■ ARTHROPLASTY

Diagnosis of prosthetic joint infection with alpha-defensin using a lateral flow device

A MULTICENTRE STUDY

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Aims

The purpose of this current multicentre study is to analyse the presence of alpha-defensin proteins in synovial fluid using the Synovasure lateral flow device and to determine its diagnostic reliability and accuracy compared with the prosthetic joint infection (PJI) criteria produced by the Musculoskeletal Infection Society (MSIS).

Patients and Methods

A cohort of 121 patients comprising 85 total knee arthroplasties and 36 total hip arthroplasties was prospectively evaluated between May 2015 and June 2016 in three different orthopaedic centres. The tests were performed on patients with a chronically painful prosthesis undergoing a joint aspiration in a diagnostic pathway or during revision surgery.

Results

Based on the MSIS criteria, 34 patients (28%) would have had a PJI, and 87 patients had no PJI. Testing with the lateral flow device had a sensitivity of 97.1% (95% confidence intervals (CI) 84.5 to 99.9) and a specificity of 96.6% (95% CI 90.3 to 99.2). The positive predictive value was 91.7% (95% CI 77.7% to 98.3), and the negative predictive value was 98.8% (95% CI 93.6 to 99.9). Receiver operator characteristics analysis demonstrated an area under the curve for the Synovasure test of 0.97 (95% CI 0.93 to 1.00).

Conclusion

Our findings suggest that the Synovasure test has an excellent diagnostic performance to confirm or reject the diagnosis of a PJI. The results are promising for the care of the painful or problematic knee and hip joint arthroplasty and the test should be considered as part of the diagnostic toolbox for PJIs.

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The development of a prosthetic joint infection (PJI) is a devastating and expensive complication associated with total joint arthroplasties.[1,2] It is responsible for 25.4% of early revisions and 7.8% of late revisions in total knee arthroplasty (TKA)[3] and for 15.6% of revisions in total hip arthroplasty (THA).[4] The incidence of PJI is rising as the number of primary arthroplasties increases.[5] Furthermore the diagnosis and appropriate treatment modalities remain a challenge.[6-8]

Currently, diagnostics tests used for the diagnosis of PJI are the C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), synovial fluid white blood cell count (SF-WBC), synovial fluid polymorphonuclear cell differential (SF-PMN), synovial fluid culture, and histopathology but none of these could be considered reliably predictive on its own merit.[9-14]

For this reason the Musculoskeletal Infection Society (MSIS) published a consensus statement in 2011 in order to help define the diagnosis of PJI.[15] The algorithm was revised in 2013.[16]

The ideal test for diagnosing PJI would be highly sensitive, specific, easy to perform and would provide results which can be readily interpreted. Evaluating the presence of synovial fluid alpha-defensins is promising as shown in recent literature.[17-20] This peptide is produced by polymorphonuclear cells as a part of the innate immunity in response to bacterial pathogens.[21] Reported results are produced by the use of a quantitative analysis with immunoassay in a laboratory setting after centrifugation of the synovial fluid.[17-20] Recently a lateral flow test kit (Synovasure PJI lateral flow; Zimmer Biomet, Warsaw, Indiana) to be used as part

