Globulin, albumin to globulin ratio, and plasma fibrinogen may be potential diagnostic biomarkers for tibial infected nonunion

Haijun Mao
Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School
Zhen Wang
Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School
Guangyue Xu (xugy0813@163.com)
Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School

Article

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Abstract

The accurate preoperative diagnosis of infected nonunion remains challenging. Here, we evaluated the diagnostic potential of novel biomarkers for tibial infected nonunion. A single-center retrospective study was conducted on 155 patients divided into two groups: 59 with tibial infected nonunion (Group A) and 96 with tibial aseptic nonunion (Group B). Preoperatively analyzed clinical parameters included plasma D-dimer, plasma fibrinogen, albumin (ALB), globulin (GLB), albumin-to-globulin ratio (AGR), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and white blood cell (WBC) count. Receiver operating characteristic (ROC) curves, sensitivity, and specificity were utilized to compare the diagnostic potential of these biomarkers. The WBC, ESR, CRP, plasma D-dimer, plasma fibrinogen, and GLB levels of Group A were significantly higher ($P < 0.05$) than in Group B. The ALB and AGR levels of Group A were significantly lower ($P < 0.05$) than in Group B. The area under the curve (AUC) of ESR, plasma fibrinogen, and AGR were greater than 0.8. An analysis of a combination of ESR, plasma fibrinogen, GLB, and AGR had the highest AUC. In patients with comorbidities, the diagnostic accuracy of ESR, plasma fibrinogen, GLB, and AGR also performed well. Higher levels of WBC, CRP, and ESR were detected in patients who had recently used antibiotics ($P < 0.05$). GLB, AGR, and plasma fibrinogen are promising biomarkers for improving the diagnosis of tibial infected nonunion. The integrated analysis of ESR, plasma fibrinogen, GLB, and AGR provided more accurate and more specific diagnosis than the four biomarkers individually.

Introduction

Postoperative tibial infected nonunion is a chronic and debilitating disorder associated with that high hospitalization costs, lengthy treatment, and increased risk of patient morbidity and mortality [1; 2; 3; 4]. It is often difficult to discriminate between infected nonunion and aseptic nonunion [5; 6]. If infected nonunion is not promptly diagnosed or managed, it can result in devastating consequences, including permanent loss of function, amputation, or death [7; 8; 9]. Therefore, the timely and accurate diagnosis of infected nonunion is critical to facilitate any necessary reoperation, which can substantially affect treatment plans [10; 11].

Blood-based assays provide a potential solution for diagnosing infected nonunion, because they can be rapidly performed in advance of intraoperative culture [12]. Traditional biomarkers, like white blood cell count (WBC), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) are important biomarkers in the diagnostic evaluation of inflammation, and can reduce the rate of missed infections [8; 13]. These biomarkers can be widely used to diagnose acute fracture-related infections, but sometimes remain normal in late or chronic infections caused by low-virulence pathogens [14]. Therefore, new biomarkers need to be evaluated for the diagnosis of tibial infected nonunion.

Albumin (ALB) is one of the main components of serum proteins, and is associated with surgical site infection in orthopedics [15; 16]. Globulin (GLB), which is a component of complements and ceruloplasmin, generally increases during the inflammatory process [17]. Hence, the albumin-to-globulin ratio (AGR),

which considers both ALB and GLB levels, is also a promising biomarker for inflammation [18]. Furthermore, several studies reported that GLB and AGR were informative biomarkers in the diagnosis of periprosthetic joint infection (PJI) [19; 20]. In addition, serum D-dimer and plasma fibrinogen may be novel biomarkers to detect infected nonunion, and they have shown good performance in past studies [21; 22]. The tibia is the most commonly involved bone in infected nonunion of open fractures [23; 24]. However, the accuracy of these blood biomarkers in diagnosing infected nonunion of tibia fracture after surgery is unknown.

In the present study, we evaluated the diagnostic accuracy of ALB, GLB, AGR, plasma D-dimer, and plasma fibrinogen in tibial infected nonunion. We hypothesize that these new blood biomarkers will associated with tibial infected nonunion fractures after surgery, and will therefore help guide orthopedic surgeons in the assessment of patients with tibial infected nonunion.

Methods And Materials

Study design and participants

This single-center retrospective study was conducted in a tertiary hospital providing specialist treatment to patients with musculoskeletal infections. Over a period of approximately 3 years (June 2018 to July 2021), a total of 211 patients with tibial fracture who underwent surgery for nonunion after failed fracture operation were eligible for inclusion in this study. Ethical approval was obtained from the Clinical Research Ethics Committee of The Affiliated Drum Tower Hospital of Nanjing University Medical School. All methods were carried out in accordance with relevant guidelines and regulation and all patients signed informed consent before data collection. The data of patients in the database were anonymous for the purpose of protecting participants' privacy, and the entire process of data collection was nonselective and consecutive. The data were obtained from the hospital electronic medical record system. Patients were excluded based on the following exclusion criteria: 1) age < 18 years (n = 3); 2) without complete blood workup or medical records (n = 7); 3) unavailability of complete examinations (n = 4). Patients were also divided into subgroups for analysis to avoid bias: concurrent infections in other organs (n = 8), venous thrombosis (n = 13), malignancies (n = 2), autoimmune diseases (n = 4), and recent use of antibiotics 2 weeks before operation (n = 15). A nonunion was determined as radiographic evidence of nonprogression of healing for at least 3 months, or lack of healing by 9 months since the initial injury [25]. According to the fracture-related infections definition criteria, multiple gross tissue specimens (≥ 5 samples) were cultured, when the same organism was grown in at least 2 cultures of the intraoperative sample, a positive diagnosis of infection was made [8]. Ultimately, the patients with tibial nonunion were separated into two groups: 59 in Group A (operation for infected tibial nonunion) and 96 in Group B (operation for aseptic tibial nonunion) (Fig. 1).

