The gap between practice and methodology: the case of esterase in Periprosthetic Joint Infection

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ABSTRACT

Introduction: the diagnosis of periprosthetic joint infection (PJI) is a clinical challenge. Among other clinical and laboratory parameters, recent recommendations for the diagnosis of PJI advocate the assessment of leukocyte esterase in synovial fluid (SF), commonly performed with urine test strips, a method that is not validated for such a matrix. To challenge this practice, we evaluated the performance of a commercial urine test strip to assess leukocyte esterase in SF compared with leukocyte counting.
Methods: between October 2017 and September 2019, SF samples were collected from patients scheduled for revision surgery for 6 painful total knee arthroplasties. International Consensus Meeting (ICM) criteria were used to classify PJI. Leukocyte esterase (visual assessment with Menarini Aution test strips) and automated leukocyte count (Sysmex 2000, XN- 8 Body Fluids Module) were assessed in 74 SF specimens.
Results: the AUC of leukocyte esterase compared with automated leukocyte counting ranged from 0.88 to 0.94. Leukocyte esterase cutoff values of 500, 250, and 75 leukocytes/µL yielded sensitivity values ranging from 0.78-1.00, 0.87-1.00, and >0.96, respectively, whereas specificity ranged from 0.76-0.94, 0.61-0.78, and <0.37. The diagnostic performance of leukocyte esterase was higher when hemolyzed samples were excluded.
Discussion: our results highlight the many limitations of using conventional urine test strips to visually assess leukocyte esterase in SF specimens, underscoring the need to develop specific assays or perform validation studies before implementing a diagnostic procedure.

Key words: *periprosthetic joint infection, sinovial fluid, esterase*

INTRODUCTION

Appropriate use of a diagnostic test depends on several aspects: a sound clinical question, a rigorous assessment of the diagnostic accuracy of the test, and, most importantly, the ability to make clinical management decisions that link the test to health outcomes. Biomarker development research and rigorous translation into practice obey a well-defined methodology (1), and cross-sector collaboration and interaction between clinical laboratory professionals and clinicians are encouraged to share this knowledge.

The diagnosis of periprosthetic joint infection (PJI), a rare but devastating complication of total joint arthroplasty (2), is a good example of a critical area where the search for a diagnostic tool outside the methodological framework found an answer. Recent recommendations for the diagnosis of PJI (3-4) have advocated the determination of leukocyte esterase in synovial fluid (SF).

Unfortunately, measurement of leukocyte esterase in SF is often performed by physicians using semi-quantitative urine reagent strips immediately after arthrocentesis, even though the test strips are manufactured and validated exclusively for urine testing, whose pH, specific gravity, and chemical composition differ from those of other biological fluids.

Such an approach can at best be described as “empirical” since no test validation has been performed. According to the standard ISO 15189:2012, whenever a test is used for a different biological matrix than the one for which the reagent was originally validated, a new evaluation should be performed to investigate the

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diagnostic performance for this new sample type. This critical aspect runs the risk of being overlooked by clinicians. The methodological shortcomings of such a practice need to be highlighted and the introduction of a possible positive or negative bias investigated (5). However, given the clinical scenario, the need for a rapid, inexpensive, and potentially bedside test is undeniable.

PJI is a serious complication of both hip and knee replacements, favouring the occurrence of other serious complications (6-7) and poor outcomes. It is associated with high morbidity, disability, and mortality, and ultimately with a significant economic burden on the healthcare system. Diagnosis of PJI is often difficult because there is no gold standard; this condition may remain undiagnosed until surgery, forcing the surgeon to choose the appropriate treatment option in the operating room without delay. The Musculoskeletal Infection Society (MSIS) proposed some diagnostic criteria in 2011 (3-4), which were then modified during an International Consensus Meeting (ICM) in Philadelphia in 2013 (8-10) and finally updated in July 2018 (11-13). According to the ICM guidelines, the definition of PJI is based on clinical signs (e.g., presence of a fistula), biochemical investigations [e.g., assessment of C reactive protein (CRP) or erythrocyte sedimentation rate (ESR)], along with cell counting and measurement of leukocyte esterase in SF (14-17). The biochemical parameters CPR and ERS are measured in peripheral blood, usually from a central facility. Cell counting in SF is time-consuming and raises pre-analytical problems that must be solved according to the data in the literature (18). Measurement of leukocyte esterase in SF is considered a minor criterion but is not based on a validated methodology, as mentioned previously. The difficulty of dealing with the only internationally available diagnostic criteria and the burden of rapid diagnosis in unclear clinical cases have led practitioners to use conventional reactive urine strips (19-20).

