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Research Paper

Limited Predictive Value of Serum Inflammatory Markers for Diagnosing Fracture-Related Infections: results of a large retrospective multicenter cohort study

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Abstract

***Introduction*:** Diagnosing Fracture-Related Infections (FRI) based on clinical symptoms alone can be challenging and additional diagnostic tools such as serum inflammatory markers are often utilized. The aims of this study were 1) to determine the individual diagnostic performance of three commonly used serum inflammatory markers: C-Reactive Protein (CRP), Leukocyte Count (LC) and Erythrocyte Sedimentation Rate (ESR), and 2) to determine the diagnostic performance of a combination of these markers, and the additional value of including clinical parameters predictive of FRI.

***Methods*:** This cohort study included patients who presented with a suspected FRI at two participating level I academic trauma centers between February 1st 2009 and December 31st 2017. The parameters CRP, LC and ESR, determined at diagnostic work-up of the suspected FRI, were retrieved from hospital records. The gold standard for diagnosing or ruling out FRI was defined as: positive microbiology results of surgically obtained tissue samples, or absence of FRI at a clinical follow-up of at least six months. The diagnostic accuracy of the individual serum inflammatory markers was assessed. Analyses were done with both dichotomized values using hospital thresholds as well as with continuous values. Multivariable logistic regression analyses were performed to obtain the discriminative performance (Area Under the Receiver Operating Characteristic, AUROC) of (1) the combined inflammatory markers, and (2) the added value of these markers to clinical parameters.

***Results*:** A total of 168 patients met the inclusion criteria and were included for analysis. CRP had a 38% sensitivity, 34% specificity, 42% positive predictive value (PPV) and 78% negative predictive value (NPV). For LC this was 39%, 74%, 46% and 67% and for ESR 62%, 64%, 45% and 76% respectively. The diagnostic accuracy was 52%, 61% and 80% respectively. The AUROC was 0.64 for CRP, 0.60 for LC and 0.58 for ESR. The AUROC of the combined inflammatory markers was 0.63. Serum inflammatory markers combined with clinical parameters resulted in AUROC of 0.66 as opposed to 0.62 for clinical parameters alone.

Conclusion: The added value of CRP, LC and ESR for diagnosing FRI is limited. Clinicians should be cautious when interpreting the results of these tests in patients with suspected FRI.

Key words: Fracture-Related Infections, Serum Inflammation Markers, White Blood Cell Count, Erythrocyte Sedimentation Rate, C-reactive Protein, Diagnostic accuracy, osteomyelitis, infection, fracture, trauma.

Introduction

Fracture-Related Infection (FRI) is a challenging complication after surgical fracture treatment (1, 2). Consequences include reoperations, prolonged treatment with antibiotics, prolonged immobilization, inability to participate in social and work-related activities, increased medical costs, loss of function and

even amputation.(3-5) As with most medical conditions, a successful treatment outcome starts with an accurate diagnosis. The fact that the clinical presentation of infection can be obscured by apparently normal wound healing is one of the difficulties of diagnosing FRI. When wound healing is compromised, and the classical infection symptoms such as pain, increased temperature, local erythema and swelling are present, FRI is usually easy to recognize. However, FRI can also present less apparent with symptoms mimicking those of delayed- or non-union, such as pain, implant failure and impaired fracture healing. It might even be present without any clinical signs and symptoms at all (1, 6, 7).

Another difficulty has been that until recently, the literature regarding the diagnosis and treatment of FRI was hampered by the lack of a clear definition (4). However, in 2017, the characteristics of a FRI were clearly defined in a consensus meeting between experts in the field of bone infection in collaboration with the Arbeitsgemeinschaft für Osteosynthese-fragen (AO Foundation) and the European Bone and Joint Infection Society (EBJIS) (2). Two levels of certainty around diagnostic features were defined. Signs that are suggestive of FRI can be clinical signs of infection (such as redness, fever and new onset of joint effusion), radiological signs (for example bone lysis, sequestration, implant loosening, nonunion and periosteal bone formation), wound drainage and elevated serum inflammatory markers. Confirmatory clinical signs are a fistula, sinus, purulent drainage or wound breakdown which communicates to the bone itself or to the fixation device. In absence of these confirmatory clinical signs, the diagnosis can be confirmed by either microbiology (with phenotypically indistinguishable pathogens identified by culture from at least two separate deep tissue/implant specimens) or histology (presence of microorganisms in deep tissue taken during an operative intervention) (2).

