O R I G I N A L   A R T I C L E

Leukocyte esterase strip test as a reliable intraoperative PJIs biomarker. Our experience

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Summary. *Backgroud and aim of work:* Prosthetic joint infection (PJI) is the most common cause of total knee replacement failure and the third most common cause of total hip replacement failure, accounting for 16.8% of all knee revisions and 14.8% of the hip revisions; nevertheless, the diagnosis of PJI is often a challenge for the orthopaedic surgeon. The aim of these study was to evaluate the reliability of the LE strip test for diagnosis of PJI. *Materials and Methods:* From December 2016 to January 2019, we enrolled 50 patients with suspected PJI; 32 females and 18 males, the average age at the time of the surgery was 76 years. Twenty-four patients underwent knee revision surgery and twenty-six hip revision surgery. In all patients during the surgery, the synovial fluid was aspirated and used for leukocyte esterase strip test. The result of the tests was compared to periprosthetic tissues culture, histological examination and sonication fluid culture for PJI. *Results:* Comparing the results obtained from the LE test with the results obtained from the other diagnostic methods, we found that the concordance between the results of the leukocyte-esterase test and those of the culture test with peri-prosthetic tissue or synovial fluid was shown to be 93%, between LE and histological examinations, the concordance was 93% and finally with the culture of the sonicated fluid the concordance was 86% of the cases. *Conclusions:* The results of our serie show a good intraoperative diagnostic accuracy of the LE test, especially in its ability to exclude the hypothesis of periprosthetic infection in case of a negative result. (www.actabiomedica.it)

Key words: leukocyte, esterase, periprosthetic, joint, infection

Introduction

Prosthetic revisions following infections in Orthopedics is an increasingly important and discussed topic (1, 2). Prosthetic joint infection (PJI) is the most common cause of total knee replacement failure and the third most common cause of total hip replacement failure, accounting for 16.8% of all knee revisions and 14.8% of the hip revisions. (3); nevertheless, the diagnosis of PJI is often a challenge for the orthopaedic surgeon.

Over the years various biomarkers have been identified and used to make a certain diagnosis of infection, the most used in the different periprosthetic infection diagnosis algorithms are the C-reactive pro-

tein (CRP) and the serum erythrocyte rate (ESR). Furthermore, new biomarkers are continuously being studied in order to achieve the right diagnosis and to choose the best surgical/medical approach, improving the decision-making process.

In the various international consensus meetings (ICM) on the PJI diagnostic criteria have been identified and divided into Major and Minor (4, 5); to make a diagnosis of infection there must be at least one major criterion or at least three minor criteria. Among the minor criteria, we find serological tests on blood and synovial fluid. Leukocyte esterase is also present among these biomarkers. CRP and ESR are the first biomarkers to be evaluated, but alone they are not sufficient: the CRP has a sensitivity of 88% and specifici-

ty of 74%, while the ESR has a sensitivity of 77% and a specificity of 70% (6). These two parameters cannot be used alone to monitor the persistence of the infection after a revision surgery (7). Today, other systems of investigation have been developed, such as α-defensin, which seems to show promising results.

In our Department, revision surgery for PJI is a common procedure. To approach PJIs, we apply a rigid protocol, known as the "Udine Strategy" (8) and since few years it has been implemented with the routine use of the Leukocyte Esterase (LE) strip test. The aim of our retrospective observational study is to show the reliability of LE in relation to ICM criteria and in comparison with other diagnostic tests and exams. Our results show that LE is a reliable method, especially for the intraoperative diagnosis of PJIs.

Materials and Methods

From December 2016 to January 2019, we enrolled 50 patients with suspected PJI; 32 females and 18 males, the average age at the time of the surgery was 76 years. Twenty-four patients underwent knee revision surgery and twenty-six hip revision surgery.

All patients underwent preoperative blood tests, ESR, CRP. During the operation, the synovial fluid was used for leukocyte esterase test and for microbiological culture examination; periprosthetic tissue samples were sent for intraoperative histological examination and for microbiological examination. The removed prosthetic components were also examined via microbiological culture after sonication.

For the LE test, we utilized "*Chemistrip 7 Urine Test Strips*" (Roche Diagnostics, Indianapolis, Indiana) – "Multistix" Siemens. According to literature, we considered the test as positive with 2+ and 3+ results, meanwhile, 1+ has been considered negative. All strips have been read at 1 and 2 minutes as recommended by the manufacturer.