Given the current state of the art, the claim that the current mode of esterase measurement in SF of PJI diagnosis provides some clarity must be questioned. If this proves to be true, an international validation protocol such as CLSI EP 12A-2E must be performed.

Therefore, we decided to take a “real life” approach and evaluate the diagnostic accuracy of a commercial urine test strip for assessing leukocyte esterase in SF, compared to SF leukocyte counting, in order to obtaining evidence and stimulate further research.

METHODS

Seventy-four consecutive patients (36 men and 38 women) with failed or painful joint arthroplasty who presented for orthopaedic consultation between October 2017 and September 2019 were included. The study was approved by the independent ethics committee on June 27, 2017. The research was conducted in accordance with the Declaration of Helsinki and national and institutional standards, and patients gave informed consent before being enrolled in the study. Residual samples from individual patients were used for the study, and a pseudo anonymization protocol was applied immediately after routine examinations. Briefly, name and surname were replaced with one or more artificial identifiers to reduce the identifiability of the data set while remaining suitable for data analysis and processing.

All patients underwent a standardized diagnostic protocol to identify those with PJI. The standard examination included clinical assessment in conjunction with measurement of CRP and ESR, joint aspiration for SF, total white blood cell (WBC) count and percentage of polymorphonuclear leukocytes (PMN), and finally SF cultures. The diagnosis of infection was based on ICM definition of PJI (3-4). To make the diagnosis, one of two major criteria or three of five minor criteria must be met (Table 1). Insufficient amount of SF (<10 ml) for culture, total white blood cell count, and percentage of PMN (neutrophils) were considered as exclusion criteria.

An experienced orthopaedic surgeon collected the SF during preoperative evaluation for failure of a knee prosthesis of uncertain origin. The SF was collected directly into K_3EDTA tubes (Becton Dickinson, Franklin Lakes, NJ) and sent to the central laboratory within 3 hours at room temperature.

Automated leukocyte counting on SF was performed using a Sysmex XN-2000. This haematology analyser is equipped with a dedicated body fluid analysis module (XN-BF from Sysmex, Inc. Kobe, Japan); quality control XN check BF (from Sysmex, Inc. Kobe, Japan) was

Table 1
International Consensus Group definition of periprosthetic joint infection.

1) There is a sinus tract communicating with the prosthesis

2) A pathogen is isolated by culture from at least two separate tissue or fluid samples obtained from the affected prosthetic joint

3) Three of the following five criteria exist:
   1. elevated serum erythrocyte sedimentation rate (ESR) and serum C-reactive protein CRP) concentration;
   2. elevated synovial leukocyte count OR ++ result on leukocyte esterase test strip;
   3. elevated synovial neutrophil percentage (PMN%)
   4. isolation of a microorganism in one culture of periprosthetic tissue or fluid;
   5. greater than five neutrophils per high-power field in five high-power fields observed from histologic analysis of periprosthetic tissue at 9400 magnification.

used as routine internal control. Analytical performance for the analysis of SF has recently been validated in comparison with optical microscopy. Detailed information on analytical and diagnostic performance can be found elsewhere (21).

Upon arrival at the laboratory, the SF was pretreated with hyaluronidase (Sigma Chemical Co., St. Louis, MO, USA). Hyaluronidase solution was prepared by dissolving 2.5 mg hyaluronidase in 5 ml 0.1 mol/L phosphate-buffered saline at pH 7.4 (final concentration, 0.5 mg/mL). Pretreatment consisted of the addition of 20 µL of hyaluronidase to 1 mL of SF followed by incubation for 5 minutes at room temperature.

Leukocyte esterase evaluation using Menarini Aution test strips (Menarini Diagnostics, Florence, Italy) was performed by laboratory personnel immediately after arrival at the central facility using untreated samples. Leukocyte esterase values were estimated by comparing the colour of the test strip with a colour scale included in the reagent package according to the manufacturer's instructions.