Elevated serum inflammatory markers are often used as diagnostic parameters for postoperative infections after orthopedic trauma surgery and are mainly investigated in PJIs (8, 9). Although they are considered to be indicative for the presence of FRI according to the aforementioned consensus meeting, research focusing on the added value of these parameters for diagnosing FRI is limited (10-13). In a recent survey amongst medical specialists involved in the care for patients with FRI, C-reactive protein (CRP) was regarded to be the most valuable tool for diagnosing FRI, followed by the Erythrocyte Sedimentation Rate (ESR) and Leucocyte Count (LC) respectively (14). However, the added value of serum inflammatory markers is still under debate. Large cohort studies which tell us whether these markers are capable of distinguishing a bacterial infection from a normal inflammatory response due to the injury, tissue damage, fracture healing, or the fracture surgery, are lacking so far (15-19). It is therefore mandatory to assess the role of these serum inflammatory markers in the decision-making process for diagnosing FRI.

The two aims of the current study were:

1) To determine the individual diagnostic performance of the three commonly used serum inflammatory markers, CRP, LC and ESR, in FRI.

2) To assess the diagnostic value of a combination of these markers, and their value in addition to clinical parameters predictive of FRI.

Patients and Methods

Study design

This is a retrospective cohort study performed at the University Medical Center Utrecht (UMCU) and the University Medical Center Groningen (UMCG), two Level I academic trauma centers in the Netherlands.

In- and exclusion criteria

In order to be able to calculate the accuracy of serum inflammatory markers in both patients with and without FRI, patients from a previous assembled database on medical imaging for suspected FRI were included. This database comprised of all patients who underwent nuclear medical imaging for suspected FRI between February 1st 2009 and December 31st 2017 of the UMCU and UMCG. In accordance with clinical practice, where serum inflammatory markers are ordered when an infection is suspected, blood sampling had to be obtained within a range of seven days around the date an FRI was first considered (mostly at the outpatient department). Cases missing inflammatory markers or outcome data due to incomplete reporting were excluded from the analyses. In uncomplicated orthopedic- and traumatologic cases, levels of CRP peak at the second postoperative day. In uneventful cases, the CRP returns to normal values between day two to twelve postoperatively (20-25). Maximum values of LC are seen on day one to three postoperatively and decline to normal values between day four to six (26). Values of ESR peak at day seven to eleven postoperatively and decrease gradually until after week six (19). Therefore, patients were excluded who underwent surgery in 14 days preceding testing for CRP, 7 days for LC and 6 weeks for ESR testing. In- and exclusion criteria are presented in Table 1.

Table 1. Inclusion and exclusion criteria.

Inclusion	Exclusion
1. Patients with a suspected Fracture-Related Infection.	1. Patients who underwent surgery in the fourteen days preceding collection of the blood sample for determining the serum inflammatory markers 2. Pathologic fractures 3. Prosthetic joint infection (PJI) 4. Hematogenous infection 5. Patients with (auto-)immune diseases 6. Patients with (pre-)malignancies 7. Concomitant use of corticosteroids 8. Evident other focus of infection 9. No reference standard available (representative cultures or at least six months follow-up)

Ethical approval

The study protocol was evaluated by the institutional review board (medical ethical research commission, METC) of the UMCU and found to be exempted from further approval requirements (METC-17-694).

Serum Inflammatory Markers

The index test comprised of CRP and LC. Analysis was done similarly in both participating centers. In the UMCU, blood was drawn into a 2.0 mL vacuum tube (BD Vacutainer; BD Medical Systems, Franklin Lakes, NJ, USA) containing K2-EDTA as an anticoagulant for blood cell analysis and a 4.0 mL vacuum tube Lithium-Heparin as an anticoagulant for CRP measurement.

