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Comparison of the prognostic values of inflammation markers in patients with acute pancreatitis

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Short title: Inflammation markers and acute pancreatitis

ABSTRACT

Objective: Inflammation-based prognostic markers (neutrophil-lymphocyte ratio [NLR], prognostic nutritional index [PNI], red cell distribution width [RDW], lymphocyte-monocyte ratio [LMR], and mean platelet volume [MPV] are associated with overall survival (OS) in some diseases. This study assessed their prognostic value in acute pancreatitis (AP) mortality.

Design: A retrospective analysis.

Setting: Patients with AP were recruited in the emergency department and healthy individuals were recruited in healthcare centre in our hospital.

Participants: A total of 359 AP patients (31 non-survivors) and 187 healthy individuals were enrolled.

Primary and secondary outcome measures: Biochemistry and haematology results of the first test after admission were collected. Mortality prediction ability was evaluated using receiver operating characteristic (ROC) curves. OS was evaluated using the Kaplan–Meier method, with differences compared using the log-rank test. Independent relationships of mortality with each predictor were estimated in Cox proportional hazard models.

Results: Compared with survivors of AP, non-survivors had higher RDW ($p<0.001$), MPV ($p=0.007$), and NLR ($p<0.001$), and lower LMR ($p<0.001$) and PNI ($p<0.001$). NLR had the largest area under the ROC curve (0.823, $p<0.001$), with a 16.69 cut-off, 83.9% sensitivity, and 74.4% specificity. Age ($p=0.005$), NLR ($p=0.048$), PNI ($p=0.025$), C reactive protein ($p=0.001$), and RDW ($p<0.001$) were independently associated with OS.

Conclusions: NLR was the most powerful marker of OS in this patient series.

Strengths and limitations of this study

- Compared with survivors of acute pancreatitis (AP), non-survivors had higher red

cell distribution width (RDW), mean platelet volume (MPV), and neutrophil-lymphocyte ratio (NLR), and lower lymphocyte-monocyte ratio (LMR) and prognostic nutritional index (PNI).

- NLR exhibited a significantly higher area under the receiver operating characteristic curve for the prediction of mortality compared with other markers.
- Age, NLR, PNI, C reactive protein, and RDW were independently associated with overall survival in AP.
- This was a retrospective analysis.

INTRODUCTION

Acute pancreatitis (AP) is a rapid-onset inflammation of the pancreas and that varies in severity from self-limiting mild illness to rapidly progressive multiple organ failure. Statistics suggest that 10–20% of patients with AP develop severe acute pancreatitis (SAP),[1] which usually has a dreadful evolution associated to a poor prognosis.[2 3] Prediction of disease severity can guide the management of patients with AP and improve their outcome. Organ failure and infected pancreatic necrosis are common causes of mortality in such patients,[4] and a new international multidisciplinary classification of SAP incorporates both events as determinants of severity.[5] The predictive values of various markers, such as Evaluation II (APACHE II), Bedside Index of Severity in Acute Pancreatitis (BISAP) scores, C-reactive protein (CRP), and procalcitonin, have been previously assessed.[6-8] A systematic review[9] concluded it was justifiable to use blood urea nitrogen after 48 h of hospital admission for predicting persistent organ failure, and procalcitonin for predicting infected pancreatic necrosis in patients with confirmed pancreatic necrosis. In clinical studies, no reliable predictor of persistent organ failure within 48 h of admission has been identified. However, most studies have focused on disease severity, and only a few have directly investigated the relationship between predictors and mortality of AP.

There is increasing evidence that presence of a systemic inflammatory response is associated with poor survival in patients with various aetiologies, including malignancy.[10-17] Many direct or combined markers of systemic inflammation are based on routine, inexpensive, and readily available laboratory tests. Mean platelet volume (MPV),[10] red cell distribution width (RDW),[11] neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), prognostic nutritional index (PNI),[12] and lymphocyte–monocyte ratio (LMR)[17] have been used to predict the prognosis of disease. Increased MPV after admission to the intensive care unit (ICU) was found to be independently associated with increased in-hospital mortality,[10] and RDW was found to be an independent marker of short- and long-term prognosis in ICU [11]. NLR at admission served as an independent predictor of 3-month mortality rates in acute-on-chronic liver failure patients.[13] PLR was a significant independent prognostic marker in patients with resected pancreatic ductal adenocarcinoma.[14] Increased pretreatment LMR was associated with a significantly more favourable prognosis in patients with solid tumours.[17] Despite this evidence, very few studies have focused on the direct relationship between inflammation-based prognostic markers and mortality of AP. A cross-sectional study found a significant association between RDW and mortality in patients with AP.[18] Another study[19] investigated the prognostic value of NLR in AP and determined an optimal ratio for prediction of severity. To the best of our knowledge, this is the first study to simultaneously compare the prognostic value of these inflammation-based prognostic markers (NLR, PNI, C-reactive protein [CRP], RDW, LMR, MPV, and PLR) of mortality in patients with AP.

