Inflammatory disorders mimicking periprosthetic joint infections may result in false positive -defensin

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Abstract: OBJECTIVES The antimicrobial peptide -defensin has recently been introduced as potential "single" biomarker with a high sensitivity and specificity for the preoperative diagnosis of periprosthetic joint infections (PJIs). However, most studies assessed the benefits of the test with exclusion of patients with rheumatic diseases. We aimed to evaluate the -defensin test in a cohort study without exclusion of cases with inflammatory diseases. METHODS Between June 2016 and June 2017, we prospectively included cases with a suspected PJI and an available lateral flow test -defensin (Synovasure®) in synovial fluid. We compared the test result to the diagnostic criteria for PJIs published by an International Consensus Group in 2013. RESULTS We included 109 cases (49 hips, 60 knees) in which preoperative -defensin tests had been performed. Thereof, 20 PJIs (16 hips, 4 knees) were diagnosed. Preoperative -defensin tests were positive in 25 cases (22.9%) with a test sensitivity and specificity of 90% and 92.1% (95% confidence interval [CI], 68.3 - 98.8% and 84.5 - 96.8%, respectively), and a high negative predictive value of 97.6% (95% CI, 91.7 - 99.4%). We interpreted seven -defensin tests as false positive, mainly in cases with inflammatory rheumatic diseases, including crystal deposition diseases. CONCLUSIONS A negative synovial -defensin test can reliably rule out a PJI. However, the test can be false positive in conjunction with an underlying non-infectious inflammatory disease. We therefore propose to use the -defensin test only in addition to MSIS criteria and assessment for crystals in synovial aspirates.

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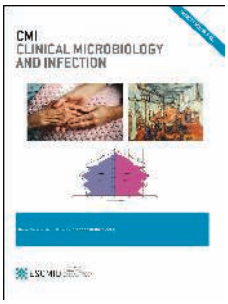

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Inflammatory disorders mimicking periprosthetic joint infections may result in false positive *α-defensin*

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Abstract

Objectives: The antimicrobial peptide *α-defensin* has recently been introduced as potential "single" biomarker with a high sensitivity and specificity for the preoperative diagnosis of periprosthetic joint infections (PJIs). However, most studies assessed the benefits of the test with exclusion of patients with rheumatic diseases. We aimed to evaluate the *α-defensin* test in a cohort study without exclusion of cases with inflammatory diseases.

Methods: Between June 2016 and June 2017, we prospectively included cases with a suspected PJI and an available lateral flow test *α-defensin* (Synovasure®) in synovial fluid. We compared the test result to the diagnostic criteria for PJIs published by an International Consensus Group in 2013.

Results: We included 109 cases (49 hips, 60 knees) in which preoperative *α-defensin* tests had been performed. Thereof, 20 PJIs (16 hips, 4 knees) were diagnosed. Preoperative *α-defensin* tests were positive in 25 cases (22.9%) with a test sensitivity and specificity of 90% and 92.1% (95% confidence interval [CI], 68.3 - 98.8% and 84.5 - 96.8%, respectively), and a high negative predictive value of 97.6% (95% CI, 91.7 - 99.4%). We interpreted seven *α-defensin* tests as false positive, mainly in cases with inflammatory rheumatic diseases, including crystal deposition diseases.

Conclusions: A negative synovial *α-defensin* test can reliably rule out a PJI. However, the test can be false positive in conjunction with an underlying non-infectious inflammatory disease. We therefore propose to use the α-defensin test only in addition to MSIS criteria and assessment for crystals in synovial aspirates.

Introduction

Successful treatment of periprosthetic joint infections (PJIs) requires an early and correct diagnosis of the infection. However, distinguishing between an infection and other causes of joint pain is often challenging. Currently, the PJI diagnosis is mainly based on preoperative and intraoperative diagnostic criteria of either the Infectious Diseases Society of America (IDSA) [1] or the Musculoskeletal Infection Society (MSIS) [2, 3]. According to these criteria, a PJI is preoperatively suspected when both serum (CRP, ESR) and synovial parameters (leucocytes, neutrophils) are elevated and/or a single positive microbiological culture is found [4]. Thus, one single biomarker allowing a high accuracy for diagnosing or excluding an infection during the preoperative work-up would be a major improvement.