The UMCG used standard 4.0 mL K2 EDTA and 4.5 mL Lithium-Heparin tubes. All blood samples were analyzed in the central diagnostic laboratories of the UMCU and UMCG (both with full ISO-15189 accreditation). C-reactive protein (CRP) was measured using a turbidimetric immunoassay on a DxAU 5811 automated chemistry analyzer (Beckman-Coulter, Brea, CA, USA). Similar analysis was done in the UMCG using a Roche CRPL3 analyzer with wide range assay (Roche, Mannheim, Germany). LC was measured using a Cell-Dyn Sapphire hematology analyzer (Abbott Diagnostics, Santa Clara, CA, USA). This analyzer uses spectrophotometry, electrical impedance and laser light scattering (multi angle polarized scatter separation, (MAPPS)) to classify blood cells (27, 28). In the UMCG, similar analysis was done using a Sysmex XN-20 Automated hematology analyzer (Sysmex, Kobe, Japan). The validity of all test results was checked with built-in quality flags, daily quality control samples and external quality assessment schemes. The ESR was measured using a method according to Westergren. The UMCU uses whole blood anticoagulated with sodium citrate 3,2% (4:1) in combination with a ESR analyzer (Monitor V100, Vital Diagnostics, SrL, Forli, Italy), in the UMCG the ESR was measured in EDTA whole blood in diluted with sodium citrate 3,2% (4:1) combination with the Starrsed interrliner (Mechatronics, Zwaag, the Netherlands) (29).

Although analyses of blood samples were done in a similar set-up, both participating centers used slightly different threshold values for the serum makers. Since statistical calculations in this paper were performed on data from both centers to improve the possible predictive performance, common threshold values used in clinical practice and reported in medical literature were used to reflect the current performance of the separate parameters. The threshold in this study for CRP was less than 5.0 mg/L and leukocyte count less than 10.0 x 10^9/L. For ESR, the threshold for men was 11 mm/h and for women 24 mm/h.

Clinical parameters

The clinical parameters included in the multivariate analysis were Gustilo-Anderson classification, ISS, diabetes mellitus, smoking status and lower extremity fractures. These parameters were used as these are known to increase the risk of a FRI (30).

Reference standard

The gold standard in the final diagnosis of FRI was based on the outcome of medical microbiology (MMB) results of at least two separate samples of deep tissue taken during a surgical intervention.(2) Two experienced trauma surgeons (GG and FIJ, >5 years board certified) assessed the validity of the MMB results. Only if two or more deep samples were taken from the suspected area of bone infection, the MMB results were regarded as relevant. Only when two or more samples were positive with both morphologically the same organism, the MMB results were regarded as positive. In case of no surgery (and therefore no intra-operative cultures), the definite diagnosis was based on a clinical follow-up of at least six months. Throughout the follow-up, a final diagnosis was made on basis of positive clinical confirmatory criteria. When the aforementioned confirmatory signs were present perioperatively, the patient was also considered to be suffering from FRI (2).

Data collection

The electronic patient files of all included patients were scrutinized on when an infectious complication was first suspected and data was collected on demographics, type of fracture according to the Müller AO Classification of Fractures (31), Gustilo Anderson classification in case of an open fracture (32), date, trauma mechanism, fracture type

and surgical management of the index trauma, laboratory findings, microbiology results, final diagnosis and clinical outcome during follow-up.

Statistical analysis

Continuous data are presented as mean and standard deviation (SD) in case of normal distributions or median and interquartile range (IQR) when not normally distributed. The baseline characteristics per center were compared to analyze whether there were any substantial differences between the centers. Hypothesis testing was done using independent t-test or Mann-Whitney U test for the continues values, and Chi-squared test or Fisher's exact test for the dichotomized values. A p-value of <0.05 was considered significant.

In the first analysis, the serum markers were dichotomized using the aforementioned threshold values, as this reflects the diagnostic performance in current clinical practice. For each parameter, true positive (TP), true negative (TN), false positive (FP) and false negative (FN) results were described. Contingency tables were constructed. Sensitivity and specificity, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratio's (LR+ and LR) were calculated. Second, to assess the maximal predictive performance, separate continuous values were used.