Demographic features and blood biomarkers

The clinical features of patients with tibial nonunion were comprehensively interpreted by the attending physician after admission. Baseline demographic features, including age, gender, BMI, smoking, and fracture position were collected. Fasting venous blood samples were obtained from patients on admission and were sent to the clinical laboratory within 2 h. These blood samples were used to test WBC, CRP, ESR, liver function tests (including ALB, GLB, AGR), and plasma coagulation examinations (including plasma D-dimer, and plasma fibrinogen). Antimicrobial treatment was stopped for at least 14 days until intraoperative specimens were collected, unless the patients needed anti-infective therapy urgently; those patients were evaluated separately.

Statistical analysis

Descriptive statistics are presented as mean ± SD (standard deviation) for normally distributed continuous variables or median (IQR) for non-normally distributed data, and frequency (percentage) for categorical variables. Kolmogorov-Smirnov (K-S) test was used to identify data as normally distributed variables or non-normally distributed data. Student's t-tests were used to analyze normal distributed numerical variables, and Mann-Whitney U test was used to analyze numerical variables with non-normal distribution or unequal variance. Pearson $\chi 2$ test or Fisher exact test were used to analyze qualitative variables. Differences with a p-value of $P < 0.05$ are considered to be significant. Receiver operating characteristic (ROC) curves were plotted to determine the diagnostic value of each biomarker for assessing tibial infected nonunion, and the area under the curve (AUC) and 95% confidence interval (CI) were calculated to compare different biomarkers. The Youden index (J = [sensitivity + specificity] – 1) was used to determine the optimal predictive cut-offs for calculating AUC. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each test were calculated. The AUC values were determined to be excellent (0.900 to 1.000), good (0.800 to 0.899), fair (0.700 to 0.799), poor (0.600 to 0.699), and having no discriminatory capacity (< 0.599). All statistical analyses were performed using STATA version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

The basic demographic data of the two groups are shown in Table I. There were no significant differences between the two groups in terms of age, gender, BMI, smoking history, or fracture position. ($P > 0.05$).

Group A had significantly higher WBC, CRP, ESR, plasma D-dimer, plasma fibrinogen, and GLB levels than Group B ($P < 0.05$; Table II). Nonetheless, the ALB and AGR levels of patients in Group A were significantly lower than those of Group B ($P < 0.05$).Table III shows the AUCs, Youden index values, optimal cutoff values, and predictive values (sensitivity, specificity, PPV, NPV) of the various biomarkers. Based on ROC curve analysis (Fig. 2), the diagnostic performance of ESR (AUC = 0.883), plasma fibrinogen (AUC = 0.819), AGR (AUC = 0.815), and GLB (AUC = 0.805) were good. Moreover, the AUC of a composite biomarker combining ESR with plasma fibrinogen, GLB, and AGR (0.926) had the highest AUC. However, other biomarkers' diagnostic accuracy were fair or poor, including WBC, CRP, plasma D-dimer, and ALB. At the optimal cutoff value, the sensitivity, specificity, PPV, and NPV were 38.98, 84.38, 60.53, and 69.23% for

WBC; 69.49, 77.08, 65.08, and 80.43% for CRP; 71.19, 90.63, 82.35, and 83.65% for ESR; 64.41, 83.33, 70.37, and 79.21% for plasma D-dimer; 72.88, 75.00, 64.18, and 81.82% for plasma fibrinogen; 88.14, 9.38, 37.41, and 56.25% for ALB; 55.93, 96.88, 91.67, and 78.15% for GLB; and 57.63, 92.71, 82.93, and 78.07% for AGR; and 76.27, 97.92, 95.75, and 87.04% for the combination of ESR with plasma fibrinogen, GLB, and AGR.

Based on the optimal threshold, table IV shows that the diagnostic performance of tested markers for patients with comorbidities, including concurrent infections in other organs, venous thrombosis, autoimmune diseases, and malignancies. The range of the diagnostic accuracy of ESR, plasma fibrinogen, and AGR was 60–100% in patients with these comorbidities. The overall diagnostic accuracies were good.

Table V shows that there were significant differences between the cases of infected tibial nonunion patients who had recently used and had not used antibiotics in terms of WBC, CRP, and ESR ($P < 0.05$). However, the levels of plasma D-dimer, plasma fibrinogen, ALB, GLB, and AGR were not significantly different between the two groups ($P > 0.05$).

Discussion

This study was carried to compare the accuracy of plasma D-dimer, plasma fibrinogen, ALB, GLB, and AGR with traditional inflammatory biomarkers (WBC, CRP, and ESR) in diagnosing tibial infected nonunion. Our data demonstrated that the blood-based biomarkers plasma fibrinogen and GLB were associated with infected tibial nonunion, and could be used as new biomarkers to diagnose infected tibial nonunion. Furthermore, the integrated analysis of four promising biomarkers (ESR, plasma fibrinogen, GLB, and AGR) provided more accurate and more specific evaluation of tibial infected nonunion than the four biomarkers individually.