Menarini Aution test strips have been specifically designed and validated for the determination of biochemical parameters, including leukocyte esterase, in urine samples. The strips consist of a plastic strip containing reactive regions. The leukocyte esterase pad contains 3-N-toluenesulfonyl-L-alanyloxyindole (0.69 mg) and 2-methoxy-4-N-(morpholine) benzene diazonium (0.38 mg). Analytical sensitivity and measurement range are reported by the manufacturer as 25 leukocytes/µL and 25-500 leukocytes/µL, respectively.

Statistical analysis

Receiver operating characteristic curve analysis (ROC) was used to investigate the diagnostic agreement between leukocyte esterase measured with Menarini Aution test strips and automated leukocyte counting on the XN-BF module. According to the international criteria (11-13), SF samples were classified as positive for infection if the cell counts yielded the following values:

- WBC ≥1.7x10^9/L
- WBC ≥3.0x10^9/L
- WBC ≥10x10^9/L
- WBC ≥1.7x10^9/L and PMN ≥65%
- WBC ≥3.0x10^9/L and PMN ≥80%
- WBC ≥10x10^9/L and PMN ≥90%

Both sensitivity and specificity of test strips were calculated taking into account these criteria, using 2×2 tables. The statistical analysis was performed with Analyze-it™, version 3.90.5 (Analyze-it Software Ltd, Leeds, UK).

RESULTS

The study population consisted of 74 patients scheduled for surgical revision of knee prostheses for suspected PJI. Two SF samples were excluded because of the high haemolysis value, which precluded leukocyte esterase measurement on the test strips. Therefore, the final sample consisted of 72 SF, on which both leukocyte esterase and automated leukocyte counting were performed and could be compared.

According to the international criteria, Table 2 reports the number (and percentage) of SF samples classified as positive or negative for PJI.

The area under the curve (AUC) of leukocyte esterase compared with automatic leukocyte counting on the XN-BF module was 0.88 for the criterion "WBC ≥10x10^9/L and PMN ≥90%"; AUC was 0.94 for the criterion "WBC ≥1.7x10^9/L and PMN ≥65%" (Table 3). When 500 leucocytes/µL, as assessed by the semiquantitative leucocyte esterase measurement, was used as cut-off, the sensitivity of the test ranged from 0.78 (95%CI 0.58-0.90) to 1.00 (95%CI 0.68-1.00) for various cell count criteria. The specificity varied from 0.76 (95%CI 0.65-0.85) to 0.94 (95%CI 0.84-0.98) (Table 4). When 250 leucocytes/µL, as assessed by the semiquantitative leucocyte esterase measurement, was used as cut-off, the sensitivity of the test ranged from 0.87 (95%CI 0.68-0.96) to 1.00 (95%CI 0.57-1.00) and the specificity ranged from 0.61 (95%CI 0.49-0.72) to 0.78 (95%CI 0.64-0.87) (Table 4).

Table 2
Number (and percentage) of synovial fluid samples classified as positive or negative for periprosthetic joint infection

Diagnostic cut-off (8,12)	Positive samples (%)	Negative samples (%)
WBC ≥1.7x10^9/L	23 (32%)	49 (68%)
WBC ≥3.0x10^9/L	15 (21%)	57 (79%)
WBC ≥10x10^9/L	8 (11%)	64 (89%)
WBC ≥1.7x10^9/L and PMN ≥65%	16 (22%)	56 (78%)
WBC ≥3.0x10^9/L and PMN ≥80%	10 (14%)	62 (86%)
WBC ≥10x10^9/L and PMN ≥90%	5 (7%)	67 (93%)

WBC, white blood cells; PMN, polymorphonuclear leukocytes.

Finally, when 75 leucocytes/µL, as assessed by the semiquantitative leucocyte esterase measurement, was used as cut-off, the sensitivity and specificity >0.96 (95%CI 0.79-0.99) and <0.37 (95%CI 0.25-0.51), respectively (Table 4). Regardless of the cut-off value for cell count diagnosis, a variable percentage of false positive cases (i.e., 26.3-66.6%) were due to haemolysis interference in leukocyte esterase assessment with urine test strips (Table 5).