The antimicrobial peptide *α-defensin* is released into the synovial fluid by neutrophil granulocytes, macrophages, and epithelial cells in response to microbial products or pro-inflammatory cytokines [5, 6] and has therefore been considered as a reliable and accurate biomarker for identifying an infection [7-11]. The point of care test "Synovasure®", sold as lateral flow test kit, appears to be a fast and easy-to-handle test, providing *α-defensin* test results within 10 minutes (www.cddiagnostics.com, 28.05.2016) [12].

In this study, we evaluated the role and reliability of the lateral flow *α-defensin* test for the diagnosis of PJI in a prospective study cohort, by comparing the results of the *α-defensin* tests with the final diagnosis obtained by using the MSIS criteria [2, 3]. Unlike most of the studies assessing the benefits of the *α-defensin* assay so far, we did not exclude cases with inflammatory rheumatic diseases aiming for a complete assessment of the test in a real-life orthopedic population.

Materials and Methods

Study design and population

We conducted a prospective study at the Orthopedic University Hospital Balgrist between June 2016 and June 2017. Clinical and epidemiological patient data were collected from the prospective database of the infectious diseases consulting service as well as from the hospital clinical information system.

We included all cases with either clinical signs suspicious for a hip or knee PJI or unexplained joint pain who received a preoperative diagnostic work-up. In these cases, we performed the serum CRP, ESR, and leucocyte counts, and synovial aspirates with leucocyte count, neutrophil granulocyte percentage, synovial CRP, microbiological culture, and an evaluation of crystal deposits. Additionally, the lateral flow *α-defensin* test (Synovasure®, Zimmer Biomet, Winterthur, Switzerland) was performed in all cases.

Cases were excluded from this study if the *α-defensin* test could not be performed either due to a medical emergency, lack of personnel trained to perform the test, or an insufficient amount of synovial aspirate available. Furthermore, we excluded cases who were treated with antibiotics for more than two weeks prior to the diagnostic work-up. In case of hemorrhagic synovial aspirates, *α-defensin* was not measured in order to avoid potential false positive results [12]. Underlying diseases such as rheumatic diseases or neoplasia were not an exclusion criterion. Patients could participate more than once if the diagnostic procedures were performed independently and were part of two completely separate incidences.

All the routine diagnostic results, except for the *α-defensin* test, were evaluated both pre- and intraoperative by two Infectious Diseases study investigators (Y.A and L.S) according to criteria of the MSIS guidelines revised at the consensus meeting in 2013. [2, 3] (Table 1). After the preoperative MSIS criteria evaluation,

cases were classified into three categories: i) PJI (sinus tract, ≥ two positive cultures with the same organism, or ≥ three minor criteria fulfilled), ii) no suspected PJI (no major criteria and less than three minor criteria), or iii) undetermined cases (incomplete preoperative diagnostics with non-interpretable synovial leucocytes due to hemolytic reaction). Final diagnosis according to the MSIS criteria was then compared to the results of the initial preoperative classification.

The lateral-flow *α-defensin* test was performed in the radiology department following the manufacturer's recommendations. We then compared the *α-defensin* test results to the final MSIS assessment. In a subgroup of cases with a subsequent septic surgery, the *α-defensin* test was repeated on the intraoperatively obtained synovial fluid aspirates. In addition, routine intraoperative diagnostics were performed and included ≥ 3 tissue biopsies, sonication fluid for microbiology, and histopathology for evaluation of tissue inflammation. The *α-defensin* test results were blinded for both the treating orthopedic and infectious diseases teams.

Microbiological evaluation and α-defensin *test*

Microbiological techniques and standard biochemical methods for the detection and identification of microorganism were performed as previously described [13]. The incubation time was seven days for synovial and sonication fluid and 10 days for tissue biopsies with a blind subculture of thioglycolate broth for another 2 to 4 days (final cultivation time of 12-14 days).

Ethics

The institutional review board of Zurich, Switzerland approved the study protocol (Kantonale Ethikkommission Number 2015-0357), and all patients signed a study specific informed consent.

Statistical analysis

The results of the *α-defensin* test were reported as either positive or negative. The sensitivity, specificity, positive and negative predictive value of the α-*defensin* test were correlated to the categorization based on the revised MSIS criteria published in 2013, which served as reference standard for the PJI diagnosis and are shown in Table 1 [2].