ALB and GLB are two easily accessible and reliable biomarkers evaluated in in liver function tests that are routinely performed before surgery, and are able to be performed rapidly and economically. ALB is a negative acute phase protein, and is considered to be a biomarker of inflammation and nutritional status [26; 27; 28]. A meta-analysis conducted by Yuwen *et al.*[15] reported that ALB levels < 3.5 g/dL was associated with an almost 2.5 fold increased risk of surgical site infection (SSI) in orthopaedics. However, Wang *et al.*[20] reported that ALB was not useful for diagnosis of patients suspected of having PJI. Similarly, Ye *et al.*[19] found that the levels of ALB were not associated with PJI. In our study, the diagnostic accuracy of ALB in tibial infected nonunion was poor, with an AUC of 0.671, sensitivity of 88.14%, specificity of 9.38%, PPV of 37.41%, and NPV of 56.25%. GLB is another major serum protein component that consists of antibodies and inflammatory cytokines, including complements, interleukin-6, and immunoglobulins [29; 30]. There is an inverse relationship between GLB and ALB in response to inflammation and infection, and the AGR could indicate an inflammatory state more accurately. GLB and AGR have been validated to have potential roles in the pathogenesis of inflammatory and infectious diseases in various studies [31; 32]. Moreover, recent studies have demonstrated that GLB and AGR are

associated with PJI and may serve as potential adjuvant biomarkers in the diagnosis of PJI [19; 20]. In accordance with these results, we also observed that high globulin levels and low AGR were independently associated with the risk of tibial infected nonunion. ROC curve analysis revealed that globulin and AGR showed acceptable predictive value for the diagnosis of tibial infected nonunion. The diagnostic value of GLB (AUC = 0.805) was slightly lower than that of AGR (0.815), which were higher than 0.8. Both biomarkers demonstrated fair sensitivity (GLB 55.93%, AGR 57.63%), but high specificity (GLB 96.88%, AGR 92.71%), reducing the misdiagnosis rate of tibial infected nonunion. Moreover, the PPV of GLB (91.67%) was the highest; suggesting that GLB may be superior as a single biomarker in predicting the diagnosis of tibial infected nonunion.

Recently, it has been demonstrated that systemic and local infections result in fibrinolytic activity [33; 34]. Moreover, coagulation-related indicators, including D-dimer and fibrinogen, are useful diagnostic markers for PJI [35; 36; 37; 38; 39]. Serum D-dimer and plasma fibrinogen have performed well in the diagnosis of infected nonunion [21; 22]. In our study, plasma fibrinogen (AUC = 0.819) demonstrated good diagnostic performance in infected nonunion, similar to the results of another recent study [21]. These data suggest that plasma fibrinogen may be a novel biomarker to diagnose tibial infected nonunion. However, the diagnostic value of plasma D-dimer was limited in our study, with a fair AUC of 0.776. Conversely, the D-dimer, assayed in serum rather than plasma, has proven to be useful for preoperative prediction of infected nonunion after ORIF [22]. Some studies demonstrated that serum D-dimer is superior to plasma D-dimer in the diagnosis of PJI [40; 41]. Consequently, high-quality prospective studies that address these research gaps are needed to validate the use of D-dimer as a biomarker for tibial infected nonunion.

WBC, CRP, and ESR are the most commonly used biomarkers of tibial infected nonunion. Unfortunately, they are usually affected by other factors such as physiological stress, treatment, and other diseases [5; 42]. Peripheral WBC is frequently normal in low-grade infections and affords little diagnostic help. The diagnostic value of WBC was limited in our study, with a fair AUC of 0.599. The standard WBC biomarker may not provide enough information to clearly distinguish between infected nonunion and aseptic nonunion. On the contrary, ESR had good performance in diagnosis of suspicious infection with higher sensitivity, which is verified by past studies [43; 44].

In subgroup analysis, we found that the diagnostic accuracy of these novel biomarkers was good in patients with tibial infected nonunion who also had other comorbidities. Considering the small number of cases, a larger number of cases should be enrolled into evaluate the diagnostic accuracies of these biomarkers and reduce potential bias. For example, recent use of antibiotics might influence inflammatory biomarkers. Our subgroup analysis showed that there were significant differences between patients with infected nonunion who recently used antibiotics vs. those who had not in a number of biomarkers, including WBC, CRP, and ESR levels ($P < 0.05$); other biomarkers were not significantly different, including plasma D-dimer, plasma fibrinogen, ALB, GLB, and AGR ($P > 0.05$). Therefore, our data suggest that plasma fibrinogen, GLB, and AGR may be better biomarkers to diagnose patients with suspected infected nonunion who have recently used antibiotics.

There are several limitations to our study. Firstly, this study was retrospective, with inherent biases. Secondly, the sample size of this study is fairly small. Finally, the different comorbidities and application of antibiotics among the patients may have influenced the observed results of the diagnostic accuracy of the biomarkers in patients with infected nonunion. Therefore, multicenter, prospective, comparative studies with larger samples are required to more thoroughly determine the accuracy of these biomarkers for predicting tibial infected nonunion.

It was a secondary analysis that recursive partitioning analysis of the inflammatory marker predictive values for infected nonunion, and is limited by the relatively small sample size of the cohort included. In order to avoid external validity, we will calibrate and externally validate this model with a future large observational cohort of patients with suspected nonunion to inform clinical utility.

