Utility of Common Biomarkers for Diagnosing Infection in Nonunion

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Objectives: To evaluate the diagnostic utility of leukocyte count (WBC), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) for distinguishing between septic and aseptic nonunions.

Design: A single-gate (cohort) design was used, using 1 set of eligibility criteria applied to a consecutive sample of nonunions.

Setting: Private quaternary referral center.

Patients/Participants: Inclusion criteria were consecutive patients (≥18 years) with a nonunion requiring surgery that allowed for direct or medullary canal tissue sampling from the nonunion site. The cohort included 204 subjects with 211 nonunions.

Intervention: Blood samples were drawn for laboratory analysis of WBC, ESR, and CRP before surgery.

Main Outcome Measurements: The reference standard used to define infection was the fracture-related infection confirmatory criteria. Measures of diagnostic accuracy were calculated. To assess the additional diagnostic gain of each index lab test while simultaneously considering the others, logistic regression models were fit.

Results: The prevalence of infection was 19% (40 of 211 nonunion sites). The positive likelihood ratios (95% confidence interval) for WBC, ESR, and CRP were 1.07 (0.38–3.02), 1.27 (0.88–1.82) and 1.57 (0.94–2.60), respectively. Multivariable modeling adjusted for the effect of preoperative antibiotics showed that WBC ($P = 0.42$), ESR ($P = 0.48$), and CRP ($P = 0.23$) were not significant predictors of infection.

Conclusions: In this consecutive sample of 211 nonunions in whom standard clinical practice would be to obtain index lab tests, our findings showed that WBC, ESR, and CRP were not significant predictors of infection.

Key Words: nonunion, biomarkers, infection, diagnostic study

Level of Evidence: Diagnostic Level II. See Instructions for Authors for a complete description of levels of evidence.

INTRODUCTION

The use of laboratory tests including leukocyte count (WBC), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) are ubiquitous in the diagnostic pathway of musculoskeletal infection and are typically obtained simultaneously. A recent consensus document recommends the use of these 3 biomarkers in adults suspected of bone infection.[1] However, research on the diagnostic utility of these biomarkers in identifying infected nonunions is limited.[2,3]

Septic nonunions are often quiescent, can be asymptomatic, and a high index of suspicion is warranted when there has been previous surgery, a history of infection, or an initial open fracture.[4] There is a spectrum of clinical presentation of septic nonunion. Some present with failed previous fixation that may harbor low virulence organisms and may appear aseptic. Others can present with soft-tissue compromise, draining wounds, and/or history of prior infection.

The importance of knowing if a nonunion is infected cannot be overstated. The treatment regimen, length and cost of treatment, and prognosis are often vastly different between septic and aseptic nonunion. Therefore, the ability to distinguish between septic and aseptic nonunion before commencing treatment is critical.

Although recent literature has suggested that WBC, ESR, and CRP are useful markers for infection in cases of fracture nonunion, as a center specialized in the treatment of nonunions, we have not routinely found these biomarkers useful in such cases. Hence, the hypothesis of the current investigation was that in a target population including a spectrum of nonunions (a consecutive sample of patients in whom standard clinical practice would be to obtain index lab tests), typical diagnostic biomarkers would not be predictive of infection.

PATIENTS AND METHODS

Inclusion criteria for this consecutive series of patients seen and operatively treated for a nonunion by the senior one of us (M.R.B.) included: (1) age ≥18 years; (2) presentation at our institution between June 6, 2015, and December 12, 2018; and (3) an operative plan that allowed for direct or

medullary canal tissue sampling from the nonunion site. A single-gate (cohort) design was used, using 1 set of eligibility criteria for all participants.[5] Potentially eligible participants were identified by the senior author at our private quaternary referral center. The study was approved by the Institutional Review Board of Texas Orthopedic Hospital.

Blood samples were drawn for laboratory analysis of WBC, ESR, and CRP within 2 weeks of surgery. Index test positivity cut-offs were determined using the laboratory reference values from our institution: WBC count (K/mm^3) >10.5 for men and >11.0 for women; ESR (mm/h) >15 for men and >20 for women; and CRP (mg/dL) ≥0.9 for both men and women. WBC values of <5.7 in men and <5.8 in women are categorized as low by our laboratory. For purposes of analysis, normal and low WBC categories were pooled. Although some subjects did receive a single prophylactic dose of antibiotics preoperatively, no other clinical intervention took place in the time interval between the index lab tests and the reference standard (deep operative culture).