Because visible haemolysis was present in 17/72 SF samples (23.6%), these samples were excluded, and ROC curve analysis was repeated. The resulting AUCs ranged from 0.90 (95%CI 0.85-0.96) to 0.97 (95%CI 0.93-1.0) (Table 3), whereas the sensitivity and specificity of various leukocyte esterase cut-offs (i.e., 75, 250, and 500 leukocyte/µL) remained essentially unchanged and 0.76 (95%CI 0.53-0.90) and 1.00 (95%CI 0.57-1.00) and 0.28 (95%CI 0.19-0.40) and 0.97 (95%CI 086-0.99), respectively (Table 6).

DISCUSSION

Joint diseases of inflammatory, autoimmune, and traumatic origin, as well as osteoarthritis, mainly due to lifestyle and life extension, are increasing worldwide (5-7, 22). Joint pain and swelling are signs that occur in any disease, regardless of aetiology (23). The diagnosis of many pathologies characterised by septic or non-septic joint effusion is essentially based on the analysis of SF. This fluid is virtually unique among the many other body fluids because of its viscosity (24-26) and presents unique challenges in performing biochemical assays, total and differential cell counts, and crystal identification (18,23,27).

Joint replacement is performed for severe osteoarthritis when the patient's symptoms interfere with daily activities and cannot be treated by other means. Unfortunately, PJI is a complication that occurs in 1-2% of primary arthroplasties (28-30) and is a major problem for both patients and orthopaedic surgeons, being one of the most devastating and costly complications after total joint arthroplasty (TJA).

Joint swelling, stiffness, and pain after arthroplasty can have a variety of causes, including infections. The diagnosis of PJI can be clear in severe or acute cases, but it is challenging in dubious cases or in late-onset infections (31-33). Orthopaedic surgeons must make decisions quickly, sometimes even while still in the operating room, based on which completely different procedures can then be performed (34-37).

The MSIS has suggested some diagnostic criteria, including "Elevated synovial leukocyte count OR ++ result on leukocyte esterase test strip" as minor criterion. Although one of two major criteria or three of five minor criteria must be met for establishing a diagnosis (Table 1), it is still debated as to whether a non-validated assay could have been included in the consensus statement (14,20,38). Test validation also includes investigation of possible interference; haemolysis, which is common in SF, has been found to be the major source of interference in our data. This must be considered as another drawback in visual assessment of test strips. The red hue of the sample obscures the colour developed in the pad, making the reading unreliable and a major source of error. This was confirmed by the variations in the AUC value when haemolysed samples were excluded from the analysis. However, this aspect has not been discussed in the literature claiming the use of urine test strips (39); moreover, this issue may be of some importance in the development of a new method.

Furthermore, orthopaedics estimated leukocyte esterase visually by comparing the colour of the test strip with the colour scale included in the reagent package. This may add an additional bias, since visual assessment is known to be highly unobjective and strictly dependent on operator skills, besides being strictly time dependent.

To verify possible inaccuracies, we decided to perform a study on the "off label" use of urine test strips, a practice already commonly diffused in orthopaedic departments (17,19).

Table 3

Receiver Operating Characteristics (ROC) curve analysis of visual assessment of leukocyte esterase with Menarini Aution test strips compared to leukocyte cut-offs (automated leukocyte count was performed using Sysmex XN-BF)

Diagnostic cut-off (8,12)	All samples (n=72)			Non-hemolyzed samples (n=55)		
	AUC	95%CI	p	AUC	95%CI	p
WBC ≥1,7x10^9/L	0.89	0.81 to 0.98	<0.0001	0.94	0.88 to 1.00	<0.0001
WBC ≥3,0x10^9/L	0.91	0.84 to 0.98	<0.0001	0.91	0.82 to 0.99	<0.0001
WBC ≥10x10^9/L	0.90	0.85 to 0.95	<0.0001	0.93	0.88 to 0.98	<0.0001
WBC ≥1,7x10^9/L and PMN ≥65%	0.94	0.89 to 0.98	<0.0001	0.97	0.93 to 1.00	<0.0001
WBC ≥3,0x10^9/L and PMN ≥80%	0.91	0.86 to 0.96	<0.0001	0.93	0.88 to 0.98	<0.0001
WBC ≥10x10^9/L and PMN ≥90%	0.88	0.83 to 0.93	<0.0001	0.90	0.85 to 0.96	<0.0001

AUC, area under the curve; CI, Confidence Interval; WBC, white blood cells; PMN, polymorphonuclear leukocytes.