Conclusion

In conclusion, our results show that GLB, AGR, and plasma fibrinogen may be reliable biomarkers to screen for tibial infected nonunion. The integrated analysis of four biomarkers (ESR, plasma fibrinogen, GLB, and AGR) may provide more accurate and more specific evaluation of tibial infected nonunion. Therefore, we recommend that surgeons begin to evaluate GLB, AGR, plasma fibrinogen, and the combination of ESR, plasma fibrinogen, GLB, and AGR as a potential screening tool for patients who might be at risk for tibial infected nonunion.

Declarations

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Author contributions

M.H: Collected the data, imaging and operation reports and wrote the initial draft of the manuscript and subsequent revisions. W.Z: Collected the data, revision of the draft, statistical analysis, and critical analysis of the results. X.G.: PI of this scientific study, study concept and design, revision of the draft, approval of the final version, critical analysis of the results. All authors read and approved the finial manuscript.

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Availability of data and materials

The final dataset will be available from the corresponding author.

Ethics approval and consent to participate

This study was approved by the ethics committee of Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, China (approval no.2021–153). All patients signed an informed consent form approved by the Institutional Review Board.

Consent for publication

Not applicable

Competing Interests

All the authors declare that there is no conflict of interest.

References

1. Chaudhary M M. Infected nonunion of tibia[J]. Indian J Orthop, 2017, 51(3): 256–268.DOI:10.4103/ortho.IJOrtho_199_16
2. Motsitsi N S. Management of infected nonunion of long bones: The last decade (1996–2006)[J]. Injury, 2008, 39(2): 155–160.DOI:10.1016/j.injury.2007.08.032
3. Berkes M, Obremskey W T, Scannell B, et al. Maintenance of hardware after early postoperative infection following fracture internal fixation[J]. J Bone Joint Surg Am, 2010, 92(4): 823–828.DOI:10.2106/jbjs.i.00470
4. Shen J, Sun D, Fu J, et al. Management of surgical site infection post-open reduction and internal fixation for tibial plateau fractures[J]. Bone Joint Res, 2021, 10(7): 380–387.DOI:10.1302/2046-3758.107.bjr-2020-0175.r2
5. Wang S, Yin P, Quan C, et al. Evaluating the use of serum inflammatory markers for preoperative diagnosis of infection in patients with nonunions[J]. Biomed Res Int, 2017, 2017: 9146317.DOI:10.1155/2017/9146317
6. Olszewski D, Streubel P N, Stucken C, et al. Fate of patients with a "surprise" positive culture after nonunion surgery[J]. J Orthop Trauma, 2016, 30(1): e19-23.DOI:10.1097/bot.0000000000000417
7. Bonnevialle P. Operative treatment of early infection after internal fixation of limb fractures (exclusive of severe open fractures)[J]. Orthop Traumatol Surg Res, 2017, 103(1s): S67-s73.DOI:10.1016/j.otsr.2016.06.019
8. Metsemakers W J, Morgenstern M, McNally M A, et al. Fracture-related infection: A consensus on definition from an international expert group[J]. Injury, 2018, 49(3): 505–510.DOI:10.1016/j.injury.2017.08.040
9. Walter N, Rupp M, Hierl K, et al. Long-term patient-related quality of life after fracture-related infections of the long bones[J]. Bone Joint Res, 2021, 10(5): 321–327.DOI:10.1302/2046-3758.105.bjr-2020-0532

10. McNally M A. Decision-making in infected nonunion: Is the surgery more important than the condition?[J]. Bone Joint J, 2016, 98-b(4): 435–436.DOI:10.1302/0301-620x.98b4.37883
11. Patzakis M J, Zalavras C G. Chronic posttraumatic osteomyelitis and infected nonunion of the tibia: Current management concepts[J]. J Am Acad Orthop Surg, 2005, 13(6): 417–427.DOI:10.5435/00124635-200510000-00006
12. Morgenstern M, Kühl R, Eckardt H, et al. Diagnostic challenges and future perspectives in fracture-related infection[J]. Injury, 2018, 49 Suppl 1: S83-s90.DOI:10.1016/s0020-1383(18)30310-3
13. Bishop J A, Palanca A A, Bellino M J, et al. Assessment of compromised fracture healing[J]. J Am Acad Orthop Surg, 2012, 20(5): 273–282.DOI:10.5435/jaaos-20-05-273
14. van den Kieboom J, Bosch P, Plate J D J, et al. Diagnostic accuracy of serum inflammatory markers in late fracture-related infection: A systematic review and meta-analysis[J]. Bone Joint J, 2018, 100-b(12): 1542–1550.DOI:10.1302/0301-620x.100b12.bjj-2018-0586.r1
15. Yuwen P, Chen W, Lv H, et al. Albumin and surgical site infection risk in orthopaedics: A meta-analysis[J]. BMC Surg, 2017, 17(1): 7.DOI:10.1186/s12893-016-0186-6
16. Yang G, Zhu Y, Zhang Y. Prognostic risk factors of surgical site infection after primary joint arthroplasty: A retrospective cohort study[J]. Medicine (Baltimore), 2020, 99(8): e19283.DOI:10.1097/md.0000000000019283
17. Li K, Fu W, Bo Y, et al. Effect of albumin-globulin score and albumin to globulin ratio on survival in patients with heart failure: A retrospective cohort study in china[J]. BMJ Open, 2018, 8(7): e022960.DOI:10.1136/bmjopen-2018-022960
18. Ukibe N R, Ndiuwem C K, Ogbu, Il, et al. Prognostic value of some serum protein fractions as early index of clinical recovery in pulmonary tuberculosis subjects[J]. Indian J Tuberc, 2020, 67(2): 167–171.DOI:10.1016/j.ijtb.2019.08.015
19. Ye Y, Chen W, Gu M, et al. Serum globulin and albumin to globulin ratio as potential diagnostic biomarkers for periprosthetic joint infection: A retrospective review[J]. J Orthop Surg Res, 2020, 15(1): 459.DOI:10.1186/s13018-020-01959-1
20. Wang H, Zhou H, Jiang R, et al. Globulin, the albumin-to-globulin ratio, and fibrinogen perform well in the diagnosis of periprosthetic joint infection[J]. BMC Musculoskelet Disord, 2021, 22(1): 583.DOI:10.1186/s12891-021-04463-7
21. Wang X J, Wang Z, Zhang Z T, et al. Plasma fibrinogen as a diagnostic marker of infection in patients with nonunions[J]. Infect Drug Resist, 2020, 13: 4003–4008.DOI:10.2147/idr.s269719
22. Wang Z, Zheng C, Wen S, et al. Usefulness of serum d–dimer for preoperative diagnosis of infected nonunion after open reduction and internal fixation[J]. Infect Drug Resist, 2019, 12: 1827–1831.DOI:10.2147/idr.s213099
23. Bhowmick K, Boopalan P, Gunasekeran C, et al. Management of chronic infected intra-articular fractures of the proximal tibia with ilizarov ring fixation[J]. J Knee Surg, 2020, 33(2): 213–222.DOI:10.1055/s-0038-1677512

