BJO

ARTHROPLASTY

Synovial fluid interleukin-6 is not superior to cell count and differential in the detection of periprosthetic joint infection

R. Mihalič,
J. Zdlovc,
P. Brumat,
R. Trebše

From Valdoltra Orthopaedic Hospital, Ankaran, Slovenia

Aims

Synovial fluid white blood cell (WBC) count and percentage of polymorphonuclear cells (%PMN) are elevated at periprosthetic joint infection (PJI). Leucocytes produce different interleukins (IL), including IL-6, so we hypothesized that synovial fluid IL-6 could be a more accurate predictor of PJI than synovial fluid WBC count and %PMN. The main aim of our study was to compare the predictive performance of all three diagnostic tests in the detection of PJI.

Methods

Patients undergoing total hip or knee revision surgery were included. In the perioperative assessment phase, synovial fluid WBC count, %PMN, and IL-6 concentration were measured. Patients were labeled as positive or negative according to the predefined cut-off values for IL-6 and WBC count with %PMN. Intraoperative samples for microbiological and histopathological analysis were obtained. PJI was defined as the presence of sinus tract, inflammation in histopathological samples, and growth of the same microorganism in a minimum of two or more samples out of at least four taken.

Results

In total, 49 joints in 48 patients (mean age 68 years (SD 10; 26 females (54%), 25 knees (51%)) were included. Of these 11 joints (22%) were infected. The synovial fluid WBC count and %PMN predicted PJI with sensitivity, specificity, accuracy, PPV, and NPV of 82%, 97%, 94%, 90%, and 95%, respectively. Synovial fluid IL-6 predicted PJI with sensitivity, specificity, accuracy, PPV, and NPV of 73%, 95%, 90%, 80%, and 92%, respectively. A comparison of predictive performance indicated a strong agreement between tests.

Conclusions

Synovial fluid IL-6 is not superior to synovial fluid WBC count and %PMN in detecting PJI.

Level of Evidence: Therapeutic Level II

Cite this article: *Bone Jt Open* 2020;1-12:737–742.

Keywords: Synovial fluid, Interleukin-6, IL-6, Periprosthetic joint infection, PJI

Introduction

Total hip arthroplasty (THA) and total knee arthroplasty (TKA) are one of the most successful and commonly performed orthopaedic surgeries.[1-3] Perirosthetic joint infection (PJI) is one of the most devastating complications related to joint arthroplasty surgery with high morbidity and substantial costs.[4] The incidence of PJI is 1% to 2% for primary and 4% for revision hip or knee arthroplasties,[5-7] a rate which will increase in the future due to the growing number of implants, increasing residency of implant, and better detection methods.[8] During the process of prosthetic joint failure evaluation, it is crucial to differentiate between septic and aseptic failure of the implant, as the treatment concepts are different.[9,10] Unfortunately, there is no single test that can confirm or rule out a PJI.[11] One of the most accurate, reproducible, and affordable tests is synovial fluid white blood cell (WBC) count and percentage of

Correspondence should be sent to René Mihalič; email: rene.mihalic@ob-valdoltra.si

doi: 10.1302/2633-1462.112.BJO-2020-0166.R1

Bone Jt Open 2020;1-12:737–742.

Table I. Patient data.

Parameter	Total	Infected	Non-infected	p-value
Patients, n (joints)	48 (49)	11 (11)	37 (38)	N/A
Mean age, yrs (SD)	68 (10)	64 (12)	69 (9)	N/A
Joint, n (%)				
Hip	24 (100)	6 (25)	18 (75)	N/A
Knee	25 (100)	5 (20)	20 (80)	N/A
Sex, n (%)				
Male	23 (100)	5 (22)	18 (78)	N/A
Female	26 (100)	6 (23)	20 (77)	N/A
Mean serum WBC, cells × 10^9/ml (SD)	7.10 (2.36)	8.67 (3.55)	6.62 (1.55)	0.102
Mean serum CRP, mg/l (SD)	33.06 (38.65)	58.32 (41.31)	7.80 (2.38)	0.005
Mean synovial WBC, cells × 10^9/ml (SD)	9.70 (29.39)	42.02 (50.01)	0.34 (0.47)	0.025
Mean synovial %PMN (SD)	31.86 (33.30)	75.00 (33.30)	19.37 (20.35)	< 0.001
Mean synovial IL-6, pg/ml (SD)	6,591.60 (20,491.51)	27,453.36 (36,146.44)	552.67 (886.07)	0.040