Table 4
Sensitivity and specificity of visual assessment of leukocyte esterase with Menarini Aution test strips compared to leukocyte cut-offs (automated leukocyte count was performed using XN-BF). All samples (n=72)

Diagnostic cut-off (10-12)	500 leukocyte/µL			
	Sensitivity (95%CI)	Specificity (95%CI)	False positive (95%CI) N samples	False negative (95%CI) N samples
WBC ≥1,7x10^9/L	0.78 (0.58-0.90)	0.94 (0.84-0.98)	0.06 (0.02-0.16) 3	0.22 (0.09-0.42) 5
WBC≥3,0x10^9/L	0.93 (0.70-0.99)	0.88 (0.77-0.94)	0.12 (0.06-0.23) 7	0.07 (0.01-0.30) 1
WBC ≥10 x10^9/L	1.00 (0.68- 1.00)	0.80 (0.68-0.88)	0.20 (0.12-0.32) 13	0.00 (0.00-0.32) 0
WBC ≥1,7x10^9/L and PMN ≥65%	0.94 (0.72- 0.98)	0.89 (0.79-0.95)	0.11 (0.05-0.22) 6	0.06 (0.01-0.28) 1
WBC ≥3,0x10^9/L and PMN ≥80%	1.00 (0.72-1.00)	0.82 (0.71-0.89)	0.18 (0.10-0.29) 11	0.00 (0.00-0.28) 0
Diagnostic cut-off (10-12)	250 leukocyte/µL			
	Sensitivity (95%CI)	Specificity (95%CI)	False positive (95%CI) N samples	False negative (95%CI) N samples
WBC ≥1,7x10^9/L	0.87 (0.68-0.96)	0.78 (0.64-0.87)	0.22 (0.13-0.36) 11	0.13 (0.04-0.32 3
WBC≥3,0x10^9/L	0.93 (0.70-0.98)	0.70 (0.57-0.80)	0.30 (0.19-0.43) 17	0.07 (0.01-0.29) 1
WBC ≥10 x10^9/L	1.00 (0.68-1.00)	0.64 (0.52-0.75)	0.36 (0.25-0.48) 23	0.00 (0.00-0.32) 0
WBC ≥1,7x10^9/L and PMN ≥65%	1.00 (0.81-1.00)	0.73 (0.60-0.83)	0.27 (0.17-0.40) 15	0.00 (0.00-0.19) 0
WBC ≥3,0x10^9/L and PMN ≥80%	1.00 (0.72-1.00)	0.66 (0.54-0.77)	0.34 (0.23-0.46) 21	0.00 (0.00- 0.28) 0
WBC ≥10 x10^9/L and PMN ≥90%	1.00 (0.57- 1.00)	0.61 (0.49-0.72)	0.39 (0.28-0.51) 26	0.00 (0.00-0.43) 0
Diagnostic cut-off (10-12)	75 leukocyte/µL			
	Sensitivity (95%CI)	Specificity (95%CI)	False positive (95%CI) N samples	False negative (95%CI) N samples
WBC ≥1,7x10^9/L	0.96 (0.79-0.99)	0.37 (0.25-0.51)	0.63 (0.49-0.75) 31	0.04 (0.01-0.21) 1
WBC ≥3,0x10^9/L	1.00 (0.80-1.00)	0.33 (0.23-0.46)	0.67 (0.57-0.77) 38	0.00 (0.00-0.20) 0
WBC ≥10 x10^9/L	1.00 (0.68-1.00)	0.30 (0.20-0.42)	0.70 (0.58-0.80) 45	0.00 (0.00-0.32) 0
WBC ≥1,7x10^9/L and PMN ≥65%	1.00 (0.81-1.00)	0.34 (0.23-0.47)	0.66 (0.53-0.77) 37	0.00 (0.00-0.19) 0
WBC ≥3,0x10^9/L and PMN ≥80%	1.00 (0.72-1.00)	0.31 (0.21-0.43)	0.69 (0.57-0.79) 43	0.00 (0.00-0.28) 0
WBC ≥10 x10^9/L and PMN ≥90%	1.00 (0.56-1.00)	0.28 (0.19-0.40)	0.72 (0.60-0.81) 48	0.00 (0.00-0.43) 0

CI, Confidence Interval; WBC, white blood cells; PMN, polymorphonuclear leukocytes.