Before surgery and for the purpose of this study, the senior author documented his clinical impression of septic versus aseptic nonunion based on the history, physical examination, imaging studies, and index lab results. The decision to administer or withhold a single, prophylactic dose of preoperative antibiotics was largely determined by this clinical impression. In keeping with standard clinical practice, when a high index of suspicion for infection was present, antibiotics were typically withheld until after the cultures were obtained.

Intraoperatively, each nonunion site was biopsied, and specimens were sent for culture. Two to 4 tissue samples were obtained for each site for each type of culture (aerobic, anaerobic, fungal, and acid-fast bacilli) and gram stain. Direct tissue sampling (n = 173) occurred when the nonunion site was operatively exposed; medullary canal tissue sampling (n = 38) occurred by reamings from the medullary canal such as during exchange nailing where the nonunion site was not directly exposed. The reference standard used to define infection followed the Musculoskeletal Infection Society (MSIS) major criteria, which are similar to the fracture-related infection (FRI) consensus definition of confirmatory criteria.[6–9] For our purposes, this included 2 positive microbial cultures of the same pathogen and/or an open/draining wound to be classified as "infected," and if a single positive culture was obtained, it was classified as "suggestive."

Statistical analyses were performed using open-source R statistical software,[10] and confidence intervals (CIs) for likelihood ratios were calculated using Simel's formula.[11] Bivariate comparisons of continuous variables were made using the Wilcoxon test. To assess the additional diagnostic gain of each index test while simultaneously considering the others, and controlling for potential confounding by other variables, logistic regression models were fit using the *rms* package.[12] When multiple tests are obtained to assist in the diagnosis of a target disorder that is not conditionally independent, multivariable logistic regression is well suited to account for this.[13,14]

WBC, ESR, and CRP are typically ordered together as measures of the underlying physiologic response to infection and consequently are not independent of one another. To that end, 2 logistic models were fit. In logistic model 1, the index lab tests were analyzed as dichotomous variables using the cut-offs as described. In logistic model 2, the index lab values were analyzed as continuous variables to determine if there was additional diagnostic information in the values beyond the cut-off points. Both models also included the administration of prophylactic antibiotics (yes/no) as a covariate. All covariates included in the models (WBC, ESR, CRP, and if a single prophylactic preoperative dose of antibiotics was administered) were specified a priori. Interquartile range odds ratios (IQRORs) are given for continuous variables in the models, which demonstrate the effect of increasing a variable from its first quartile to its third quartile.

Bayes' rule is often used in diagnostic research for prediction of the likelihood of a target disease.[15] Further explanation of this and terminology regarding the different indices of diagnostic performance is given in a **Supplemental Digital Content 1** (see **Appendix**, http://links.lww.com/JOT/B169).

RESULTS

Of 208 subjects with 215 nonunions, there were 4 subjects with 4 nonunions whose index procedure and tissue analyses were performed at an outside institution. For consistency in the clinical reference standard (operative cultures), these 4 cases were excluded. Hence, the cohort consisted of 204 subjects (105 men and 99 women) with 211 nonunions (5 subjects had 2 nonunion sites and 1 had 3). The median age of the cohort was 56 years. The side of the nonunion was right in 109 cases. For baseline characteristics of the cohort, **Supplemental Digital Content 2** (see **Table**, http://links. lww.com/JOT/B170). Fig. 1 shows the flow of participants through the study, the index tests evaluated, and the target condition of infection determined by MSIS major/FRI confirmatory criteria. This flow diagram follows the Standards for Reporting of Diagnostic Accuracy Studies guidelines for reporting diagnostic accuracy studies.[5]

The distribution of the 211 nonunion sites was as follows: clavicle (16), humerus (34), "elbow" (failed elbow fusion or olecranon nonunion) (3), forearm (9), femur (45), "knee" (failed knee fusion or plateau nonunion or patella nonunion) (6), tibia (63), fibula (8), and "ankle" (failed ankle fusion or nonunion of malleoli or plafond nonunion) (27); hence, 149 in the lower extremities and 62 in the upper extremities. The distribution of type[16] of nonunion in the cohort was as follows: hypertrophic (n = 29), oligotrophic (89), atrophic (80), and synovial pseudoarthrosis (13). The median interval from injury to our index surgical intervention was 0.95 years.