IL-6, interleukin-6; N/A, not applicable; WBC, white blood cells.

polymorphonuclear cells (%PMN).[8,12,13] Current research of synovial fluid has drawn attention to improved methods of PJI detection with reported higher diagnostic accuracy than synovial fluid WBC count and %PMN analysis.[11,14-21] Since the synovial fluid WBC count and %PMN are increased in the presence of PJI and because in inflammatory conditions leucocytes tend to produce more pro-inflammatory proteins, like interleukins (IL),[22] we hypothesized that increased concentrations of ILs in the synovial fluid could be even more accurate than WBC count and %PMN in the detection of PJI. After the literature review, we decided to analyze the concentration of the synovial fluid interleukin 6 (IL-6) in painful failed artificial hip or knee joints[14,23-25] and to compare its PJI detection strength to the synovial fluid WBC count with %PMN. IL-6 is one of the key cytokines inducing inflammation for septic reasons and is strongly upregulated when a septic condition occurs.[26] There are also reports that local expressions of IL-6 in patients with PJI differ significantly from those with aseptic failure.[27] However, it remains unclear whether the diagnostic value for PJI of IL-6 is superior to synovial fluid WBC count and %PMN.

The aim of our study was to define the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of synovial fluid WBC count, %PMN and IL-6 in the detection of PJI, and to compare the predictive performance of all three diagnostic tests in the detection of PJI.

Methods

Study design. In the 21-month period (from March 2012 to January 2014), we prospectively included a consecutive series of 50 joints in 49 unselected patients undergoing joint revision surgery for any reason except for periprosthetic fracture. A total of 49 joints in 48 patients undergoing artificial hip (n = 24) or knee (n = 25) revision surgery were included. The mean age of the cohort was 68 years (SD 10) and 25 patients (53%) were female. All surgeries were performed by the senior author (RT). In 48 patients, hip or knee revision surgery was performed, and in one patient surgery was performed on both knees. One patient was subsequently excluded because the revision surgery was performed on the shoulder. A final cohort of 49 joints was analyzed. The patient data included: affected joint, age, sex, serum WBC with %PMN, serum CRP, synovial fluid WBC, %PMN, and IL-6 concentration (Table I). In the perioperative phase (up to three weeks before surgery or intraoperatively), an arthrocentesis of the affected joint was performed, and the collected synovial fluid was sent for microbiological analysis, determination of the WBC count, %PMN, and IL-6 concentration. During the surgery, a minimum of four samples were collected for microbiological analysis, along with one sample for histopathological analysis. According to the selected criteria for PJI, patients were diagnosed as having a PJI or an aseptic failure (infected and noninfected group). According to the predefined cut-off values of all three synovial fluid tests, the samples were labeled as positive or negative for infection. The PJI diagnostic accuracy of all three tests was then compared.

The study was approved by the Institutional Review Board (IRB No. 2/2020).

Synovial fluid handling and testing. Synovial fluid WBC count and %PMN were manually determined under the microscope with the Neubauer Improved cell counting chamber (BRAND Merck, Darmstadt, Germany) by an experienced medical biochemist (DT).

For IL-6 measurement, synovial fluid samples were centrifuged at 1500 rpm for ten minutes, within an hour after collection. The resulting supernatant was stored at -35° C and sent for testing. IL-6 concentration in synovial fluid were determined on the Immulite 2000 System (Siemens Healthcare Diagnostics Products, Firmley, UK) with the chemiluminescent immunoassay (CLIA) method.

Synovial fluid cut-off values. We used the same cut-off values for WBC count and %PMN as defined by Trampuz

Table II. Infected joints.