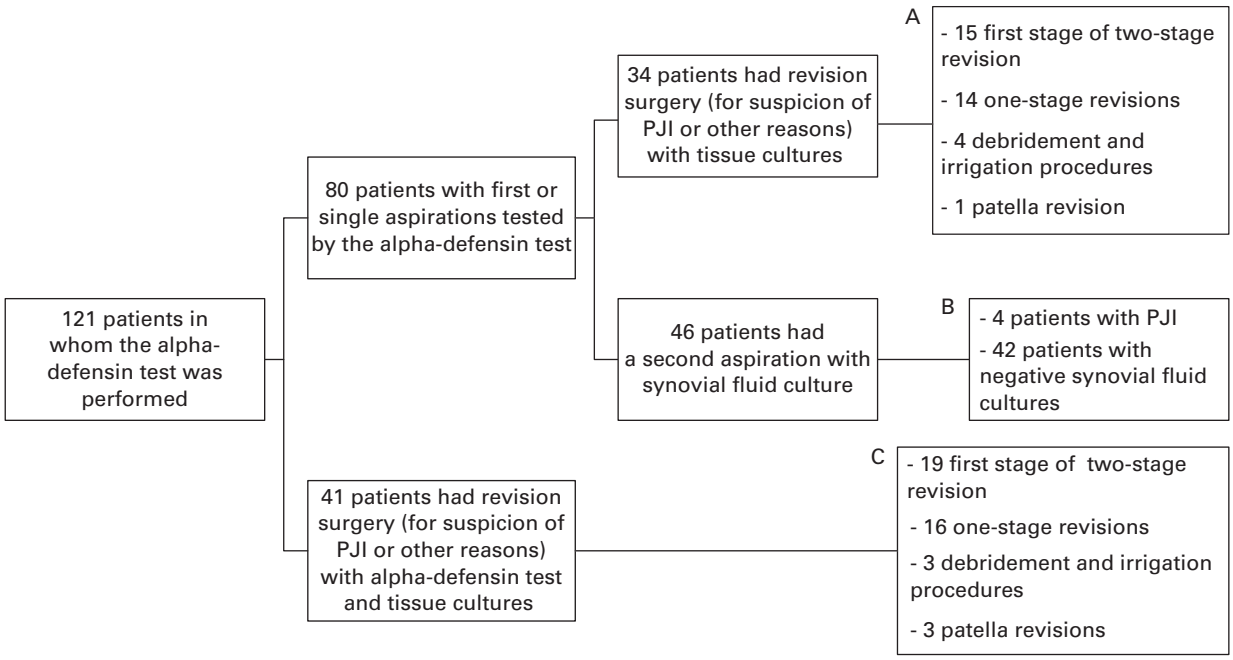

Fig. 1

Flowchart demonstrating how the synovial fluid for alpha-defensin testing was acquired. Boxes A and C present which types of revision surgery were performed. Box B indicates the outcome of the 46 patients who had only cultures from two synovial fluid aspirations (PJI, prosthetic joint infection).

of the pre-operative investigations or during surgery, has been made commercially available.

The aim of this prospective multicentre study was to analyse the diagnostic accuracy and reliability of the Synovasure lateral flow device in comparison with the MSIS criteria. A second goal was to match the results of this assay with other diagnostic tests.

Patients and Methods

This study was conducted in three different hospital facilities. Approval was obtained by the institutional ethical review board in each centre. Inclusion criteria were: a chronic painful total knee or hip prosthesis, symptoms present more than four weeks after trauma or surgery, adequate volume of collected available synovial fluid and sufficient clinical data to perform the lateral flow and other lab tests and to determine the MSIS criteria. Patients with inflammatory conditions, metallosis or recent antibiotic use were not excluded. Exclusion criteria were aspirations of native joints, uni-compartmental arthroplasties and spacers.

The synovial fluid was obtained by a joint aspiration or by fluid collection during surgery. The Synovasure test was routinely carried out in the operating room. All staff involved in handling collector vials and test kits had been adequately trained.

If the Synovasure test was used during revision surgery, the surgeon's intention-to-treat was recorded before the test results were made available. Allowance was made for the surgeon to change the operative plan if desired.

A total of 121 patients (40 in two centres and 41 in the other centre) fulfilled the inclusion criteria for the study. The mean age of the included patients was 63.5 years (36 to 88). In 85 patients the implant involved was a TKA and in 36 patients a THA.

All tests were performed between May 2015 and June 2016 (Fig. 1). In 80 patients, a joint aspiration was performed. In the other 41 patients, the synovial fluid was collected during revision surgery. The latter patients had already undergone aspiration during pre-operative investigation but without use of the alpha-defensin test. Of these, 19 patients had a one-stage revision, 16 patients the first stage of a two-stage revision. The other six patients had a revision of the patellar button (three patients) or a debridement and irrigation procedure (D&I) (three patients). Specimens were collected from TKAs with needle aspiration or aspiration before arthrotomy. In THA the specimen was obtained with image intensifier guided aspiration or aspiration through the joint capsule before arthrotomy to avoid blood soiling or contamination by skin flora. The synovial fluid was deposited in the dedicated receiver and a microsafe tube was filled with 15 µl of fluid by dipping it in the receiver. This fluid was added to the dilution buffer. The dilution of synovial fluid and buffer was finalised by shaking the bottle. Finally, three drops of the diluted synovial fluid were applied to the test cassette. The result was read between ten and 20 minutes after deposition of the drops. The test was positive when the control line and the alpha-defensin line were both visible. Even the slightest line was

