After centrifugation, when grades 2 and 3 (+ and ++, respectively) were used as the positive threshold, the sensitivity and specificity were 92.5% (80.9%-97.6%) and 100% (94.3%-100%), respectively.

Conclusions: The influence of centrifugation should be considered when interpreting the LE strip test results. For cases without centrifugation, we recommended using ++ as the positive threshold, while for cases using centrifugation, the threshold should be reduced to both ++ and +.

Periprosthetic joint infection (PJI) is a devastating complication of total joint arthroplasty. An estimated 1%-3% of patients who undergo total joint arthroplasty subsequently develop a PJI [1–3]. Although several tests are available, the diagnosis of PJI remains challenging because in clinical practice, clinical history and examination cannot always distinguish between septic and aseptic causes of failure. Only a few PJI cases have a typical sinus tract communicating with the joint. In addition, serum markers, such as C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR), often have limited diagnostic efficiency in some cases [4,5]. Owing to the existence of a biofilm in most chronic PJI cases and to the administration of antibiotics to patients before obtaining fluid or tissue samples, culture-negative cases are common [6,7]. Thus, in recent years, an increasing number of studies have focused on local synovial fluid biomarkers, such as synovial CRP, leukocyte esterase (LE), and human α-defensin [8].

LE is an enzyme present within granulocytes and secreted by activated neutrophils that will be recruited to the infected site. Parvizi et al [9] first demonstrated that an LE strip was extremely valuable in identifying the presence of PJI in patients who previously underwent total knee arthroplasty. Several studies have also

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confirmed this utility [10–15]. However, the threshold used for the LE test is inconsistent; some studies consider "++" a positive result [14], while other studies consider both "+" and "++" a positive result [11,13]. Even in the modified Musculoskeletal Infection Society (MSIS) criteria [16], "++" and "+" are both listed in the "Threshold for the Minor Diagnostic Criteria". Therefore, further investigation into the appropriate threshold for the LE test is required.

Moreover, according to previous literature and our experience, approximately one-quarter to one-third of LE strips cannot be read due to the color interference caused by erythrocytes in blood-synovial fluid before LE strip testing [9,10]. In a technical note, Aggarwal et al [17] recommended the use of centrifugation in blood joint aspiration cases and stated that the accuracy of LE testing is not affected by centrifugation. However, in that study, the synovial fluid samples were thoroughly hand mixed with the patient's own blood, and the blood volume and sample size were not mentioned. To date, few studies have further evaluated the influence of centrifugation on LE strip test results. Thus, whether centrifugation has an effect on LE strip test results requires further investigation.

The purposes of this study were to further confirm the validity of LE strip test results following the centrifugation of synovial fluid samples and to discuss the proper thresholds for the LE strip test with and without synovial fluid centrifugation.

Patients and Methods

Our institution's research ethics board approved this study. From April 2016 to October 2017, surgeons of the outpatient clinic recommended joint aspiration for patients who met the following criteria: (1) patients after total knee or hip arthroplasty who needed a diagnostic aspiration for any sign of infection, such as fever, swelling, or an elevated level of ESR or CRP and (2) patients who were going to undergo a hip or knee revision surgery. In total, 168 patients accepted the suggestion and showed up to the clinic operation room for an aspiration. These patients gave informed consent to use their synovial fluid sample and clinical data. Patients who met either of the following criteria were excluded: (1) no valid synovial fluid samples (28 patients) or (2) lost to follow-up or insufficient information to determine whether an infection was present (7 patients). In total, 35 patients were excluded, and 133 patients were included in the final cohort.

Of those 133 patients, 53 were classified as infected, and the other 80 patients were classified as noninfected according to the modified MSIS criteria for PJI [16]. The patient characteristics are shown in Table 1. The 2 groups did not significantly differ in terms of male/female ratio, age, or body mass index.

Table 1
Patient Characteristics.

Variable	PJI (n = 53)	Non-PJI (n = 80)	*P*
Sex (male/female)	23/30	31/49	.719
Age (y) mean ± SD (range)	63.9 ± 14.4 (18-85)	65.7 ± 9.7 (34-87)	.414
BMI (kg/m^2)	25.18 ± 3.35 (16.90-34.60)	25.40 ± 3.88 (19.56-39.96)	.740
Hip/knee prosthesis	26/27	44/36	.595
Serum CRP (mg/L)	39.64 ± 50.48 (1.00-257.00)	9.30 ± 14.00 (1.00-64.84)	.000[a]
Serum ESR (mm/h)	49.02 ± 25.68 (6-97)	18.49 ± 16.98 (1.00-75.00)	.000[a]

BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PJI, periprosthetic joint infection; SD, standard deviation.
[a] $P < .05$.

After aspiration, one drop of synovial fluid was immediately applied to the LE strip test pads (AUTION Sticks, 10PA; ARKRAY, Japan). The LE strip test result was read based on the change in the strip pad color approximately 3 minutes later. Five different color grades are shown on the color chart (-neg, 25, 75, 250, and 500) (Fig. 1). The interpretation of these results has not been consistent in previous studies; some studies considered "++" (equal to 500) as a positive result [14], while other studies considered both "+" and "++" (equal to 250 and 500, respectively) as positive results [11,13]. Therefore, we divided the results into 3 grades. Grade 3 (dark purple, 500, equal to ++) was positive, grade 2 (light purple, 250, equal to +) was intermediate, and grade 1 (other colors included, −neg, 25, and 75) was negative (Fig. 1).

Of the 133 cases, the LE strip test results in 23 cases could not be read without centrifugation because of the color disturbance caused by blood contamination. Thus, the blood-synovial fluid was transferred from the syringe to a common centrifuge tube without any special agents, such as anticoagulant, immediately and was subjected to centrifugation (SCILOGEX D3024, 6600 revolutions per minute, 180 s) as recommended by Aggarwal et al [17].

In addition, to evaluate the influence of centrifugation, the remaining 110 synovial fluid samples that were not contaminated by blood were also centrifuged. After the synovial fluid was centrifuged, we collected the supernatant in 2-mL cryogenic vials and then applied one drop to another LE strip. The supernatants we collected in these cases ranged in volume from approximately 0.5-10 mL and were stored in one to five 2-mL cryogenic vials. Following the same procedure as for the samples that were not centrifuged, the LE strip test results were read based on the change in the strip pad color after approximately 3 minutes.

The LE results for samples before and after centrifugation were read by 3 independent observers who were blinded to any diagnostic information about the patients. Although these observers were not the same for all patients, all the observers were well trained and had experienced at least 30 cases before participating in this study. If there were any inconsistencies in the recorded results among the 3 observers, the grade assigned by the majority of the observers was considered the final result.

Statistical Analysis

For statistical analyses, values of $P < .05$ were considered significant. Parametric data were assessed via the *t* test, whereas nonparametric categorical variables were analyzed with the paired chi-square test to make comparisons between the 2 groups (IBM SPSS Statistics, version 21.0.0.0). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and their corresponding 95% confidence intervals for the LE strip test results were also calculated.

Results

Among the 133 patients, serum CRP and the ESR were higher in the PJI group than those in the non-PJI group, and these differences were statistically significant ($P < .05$). The LE strip test results for synovial fluid before and after centrifugation are shown in Table 2.

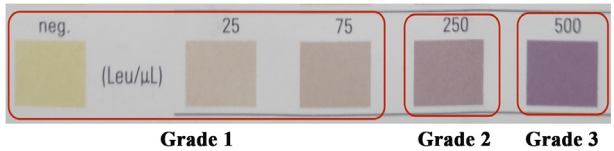

Fig. 1. Definitions of the color grades.