Pt	Procedure	Microorganism	Serum WBC, cells × 10^9/ml	Serum CRP, mg/l	Synovial WBC, cells × 10^9/ml	%PMN	Synovial IL-6, pg/ml	Positive samples	Histopathology sample
1	DAIR	*S. aureus*	17.6	133.6	165	95	126,528	6/6	Positive
2	DAIR	*S. aureus*	7.2	82.9	27.25	82	17,400	3/6	Positive
3	DAIR	*C. acnes* and CoNS	5	11.6	0.63	3	101	6/6	Negative
4	Removal and spacer	*C. acnes* and CoNS	7.7	≤ 5	0.15	7	153	3/6	Negative
5	DAIR	*S. epidermidis*	6.7	77.7	10.1	95	66,972	2/6	Positive
6	Two-stage revision with a spacer	MRSA	12.8	63.1	119.7	96	679	6/6	Positive
7	Two-stage revision without a spacer	MRSE	6.4	14.5	30.5	88	12,580	2/6	Positive
8	Removal	*E. faecalis*	6.1	63.4	47	86	15,080	3/6	Positive
9	Removal	*E. coli* (ESBL)	9.2	106.5	22.8	95	25,498	6/6	Positive
10	Removal	*S. sanguinis*	6.2	24.5	27	92	27,006	6/6	Positive
11	Removal	N/A	10.5	5.4	12.1	86	9,990	0/6	Positive

C. acnes, *Cutibacterium acnes*; CoNS, Coagulase-negative staphylococci; DAIR, debridement, antibiotics, and implant retention; *E.coli*, *Escherichia coli*; *E. faecalis*, *Enterococcus faecalis*; ESBL, extended spectrum beta lactamase; IL-6, interleukin-6; MRSA, methicillin-resistant *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*; *S. Sanguinis*, *Staphylococcus sanguinis*; WBC, white blood cells.

et al[12] on 133 failed TKAs for both failed TKAs and THAs. We based the implementation of the same cut-off values in our study on 196 failed THAs as presented at the 16th EFORT Annual Congress[28] where we calculated similar optimal cut-offs as Trampuz et al.[12] Calculated cut-off values for WBC count and %PMN were set at 1,7 × 10^9 cells/ml and ≥ 65% PMN, respectively.

For the IL-6 concentration, the cut-off value was set at 2,300 pg/ml, according to the calculations of Xie et al,[25] who performed a meta-analysis of 17 articles reporting the optimal synovial fluid IL-6 concentrations in the detection of PJI.

Microbiological analysis. All the materials were stored in sterile plastic containers and transported to the laboratory immediately after sampling. All samples were cultured for 14 days on solid and liquid media.

Histopathological analysis. Histopathological samples were analyzed under the microscope at the magnification of 400. The result was considered as positive if a mean of > 5 PMNs was observed on at least ten high-power fields (HPF).[29]

PJI criteria. For the study purpose, we needed to modify the conventional criteria for PJI including only the presence of sinus tract, inflammation in histopathological samples, and growth of the same microorganism in at least two or more samples of periprosthetic tissue or synovial fluid.[5,12,30] We could not use the criteria proposed by the Musculoskeletal Infection Society (MSIS),[31] upgraded and validated by Parvizi et al[32] and confirmed at the International Consensus Meeting (ICM) on Musculoskeletal Infection in 2018, because synovial fluid WBC count and %PMN represent an essential part of the criteria. Consequently, we needed to apply neutral criteria for unbiased comparison of the predictive value of the synovial fluid IL-6 against WBC count and %PMN.

Statistical analysis. Synovial fluid WBC count with %PMN and synovial fluid IL-6 concentration were compared between samples with present or absent PJI using the Mann-Whitney U test. Receiver operating characteristic (ROC) curves were constructed to assess the diagnostic performance of synovial fluid WBC with %PMN and synovial IL-6. Clinically relevant values were used as cut-off values (IL-6 2,300 pg/ml; WBC 1.7 × 10^9 cells/ml; PMN ≥ 65% PMN). ROC curves were compared using DeLong's method. The agreement between both diagnostic tests was assessed by chi-squared test and Cohen's kappa. Continuous variables were compared using paired *t*-tests. $p < 0.05$ was considered significant.