Third, to assess the diagnostic performance of the combination of the inflammatory markers, a multivariable logistic regression model including the inflammatory markers was fitted. Subsequently, two models were fitted to determine the added value of the inflammatory markers to the clinical parameters. The first one included the clinically predetermined parameters. The second one included these parameters, and also the combined inflammatory markers. To reduce the risk of overfitting, a maximum of one predictor per 5-10 events was used.

The diagnostic performance of these continuous models was assessed using the AUROC as a measure of discrimination. The Q-point method, which determines the threshold value closest to the upper left corner of the AUROC, was used to deduct the optimal threshold, for which the sensitivity and specificity were calculated.

Sensitivity analyses were performed to (1) assess whether the diagnostic performance of the multivariable logistic regression analysis differs per center, (2) whether the time interval (<14 days versus ≥ 14 days between inflammatory markers and intra-operative cultures) affects the diagnostic performance and (3) to assess whether the linearity assumption of the combined markers with the (logit) outcome affects the performance, through log-transforming the variables.

All data analyses were performed using the Statistical Package for Social Sciences (SPSS®) statistics for Windows (version 20.0.0.0, IBM, Armonk, NY, USA). Where applicable, the reporting of this study followed the Transparent Reporting of a multivariable Prediction Model for individual diagnosis or prognosis (TRIPOD statement) (33).

Results

The cohort consisted of 365 patients who underwent medical imaging for suspected FRI. A total of 197 patients were excluded from analyses due to missing data on serum inflammatory markers (n=171) or other parameters. After exclusion, a total of 168 patients were included in this study. Basic demographics and clinical characteristics of the included patients from both participating centers are shown in Table 2. The cohort consisted predominantly of male patients (n=115, 68.5%) with a median age of 54 (IQR 40-62). Fractures were most commonly located in the lower extremity (n=140, 83.4%). The study population consisted of patients who were suspected to suffer from long standing FRI. The median interval between initial fracture surgery and nuclear imaging for a suspected FRI was 480 (IQR 229-1312) days.

FRI in study population

Overall, FRI was present in 61 patients (36%). In the cohort, 41 patients were diagnosed with FRI on basis of MMB results. Twenty patients with negative or without MMB results developed FRI during the follow up. The median clinical follow up in the cohort was 53 (IQR 45-134) weeks. Median interval between blood sampling for laboratory analysis and operatively obtained samples for MMB was 49 (IQR 19-85) days.

Diagnostic performance of serum inflammatory markers

Details on the serum markers are shown in Table 3. For CRP, there were 49 TP, 36 TN, 69 FP and 10 FN results. This corresponds to 83% sensitivity and 34% specificity. When considering CRP as a continuous variable, an AUROC of 0.64 (0.55-0.72) was found. The optimum threshold was 10.5 mg/L, with a corresponding 61.0% sensitivity and 62.9% specificity. For leukocyte count, there were 22 TP, 72 TN, 26 FP and 35 FN results. This resulted in a 39% sensitivity and 74% specificity. When analyzed as a continuous variable the AUROC was 0.60 (0.50-0.69). The optimum threshold was 8.6 $x10^9$/L, with a corresponding 60.0% sensitivity and 61.2% specificity. Regarding ESR, there were 18 TP, 35 TN, 11 FP and 22

FN results. This is consistent with 45% sensitivity and 76% specificity. When analyzed as a continuous variable, the AUROC was 0.58 (0.46-0.71). At the optimum threshold (10.0), sensitivity was 72.4% specificity 50.1%. The results are presented in Table 4 and Table 5.

Table 2. Baseline characteristics of study population.