RESULTS

We initially evaluated 149 cases (72 hip, 77 knee) which occurred in 148 cases with available preoperative synovial fluid aspirates. Thereof, 40 cases had to be excluded from our analysis, resulting in a final number of 109 cases (49 hip and 60 knee) (Figure 1). One patient had two independent occurrences of PJI episodes. They occurred four months apart and their diagnostic work-ups did not interfere with the aim and the exclusion criteria of this study.

Case characteristics and standard diagnostic findings

The median age at the time of the first diagnostic evaluation was 68 years in the 49 hip cases (range 41-88) and 63 years in the 60 knee cases (range 48-85). Diagnostic characteristics of serum and synovial parameters of all included cases are summarized in Table 2. In addition to the preoperative MSIS-based diagnostics, we assessed the presence of crystals in the obtained synovial fluids. We found calcium pyrophosphate crystal deposits in the aspirates of one hip and eight knees, cholesterol crystals in the aspirate of one hip, and hydroxyapatite crystals in the aspirate of one knee.

Based on the preoperative diagnostic findings, we identified 18 cases as 'suspected PJIs', 87 cases as 'no suspected PJIs', and four cases as 'undetermined cases'. In 17 out of the 18 cases with preoperatively diagnosed PJI, infection was confirmed intraoperatively by either positive microbiological cultures or documentation of an acute inflammation in histopathology. One case diagnosed with a definitive PJI based on the presence of a sinus tract refused any further surgery and was treated with suppressive antibiotics.

Of the 87 cases with no suspected PJI's, a revision surgery was performed in 46 cases, mainly due to suspected aseptic loosening, arthrofibrosis, instability, ossifications, periprosthetic fracture, or metallosis. In one out of these 46 cases, a PJI due to coagulase-negative staphylococci (CNS) was diagnosed intraoperatively, which had been missed preoperatively. In the remaining 41 cases without surgery and therefore no intraoperative diagnostics, the final diagnosis was based on preoperative exclusion of a PJI and the favorable clinical course with physical or anti-inflammatory treatment.

Out of the four cases in whom classification into 'PJI' or 'no suspected PJI' was not feasible (lack of synovial leucocyte counts due to cell hemolysis later in the laboratory), three cases received intraoperative diagnostics. In one of these three cases, we diagnosed a PJI based on growth of CNS in the biopsy and sonication cultures. In the two remaining cases, an infection was finally excluded and aseptic loosening of the prosthesis diagnosed. In the one case without intraoperative diagnostics, a complex regional pain syndrome of the knee was diagnosed, which improved with anti-inflammatory treatment.

In summary, MSIS criteria detected 20 PJIs (16 hip, 4 knee) and 89 no PJIs (33 hip, 56 knee) (Figure 1). Infections were mainly caused by coagulase-negative staphylococci (n=7) and *Staphylococcus aureus* (n=3) (Table 3). In the 89 no PJI

cases, the most common cause of pain was aseptic loosening of the prosthesis (n=27), followed by muscular insufficiency (n=25) (Table 3). Preoperative MSIS criteria detected PJIs in 18 out of 19 cases (sensitivity of 94.7%) and excluded an infection in 86 out of 87 cases (specificity of 98.9%) after exclusion of the four cases, which had been initially characterized as 'undetermined cases'.

Interpretation and reliability of the α-defensin *test*

The preoperative *α-defensin* test was positive in 25 of the total 109 cases (22.9%) (17 hip, 8 knee). Of the 20 PJI cases diagnosed based on the MSIS criteria, 18 were correctly detected by the preoperative *α-defensin* test, while two hip PJIs (one case with a sinus tract and a positive *Candida tropicalis* culture, the other case with a positive CNS culture) would have been missed (sensitivity 90%). Among the 89 no PJI cases, a correct negative preoperative *α-defensin* test result was found in 82 cases, while a false positive test result was found in seven cases (specificity 92.1%). Among these, we diagnosed calcium pyrophosphate dehydrate crystal deposition disease (CPPD) (n=2), rheumatoid arthritis (n=1), psoriasis arthropathy with additional diagnosis of hydroxyapatite crystal deposition disease (n=1), aseptic loosening (n=2), and one case with muscular insufficiency *(Table 3 and Appendix S2).*

Based on these findings, we calculated a negative predictive value of 97.6% (82/84, 95% CI, 91.7 to 99.4%), a positive predictive value of 72.0% (18/25, 95% CI, 55.4 to 84.2%), and an accuracy of 91.7% (100/109, 95% CI, 84.9 to 96.2%). A sub-analysis of the cases with preoperative *α-defensin* and intraoperative diagnostics (n=66), showed similar statistical results. In particular, "specificity" and "negative predictive value" reflected the findings of the overall study population (Appendix S1).