Of the 211 nonunion sites, 62 followed an open fracture, 141 a closed fracture, and the status of the soft tissue at initial injury was unknown in 8 cases. Of the 211 nonunion sites, 43 had a history of previous positive deep cultures, 161 had no such history, and the history was obscure in 7 cases. On presentation 24 nonunion sites were not associated with a previous operative exposure, 160 had a healed surgical wound, and 27 had a nonunion site associated with a draining wound. Eighty-nine percent of all nonunions had undergone one or more previous operative procedures.
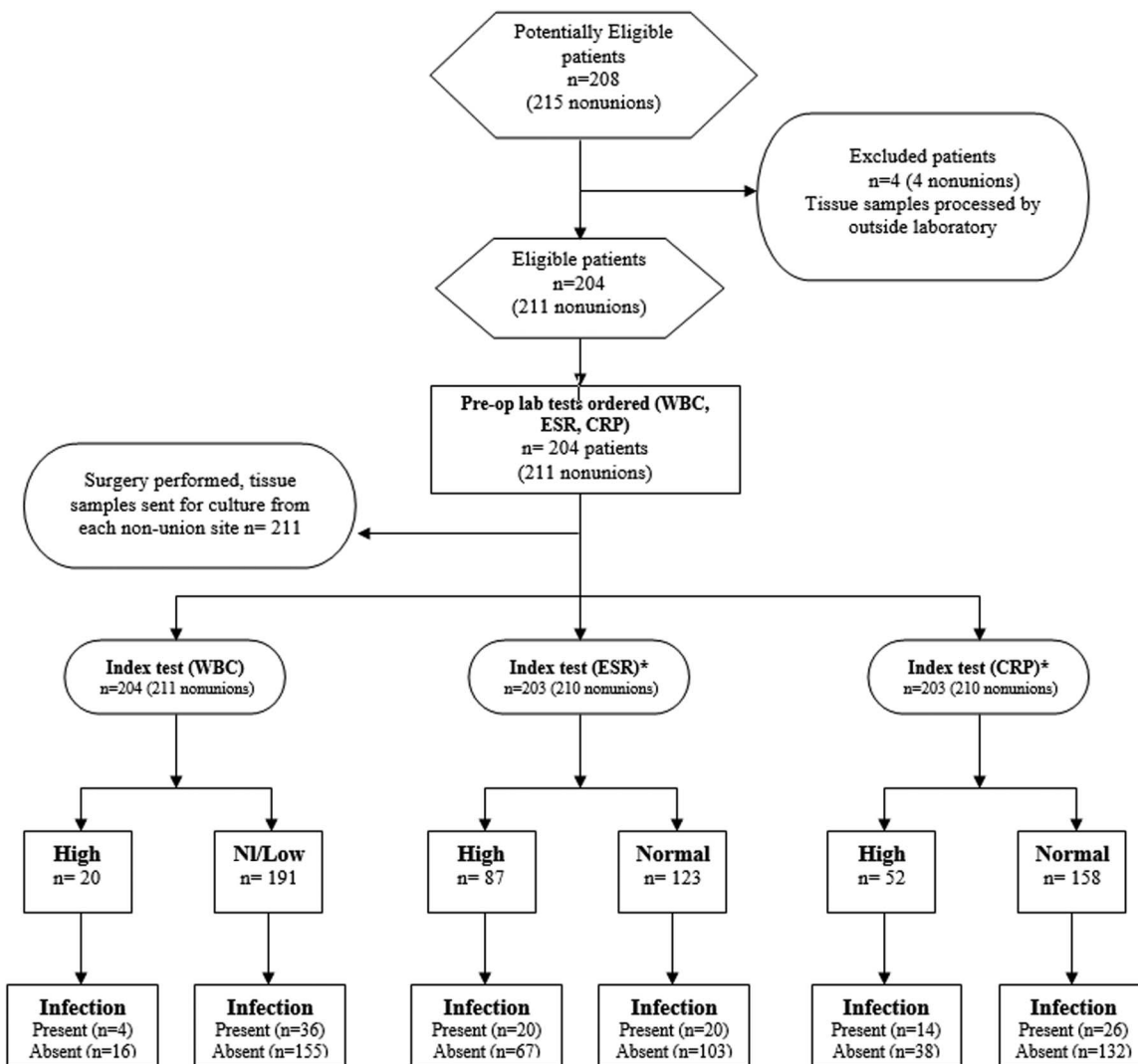
FIGURE 1. STARD flow diagram showing the flow of participants through the study, the index lab tests evaluated, and the target condition of infection determined by the clinical reference standard of microbial culture. *Note: ESR and CRP were missing for 1 male subject with 1 nonunion site involving the femur. STARD, Standards for Reporting of Diagnostic Accuracy Study.