	Both centers	UMCU (n=41)	UMCG (n=127)	*p*-value
Age (median (IQR))	54 (40-64)	58 (47-63)	54 (38-64)	0.27
Age at onset (median (IQR))	51 (36-59)	53 (45-59)	51 (36-62)	0.26
Sex				
Male	115 (68.5%)	26 (63.4%)	89 (70.1%)	0.44
Comorbidities				
Diabetes mellitus	13 (7.7%)	5 (12.2%)	8 (6.3%)	0.31
Psychiatric disorder	11 (6.5)	2 (4.9%)	9 (7.1%)	0.47
Obesity	21 (12.5%)	2 (4.9%)	19 (15.0%)	0.11
Osteoporosis	5 (3.0%)	5 (12.2%)	0 (0%)	0.35
Hypothyroidism	3 (1.8%)	1 (2.4%)	2 (1.6%)	0.57
Risk factors				
Smoking	63 (37.5%)	14 (34.1%)	49 (38.6%)	0.71
NSAIDs	31 (18.5%)	5 (12.2%)	26 (20.5%)	0.26
Soft drugs	6 (3.6%)	2 (4.9%)	4 (3.1%)	0.64
Hard drugs	6 (3.6%)	2 (4.9%)	4 (3.1%)	0.64
Alcohol abuse	7 (4.2%)	2 (4.9%)	5 (3.9%)	0.68
ASA classification				0.40
I	58 (35.5%)	14 (34.1%)	44 (39.3%)	
II	72 (42.9%)	20 (48.8%)	52 (46.4%)	
III	20 (11.9%)	4 (9.8%)	16 (14.3%)	
IV	1 (0.6%)	1 (2.4%)	0 (0.0%)	
Unknown	17 (10.1%)	2 (4.9%)	15 (11.8%)	
BMI, n = 150 (mean (SD))	28,18 (5.38)	26.91 (4.68)	28,77 (5.54)	0.06
Unknown (n=)	18 (10.7%)	1 (2.4%)	17 (13.4%)	
ISS				**<0.001**
<16	114 (67.9%)	16 (39.0%)	99 (78.0%)	
>16	39 (23.2%)	18 (43.9%)	21 (16.5%)	
Unknown	15 (8.9%)	7 (17.1%)	7 (5.5%)	
Fracture location				**0.002**
Upper extremity	18 (10.7%)	1 (2.4%)	17 (13.4%)	
Lower extremity	140 (83.3%)	33 (80.5%)	107 (84.3%)	
Spine	7 (4.2%)	5 (12.2%)	2 (1.6%)	
Pelvis	3 (1.8%)	2 (4.9%)	1 (0.8%)	
Fracture type				0.85
Open	80 (47,6%)	18 (43.9%)	62 (48.8%)	
Closed	79 (47.0%)	16 (39.0%)	63 (49.6%)	
Unknown	9 (5.4%)	7 (17.1%)	2 (1.6%)	
Gustilo-Anderson Classification (32)				**0.04**
Grade 1	16 (9.5%)	3 (7.3%)	13 (10.2%)	
Grade 2	12 (7.1%)	0 (0.0%)	12 (9.4%)	
Grade 3	43 (19.7%)	11 (26.8%)	22 (17.4%)	
Unknown	19 (11.3%)	4 (9.8%)	15 (11.8%)	

Table 3. CRP, LC and ESR.

	FRI				No FRI			
	TP	TN	Median	IQR	FP	FN	Median	IQR
CRP	49	36	15.0 mg/L	5.0-60.0 mg/L	69	10	7.0 mg/L	4.1-18.5 mg/L
LC	22	72	9.3 x10^9/L	7.1-12.4 x10^9/L	26	35	8.1 x10^9/L	6.7-10.2 x10^9/L
ESR	18	35	18.0 mm/h	7.0-36.0 mm/h	11	22	11.0 mm/h	5-31.5 mm/h

Table 4. Diagnostic accuracies for CRP, LC and ESR.