24. Thakore R V, Francois E L, Nwosu S K, et al. The gustilo-anderson classification system as predictor of nonunion and infection in open tibia fractures[J]. Eur J Trauma Emerg Surg, 2017, 43(5): 651–656.DOI:10.1007/s00068-016-0725-y
25. Bell A, Templeman D, Weinlein J C. Nonunion of the femur and tibia: An update[J]. Orthop Clin North Am, 2016, 47(2): 365–375.DOI:10.1016/j.ocl.2015.09.010
26. Don B R, Kaysen G. Serum albumin: Relationship to inflammation and nutrition[J]. Semin Dial, 2004, 17(6): 432–437.DOI:10.1111/j.0894-0959.2004.17603.x
27. Eckart A, Struja T, Kutz A, et al. Relationship of nutritional status, inflammation, and serum albumin levels during acute illness: A prospective study[J]. Am J Med, 2020, 133(6): 713–722.e717.DOI:10.1016/j.amjmed.2019.10.031
28. Deng S, Gao J, Zhao Z, et al. Albumin/procalcitonin ratio is a sensitive early marker of nosocomial blood stream infection in patients with intra-cerebral hemorrhage[J]. Surg Infect (Larchmt), 2019, 20(8): 643–649.DOI:10.1089/sur.2018.260
29. Zhou T, Yu S T, Chen W Z, et al. Pretreatment albumin globulin ratio has a superior prognostic value in laryngeal squamous cell carcinoma patients: A comparison study[J]. J Cancer, 2019, 10(3): 594–601.DOI:10.7150/jca.28817
30. Wu P P, Hsieh Y P, Kor C T, et al. Association between albumin-globulin ratio and mortality in patients with chronic kidney disease[J]. J Clin Med, 2019, 8(11).DOI:10.3390/jcm8111991
31. Hoffman L K, Ghias M H, Cohen S R, et al. Polyclonal hyperglobulinaemia and elevated acute-phase reactants in hidradenitis suppurativa[J]. Br J Dermatol, 2018, 178(2): e134-e135.DOI:10.1111/bjd.15958
32. Schmilovitz-Weiss H, Cohen M, Pappo O, et al. Serum globulin levels in predicting the extent of hepatic fibrosis in patients with recurrent post-transplant hepatitis c infection[J]. Clin Transplant, 2007, 21(3): 391–397.DOI:10.1111/j.1399-0012.2007.00657.x
33. Stark K, Massberg S. Interplay between inflammation and thrombosis in cardiovascular pathology[J]. Nat Rev Cardiol, 2021, 18(9): 666–682.DOI:10.1038/s41569-021-00552-1
34. Han H, Yang L, Liu R, et al. Prominent changes in blood coagulation of patients with sars-cov-2 infection[J]. Clin Chem Lab Med, 2020, 58(7): 1116–1120.DOI:10.1515/cclm-2020-0188
35. Xu H, Shang G, Wang Y, et al. Plasma fibrinogen is a reliable marker for diagnosing periprosthetic joint infection and determining the timing of second-stage revision[J]. Int J Infect Dis, 2021, 108: 220–225.DOI:10.1016/j.ijid.2021.05.068
36. Yang F, Zhao C, Huang R, et al. Plasma fibrinogen in the diagnosis of periprosthetic joint infection[J]. Sci Rep, 2021, 11(1): 677.DOI:10.1038/s41598-020-80547-z
37. Wu H, Meng Z, Pan L, et al. Plasma fibrinogen performs better than plasma d-dimer and fibrin degradation product in the diagnosis of periprosthetic joint infection and determination of reimplantation timing[J]. J Arthroplasty, 2020, 35(8): 2230–2236.DOI:10.1016/j.arth.2020.03.055
38. Hu Q, Fu Y, Tang L. Serum d-dimer as a diagnostic index of pji and retrospective analysis of etiology in patients with pji[J]. Clin Chim Acta, 2020, 506: 67–71.DOI:10.1016/j.cca.2020.03.023