Table 5
Description of false positive samples with application of the three leukocyte esterase cut-off

Diagnostic cut-off (8,12)	Leukocyte esterase cut-off					
	500 leukocyte/µL		250 leukocyte/µL		75 leukocyte/µL	
	False positive (95% CI) N. samples	N. Sample with hemolysis (approx%)	False positive (95%CI) N. samples	N. Sample with hemolysis (approx%)	False positive (95% CI) N. samples	N. Sample with hemolysis (approx%)
WBC 1,7x10^9/L	0.06 (0.02-0.16) 3	2 (67%)	0.22 (0.13-0.36) 11	7 (64%)	0.63 (0.49-0.75) 31	10 (33%)
WBC ≥3,0x10^9/L	0.12 (0.06-0.23) 7	2 (29%)	0.30 (0.19-0.43) 17	7 (41%)	0.67 (0.57-0.77) 38	10 (27%)
WBC ≥10x10^9/L	0.20 (0.12-0.32) 13	6 (46%)	0.36 (0.25-0.48) 23	11 (48%)	0.70 (0.58-0.80) 45	14 (31%)
WBC ≥1,7x10^9/L and PMN ≥65%	0.11 (0.05-0.22) 6	4 (67%)	0.27 (0.17-0.40) 15	9 (60%)	0.66 (0.53-0.77) 37	12 (33%)
WBC ≥3,0 x10^9/L and PMN ≥80%	0.18 (0.10-0.29) 11	4 (36%)	0.34 (0.23-0.46) 21	9 (43%)	0.69 (0.57-0.79) 43	12 (28%)
WBC ≥10 x10^9/L and PMN ≥90%	0.24 (0.15-0.35) 16	6 (38%)	0.39 (0.28-0.51) 26	11 (43%)	0.72 (0.60-0.81) 48	12 (25%)

CI, Confidence Interval; WBC, white blood cells; PMN, polymorphonuclear leukocytes.

Table 6
Sensitivity and specificity of visual assessment of leukocyte esterase with Menarini Aution test strips compared to leukocyte cut-offs (automated leukocyte count was performed using XN-BF). Non-hemolyzed samples (n=55).

Diagnostic cut-off (8,12)	500 leukocyte/µL			
	Sensitivity (95% CI)	Specificity (95%CI)	False positive (95%CI) N. samples	False negative (95%CI) N. samples
WBC ≥1,7x10^9/L	0.76 (0.53-0.90)	0.97 (0.86-0.99)	0.03 (0.005-0.13) 1	0.23 (0.1-0.5) 4
WBC ≥3,0x10^9/L	0.90 (0.6-0.98)	0.89 (0.76-0.95)	0.11 (0.05-0.23) 5	0.10 (0.02-0.4) 1
WBC ≥10x10^9/L	1.00 (0.65-1.00)	0.85 (0.73-0.93)	0.15 (0.07-0.27) 7	0.0 (0.0-0.35) 0
WBC ≥1,7x10^9/L and PMN ≥65%	0.94 (0.72-0.99)	0.89 (0.78-0.95)	0.11 (0.05-0.21) 6	0.06 (0.01-0.28) 1
WBC ≥3,0x10^9/L and PMN ≥80%	1.0 (0.72-1.00)	0.82 (0.71-0.90)	0.17 (0.10-0.29) 11	0.00 (0.0-0.27) 0
WBC ≥10x10^9/L and PMN ≥90%	1.00 (0.57-1.00)	0.76 (0.65-0.85)	0.24 (0.15-0.35) 1 6	0.00 (0.0-0.43) 0

CI, Confidence Interval; WBC, white blood cells; PMN, polymorphonuclear leukocytes.