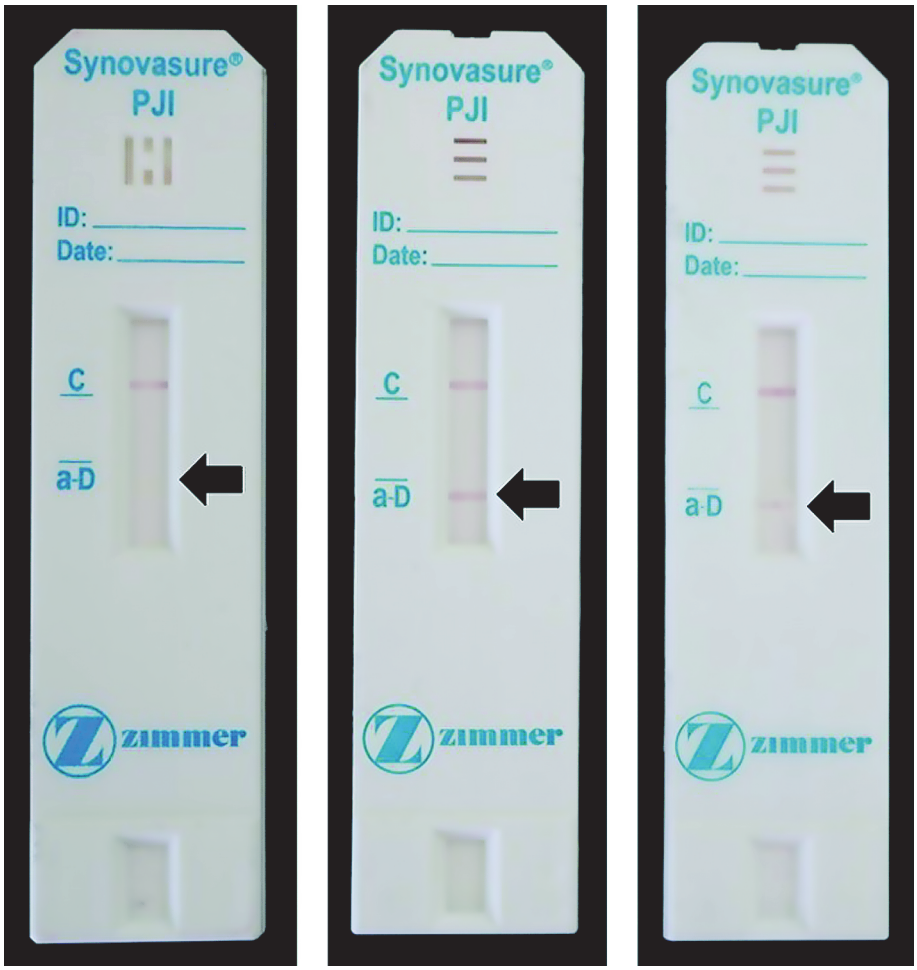

Fig. 2

Three test results are shown: negative test result (left); positive test with a clear test-line comparable with the control-line (middle); colour intensity of test-line significantly less than that of control-line. Nevertheless, positive test result because the two lines did appear (right).

considered representative. A negative test result was confirmed when only the control line was present (Fig. 2).

Whenever possible, three to six microbiological cultures of synovial tissue were collected at the time of surgery (75 patients). Of the 80 patients who underwent aspiration, 34 underwent further surgery at follow-up (15 first stages of a two-stage revision, 14 one-stage revisions, four D&I and one revision of the patellar component). The 46 patients who did not undergo secondary surgery, had a repeat aspiration for culture of the synovial fluid (Fig. 1). Ultimately four of these patients had positive cultures with identical micro-organisms in both synovial fluid cultures. Despite the presence of a PJI they were deemed unfit for further surgery: one patient died, the three other patients were treated with suppressive antibiotics and monitored by regular follow-up visits.