Test	CRP	LC	ESR
Sensitivity (95% CI)	83.1% (71.0%-91.6%)	38.6% (22.0%-52.4%)	45.0% (29.3% - 61.5%)
Specificity (95% CI)	34.3% (25.3%-44.2%)	73.5% (63.6%-81.9%)	76.1% (61.2% - 87.4%)
PPV (95% CI)	41.5% (37.2%-46.0%)	45.8% (34.7%-57.4%)	62.1% (46.8% - 75.2%)
NPV (95% CI)	78.3% (65.9%-87.0%)	67.3% (61.9%-72.3%)	61.4% (53.5% - 68.7%)
LR+ (95% CI)	1.26 (1.06-1.51)	1.45 (0.91-2.31)	1.88 (1.01 - 3.49)
LR- (95% CI)	0.49 (0.26-0.92)	0.84 (0.66-1.06)	0.72 (0.52 - 1.00)
Accuracy	51.8% (43.9%-59.7%)	60.7% (52.5%-68.4%)	79.6% (64.7% - 90.2%)

Table 5. Diagnostic accuracies for continuous variables CRP, LC, ESR and CRP + LC.

Test	CRP	LC	ESR	CRP + LC
AUROC	0.64 (95% CI 0.55-0.72)	0.60 (95% CI 0.50-0.69)	0.58 (95% CI 0.46-0.71)	0.63 (95% CI 0.54-0.73)
Sensitivity	61.0%	60.0%	72.4%	60.0%
Specificity	62.9%	61.2%	50.1%	63.9%

Table A. Models multivariable logistic regression analyses.

	AUROC	95% CI	n =	Intercept	Gustilo 0-1+2	Gustilo 0-3	DM	ISS	Smoking	Lower extremity	LC	CRP
1	0.63	0.54 - 0.73	152	-1.179	N/A	N/A	N/A	N/A	N/A	N/A	0.005	0.048
2	0.62	0.51 - 0.72	134	0.357	0.496	0.212	-0,158	-0.066	0.282	-0.811	N/A	N/A
3	0.66	0.55 - 0.77	123	-1.050	0.479	-0.101	0,804	-0.139	0.370	-0.746	0.044	0.007

Multivariable logistic regression analysis

ESR was left out of these analyses as this marker was missing in half of the patients (n=86, 51.2%). The AUROC of CRP and LC combined was 0.63 (95% CI 0.54-0.73). At the Q-point, there were 33 TP, 62 TN, 35 FP and 22 FN, with a sensitivity and specificity of 60% and 64% (Table 4 and Table 5). The model with clinical parameters and combined inflammatory markers had an AUROC of 0.66 (95% CI 0.55-0.77), as compared to 0.62 (95% CI 0.51-0.72) without inflammatory markers.

The AUROC of the combined markers per center was 0.63 (0.54-0.73) for the UMCG, and 0.68 (0.51-0.87) for the UMCU. The AUROC was 0.64 (0.34-0.93) <14 days and 0.61 (0.48-0.75) ≥14 days. The AUROC of the model with log-transformed CRP and LC was 0.63 (0.54-0.73).

Discussion

This study focused on the diagnostic accuracy of the serum inflammatory markers CRP, LC and ESR in patients who were suspected of FRI. It is the first study to include clinical parameters proven to be predictive of FRI in its analysis. Although most clinicians regard serum inflammatory markers to be part of the general work-up of suspected FRI, the results of this study indicate that they should be cautious when interpreting their results, as was published in the Consensus definition on FRI (2).

The majority of the literature on inflammatory markers in orthopedic infection has focused on periprosthetic joint infections (PJI) and osteomyelitis of the diabetic foot (34-37). CRP has been proven to be useful in both (38, 39). Moreover, the value of LC is less well established. (9, 40) In early postoperative infections after fracture surgery, continuous elevation or a secondary rise might be expected in CRP and LC (24, 41). Levels of serum CRP, LC and ESR have been shown to be significantly lower in FRI than in hematogenous osteomyelitis and osteomyelitis of the diabetic foot (42, 43).