Table 6 - continues

Diagnostic cut-off (8,12)	250 leukocyte/µL			
	Sensitivity (95% CI)	Specificity (95%CI)	False positive (95%CI) N samples	False negative (95%CI) N samples
WBC ≥1,7x10^9/L	0.88 (0.66-0.97)	0.89 (0.76-0.96)	0.105 (0.04-0.24) 4	0.12 (0.03-0.34) 2
WBC ≥3,0x10^9/L	0.90 (0.6-0.98)	0.78 (0.64-0.87)	0.22 (0.12-0.36) 10	0.1 (0.02-0.4) 1
WBC ≥10x10^9/L	1 (0.65-1.0)	0.75 (0.61-0.85)	0.25 (0.15-0.39) 12	0.0 (0.0-0.35) 0
WBC ≥1,7x10^9/L and PMN ≥65%	1.0 (0.81-1.0)	0.73 (0.60-0.83)	0.270.17-0.40) 15	0.0 (0.0-0.19) 0
WBC ≥3,0x10^9/L and PMN ≥80%	1.0 (0.72-1.0)	0.66(0.54-0.77)	0.34 (0.23-0.46) 21	0.0 (0.0-0.27) 0
WBC ≥10x10^9/L and PMN ≥90%	1.0 (0.57-1.0)	0.61 (0.49-0.72)	0.39 (0.28-0.51) 26	0.0 (0.0-0.43) 0
Diagnostic cut-off (8,12)	75 leukocyte/µL			
	Sensitivity (95% CI)	Specificity (95%CI)	False positive (95%CI) N. samples	False negative (95%CI) N. samples
WBC ≥1,7x10^9/L	1.00 (0.82-1.00)	0.45 (0.30-0.60)	0.55 (0.4-0.7) 21	0 (0.0-0.18) 0
WBC ≥3,0x10^9/L	1.00 (0.72-1.0)	0.38 (0.25-0.52)	0.62 (0.47-0.75) 28	0.0 (0.0-0.28) 0
WBC ≥10x10^9/L	1.00 (0.65-1.0)	0.35 (0.23-0.50)	0.65 (0.50-0.77) 31	0.0 (0.0-0.35) 0
WBC ≥1,7x10^9/L and PMN ≥65%	1.00 (0.81-1.0)	0.34 (0.23-0.47)	0.66 (0.53-0.77) 37	0.0 (0.0-0.19) 0
WBC ≥3,0x10^9/L and PMN ≥80%	1.00 (0.72-1.0)	0.30 (0.20-0.43)	0.69 (0.57-0.79) 43	0.0 (0.0-0.28) 0
WBC ≥10x10^9/L and PMN ≥90%	1.00 (0.57-1.0)	0.28 (0.19-0.40)	0.72 (0.6-0.81) 48	0.0 (0.0-0.43) 0

CI, Confidence Interval; WBC, white blood cells; PMN, polymorphonuclear leukocytes.

Although our data overall show that various semi-quantitative leukocyte esterase values (i.e., between 75-500 leukocyte/µL) may have a low diagnostic sensitivity of some utility, the diagnostic specificity compared with automated leukocyte counting varies considerably and appears to be inadequate. Assessment of leukocyte esterase with urine test strips provides only marginally useful clinical information when the leukocyte count is 500/µL; when the cell count is 250 leukocyte/µL, the information obtained is clinically useless. Further research is needed to meet clinical needs and to introduce a usable test for measuring leukocyte esterase in SF. A point-of-care device specifically designed for SF would be very attractive.

Our study has some shortcomings. For example, we included a limited number of samples. Although sample size is known to affect the diagnostic accuracy of the test, our study should stimulate debate among professionals about an indication that has not been based on sound methodology to the best of our knowledge.

CONCLUSIONS

Automated SF leukocyte counting performed with validated haematology analyser provides a reliable estimate of leukocyte count in SF and appears to be suitable as minor criterion. Clinical laboratories collaborating with orthopaedic departments may consider implementing the procedure and organise the workflow and TAT according to clinical needs. Given the biological plausibility of esterase measurement as an indicator of joint infection severity, the need to develop a specific rapid test must be emphasised. In the meantime, rigorous validation studies of urine devices would allow a better definition of biases, interferences, and all the other analytical parameters needed to determine the diagnostic accuracy of the test.

CONFLICT OF INTEREST

None

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