For 26 patients no distinct cause of the chronic painful prosthesis could be determined. These patients are monitored by regular follow-up visits. One patient underwent a percutaneous release of the medial collateral ligament. Of the remaining 15 patients, 11 patients had a chronic instability, one patient had an aseptic loosening and three patients had an avascular necrosis of the patella. These patients declined further surgery. All microbiological cultures were cultivated for a period of 14 days.

The diagnosis of infection was made based on the MSIS definition of PJI.[15] An experienced pathologist specialised in musculoskeletal diseases was not available during surgery in two of the three centres for frozen section investigation. Therefore, we were obliged to modify the diagnostic requirement for PJI by MSIS criteria: a patient was deemed infected when at least one of the two major criteria was positive or a minimum of three of the five minor criteria were positive (Table I). To determine these modified MSIS criteria all 121 patients had a complete set of CRP, ESR, SF-WBC and SF-PMN. The presence or absence of pus was documented in all cases (aspiration or surgery).

Following the modified MSIS criteria, 34 patients (28.1%) were diagnosed with PJI whereas 87 patients were not. The 32 infected patients had positive cultures

Table I. The modified Musculoskeletal Infection Society (MSIS) criteria

	Criteria
Major criteria	Two positive periprosthetic cultures with phenotypically identical organisms
	A sinus tract communicating with the joint
Minor criteria	Elevated serum C-reactive protein (> 10 mg/L) or erythrocyte sedimentation rate (> 30 mm/hr)
	Elevated synovial fluid white blood cell (> 3000 cells/μl) count
	Elevated synovial fluid polymorphonuclear neutrophil percentage (> 80%)
	Purulence in the affected joint
	A single positive culture

Table II. The microbiological data from the 34 infected cases according to the Musculoskeletal Infection Society (MSIS) criteria. In five cases two or more species were grown from the tissue cultures

Micro-organism	n
MRSE	13
MRSA	1
MSSA	2
MSSE	4
Enterococcus faecalis	4
Escherichia coli	2
Streptococcus agalactiae	4
Pseudomonas aeruginosa	1
Klebsiella pneumoniae	2
Propionibacterium acnes	2
Corynebacteria	1
Culture negative PJI	2

MRSE, methicillin resistant *staphylococcus epidermidis*; MRSA, methicillin resistant *staphylococcus aureus*; MSSA, methicillin sensitive *staphylococcus aureus*; MSSE, methicillin sensitive *staphylococcus epidermidis*; PJI, prosthetic joint infection

Table III. The results of the Synovasure test with the Musculoskeletal Infection Society (MSIS) criteria. There was one false-negative result and three false-positive results

MSIS criteria Synovasure test	Positive	Negative	Total
Positive	33	3	36
Negative	1	84	85
Total	34	87	121

suggestive for PJI, and two patients were culture negative for PJI despite meeting the modified MSIS criteria. The microbiological data are shown in Table II.

The results of the Synovasure test, ESR, CRP, combined ESR and CRP, SF-WBC, SF-PMN and the culture of the aspirated fluid were compared with the modified MSIS criteria for PJI. The intention-to-treat decision of the surgeon in charge was compared with the MSIS criteria and the Synovasure test result.

Statistical analysis. Statistical power analysis aided us in determining that a series of 40 patients would be required in each centre. Statistical analysis was performed by using SPSS Statistics 24 software (IBM Corp., Armonk, New York). The sensitivity, specificity, overall diagnostic accuracy, positive and negative predictive values, positive likelihood ratio (LR+) and negative likelihood ratio (LR-), receiver operator curves (ROCs) and the area under the curve (AUC) were calculated for the alpha-defensin test using the modified MSIS criteria as the reference standard. For ROCs we obtained the AUC which describes the discriminative ability of a diagnostic test. Statistical significance of AUC analysis has been reached if the area of the calculated test is significantly different to 0.5. The sensitivity, specificity, predictive values and likelihood ratios of the alpha-defensin test were compared with synovial fluid cultures, SF-WBC, SF-PMN, ESR, CRP, combined ESR and CRP with the McNemar's chi-squared test. A p-value < 0.05 was considered significant.