Fifteen (22.7%) out of the overall 66 cases with intraoperative standard diagnostics had an additional intraoperative *α-defensin* test. Both sensitivity (77.8%, 7/9) and specificity (50.0%, 3/6) of *α-defensin* was lower than in the preoperative setting.

In general, we found that an increasing synovial leucocyte count went along with an increasing probability of having a positive *α-defensin* test. In one case (knee 10, Appendix S2), suffering from psoriasis arthropathy and hydroxyapatite disease, the *α-defensin* test was negative despite 10'500 synovial leucocytes in the initial preoperative joint aspiration. In the second joint aspiration, a clearly positive *α-defensin* test result and 18'000 synovial leucocytes were found. The increase in synovial leucocytes in the second aspirate went along with a more active rheumatic disease.

Comparison of the positive α-defensin *test results with the synovial CRP values*

In 100 out of 109 cases (91.7%), the synovial CRP was measured in addition for comparison reasons to the *α-defensin* test. As recently published [8], a synovial CRP value of 3 mg/l was considered a suitable threshold allowing to distinguish between a joint infection and other causes of joint pain. Applying this suggested threshold to our hip cases confirmed that in all three false positive *α-defensin* tests, the synovial CRP was 0 mg/l, whereas in all except one of the 11 true positive *α-defensin* tests, the synovial CRP was above 3 mg/l (Table 4). However, among the knee cases, applying the synovial CRP threshold of 3 mg/l could not reliably detect false positive α-*defensin* test results, as in 50% of the knee cases the CRP values were still above the threshold. Furthermore, one case diagnosed as a culture-negative PJI had a low

CRP of 1 mg/l, while in the other two cases, the CRP values were highly elevated with 44.8 and 22.3 mg/l.

Discussion

We found a high negative predictive value of the preoperative *α-defensin* test allowing to exclude a PJI in this prospective study. However, with a specificity of 92.2%, we did not find any additional advantage of a positive *α-defensin* test at the time when preoperative diagnostics was performed as compared to using the established consensus meeting criteria.

By applying the *α-defensin* test, we could rule out a PJI with a high probability if the test result came back negative. We only found two false negative *α-defensin* tests: One case with a *Candida* sp. infection presented with a sinus tract infection where *Candida* sp. should have been detected by the *α-defensin* test [12]. However, *Candida* sp. has been previously described as reason for false-negative *α-defensin* results [14]. The second case presented with a *S. epidermidis* PJI and a large muscular abscess. Previously, a low sensitivity of the *α-defensin* test in infections due to low-grade microorganisms has been described in one publication including 50 patients [15].

Based on the test results in our cohort, we calculated a low specificity of 92.1% for the *α-defensin* test. This is in line with previously published reports, where a low test specificity was mainly described in the context of metallosis or polyethylene wear of the prosthesis components [14], hemolytic blood in the synovial fluid [4], and in one case report with an episode of acute gout [16]. However, most false positive results in our cohort were due to inflammatory diseases, including CPPD, rheumatoid arthritis, and psoriasis arthropathy. The synovial fluid in these cases also showed elevated neutrophils as in PJI. This is the first description of such an influence of inflammatory diseases on the test results of *α-defensin* [12], especially since criteria on crystal deposition or rheumatic diseases are not included in the MSIS algorithm so far.

In three cases with a false positive *α-defensin* test result, an inflammatory disorder as well as a bloody joint aspiration were both ruled out as potential reasons for false positivity. The *α-defensin* test in all these cases was only weak positive, however, the significance of this finding needs further investigation. In case of false positive *α-defensin* tests in synovial aspirates of the hip, we could demonstrate a good correlation to low synovial CRP values (below the recommended cut-off of 3 mg/l [8]), whereas this association could not be shown in synovial aspirates of the knee.