39. Grzelecki D, Walczak P, Grajek A, et al. Elevated plasma d-dimer concentration has higher efficacy for the diagnosis of periprosthetic joint infection of the knee than of the hip-a single-center, retrospective study[J]. J Orthop Res, 2021, 39(2): 291–298.DOI:10.1002/jor.24897
40. Li C, Margaryan D, Ojeda-Thies C, et al. Meta-analysis of serum and/or plasma d-dimer in the diagnosis of periprosthetic joint infection[J]. J Orthop Surg Res, 2020, 15(1): 298.DOI:10.1186/s13018-020-01808-1
41. Xu H, Xie J W, Yang J L, et al. Role of d-dimer and fibrinogen in the diagnosis of periprosthetic joint infection: A systematic review and meta-analysis[J]. Orthop Surg, 2021, 13(3): 692–700.DOI:10.1111/os.12969
42. Metsemakers W J, Kuehl R, Moriarty T F, et al. Infection after fracture fixation: Current surgical and microbiological concepts[J]. Injury, 2018, 49(3): 511–522.DOI:10.1016/j.injury.2016.09.019
43. Vasso M, Schiavone Panni A. Low-grade periprosthetic knee infection: Diagnosis and management[J]. J Orthop Traumatol, 2015, 16(1): 1–7.DOI:10.1007/s10195-014-0294-y
44. Garvin K L, Konigsberg B S. Infection following total knee arthroplasty: Prevention and management[J]. J Bone Joint Surg Am, 2011, 93(12): 1167–1175.DOI:10.2106/00004623-201106150-00012

Tables

Table I. Patient characteristics for the two groups

	Group A (n = 59)	Group B (n = 96)	*P*-value
Number of women	7/52（11.9%）	20/76（20.8%）	0.193†
Age (year, mean ± SD)	47.8 ± 15.2	45.7± 13.0	0.381#
BMI (kg/m^2, mean ± SD)	25.1±3.6	24.8±3.2	0.487#
Smoking history	12/47（21.1%）	18/78（17.3%）	0.836†
Position (left)	30/29 (50.8%)	53/43 (55.2%)	0.662†
Fracture position (actual sites)			0.476†
Proximal	8/51(13.6%)	19/77(19.8%)	
Mid-diaphysis	30/29(50.8%)	50/46(52.1%)	
Distal	21/38(35.6%)	27/69(28.1%)	

Notes: Group A = tibial infected nonunion; Group B = tibial aseptic nonunion; # Independent-samples *t*-test; † Chi-squared test (linear by linear); *P < 0.05 indicates statistical significance.

Abbreviation: BMI = body mass index.

Table II. Comparison of the tested markers in the two groups

	Group A (n = 59)	Group B (n = 96)	*P*-value
WBC (10^9/μL)			
Median	6.6	6.2	0.038*
P25, P75	5.5 ~ 8.2	5.4 ~ 7.2	
CRP (mg/L)			<0.001*
Median	7.0	3.5	
P25, P75	3.8~ 17.5	2.5 ~ 4.9	
ESR (mm/hr)			<0.001*
Median	25.0	6.5	
P25, P75	13.0 ~ 39.0	5.0 ~ 11.0	
Plasma D-dimer (mg/L)			<0.001*
Median	1.19	0.34	
P25, P75	0.53 ~ 2.9	0.17 ~ 0.75	
Plasma fibrinogen (mg/L)			<0.001*
Median	3.4	2.4	
P25, P75	2.8 ~ 5.1	2.1 ~ 2.9	
ALB (g/L)			<0.001*
Median	38.9	41.2	
P25, P75	37.7 ~ 41.1	39.2 ~ 43.1	
GLB (g/L)			<0.001*

Median	32.2	24.2	
P25, P75	26.4 ~ 39.2	21.1 ~ 27.2	
AGR			<0.001*
Median	1.30	1.71	
P25, P75	0.98 ~ 1.56	1.48 ~ 1.91	

Notes: Group A = tibial infected nonunion; Group B = tibial aseptic nonunion; All P-values calculated using Mann-Whitney U test; *P < 0.05 indicates statistical significance.

Abbreviation: WBC = white blood cell; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; ALB = albumin; GLB = globulin; AGR = albumin-to-globulin ratio

Table III. The diagnostic value of tested markers in patients with tibial infected nonunion.

Variables	AUC (95%CI)	Optimal Cutoff Value	Sensitivity (%)	Specificity(%)	PPV (%)	NPV (%)
WBC	0.599 (0.504-0.695)	7.65×10^9/L	38.98	84.37	60.53	69.23
CRP	0.748 (0.665-0.832)	4.95 mg/L	69.49	77.08	65.08	80.43
ESR	0.883 (0.830-0.937)	17.5 mm/h	71.19	90.63	82.35	83.65
Plasma D-dimer	0.776 (0.700-0.852)	0.95 mg/L	64.41	83.33	70.37	79.21
Plasma fibrinogen	0.819 (0.752-0.887)	2.85 g/L	72.88	75.00	64.18	81.82
ALB	0.671 (0.580-0.762)	44.45 g/L	88.14	9.38	37.41	56.25
GLB	0.805 (0.726-0.883)	31.5 g/L	55.93	96.88	91.67	78.15
AGR	0.815 (0.742-0.889)	1.35	57.63	92.71	82.93	78.07
ESR+ Plasma Fibrinogen +GLB+AGR	0.926 (0.880-0.972)	0.41	76.27	97.92	95.75	87.04

Abbreviation: WBC = white blood cell; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; ALB = albumin; GLB = globulin; AGR = albumin-to-globulin ratio; AUC = areas under the curve; CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value.