Results

The Synovasure test showed a sensitivity of 97.1% (95% confidence interval (CI) 85.7 to 99.9) and a specificity of 96.5% (95% CI 90.3 to 99.2). The positive predictive value was 91.7% (95% CI 77.7 to 98.3), and the negative predictive value was 98.8% (95% CI 93.6 to 99.9). The LR+ was 28.2 (95% CI 9.2 to 85.7). The LR- was 0.03

Table IV. The sensitivity and specificity results. Combination of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) resulted in a positive test if either one of both was elevated. The p-value shows statistical significance compared with the Synovasure test (McNemar's chi-squared test)

Results	Sensitivity (%) (95% CI)	p-value	Specificity (%) (95% CI)	p-value
Synovasure	*97.1* (84.7 to 99.9)		*96.6* (90.2 to 99.3)	
CRP	*70.6* (52.2 to 84.9)	< 0.001	*81.6* (71,9 to 89.1)	< 0.001
ESR	*47.6* (25.7 to 70.2)	< 0.001	*82.8* (70.6 to 91.4)	0.002
CRP/ESR	*75.0* (50.9 to 91.3)	0.016	*77.2* (64.2 to 87.3)	< 0.001
SF-WBC	*89.3* (71.8 to 97.7)	0.25	*96.2* (89.4 to 99.2)	1
SF-PMN	*89.3* (71.8 to 97.7)	0.062	*92.2* (82.7 to 97.4)	0.031
Culture	*82.4* (65.5 to 93.2)	0.004	*98.8* (93.8 to 99.9)	0.5

SF-WBC, synovial fluid white blood cell count; SF-PMN, synovial fluidpolymorphonuclear neutrophil percentage; CI, confidence intervals

Table V. Overall diagnostic accuracy, predictive values and likelihood ratios of the different tests

Results (%) (95% CI)	ACC	PPV	NPV	LR+	LR-
Synovasure	*96.7* (82.5 to 98.7)	*91.7* (77.5 to 98.3)	*98.8* (93.6 to 100)	*28.2* (9.2 to 85.7)	*0.03* (0 to 0.2)
CRP	*78.5* (65.7 to 89.3)	*60* (43.3 to 75.1)	*87.6* (78.5 to 93.9)	*3.8* (2.3 to 6.3)	*0.4* (0.2 to 0.6)
ESR	*73.4* (54.5 to 85.3)	*50* (27.2 to 72.8)	*81.4* (69.1 to 90.3)	*2.8* (1.3 to 5.7)	*0.6* (0.4 to 1.0)
CRP/ESR	*76.6* (55 to 88.3)	*53.6* (40.2 to 66.5)	*89.8* (80.3 to 95.0)	*3.3* (1.9 to 5.7)	*0.3* (0.2 to 0.7)
SF-WBC	*94.4* (83.5 to 98.3)	*89.3* (71.8 to 97.7)	*96.3* (89.4 to 99.2)	*23.8* (7.8 to 72.8)	*0.1* (0.04 to 0.3%)
SF-PMN	*91.3* (81.2 to 97.7)	*83.3* (65.3 to 94.4)	*95.2* (86.5 to 99.0)	*11.4* (4.9 to 26.8)	*0.1* (0.04 to 0.3)
Culture	*94.2* (82.8 to 97.9)	*96.6* (82.2 to 99.9)	*93.5* (86.3 to 97.6)	*71.6* (10.1 to 506.1)	*0.2* (0 to 0.4)

CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; SF-WBC, synovial fluid white blood cell count; SF-PMN, synovial fluidpolymorphonuclear neutrophil percentage; ACC, overall diagnostic accuracy; PPV, positive predictive value; NPV, negative predictive value; LR +, positive likelihood ratio; LR-, negative likelihood ratio