Biomarkers and Infection

Using the MSIS major/FRI confirmatory criteria, the prevalence of infection in the cohort was 19% (40 of 211 nonunion sites). The diagnosis of infection was made by ≥ 2 positive cultures in 34 cases and based on an open/draining wound in an additional 6 cases who did not have ≥ 2 positive cultures. The mean age in infected cases was 53 years, and the mean age in aseptic cases was 56 years ($P = 0.14$). There were 4 positive gram stains, and all 4 were ultimately classified as septic nonunions based on ≥ 2 positive deep cultures that identified phenotypically indistinguishable pathogens.

The number of infections by anatomic location was as follows: elbow (1), clavicle (3), humerus (1), ankle (9), femur (3), fibula (5), and tibia (18). The distribution of cultured organisms by anatomic site and administration of a single preoperative prophylactic dose of antibiotics is shown in Fig. 2. Nearly half of the subjects classified as infected

(47%) received a single prophylactic dose of antibiotics preoperatively.

The median and IQR values for the overall cohort for WBC, ESR, and CRP were 7.2 (5.7–8.9), 14 (7–27), and 0.2 (0.2–0.8), respectively. Table 1 lists descriptive statistics for index lab tests given as continuous and dichotomous (binary) variables, the cumulative number of positive index tests, and the biopsy method stratified by FRI criteria. Table 2 shows the diagnostic accuracy metrics calculated for index lab tests defining infection using the MSIS major/FRI confirmatory criteria. Table 3 shows the diagnostic metrics for index lab tests using a combination of FRI confirmatory and suggestive criteria as a positive result, essentially this is the diagnostic utility of the lab tests associated with a positive culture (≥ 1). Overall, the diagnostic utility of WBC, ESR, and CRP in this cohort was low (likelihood ratios close to 1). The diagnostic odds ratios (DORs) (and 95% CI) for these 3 tests were 1.10 (0.34–3.41), 1.54 (0.77–3.07), and 1.87 (0.89–3.93), respectively.
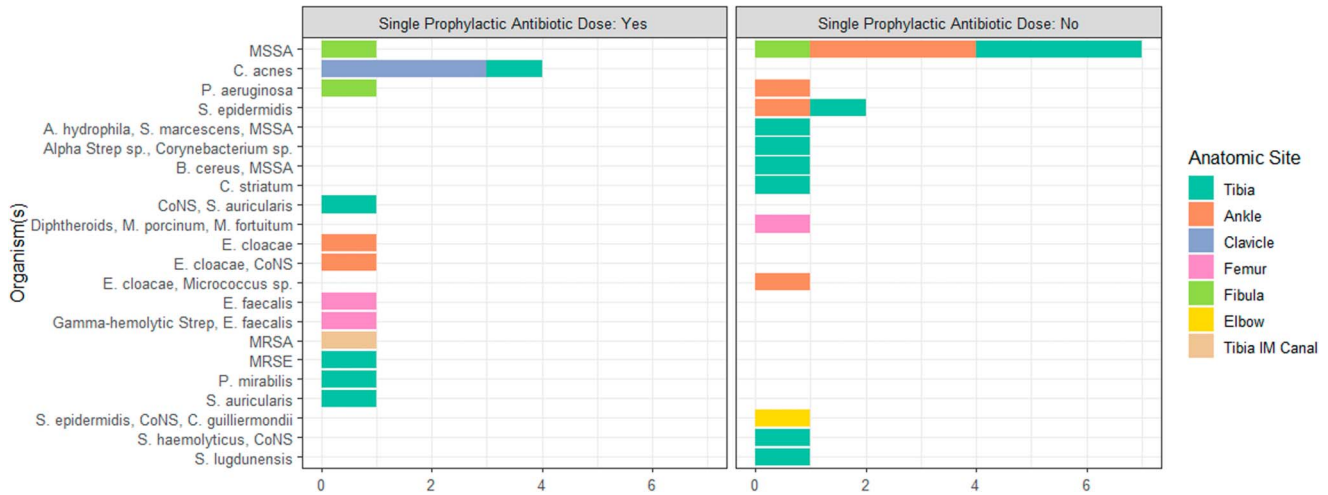
FIGURE 2. The frequency of cultured organisms in the 34 infected cases using MSIS/FRI criteria of ≥2 positive cultures. Note: This figure does not include the 6 cases classified as infected by MSIS/FRI criteria with an open/draining wound without ≥2 positive cultures. **Editor's Note**: A color image accompanies the online version of this article.