Although only assessed in a small number of cases, the intraoperative *α-defensin* test was inferior as compared to the preoperative *α-defensin* test. This result is in line with a study by Kasparek et al., who demonstrated a low sensitivity of 67% [17]. In one case (Appendix S2, Knee 37) with a “culture negative PJI”, the *α-defensin* result switched from positive preoperatively to negative intraoperatively after two days of antibiotic treatment, even though a previous antibiotic treatment has not been considered a concern so far [12, 18].

A limitation of our study is the small number of preoperative *α-defensin* tests in cases with culture-negative PJI, but also the small number of the different cultivated bacterial microorganisms. Furthermore, we did not yet present data of shoulder PJI in which low-virulent pathogens such as CNS and *Cutibacterium acnes* predominate.

In summary, the lateral flow-test *α-defensin* is a biomarker test with a high negative predictive value, allowing to exclude a PJI. However, due to false positive test results in the presence of underlying inflammatory non-infectious conditions, the medical case history has to be carefully evaluated in order to avoid unnecessary additional interventions. In addition, checking for crystals in synovial aspirates and ruling out an inflammatory rheumatic diseases in routine diagnostic of patients with suspected PJI will help further optimizing patient care.

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Conflict of interest disclosure

This study was an investigator-initiated trial, and only the Synovasure® tests were provided by the company Zimmer Biomet (Synovasure®, Zimmer Biomet, Winterthur, Switzerland). The company had no influence of the study design, in- and exclusion of patients, and study results.

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Tables and Figures

Table 1. MSIS definition of PJI according to the consensus meeting 2013 in Philadelphia [2, 3]. A PJI is confirmed if either 1 major or 3 of 5 minor criteria are fulfilled.

Diagnostics	**Major criteria**	**Minor criteria**
Symptoms	Sinus tract	
Microbiology	Detection of pathogen in ≥ 2 diagnostic materials (aspiration, intraoperative biopsy or sonication).	
Laboratory		ESR > 30 mm/h **and** CRP > 10 mg/l
Joint puncture		Leucocytes in joint aspiration > 3000/µl[i]
Joint puncture		Percentage (%) of neutrophil granulocytes in joint aspiration ≥ 80%
Histopathology		Acute inflammation (neutrophil granulocytes) in periprosthetic tissue
Microbiology		Detection of pathogen in only 1 diagnostic material

One positive culture of the synovial fluid with > 50 CFU/ml (colony forming units / ml) counts as relevant.

Table 2. Diagnostic characteristics of serum and synovial parameters of the included 109 cases (49 hips, 60 knees) for MSIS evaluation, finally diagnosed as 20 PJIs and 89 non-PJIs.

Characteristics	**PJI** **N=20**	**No PJI** **N=89**
Serum laboratory findings		
Leucocytes/µl, median (range)	8.2 (3.7 – 12.3)	7.9 (3.5 – 13.8)
CRP (mg/l), median (range)	33.6 (2.6 – 273.5)	2.7 (0.1 – 216.8)
ESR (mm/h), median (range)	46 (9 – 78)	19.5 (2 – 105)
Synovial fluid		
Leucocytes, N (%)[a]	18 (90)	82 (92.1)
Median (range)	34.650 (700 – 230.600)	200 (0 – 53.000)
Neutrophil granulocytes, N (%)	18 (90%)	37 (41.6)
Ca. 100%	2	1
Ca. 80%	14	3
>50%	2	7
<50%	0	26
Crystal deposits, N (%)	17 (85)	85 (95.5)
+ Calcium pyrophosphate	0	9
+ Hydroxyapatite	0	1
+ Cholesterol	0	1
Microbiological culture, N (%)	19 (95)	89 (100)
Positive, N (%)	16 (84.2)	2 (2,2)
CRP, N (%)	16 (80)	84 (94.4)
Median (range)	14.4 (1-51.6)	0 (0 – 95.4)

Table 3. Final diagnosis of 109 cases with 20 PJIs and 89 no PJIs and correlation with *α-defensin* test result