A comparison was made between the LE test and the various diagnostic methods used (intraoperative histological examination with PMNs count, microbiological examination of culture and previous examination) both in a descriptive way, evaluating the percentage of cases in which the tests agreed both positively and negatively.

Results

Of the 50 cases examined, the LE test on synovial fluid was positive in 16, the intraoperative histological examination for leukocyte count was positive in 10 and in 3 was not performed, culture microbiological tests on periprosthetic tissue were positive in 12 and not performed in 1, the microbiological culture tests after sonication of the explanted prosthetic components were positive in 12, not performed in 1 and in 2 cases the results were positive only in one of the explanted components (1 head and 1 in the stem in 2 revisions of total hip prosthesis).

None of the LE tests was excluded due to blood contamination.

Comparing the results obtained from the LE test with the results obtained from the other diagnostic methods, we found that the concordance between the results of the leukocyte-esterase test and those of the culture test with peri-prosthetic tissue or synovial fluid was shown to be 93%, in 41 of the 44 cases examined the results were overlapping (Table 1). Performing the same operation between the leukocyte-esterase test and the intraoperative histological examinations, the concordance was 93%, being positive in 42 of 45 comparable patients (Table 2). Finally, we found that the LE test produced results which agree with the culture of the sonicated fluid in 86% of the cases, in 43 of 49 comparable cases (Table 3).

Using the criteria of the international consensus (4) the diagnosis of periprosthetic infection was made in 13 patients, 12 of these met the major criteria, presenting two or more positive cultures for the same micro-organism; 3 of these also presented with a fistula. One patient instead met three minor criteria, presenting positivity to the leukocyte-esterase test and to the synovial leukocyte count, to the percentage of PMNs neutrophils in synovial fluid and to PCR. Based on this, the accuracy of the leukocyte-esterase test was evaluated, showing a sensitivity of 100%, a specificity of 94%, a positive predictive value (PPV) of 84% and a negative predictive value (NPV) of 100 %. The same was also done for the other examined tests.

According to our data, the microbiological cultures reported a sensitivity of 80%, specificity of 100%, PPV of 100% and NPV of 92,86%, the intraoperative

Table 1. Comparison between cultural exam and LE test

	Cultures +	Cultures -	Total	
LE +	10	3	13	p = 0,023
LE -	0	31	31	
Total	10	34	44	

In green the number of patients in which both the exams (cultural exam, histological exam and cultures after sonication) and the LE test are negative, in red the number of patients in which both the exams and the LE test are positive.

Table 2. Comparison between intraoperative histological exam and LE test

	Histological +	Histological -	Total	
LE +	10	3	13	p = 0,03
LE -	0	32	32	
Total	10	35	45	

In green the number of patients in which both the exams (cultural exam, histological exam and cultures after sonication) and the LE test are negative, in red the number of patients in which both the exams and the LE test are positive.

Table 3. Comparison between sonication exam and LE test

	Sonication +	Sonication -	Total	
LE +	10	6	16	p = 0,025
LE -	2	33	35	
Total	12	39	51	

In green the number of patients in which both the exams (cultural exam, histological exam and cultures after sonication) and the LE test are negative, in red the number of patients in which both the exams and the LE test are positive.

histological examination a sensitivity of 80%, specificity of 100%, PPV of 100% and NPV of 92%, and the sonication of prosthetic components a sensitivity of 84%, specificity of 91%, PPV of 84% and NPV of 92%.

Discussion

Peri-prosthetic infections are a serious complication of joint replacement procedures, for this reason, it is necessary to make an early and accurate diagnosis, so to proceed, as soon as possible, with a specific multidisciplinary approach. An only-surgical or only-medical approach has been found to be ineffective; therefore, a structured cooperation of the orthopaedic surgeon and the infectivologist is crucial in the diagnosis and in the choice of the appropriate therapy.

We are trying to improve the promptness and accuracy of the diagnostic process, we are, therefore, studying and looking for new biomarkers, both in serum and in synovial fluid, usable in clinical practice. In the serum, besides CRP and ESR, IL6 and IL4 are currently being considered. In the synovial fluid, we find IL-6, IL-1b, IL-17, α-defensin and LE test (9,10). But while the various IL research tests are expensive, the LE test is very cheap because it uses common urine test strips.