All calculations were performed using IBM SPSS Statistics software package v. 25 (IBM, Armonk, New York, USA) and pROC package in R software v. 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Of the 49 joints included in this study, 11 (22%) were infected, ten had positive microbiological samples, and one had positive histopathology with negative microbiological results. Five were acute infections (four of them acute haematogenous) and six were chronic, with symptoms persisting the whole time after the index procedure. No patient had a sinus tract. The most frequent causative microorganisms were *Staphylococcus aureus* (n = 2) and *Cutibacterium acnes* in combination with coagulase-negative *staphylococci* (CoNS, other than *Staphylococcus epidermidis*) (n = 2), followed by *S. epidermidis* (n = 1), methicillin resistant *S. aureus* (n = 1), methicillin resistant *S. epidermidis* (n = 1), *Enterococcus faecalis* (n = 1), *Escherichia coli* (n = 1), and *Streptococcus sanguinis* (n = 1) (Table II).

Serum CRP, synovial fluid WBC count, %PMN, and IL-6 concentration were statistically significantly higher in

Table III. Synovial interleukin-6 and synovial white blood cell count.

Predictor	AUROC (95% CI)	Clinically relevant cut-off value	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Accuracy, % (95% CI)	p-value (AUROC comparison)
Synovial IL-6	0.861 (0.704 to 1.000)	2300	73 (45 to 100)	95 (87 to 100)	80 (58 to 100)	92 (85 to 100)	90 (82 to 98)	0.171
Synovial WBC	0.944 (0.853 to 1.000)	1.70	82 (55 to 100)	97 (92 to 100)	90 (71 to 100)	95 (88 to 100)	94 (88 to 100)	

AUROC, area under the ROC curve; CI, confidence interval; IL-6, interleukin-6; NPV, negative predictive value; PPV, positive predictive value; WBC, white blood cells.

Table IV. Synovial interleuking-6 and synovial percentage of polymorphonuclear cells.

Infected	AUROC (95% CI)	Clinically relevant cut-off value	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Accuracy, % (95% CI)	p-value (AUROC comparison)
Synovial IL-6	0.861 (0.704 to 1.000)	2,300	73 (45 to 100)	95 (87 to 100)	80 (58 to 100)	92 (85 to 100)	90 (82 to 98)	0.171
Synovial %PMN	0.944 (0.853 to 1.000)	65	82 (55 to 100)	97 (92 to 100)	90 (71 to 100)	95 (88 to 100)	94 (88 to 100)	

AUROC, area under the ROC curve; CI, confidence interval; IL-6, interleukin-6; NPV, negative predictive value; %PMN, percentage of polymorphonuclear cells; PPV, positive predictive value.

the infected group ($p = 0.005$; $p = 0.025$; $p < 0.001$, $p = 0.040$, respectively) but there was no statistically significant difference in serum WBC count between infected and non-infected groups ($p = 0.102$) (Table I).

There was no difference in the detection potential of synovial fluid WBC count and %PMN in our cohort. Both markers were either above or below the preset synovial fluid cut-off values regardless of the reason for failure. The synovial fluid WBC count and %PMN predicted PJI with a sensitivity, specificity, accuracy, PPV, and NPV of 82%, 97%, 94%, 90%, and 95%, respectively. Synovial IL-6 predicted PJI with sensitivity, specificity, accuracy, PPV, and NPV of 73%, 95%, 90%, 80%, and 92%, respectively (Tables III and IV).

There was a strong agreement between both tests (Kappa = 0.749). However, there was a non-significant trend of a better diagnostic value of synovial fluid WBC count and %PMN, compared with synovial IL-6 ($p = 0.171$).