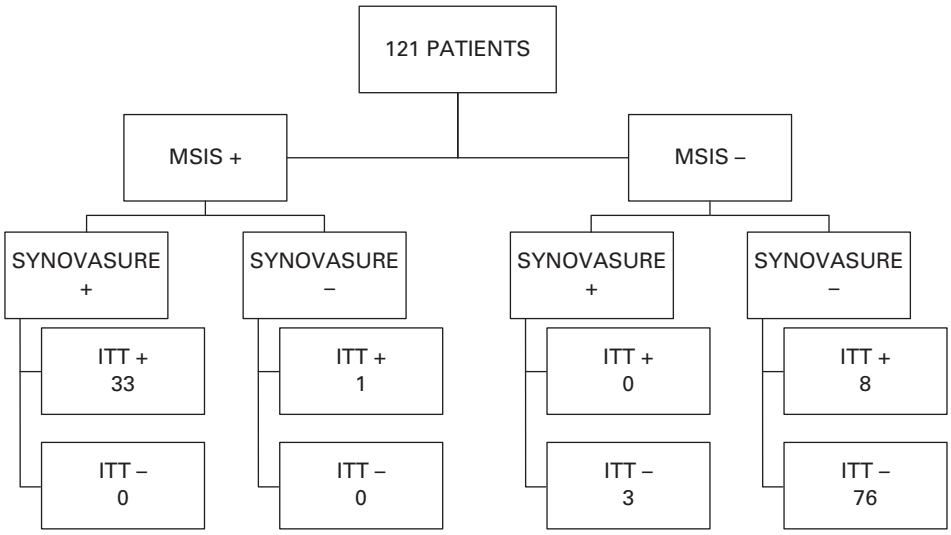

Fig. 3

Musculoskeletal Infection Society (MSIS) criteria compared with intention-to-treat (ITT) decision and Synovasure result (+, treat as an infection; -, treat as non-infected).

(95% CI 0.0 to 0.21). The test correctly identified 33 of the 34 infected patients and 84 of 87 as not infected. In one case, it showed a false-negative result, and in three cases the test was false-positive (Table III). One of these patients with a false-positive result had a metal-on-metal THA. ROC analysis demonstrated an AUC for the Synovasure test of 0.97 (95% CI 0.93 to 1.00) ($p < 0.001$).

The results of the different tests are presented in Tables IV and V. The only test that showed a similar sensitivityand specificity to the Synovasure test was the SF-WBC ($p = 0.25$ and $p = 1$, respectively).

Regarding the analysis of the intention-to-treat by the surgeon we found 12 mismatches with the result of the Synovasure test (Fig. 3). In eight cases the Synovasure test cor-

rectly predicted the presence or absence of infection whilst the surgeon thought differently, whereas the clinical suspicion of the surgeon was correct in four mismatch cases.

Discussion

Diagnosing a PJI remains a challenge in patients with a chronically painful knee or hip joint arthroplasty and a correct diagnosis is of the utmost importance. Our findings demonstrate that the Synovasure lateral flow test has a high diagnostic performance.

To our knowledge there are two other studies evaluating the Synovasure lateral flow test as such. Kasparek et al[22] found that the test was less discriminating with a sensitivity of 67% (95% CI 35 to 89) and a specificity of 93% (95% CI 75 to 99) clearly not backing up previously reported laboratory studies.[17-20] However this particular study had a relatively small group of 40 patients. The authors stated that a possible reason for false results could be the contamination of the sample with blood and cellular debris. They concluded that centrifugation of the samples in combination with laboratory immune assays could possibly be more accurate than the Synovasure lateral flow test. Our superior results for the Synovasure test were obtained without centrifugation of the synovial fluid.

In another single centre study (49 patients) Sigmund et al[23] found a sensitivity of 69% (95% CI 46 to 92) and a specificity of 94% (95% CI 86 to 100) in line with the results of Kasparek et al.[22] A possible explanation for the higher rate of false negative results (four patients) is the inclusion of 15 cases (30% of the study population) with a spacer *in situ* (11 re-implantations at second-stage revision and four spacer exchanges). Presence of a spacer in the studied joint was an exclusion criterion in our series because we believe that the inflammatory state post-operatively can influence the test results. Likewise, the MSIS criteria were not developed for evaluation of joints with a spacer. Other causes for this difference could be the testing of patients with an arthroplasty at a location different from hip or knee (one total elbow arthroplasty, one total shoulder arthroplasty, one total femoral arthroplasty) or the higher proportion of THAs (30 patients) than TKAs (17 patients). We only included patients with a THA (36 patients) or a TKA (85 patients). We consider it more difficult to acquire adequate amounts of synovial fluid without blood contamination during needle aspiration of a THA. Lastly the authors used a different modification of the MSIS criteria making a thorough comparison with our series difficult.