Simultaneous Effects of Multiple Factors on Diagnosis of Infection

Logistic Model 1: Lab Values Treated as Dichotomous Independent Predictors

WBC (OR = 0.55, 95% CI = 0.13–2.37, $P = 0.42$), ESR (OR = 0.70, 95% CI = 0.27–1.86, $P = 0.48$), and CRP (OR = 1.86, 95% CI = 0.67–5.18, $P = 0.23$) were not significant predictors of septic nonunion. For clarity, although withholding a single preoperative dose of antibiotics was associated with a higher odds of infection (OR = 29.82, 95% CI = 10.30–86.35, $P < 0.001$), the model clearly showed that WBC, ESR, and CRP were not significant predictors of infection.

Logistic Model 2: Lab Values Treated as Continuous Independent Predictors

WBC (IQROR = 0.77, 95% CI = 0.46–1.28, $P = 0.31$), ESR (IQROR = 1.36, 95% CI = 0.87–2.12, $P = 0.18$), and CRP (IQROR = 0.97, 95% CI = 0.84–1.13, $P = 0.71$) were

TABLE 1. Biomarkers and Tissue Sampling Method Stratified by the Infection Status Using FRI Consensus Nomenclature: Median (IQR) or n (% Rounded to the Nearest Percent)

	Not Infected (n = 155)	Suggestive* (n = 16)	Infected† (n = 40)
WBC	7.3 (5.8–9.1)	6.5 (5.5–7.8)	7.2 (5.7–9.1)
WBC (binary)			
High	16 (10%)	0	4 (10%)
Nl/Low	139 (90%)	16 (100%)	36 (90%)
ESR	13 (6.2–26.8)	15.0 (8.5–27.0)	17.5 (9.2–40.0)
ESR (binary)			
High	59 (38%)	8 (50%)	20 (50%)
Normal	95 (62%)	8 (50%)	20 (50%)
CRP	0.2 (0.2–0.7)	0.2 (0.2–0.7)	0.2 (0.2–1.5)
CRP (binary)			
High	35 (23%)	3 (19%)	14 (35%)
Normal	119 (77%)	13 (81%)	26 (65%)
Cumulative number of positive index tests			
None	80 (52%)	7 (44%)	16 (40%)
1 positive	42 (27%)	7 (44%)	10 (25%)
2 positive	28 (18%)	2 (12%)	14 (35%)
3 positive	4 (3%)	0	0
Biopsy method			
Direct	121 (78%)	14 (88%)	38 (95%)
Medullary	34 (22%)	2 (12%)	2 (5%)

*Single positive culture.
†Had ≥2 positive deep cultures that identified phenotypically indistinguishable pathogens and/or an open/draining wound.

TABLE 2. Indices of Diagnostic Performance of Lab Tests in the Diagnosis of Infection Based on MSIS Major/FRI Confirmatory Criteria (2 Positive Cultures of the Same Organism and/or an Open/Draining Wound)

	Se	Sp	PPV	NPV	LR+	LR−	DOR
WBC	0.10 (0.03–0.24)	0.91 (0.85–0.95)	0.20 (0.06–0.44)	0.81 (0.75–0.86)	1.07 (0.38–3.02)	0.99 (0.89–1.11)	1.10 (0.34–3.41)
ESR	0.50 (0.34–0.66)	0.61 (0.53–0.68)	0.23 (0.15–0.33)	0.84 (0.76–0.90)	1.27 (0.88–1.82)	0.83 (0.59–1.15)	1.54 (0.77–3.07)
CRP	0.35 (0.21–0.52)	0.78 (0.71–0.84)	0.27 (0.16–0.41)	0.84 (0.77–0.89)	1.57 (0.94–2.60)	0.84 (0.66–1.07)	1.87 (0.89–3.93)

The prevalence of infection using these criteria was 19% (40/211).