Discussion

PJI detection remains one of the most challenging acts in the perioperative evaluation of painful artificial joints, especially if there are no other clinical or biochemical signs indicating PJI. The evaluation protocol requires a thorough patient history, a clinical examination, and the use of multiple diagnostic tests.[4] In the perioperative evaluation, the aspiration of the affected joint should be a standard diagnostic tool.[33] The recent literature has not yet highlighted an optimal biomarker which could, as a single point-of-care test, detect PJI. Mostly, combinations of several diagnostic tools improve PJI detection accuracy. Recent efforts in the detection of PJI are focused on the identification of more accurate biomarkers in synovial fluid because the PJI is arising in the local environment of the affected joint and only progresses to the systemic level when the concentration of planktonic microorganisms in synovial fluid outperforms the capacity of the local host immunity.[34] It is proven that the main production of biomarkers occurs in the affected joint and the analysis of local biomarkers may therefore provide better diagnostic performance than analysis of the serum biomarkers.[34,35] Consequently, new promising synovial fluid biomarkers for the detection of PJI have been continuously introduced, such as synovial fluid α defensin,[17,36,37] leucocyte esterase,[15] and interleukins, especially IL-6.[10,14,23,38,39] In the reported trials, the synovial IL-6 has shown high levels of specificity (85% to 100%) and sensitivity (62% to 100%),[10,14] with high PPV (85% to 100%) and high NPV (89% to 100%).[14,23,39] High NPV is especially important because the negative result indicates that the failure is unlikely due to PJI, which leads to less complex and better-suited treatment. Thus, it seems that IL-6 could have had an important role in the perioperative evaluation of painful artificial joints. Therefore, we decided to test if synovial fluid IL-6 concentration is superior to the synovial fluid WBC count with %PMN in detection of PJI.

No difference and a strong agreement between both diagnostic tests were observed in our study. However, based on the comparison of the diagnostic performance, it seems that the synovial fluid IL-6 is not superior to synovial fluid WBC count with %PMN. Considering the trend of a better diagnostic performance of the latter, we think that synovial fluid WBC count with %PMN is more reliable in the detection of PJI than synovial fluid IL-6.

Deirmengian et al[14] identified several synovial fluid biomarkers, including IL-6, with substantially elevated concentrations in patients with hip or knee PJI. At a cut-off value of 13,350 pg/ml, the synovial IL-6 had a sensitivity and specificity of 100%. In a study by Jacovides et al,[23] synovial IL-6 was strongly linked to hip and knee PJI. At a cut-off value of 4,270 pg/ml, the synovial IL-6 had a sensitivity of 87% and a specificity of 100%.[23] Gollwitzer et al[38]

also assessed the diagnostic efficacy of synovial fluid IL-6. They reported that using a cut-off value of 1,896.6 pg/ml resulted in a sensitivity of 60% and a specificity of 95%.[38] More recently, Gallo et al[39] assessed the diagnostic power of synovial fluid IL-6 concentration in patients with failed hip or knee arthroplasty. At a calculated cut-off value of 20,988 pg/ml, the synovial fluid IL-6 level had a sensitivity of 68%, and a specificity of 98%.[39] It is interesting that different authors reported similar results regarding the diagnostic performance of synovial fluid IL-6 at different cut-off values, which could be another indicator that the test is unreliable for accurate detection of PJI.

In the presented study we observed an interesting and, in our opinion, an important finding. The false-negative result of all three synovial fluid tests was related to the growth of *C. acnes* in combination with Coagulase-negative staphylococci (CNS), growing in six out of six samples in the first joint, and three out of six samples in another joint. The synovial fluid WBC count, %PMN, and IL-6 concentrations were 0.63 × 10^9 cells/ml, 3% and 101 pg/ml, respectively, in the first case and 0.15 × 10^9 cells/ml, 7% and 153 pg/ml, respectively, in the second case. However, all serum biomarkers (WBC count and CRP), as well as histopathological samples, were evidently negative for PJI (Table II). The other nine infected joints, independently if the reason for failure was acute or chronic PJI, had remarkably high levels of synovial fluid WBC count, %PMN, and IL-6, except for one MRSA infection where the IL-6 concentration was far below preset cut-off value.

A similar observation was made by Frangiamore et al,[40] who also reported two false-negative results where cultures were positive for *C. acnes*. These important findings indicate a lack of efficient synovial fluid and serum tests, which could detect some slow-growing organisms, particularly *C. acnes*. The studied tests are probably less suitable for instances where *C. acnes* is a common PJI-causing organism such as shoulder arthroplasty.