In addition, in regards to both these papers[22,23] we also postulate that in our three centres the alpha-defensin test was conducted by thoroughly trained medical staff in a standardised fashion. Moreover, according to manufacturer's instructions, blood contamination of the synovial fluid could indeed potentially influence the result due to dilution of the alpha-defensin synovial fluid level. For the same reason, centrifugal treatment of the sample is not allowed because of dilution of the synovial fluid with haematogenous cellular fluid and plasma.

A survey of the present literature revealed promising results of alpha-defensin testing as a screening tool comparable with the MSIS criteria.[17,19] Furthermore, alpha-defensin testing appears to provide consistent results, regardless of the type and virulence of the responsible micro-organism and test result does not seem to be influenced by preceding administration of antibiotics to the patient.[24,25] In the past, other lab tests including ESR, CRP, SF-WBC, SF-PMN and cultures have failed to achieve this.[9-13]

Our results are encouraging given the potential usage as a reliable and accurate single screening test because of equivalent sensitivity and specificity compared with the MSIS criteria. Apart from the SF-WBC, the Synovasure test out-performed our other diagnostic tests.

SF-WBC testing presents some difficulties: first, there is no consensus in the literature concerning the optimal cut-off values.[9,10,15,16] Secondly, it incurs a delay while the specimen is transported to the laboratory and the investigation is performed. Thirdly, larger volumes of synovial fluid are generally required. However, the commercial price of the Synovasure test is currently relatively high in comparison with other diagnostic tests. The mean price in Europe is around $300. Economical cost-to-benefit analysis is obviously required.

In this study the surgeon in charge was asked to use available clinical data to assess whether the prosthesis was infected or not and to clarify his/her intention-to-treat preoperatively, before assessing biofilm and before the Synovasure test results were made available. However, throughout the study the surgeons did not change their surgical plan when the result of the Synovasure test was revealed.

Following this, seven patients incorrectly underwent two-stage revision and one patient a D&I because of clinical suspicion of PJI which could have been avoided if the Synovasure test result was believed. In one patient, the Synovasure test was a false-negative in the presence of an infection which was correctly presumed by the treating surgeon. Other studies have shown however that clinical decision-making is not very reliable.[26] Based on this information we judge the Synovasure test as potentially valuable to guide treatment options.

A limitation of this study is the lack of quantitative measurements of alpha-defensin levels preventing any comparison with studies using previously reported laboratory assays. Although 75 of the 121 patients had surgery with collection of tissue cultures, the other 46 patients only had two independent synovial fluid cultures obtained. This is a second limitation because the MSIS criteria ideally require collection of three to six tissue cultures. Another limiting factor is the lack of histopathology to define these criteria. Therefore we preferred to retain the minor criterion of purulence around the prosthesis which was eliminated in a reassessment of the MSIS criteria in 2013.[16] We note Bingham et al[19] also preferred to retain this criterion because of the same problem. A final weakness is that

patients who had antibiotic treatment were not excluded, possibly influencing results of SF-WBC, SF-PMN and cultures. This could possibly result in more false-positive results. However, the three patients with a false-positive test in our series were not on antibiotics during the collection of the data. Elsewhere Shahi et al[25] provided ample evidence that the alpha-defensin test results by quantitative analysis were not influenced by the use of antibiotics beforehand.

We conclude that our multicentre study confirms that the Synovasure lateral flow test has a promising diagnostic potential as a screening tool but also during surgery. These results are promising for the care of the painful or problematic knee and hip joint arthroplasty but given the high cost of this test at face value, further economic analysis is necessary to evaluate cost-effectiveness.

Take home message:

- The Synovasure lateral flow test is an appropriate tool to confirm or reject the diagnosis of periprosthetic infection.
- Alpha-defensin outperforms other diagnostic tests in this multicentre trial.

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P. Berger: Contribution to design of research protocol, Data collection, Paper writing, Paper editing.
M. Van Cauter: Data collection.
R. Driesen: Contribution to design of research protocol, Data collection.
J. Neyt: Contribution to design of research protocol, Data collection, Approval of submitted and reviewed paper.
J. Bellemans: Data collection.

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