We estimated Se, Sp, PPV, NPV, positive and negative Likelihood Ratios (LR+ and LR−), and DOR with their corresponding CIs (95% CI) for index lab tests by constructing 2 × 2 contingency tables using the *epiR* package. Exact binomial confidence limits were calculated for proportions (Se, Sp, PPV, and NPV), and CIs for likelihood ratios were calculated using Simel's formula.

not significant predictors of infection. Although withholding preoperative antibiotics was associated with a higher odds of septic nonunion (OR = 29.37, 95% CI = 10.26–84.09, $P <$ 0.001), this model also showed that WBC, ESR, and CRP treated as continuous variables were not significant predictors of infection.

DISCUSSION

In this study of 204 consecutive patients with 211 nonunions who all received the same index tests and reference standard, we did not find typical preoperative biomarkers (WBC, ESR, and CRP) to be predictive of infection. The reasons our findings differ from the 2 recent studies that have investigated these biomarkers in the setting of nonunion [2,3] are likely because of differences in study design, methodology, and analyses.

Both previously published studies retrospectively reviewed smaller sample sizes of nonconsecutive patients and included only "high-risk" subjects [the prevalence of infection in these studies were 33% (2) and 83% (3)]. Inclusion of only high-risk patients introduces selection/spectrum bias by primarily focusing on a target-positive population that leads to overestimation of performance of diagnostic tests.[17] Both studies also seem to have used a sequential application of Bayes' theorem to calculate positive predictive values (PPVs) based on the cumulative number of positive tests, and such Bayesian chain calculations to estimate diagnostic probabilities for several tests that are not conditionally independent is inappropriate.[13,14] Also, keep in mind that as the prevalence increases so does the PPV. Stucken et al [2] reported the predicted probability of infection if 1, 2, or all 3 index tests (WBC, ESR, and CRP) were positive to be 19%, 56%, and 100%, respectively. This approach undoubtedly led to biased estimates that are overly optimistic because the tests are not conditionally independent (eg, subjects with an elevated ESR are more likely to have an elevated CRP); positive correlations that exist among the index tests make the effect of the second test smaller once the first test result is already known. Because of the simplicity and statistical incorrectness of this approach, it is sometimes referred to as "Naïve" Bayes.[15] Stucken et al also defined infection by a single positive culture, while we have used the MSIS/FRI criteria to define the reference standard of infection. Although, we also analyzed the diagnostic utility of the lab tests using a positive culture (≥1) as the outcome (Table 3) and found they had

poor diagnostic utility. Another difference between the current study and Stucken et al's study is that we obtained 2–4 tissue samples for culture, whereas they reported taking 4–6 samples. Although these differences might explain, in part, the differences in the results, it seems more likely that the differences are due to differences in patient selection and spectrum of disease. The current study sought to include a broader spectrum of nonunions, a consecutive sample of all comers, where the diagnosis of infection was less clear and in whom standard clinical practice is to obtain index lab tests, rather than selecting high-risk subjects some of whom are already above the treatment threshold for infection at initial presentation and are therefore more likely to have elevated lab tests.

The role of preoperative antibiotics to decrease surgical site infection is well established;[18] however, when a diagnosis of infection is unclear, prophylactic antibiotics are often withheld until tissue samples are obtained because they could inhibit organism growth and lead to false-negative culture results.[19] This approach was closely followed in the current study; 73% of subjects with a clinical impression of septic nonunion compared with only 3% believed to have an aseptic nonunion had antibiotics withheld. In the 2 recent studies that investigated the diagnostic utility of laboratory tests in the setting of nonunion, one study makes no mention of the use of prophylactic antibiotics,[2] whereas the other excluded anyone who received preoperative antibiotics.[3] Current evidence suggests that exposure to a single dose of prophylactic antibiotics before intraoperative cultures have minimal, if any, impact on culture results.[20–23] If there were some false negative culture results in our study because of the administration of preoperative antibiotics, unless these were unequally distributed among those with abnormal and normal index lab values, it is unlikely to have biased our findings; and even if this were the case, the use of multivariable regression would control for this which is why this variable was included in the models. The apparent suppression effect of antibiotics that we found in our regression models is likely confounded by the clinical impression which was the key factor in determining the use of prophylactic antibiotics, hence, they are correlated; and the magnitude of effect for the clinical impression was quite large (DOR = 41, 95% CI: 15–116) similar to that found for antibiotics. Had there been sufficient sample size to adjust for the clinical impression in the models we would have likely seen little, if any, effect of antibiotics. Furthermore, as shown in Fig. 2, many subjects had a positive