Table IV. Diagnostic performance of tested markers for patients with comorbidities.

Comorbidities and Numbers		Concurrent infections in other organs (n = 8)	Venous thrombosis (n = 13)	Autoimmune diseases (n = 4)	Malignancies (n = 2)
ESR	Group A	7(6)	5(4)	1(1)	2(2)
	Group B	1(0)	8(8)	3(2)	0
Plasma fibrinogen	Group A	7(6)	5(2)	1(0)	2(2)
	Group B	1(0)	8(7)	3(3)	0
GLB	Group A	7(6)	5(4)	1(1)	2(2)
	Group B	1(1)	8(7)	3(3)	0
AGR	Group A	7(7)	5(3)	1(1)	2(2)
	Group B	1(1)	8(8)	3(3)	0

Notes: Group A = tibial infected nonunion; Group B = tibial aseptic nonunion.

Abbreviation: ESR = erythrocyte sedimentation rate; ALB = albumin; GLB = globulin; AGR = albumin-to-globulin ratio

Table V. Comparison of tested markers between patients with tibial infected nonunion who used antibiotics two weeks before and patients with tibial infected nonunion who had not used antibiotics recently

Variables	Normal range	Tibial Infected Nonunion Recent Use of Antibiotics (n = 15) (median, P25–75)	Tibial Infected Nonunion Recent No Use of Antibiotics (n = 59) (median, P25–75)	*P*-value
WBC(10^9/μL)	3.5-9.5	5.6(5.1-6.4)	6.6 (5.5-8.2)	0.029*
CRP(mg/L)	0-8	4.4(2.8-6.1)	7.0(3.8-17.5)	0.035*
ESR(mm/hr)	0-15	15.0(12.0-18.0)	25.0(13.0-39.0)	0.006*
Plasma D-dimer (mg/L)	0-0.5	0.9(0.7-1.5)	1.2(0.5-2.9)	0.273
Plasma Fibrinogen(mg/L)	2-4	3.6(2.9-4.5)	3.4(28-5.1)	0.777
ALB (g/L)	40-55	39.4(37.9-41.8)	38.9(37.7-41.1)	0.727
GLB (g/L)	20-40	38.5(27.3-39.5)	32.2(26.4-39.2)	0.224
AGR	1.2-2.4	1.1(1.0-1.4)	1.3(1.0-1.6)	0.378

Notes: All P-values calculated using the Mann-Whitney U test; *$P < 0.05$ indicates statistical significance.

Abbreviation: WBC = white blood cell; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; ALB = albumin; GLB = globulin; AGR = albumin-to-globulin ratio.