In the study, the diagnostic accuracy of the LE test was evaluated by comparing it with the microbiological culture examination, the microbiological examination after sonication of the prosthetic components removed, and the intraoperative histological examination with PMNs count. While the first two tests require time for their execution, on average from 7 to 14 days, the intraoperative histological examination takes few minutes, and it can, therefore, influence the operator in the intraoperative surgical choice, facilitating the decision between prosthesis removal and reimplantation or the positioning of a cemented spacer.

The leukocyte-esterase has been extensively studied in the literature; this is an enzyme produced by PMNs during infection (11). For the test, we used the Chemstrip 7 urine test strip, simple and with very low costs. The sensitivity and specificity of the LE test for the diagnosis of PJI is 81% and 97% respectively, but this test also has the disadvantage of being illegible if contaminated with blood (12, 13).

In our centre, a rigid protocol is applied to deal with suspicion of PJI (8), the «Udine Strategy» which was implemented with the use of the LE test. After the introduction of this additional test, the results were good, and we found it to be a reliable intraoperative exam, which can help us in decision-making, complementing the information we receive from other intraoperative exams (14). The results of the LE test obtained show a significant concordance with culture tests (93%), with sonicates (84%) and with intraoperative histological examinations (93%).

These results show a good intraoperative diagnostic accuracy of the LE test, especially in its ability to exclude the hypothesis of periprosthetic infection

Table 4. Table of content of the results obtained with the different exams for each patient

	culture	sonication	Frozen section exam (>5° PMN/HRF=pos)	L.E. test
1	neg	neg	neg	neg
2	neg	neg	pos	pos
3	neg	neg	neg	neg
4	neg	neg	neg	neg
5	neg	neg	neg	neg
6	pos	pos	pos	pos
7	neg	neg	neg	neg
8	neg	neg	neg	pos
9	neg	neg	neg	neg
10	pos	pos	pos	pos
11	N.E.	N.E.	N.E.	neg
12	neg	neg	neg	neg
13	neg	neg	N.E.	neg
14	pos	pos	neg	pos
15	neg	neg	neg	neg
16	neg	neg	neg	neg
17	neg	neg	neg	neg
18	neg	neg	neg	neg
19	pos	pos	pos	pos
20	neg	neg	neg	neg
21	neg	neg	neg	neg
22	neg	neg	neg	neg
23	neg	neg	neg	neg
24	neg	neg	neg	neg
25	neg	neg	neg	neg
26	neg	neg	neg	neg
27	neg	pos	neg	neg
28	neg	neg	neg	neg
29	neg	neg	neg	neg
30	neg	neg	neg	neg
31	pos	pos	pos	pos
32	neg	neg	neg	neg
33	neg	neg	neg	neg
34	neg	neg	neg	neg
35	neg	neg	neg	neg
36	pos	pos	pos	pos
37	pos	pos	pos	pos
38	neg	neg	neg	pos
39	pos	pos	pos	pos
40	neg	neg	neg	neg
41	neg	neg	neg	neg
42	pos	pos	pos	pos
43	neg	neg	neg	neg
44	N.E.	N.E.	N.E.	pos
45	pos	pos	N.E.	N.E.
46	neg	neg	neg	neg
47	pos	pos	pos	pos
48	neg	N.E.	N.E.	pos
49	pos	pos	neg	neg
50	neg	neg	neg	pos

Notes: "neg" stands for negative, "pos" stands for positive, N.E. stands for not executed.

in case of a negative result. Again, a doubt may arise when synovial fluid is not obtainable or when it is contaminated with blood.

Many authors have studied α-defensin, as a biomarker for PJIs, which has been shown to have the highest diagnostic accuracy ratio (15). This biomarker is released by activated neutrophils (16), and its sensitivity is 100% and the specificity of 96%, as shown in a recent meta-analysis (12). An important disadvantage is the cost of this test, much higher than the LE test. We believe that, nowadays, the LE test still has a better price/performance ratio, it gives us the possibility to obtain reliable information in a short time and at a very limited cost.

A major limitation of our study is certainly the limited number of patients that have been enrolled, which could reflect a selected sub-population of patients and could, therefore, invalidate the investigation. In this regard, it would be necessary to increase the observation time so as to enrol a greater number of patients.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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