Final diagnosis	**Total N (%)**	**Hip N**	**Knee N**	**Positive *α-defensin* , N**
PJI	20 (18.0%)	16	4	18
Monomicrobial infection	16	14	2	14
Staphylococcus aureus	3	3	0	3
Coagulase-negative staphylococci	7	5	2	**6**
Streptococcus dysgalactiae	1	1	0	1
Enterococcus faecalis	1	1	0	1
Escherichia coli	1	1	0	1
Propionibacterium avidum	1	1	0	1
Proteus vulgaris	1	1	0	1
Candida tropicalis	1	1	0	**0**
Polymicrobial infection[1]	1	1	0	1
Culture negative infections[2]	3	1	2	3

No PJI	**89 (81.7%)**	33	56	7
Aseptic loosening	27	17	10	**2**[5]
Muscular insufficiency/tendinopathy	25	9	16	**1**[5]
Arthrofibrosis	9	0	9	0
New fracture or delayed union	4	1	3	0
Mechanic[3]	6	2	4	0
Crystal deposition disease	4	1	3	**2**
Psoriasis, rheumatic arthritis	2	0	2	**2**
Metallosis	0	1	0	**0**
Wound healing disorder without PJI	2	2	0	0
Complex Regional Pain Syndrome (CRPS)	1	0	1	**0**
Pain unknown origin	4	0	4	0
Other[4]	4	0	4	0

No, number

[1] *S. caprae/capitis and S. epidermidis;*

[2] due to preoperative antibiotic treatment. In all, 16s rDNA PCR, mycobacterial culture and PCR, and serology for atypical bacteria *(Francisella tularensis, Coxiella burnetti, Bartonella, Brucella)* were negative*;*

[3] ossifications, retropatellar arthrosis, rotation failure*;*

[4] other diagnosis included lumbago with radiculopathy, scar pain with complete improvement after chiropractic; potential contact allergy to “Vanadium chloride” in the prosthesis;

[5] Synovasure test result was weak positive

Table 4. Comparison of positive α-defensin test results with synovial CRP values of the preoperative diagnostic work-up in 17 hip and 8 knee cases (true positives shaded in gray).

	Case Nr	*α-defensin* Test	Synovial CRP (mg/l)	Final diagnosis
	HIP			
1	H12	False positive	0	Aseptic loosening
2	H20	False positive	0	Aseptic loosening
3	H36	False positive	0	Aseptic loosening and CPPD
4	H1	True positive	9.7	PJI (polymicrobial, *S. caprae/capitis, S. epidermidis)*)
5	H6	True positive	3.8	PJI (*S. aureus*)
6	H10	True positive	13.6	PJI (CNS)
7	H17	True positive	15.1	PJI (CNS)
8	H25	True positive	Not done	PJI (*S. dysgalactiae*)
9	H34	True positive	18.5	PJI (*E. faecalis*)
10	H40	True positive	1.3	PJI (*P. avidum*)
11	H52	True positive	Not done	PJI (*E. coli*)
12	H62	True positive	10.4	PJI (CNS)
13	H63	True positive	6.1	PJI (*S. aureus*)
14	H64	True positive	37.6	Culture-negative PJI
15	H65	True positive	Not done	PJI (CNS)
16	H71	True positive	23.6	PJI (*Proteus vulgaris*)
17	Relapse H6	True positive	24.6	PJI (*S. aureus*)
	Knee			
1	K2	False positive	95.4	CPPD
2	K10	False positive	1	Psoriasis arthropathy,

				hydroxyapatite disease
3	K63	False positive	7.5	Muscular insufficiency/rupture
4	K75	False positive	2.7	Rheumatoid arthritis
5	K3	True positive	1	Culture negative PJI (3 minor criteria: acute inflammation in histopathology, elevated synovial leucocytes, growth of *S. epidermidis* in 1 sample)
6	K37	True positive	44.8	Culture negative PJI (3 minor criteria: elevated CRP/ESR, elevated synovial leucocytes, 80% neutrophil granulocytes)
7	Relapse K49	True positive	Not done	PJI (CNS)
8	K74	True positive	22.3	PJI (CNS)

H, hip; K, knee; CNS, coagulase-negative staphylococci; CPPD, calcium pyrophosphate dehydrate crystal deposition disease; PJI, periprosthetic joint infection.

Figure legends

Figure 1. Flowchart of 109 cases with interpretation of preoperative and final diagnostic criteria according to MSIS criteria after either intraoperative diagnostics with tissue cultures for microbiology and histopathology or taking into account the clinical course without surgery.