Figures

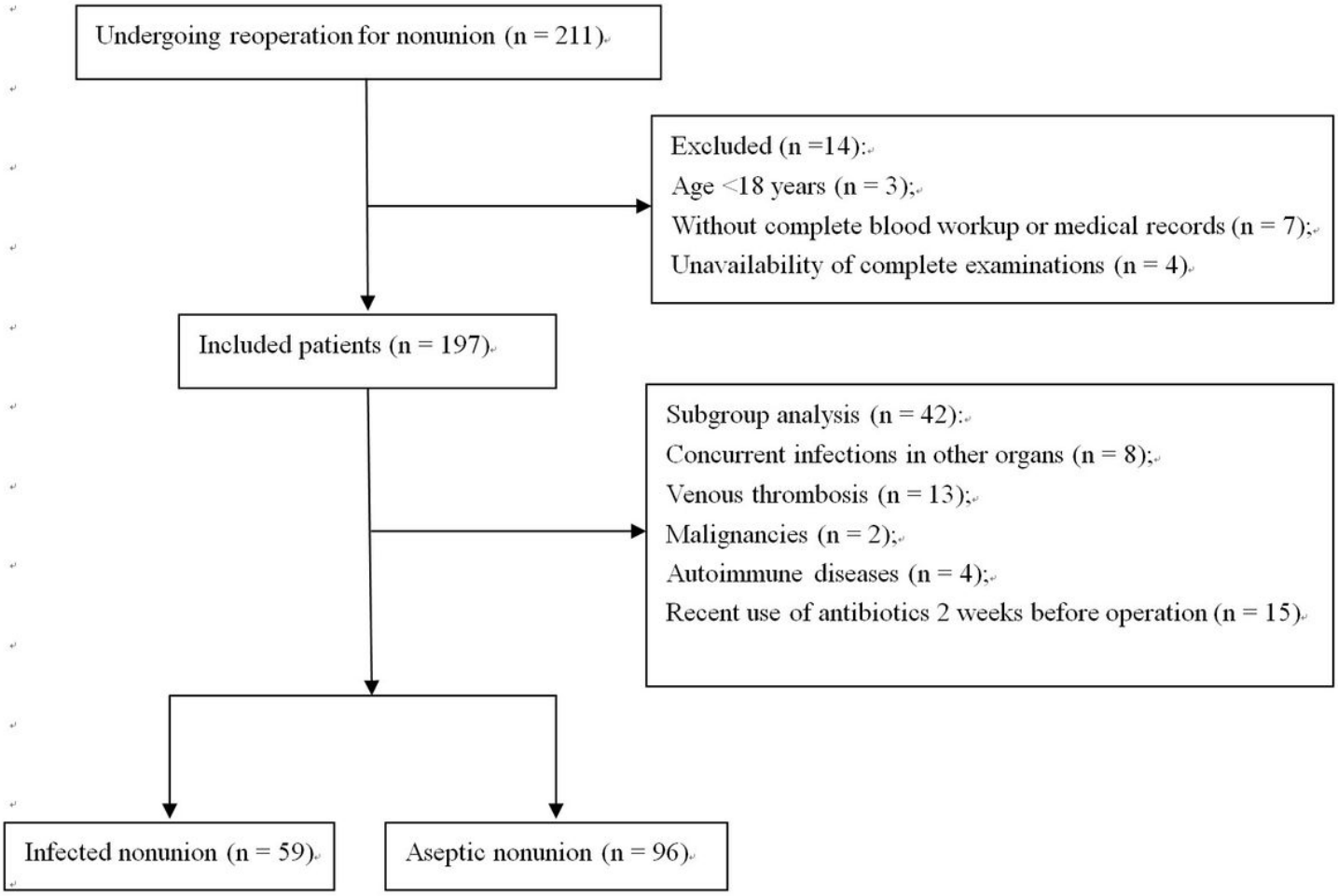

Figure 1

Flow diagram of the study design.

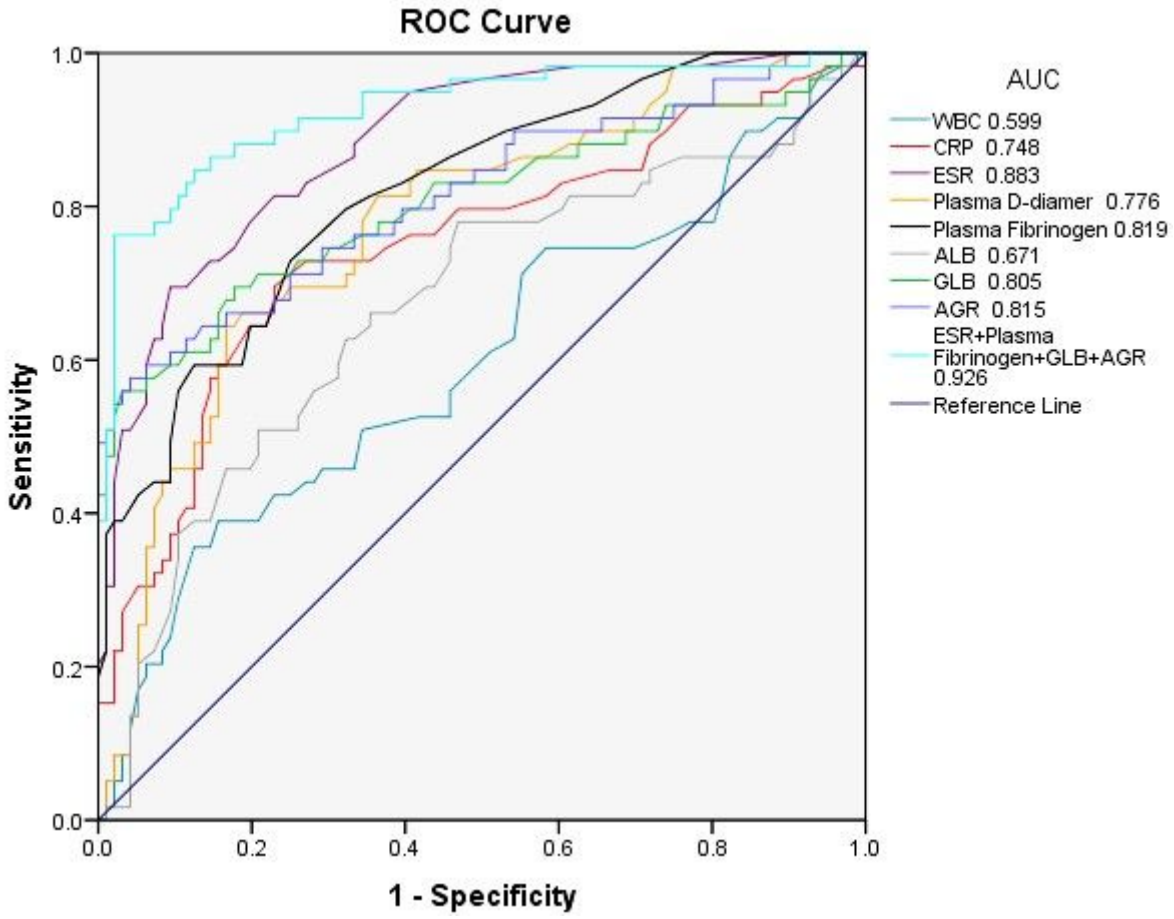

Figure 2

The ROC curves of biomarkers in the diagnosis of tibial infected nonunion.

Notes: WBC = white blood cell; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; ALB = albumin; GLB = globulin; AGR = albumin-to-globulin ratio;