There are some limitations to the study. First, the sample size was small. Despite the small number of patients, we were able to show that there is no difference in diagnostic performance between both tests. Second, synovial fluid WBC count and %PMN are standard diagnostic tools in different definition criteria for PJI.[30-32] Consequently, these could not be used as standard diagnostic criteria for this study, since we were comparing their diagnostic performance with that of IL-6. Application of the modified criteria in this study could affect a proper classification of analyzed joints into the infected or non-infected group, and in small samples, this could significantly affect the final statistical result.

In conclusion, the diagnosis of PJI in patients undergoing revision arthroplasty remains a challenge, especially in chronic or low-grade cases, where the causative microorganism is of low virulence. However, the current guidelines for the diagnosis of PJI are not suited for such patients. The presented study demonstrates that synovial IL-6 has no added value in the diagnostic process of PJI and could be abandoned as a standard biomarker in the evaluation process of failed artificial joints. The scientific research should focus more on the identification of eventual synovial fluid biomarkers produced by bacteria or those able to detect the biofilm, to avoid failures of the current non-specific biomarkers.

References

1. **Learmonth ID**, **Young C**, **Rorabeck C**. The operation of the century: total hip replacement. *The Lancet*. 2007;370(9597):1508–1519.
2. **Ranawat CS**. History of total knee replacement. *J South Orthop Assoc*. 2002;11(4):218–226.
3. **Zacharia B**, **Paul M**, **Thanveeruddin Sherule M**. Patient-Based outcome analysis is important to determine the success of total knee arthroplasty: result of a focus group discussion. *Med Devices*. 2016;9:125–130.
4. **Tande AJ**, **Patel R**. Prosthetic joint infection. *Clin Microbiol Rev*. 2014;27(2):302–345.
5. **Zimmerli W**, **Trampuz A**, **Ochsner PE**. Prosthetic-joint infections. *N Engl J Med*. 2004;351(16):1645–1654.
6. **Kurtz S**, **Ong K**, **Lau E**, **Mowat F**, **Halpern M**. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. *J Bone Joint Surg Am*. 2007;89-A(4):780–785.
7. **Ong KL**, **Kurtz SM**, **Lau E**, **Bozic KJ**, **Berry DJ**, **Parvizi J**. Prosthetic joint infection risk after total hip arthroplasty in the Medicare population. *J Arthroplasty*. 2009;24(6 Suppl):105–109.
8. **Trampuz A**, **Zimmerli W**. Prosthetic joint infections: update in diagnosis and treatment. *Swiss Med Wkly*. 2005;135(17-18):243–251.
9. **Johnson AJ**, **Zywiel MG**, **Stroh A**, **Marker DR**, **Mont MA**. Serological markers can lead to false negative diagnoses of periprosthetic infections following total knee arthroplasty. *Int Orthop*. 2011;35(11):1621–1626.
10. **Randau TM**, **Friedrich MJ**, **Wimmer MD**, **et al**. Interleukin-6 in serum and in synovial fluid enhances the differentiation between periprosthetic joint infection and aseptic loosening. *PLoS One*. 2014;9(2):e89045.
11. **Shahi A**, **Parvizi J**. The role of biomarkers in the diagnosis of periprosthetic joint infection. *EFORT Open Rev*. 2016;1(7):275–278.
12. **Trampuz A**, **Hanssen AD**, **Osmon DR**, **Mandrekar J**, **Steckelberg JM**, **Patel R**. Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. *Am J Med*. 2004;117(8):556–562.
13. **Schinsky MF**, **Della Valle CJ**, **Sporer SM**, **Paprosky WG**. Perioperative testing for joint infection in patients undergoing revision total hip arthroplasty. *J Bone Joint Surg Am*. 2008;90-A(9):1869–1875.
14. **Deirmengian C**, **Hallab N**, **Tarabishy A**, **et al**. Synovial fluid biomarkers for periprosthetic infection. *Clin Orthop Relat Res*. 2010;468(8):2017–2023.
15. **Parvizi J**, **Jacovides C**, **Antoci V**, **Ghanem E**. Diagnosis of periprosthetic joint infection: the utility of a simple yet unappreciated enzyme. *J Bone Joint Surg Am*. 2011;93-A(24):2242–2248.
16. **Ahmad SS**, **Shaker A**, **Saffarini M**, **Chen AF**, **Hirschmann MT**, **Kohl S**. Accuracy of diagnostic tests for prosthetic joint infection: a systematic review. *Knee Surg Sports Traumatol Arthrosc*. 2016;24(10):3064–3074.
17. **Bonanzinga T**, **Ferrari MC**, **Tanzi G**, **Vandenbulcke F**, **Zahar A**, **Marcacci M**. The role of alpha defensin in prosthetic joint infection (PJI) diagnosis: a literature review. *EFORT Open Rev*. 2019;4(1):10–13.
18. **Sigmund IK**, **Holinka J**, **Lang S**, **et al**. A comparative study of intraoperative frozen section and alpha defensin lateral flow test in the diagnosis of periprosthetic joint infection. *Acta Orthop*. 2019;90(2):105–110.
19. **Goswami K**, **Parvizi J**, **Maxwell Courtney P**. Current recommendations for the diagnosis of acute and chronic PJI for hip and Knee-Cell counts, alpha-defensin, leukocyte esterase, next-generation sequencing. *Curr Rev Musculoskelet Med*. 2018;11(3):428–438.
20. **Lee YS**, **Koo K-H**, **Kim HJ**, **et al**. Synovial fluid biomarkers for the diagnosis of periprosthetic joint infection: a systematic review and meta-analysis. *J Bone Joint Surg Am*. 2017;99-A(24):2077–2084.
21. **Wouthuyzen-Bakker M**, **Ploegmakers JJW**, **Kampinga GA**, **Wagenmakers-Huizenga L**, **Jutte PC**, **Muller Kobold AC**. Synovial calprotectin: a potential biomarker to exclude a prosthetic joint infection. *Bone Joint J*. 2017;99-B(5):660–665.