Studies on the diagnostic value of serum inflammatory markers in FRI are limited, and their methodology is heterogeneous. Different serum marker thresholds are used, and study populations vary. As in the current study, the study population of Buhl et al. consisted of patients who underwent nuclear medical imaging for suspected FRI or infected prosthesis.(44) They reported a sensitivity and specificity for ESR of 84% and 29% respectively, and 56% and 35% for CRP. These results differ from those in the current study. This may be due to PJI being excluded in the current study and the use of different thresholds. Most studies on serum markers in FRI have focused on subgroups of FRI, such as infected non-union or patients undergoing conversion surgery. One study reported on the value of CRP and ESR in diagnosing infection in patients undergoing conversion from internal fixation of a femoral neck fracture to total hip arthroplasty (45). The authors reported a higher diagnostic accuracy than the current study, with an AUROC of 0.89 for both markers. Unfortunately, their study has a high risk of overfitting due to the inclusion of only six patients with FRI. Therefore, the true AUROC, obtained after (internal and) external validation, will be much lower (46). Several studies have focused on the value of inflammatory markers in diagnosing infection in patients presenting with mal- or non-union (11-13). The diagnostic accuracy of individual serum inflammatory markers in this sub-group of FRI is low. Some of these studies have looked at the diagnostic accuracy of combined serum markers. Similar to the results of the current study, combining markers was found to increase the diagnostic accuracy for FRI only marginally.(11, 13)

With an accuracy of 79.6%, the diagnostic value of ESR in the current study appears to be high. However, the large overlap in the IQR of the FRI and non-FRI groups shows the discriminative value of ESR to be low.

The differences in results between the literature and the current study may be caused by several factors. Most importantly, several different thresholds are used to define elevation of serum inflammatory markers. This makes a valid comparison of results impossible, especially when only sensitivity and

specificity are reported. Furthermore, FRI is a heterogeneous disease, with tissue involvement varying in location and severity. Some studies focus on all patients with FRI, others choose subgroups to increase population homogeneity. These differences in study populations further complicate comparing results and it is therefore imperative that international lab protocols are being developed and uniform diagnostic criteria including threshold values and timing for obtaining serum inflammatory markers regarding FRI are being established and implemented. Finally, most studies have looked at serum markers taken between 1 to 14 days prior to obtaining intra-operative cultures. The current study focused on inflammatory markers when infection was first suspected, with a median of 48.5 days between index- and reference test. This is in concordance with clinical practice, as the clinician will obtain serum inflammatory markers at the time an FRI has to be confirmed or ruled out. The actual surgery often follows at a later point, when additional diagnostic work, such as imaging, has been completed. This difference may have influenced the results.

Strengths of this study are that it is one of the largest cohorts investigating the diagnostic performance of individual and combined serum inflammatory markers in FRI. The inclusion of combined markers is important, as in clinical practice, inflammatory markers are never interpreted individually. Furthermore, they are always interpreted in combination with clinical parameters. Therefore, information from multiple markers was combined with clinical parameters that are associated with FRI to estimate the probability of infection.

This study does have some limitations. First of all, all patients with suspected FRI were collectively analyzed, and thus these results may not be applicable to all possible subgroups. Furthermore, due to its retrospective nature, there was no uniform time interval between index- and reference test. However, this is in accordance with clinical practice. In addition, the laboratory measurements have been performed using different methods, however due to laboratory standardization and internal and external quality control schemes differences due to measurement methods are negligible. Also, the outcome of this study might be affected by selection bias as the patients undergoing advanced nuclear imaging could have been selected based on the outcome of their serum inflammatory marker testing. This could potentially alter the true NPV of the markers.

Conclusion

The outcome of this retrospective study indicates that the added diagnostic value of CRP, LC and ESR seems to be limited for FRI. FRI can still be present when serum inflammatory markers are within normal range. Therefore, clinicians should be cautious when interpreting the results of these tests in patients with suspected FRI.

Abbreviations

AO: Arbeitsgemeinschaft für Osteosynthese-fragen; AUROC: area under the receiver operating characteristic; CI: confidence interval; CRP: c-reactive protein; ESR: erythrocyte sedimentation rate; FN: false negative; FP: false positive; FRI: Fracture-Related Infection; IQR: interquartile range; ISS: injury severity score; LC: leukocyte count; LR-: negative likelihood ratio; LR+: positive likelihood ratio; METC: medical ethical research commission; MMB: medical microbiology; NPV: negative predictive value; PJI: periprosthetic joint infection; PPV: positive predictive value; TN: true negative; TP: true positive; UMCG: university medical center Groningen; UMCU: university medical center Utrecht.

Competing Interests

The authors have declared that no competing interest exists.

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