TABLE 3. Indices of Diagnostic Performance of Lab Tests Associated With a Positive Culture Including MSIS Inconclusive/FRI Suggestive Criteria (a Single Positive Culture) in Addition to MSIS Major/FRI Confirmatory Criteria

	Se	Sp	PPV	NPV	LR+	LR−	DOR
WBC	0.07 (0.02–0.17)	0.90 (0.84–0.94)	0.20 (0.06–0.44)	0.73 (0.66–0.79)	0.69 (0.24–1.98)	1.04 (0.95–1.13)	0.67 (0.21–2.09)
ESR	0.50 (0.36–0.64)	0.62 (0.54–0.69)	0.32 (0.23–0.43)	0.77 (0.69–0.84)	1.31 (0.94–1.81)	0.81 (0.61–1.08)	1.61 (0.87–2.98)
CRP	0.30 (0.19–0.44)	0.77 (0.70–0.84)	0.33 (0.20–0.47)	0.75 (0.68–0.82)	1.34 (0.82–2.18)	0.90 (0.74–1.09)	1.48 (0.75–2.93)

The prevalence of a positive culture when combining confirmatory and suggestive criteria was 27% (56/211).

We estimated Se, Sp, PPV, NPV, positive and negative likelihood ratios (LR+ and LR−), and DOR with their corresponding CIs (95% CI) for index lab tests by constructing 2×2 contingency tables using the *epiR* package. Exact binomial confidence limits were calculated for proportions (Se, Sp, PPV, and NPV), and CIs for likelihood ratios were calculated using Simel's formula.

culture despite receiving a single prophylactic dose of antibiotics preoperatively.

Sensitivity (Se) and specificity (Sp) are commonly reported measures of diagnostic test accuracy, despite the fact that they actually have no diagnostic relevance as they are "reverse probabilities" [24] conditional on knowing the disease status. Although they do have the advantage of not being influenced by prevalence, they are influenced by disease spectrum, patient characteristics, and vary among different patient populations.[24,25] Nonetheless, we have included them because of their widespread use and for comparison with recent nonunion diagnostic research studies. Of the 4 classic indices used to describe diagnostic tests, we agree with Moons and Harrell that post-test probabilities [PPV, negative predictive value (NPV)] should be emphasized[24] over Se and Sp because they approach the data from the direction of the test results, which is how such data are used clinically to determine the probability of disease and they allow for the extrapolation of test characteristics to populations of patients. In the current investigation, the PPVs of WBC, ESR, and CRP in diagnosing infection in nonunion was 0.20, 0.29, and 0.33, respectively, indicating low diagnostic utility. Better still are the use of likelihood ratios (LRs) and DORs (Tables 2 and 3) which are independent of prevalence, intuitive, and are applicable to specific patients. The positive LRs for all 3 tests were close to 1 meaning the tests would yield minimal increases in the probability of infection.

When dichotomizing test results, diagnostic information from values above the cut-off can be lost.[13] Multilevel likelihood ratios can be used to estimate the LRs for different intervals of test results. We did not calculate multilevel LRs for the index tests because the frequencies of values above the index cut-off points were too few to allow for stable estimates, which is a limitation of the current study. However, given that the results from the models did not show that the index tests were predictive of infection, particularly when analyzed as continuous data, we would not expect to see dramatic increases in the LRs at intervals above the cut-off points. Another potential limitation of our study was using the nonunion site as the unit of analysis rather than the patient,[26] although, this approach is consistent with prior research on the topic.[2]

In summary, whereas Stucken et al [2] and Wang et al [3] reported utility of WBC, ESR, and CRP in the diagnosis of infection in nonunion, the results of this consecutive series of nonunions, in whom standard clinical practice would be to obtain index lab tests, using more appropriate sampling and statistical methods found that these biomarkers were of little utility in discriminating septic from aseptic nonunion.