22. **Deirmengian C**, **Lonner JH**, **Booth RE**. The mark coventry Award: white blood cell gene expression: a new approach toward the study and diagnosis of infection. *Clin Orthop Relat Res*. 2005;440:38–44.
23. **Jacovides CL**, **Parvizi J**, **Adeli B**, **Jung KA**. Molecular markers for diagnosis of periprosthetic joint infection. *J Arthroplasty*. 2011;26(6 Suppl):99–103.
24. **Lenski M**, **Scherer MA**. Synovial IL-6 as inflammatory marker in periprosthetic joint infections. *J Arthroplasty*. 2014;29(6):1105–1109.
25. **Xie K**, **Dai K**, **Qu X**, **Yan M**. Serum and synovial fluid interleukin-6 for the diagnosis of periprosthetic joint infection. *Sci Rep*. 2017;7(1):1496.
26. **Tanaka T**, **Narazaki M**, **Kishimoto T**. Il-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol*. 2014;6(10):a016295.
27. **Worthington T**, **Dunlop D**, **Casey A**, **Lambert R**, **Luscombe J**, **Elliott T**. Serum procalcitonin, interleukin-6, soluble intercellular adhesin molecule-1 and IgG to short-chain exocellular lipoteichoic acid as predictors of infection in total joint prosthesis revision. *Br J Biomed Sci*. 2010;67(2):71–76.
28. **Mihalič R**, **Bedenčič K**, **Trebše R**. New cut-off values for synovial fluid cell count and differential for the diagnosis of prosthetic hip infection. 16th EFORT annual Congress. *Prague*. 2015.
29. **Mirra JM**, **Amstutz HC**, **Matos M**, **Gold R**. The pathology of the joint tissues and its clinical relevance in prosthesis failure. *Clin Orthop Relat Res*. 1976;117:221–240.
30. **Osmon DR**, **Berbari EF**, **Berendt AR**, **et al**. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the infectious diseases Society of America. *Clin Infect Dis*. 2013;56(1):e1–e25.
31. **Parvizi J**, **Zmistowski B**, **Berbari EF**, **et al**. New definition for periprosthetic joint infection: from the Workgroup of the musculoskeletal infection Society. *Clin Orthop Relat Res*. 2011;469(11):2992–2994.
32. **Parvizi J**, **Tan TL**, **Goswami K**, **et al**. The 2018 definition of periprosthetic hip and knee infection: an evidence-based and validated criteria. *J Arthroplasty*. 2018;33(5):1309–1314.
33. **Hassebrock JD**, **Fox MG**, **Spangehl MJ**, **Neville MR**, **Schwartz AJ**. What is the role of repeat aspiration in the diagnosis of periprosthetic hip infection? *J Arthroplasty*. 2019;34(1):126–131.
34. **Ricciardi BF**, **Muthukrishnan G**, **Masters E**, **Ninomiya M**, **Lee CC**, **Schwarz EM**. Staphylococcus aureus evasion of host immunity in the setting of prosthetic joint infection: biofilm and beyond. *Curr Rev Musculoskelet Med*. 2018;11(3):389–400.
35. **Qu X**, **Zhai Z**, **Wu C**, **et al**. Preoperative aspiration culture for preoperative diagnosis of infection in total hip or knee arthroplasty. *J Clin Microbiol*. 2013;51(11):3830–3834.
36. **Deirmengian C**, **Kardos K**, **Kilmartin P**, **Gulati S**, **Citrano P**, **Booth RE**. The alpha-defensin test for periprosthetic joint infection responds to a wide spectrum of organisms. *Clin Orthop Relat Res*. 2015;473(7):2229–2235.
37. **Bonanzinga T**, **Zahar A**, **Dütsch M**, **Lausmann C**, **Kendoff D**, **Gehrke T**. How reliable is the alpha-defensin immunoassay test for diagnosing periprosthetic joint infection? A prospective study. *Clin Orthop Relat Res*. 2017;475(2):408–415.
38. **Gollwitzer H**, **Dombrowski Y**, **Prodinger PM**, **et al**. Antimicrobial peptides and proinflammatory cytokines in periprosthetic joint infection. *J Bone Joint Surg Am*. 2013;95-A(7):644–651.
39. **Gallo J**, **Svoboda M**, **Zapletalova J**, **Proskova J**, **Juranova J**. Serum IL-6 in combination with synovial IL-6/CRP shows excellent diagnostic power to detect hip and knee prosthetic joint infection. *PLoS One*. 2018;13(6):e0199226.
40. **Frangiamore SJ**, **Saleh A**, **Kovac MF**, **et al**. Synovial fluid interleukin-6 as a predictor of periprosthetic shoulder infection. *J Bone Joint Surg Am*. 2015;97-A(1):63–70.