REFERENCES

1. Glaudemans AW, Jutte PC, Cataldo MA, et al. Consensus document for the diagnosis of peripheral bone infection in adults: a joint paper by the EANM, EBJIS, and ESR (with ESCMID endorsement). *Eur J Nucl Med Mol Imaging.* 2019;46:957–970.
2. Stucken C, Olszewski DC, Creevy WR, et al. Preoperative diagnosis of infection in patients with nonunions. *J Bone Joint Surg Am.* 2013;95: 1409–1412.
3. Wang S, Yin P, Quan C, et al. Evaluating the use of serum inflammatory markers for preoperative diagnosis of infection in patients with nonunions. *Biomed Res Int.* 2017;2017:9146317.
4. Olszewski D, Streubel PN, Stucken C, et al. Fate of patients with a "surprise" positive culture after nonunion surgery. *J Orthop Trauma.* 2016;30:e19–e23.
5. Cohen JF, Korevaar DA, Altman DG, et al. STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. *BMJ Open.* 2016;6:e012799.
6. Workgroup Convened by the Musculoskeletal Infection Society. New definition for periprosthetic joint infection. *J Arthroplasty.* 2011;26: 1136–1138.
7. 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;469:2992–2994.
8. Obremskey WT, Metsemakers WJ, Schlatterer DR, et al. Musculoskeletal infection in orthopaedic trauma: assessment of the 2018 international consensus meeting on musculoskeletal infection. *J Bone Joint Surg Am.* 2020;102:e44.
9. Metsemakers WJ, Morgenstern M, McNally MA, et al. Fracture-related infection: a consensus on definition from an international expert group. *Injury.* 2018;49:505–510.
10. R Core Team. *C: A Language and Environment for Statistical Computing. [Internet].* Vienna, Austria: R Foundation for Statistical Computing; 2019. Available at: https://www.R-project.org/. Accessed June 13, 2019.
11. Simel DL, Samsa GP, Matchar DB. Likelihood ratios with confidence: sample size estimation for diagnostic test studies. *J Clin Epidemiol.* 1991; 44:763–770.
12. Harrell FE, Jr. *rms: Regression Modeling Strategies [Internet].* 2019. Available at: https://CRAN.R-project.org/package=rms. Accessed October 29, 2019.
13. Fischer JE, Bachmann LM, Jaeschke R. A readers' guide to the interpretation of diagnostic test properties: clinical example of sepsis. *Intensive Care Med.* 2003;29:1043–1051.
14. Moons KG, van Es GA, Deckers JW, et al. Limitations of sensitivity, specificity, likelihood ratio, and Bayes' theorem in assessing diagnostic probabilities: a clinical example. *Epidemiol Camb Mass.* 1997;8:12–17.

15. Steyerberg EW. *Clinical Prediction Models: A Practical Approach to Development, Validation, and Updating*. Berlin, Germany: Springer Science & Business Media; 2008.

16. Brinker MR, O'Connor DP. Nonunions: evaluation and treatment. In: *Skeletal Trauma: Basic Science, Management, and Reconstruction*. Philadelphia, PA: Elsevier Saunders; 2009:637–718.

17. Haynes RB, David SL, Gordon GH, et al. *Clinical Epidemiology: How to Do Clinical Practice Research*. 3rd ed. Philadelphia, PA: LWW; 2005.

18. Prokuski L. Prophylactic antibiotics in orthopaedic surgery. *J Am Acad Orthop Surg*. 2008;16:283–293.

19. Leekha S, Terrell CL, Edson RS. General principles of antimicrobial therapy. *Mayo Clin Proc*. 2011;86:156–167.

20. Burnett RS, Aggarwal A, Givens SA, et al. Prophylactic antibiotics do not affect cultures in the treatment of an infected TKA: a prospective trial. *Clin Orthop*. 2010;468:127–134.

21. Wouthuyzen-Bakker M, Benito N, Soriano A. The effect of preoperative antimicrobial prophylaxis on intraoperative culture results in patients with a suspected or confirmed prosthetic joint infection: a systematic review. *J Clin Microbiol*. 2017;55:2765–2774.

22. Trionfo A, Thoder JJ, Tosti R. The effects of early antibiotic administration on bacterial culture growth from hand abscesses. *Hand (N Y)*. 2016;11:216–220.

23. Al-Mayahi M, Cian A, Lipsky BA, et al. Administration of antibiotic agents before intraoperative sampling in orthopedic infections alters culture results. *J Infect*. 2015;71:518–525.

24. Moons KG, Harrell FE. Sensitivity and specificity should be de-emphasized in diagnostic accuracy studies. *Acad Radiol*. 2003;10:670–672.

25. Dujardin B, Van den Ende J, Van Gompel A, et al. Likelihood ratios: a real improvement for clinical decision making? *Eur J Epidemiol*. 1994;10:29–36.

26. Bryant D, Havey TC, Roberts R, et al. How many patients? How many limbs? Analysis of patients or limbs in the orthopaedic literature: a systematic review. *J Bone Joint Surg Am*. 2006;88:41–45.