Author information:
- R. Mihalič, MD, Consultant Orthopaedic Surgeon
- P. Brumat, MD, Orthopaedic Surgery Resident
  Service for Bone Infections, Valdoltra Orthopaedic Hospital, Ankaran, Slovenia.
- J. Zdovc, Mag Pharm, Teaching Assistant, Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia.
- R. Trebše, MD, PhD, Consultant Orthopaedic Surgeon, Head of Service for Bone Infections, Associate Professor, Service for Bone Infections, Valdoltra Orthopaedic Hospital, Ankaran, Slovenia; Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia.

Author contributions:
- R. Mihalič: Designed the study, Collected and analyzed the data, Wrote the manuscript.
- J. Zdovc: Analyzed the data, Wrote the manuscript.
- P. Brumat: Analyzed the data, Wrote the manuscript.
- R. Trebše: Designed the study, Wrote the manuscript.

Funding statement:
- This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ICMJE COI statement:
- R. M., J. Z. and P. B. declare no conflict of interests. R.T. is the current president of The European Bone and Joint Infection Society (EBJIS).

Acknowledgements
- We thank Dunja Terčič, chief of Biochemical Laboratory at Valdoltra Orthopaedic Hospital, for her efforts in synovial fluid analysis.

Ethical review statement
- This study was approved by the Institutional Review Board (IRB No. 2/2020).