MATERIALS AND METHODS

Participants

This retrospective analysis consecutively enrolled a series of patients with AP who were admitted to the emergency department of our hospital between 1 July 2013 and 18 August 2015. A diagnosis of acute pancreatitis required two of three features: (1) prolonged abdominal pain characteristic of AP, (2) threefold elevation of serum amylase and/or lipase levels above the normal range, and (3) characteristic findings of AP on abdominal ultrasonography and/or computed tomography scan.[1] Mild acute pancreatitis (MAP) was defined as absence of organ failure and absence of local or systemic complications.[1] Moderately severe acute pancreatitis (MSAP) was defined as no evidence of persistent organ failure, but presence of local or systemic complications and/or organ failure that resolved within 48 h. SAP was defined as persistent organ failure (>48 h).[1] Patients with recurrent pancreatitis were enrolled only at first admission. Patients with traumatic pancreatitis, autoimmune pancreatitis, diabetes mellitus, tumour, or liver failure were excluded. All enrolled patients were followed up for 100 days or until death. Healthy controls matched for age and sex, without chronic diseases or abnormal physical examination results, were also recruited from the physical examination centre.

Ethics statement

Each participant gave written informed consent after being provided with an explanation of the study. The Ethics Committee of The First Affiliated Hospital of Zhejiang University College of Medicine approved the consent procedure and experiment periods. The study was conducted in accordance with the ethical principles of the Helsinki Declaration.

Demographic information and laboratory analysis

Demographic information, including age, sex, aetiology, and complication, was collected from medical records. Complete blood counts (CBCs) with differentials were performed in samples of peripheral blood collected in ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes with an XE-2100 haematology autoanalyzer (Sysmex Corporation, Kobe, Japan). White blood cell count (WBC), neutrophil count, lymphocyte count, monocyte count, platelet count (PLT), RDW, and MPV were obtained during the emergency visit, with a turnaround time of less than 30 min. Additional laboratory data, including albumin, CRP, and amylase, were obtained within 12 h of admission using a Hitachi 7600 chemistry analyser (Hitachi High-Technologies, Tokyo, Japan) and Roche reagents (Roche Diagnostics, Indianapolis, IN, USA).

We assessed the prognostic value of general inflammation-based prognostic markers (NLR, PNI, CRP, RDW, LMR, MPV, and PLR) for predicting the mortality of AP. MPV and RDW were obtained directly from the CBCs. NLR, PLR, and LMR were ratios of two types of blood cell. PNI = albumin (g/l) + 5 × total lymphocyte count (10^9/l).

Statistical analysis

The Kolmogorov–Smirnov and Shapiro–Wilk tests were used to determine whether continuous variables were normally distributed. Based on the result, continuous variables were expressed as either mean ± standard deviation (SD), or median and range. Categorical variables were expressed as numbers. The significance of differences in sex and aetiology were compared using the χ^2 test. The significance of differences in the haematology and biochemistry results obtained in healthy

participants, and in patients with MAP, MSAP, and SAP, were determined using one-way analysis of variance (ANOVA) for normally distributed variables, and the Kruskal–Wallis H test for non-normally distributed variables. The significance of differences in haematology and biochemistry results between non-survivors and survivors of AP were determined using an independent sample *t*-test for normally distributed variables and Mann–Whitney U test for non-normally distributed variables. Similar statistical methods were used to determine the between-group differences (e.g., healthy control *vs.* MAP; MAP *vs.* MSAP; MSAP *vs*. SAP). Receiver operator characteristic (ROC) curves were plotted, and areas under the ROC curves (AUCs) were calculated to evaluate the discrimination threshold of each marker. Appropriate cut-off points for the optimal combination of sensitivity and specificity were determined using the Youden index. AP patients were stratified into groups by cut-off values. Overall survival (OS) curves were calculated using the Kaplan–Meier method, and differences in survival rates were compared using the log-rank test. Univariate and multivariate Cox proportional hazard models were used to estimate the significance and independence of the relationship of each inflammation-based prognostic marker and mortality. Hazard ratios (HRs) and 95% confidence intervals (CIs) of each independent risk factor were calculated. Age, NLR, PNI, CRP, RDW, LMR, MPV, and PLR were included in this model. A p-value <0.05 was considered statistically significant. Statistical analyses were performed with SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

A total of 546 individuals (197 MAP, 76 MSAP, 86 SAP, and 187 healthy control participants) were enrolled in the study. Forty-five patients with traumatic pancreatitis (n=1), autoimmune pancreatitis (n=5), diabetes mellitus (n=7), tumour (n=7), liver failure (n=2), or incomplete medical records or who were lost to follow-up (n=23) were excluded from the analysis. Tables 1 and 2 show the baseline characteristics of the patients. There were no significant differences in age (p=0.454) or sex (p=0.981) among the four groups (MAP, MSAP, SAP, and healthy participants). There were significant differences in RDW (p<0.001), NLR (p<0.001), PLR (p<0.001), LMR (p<0.001), and PNI (p<0.001) among the four groups. The three AP groups did not differ significantly in aetiology (p=0.875). As the illness worsened, CRP, RDW, and NLR gradually increased, but PNI decreased (all p<0.05; Table 1). PLR (p=0.026) and MPV (p=0.017) increased significantly and LMR decreased significantly (p<0.001) in MSAP as compared with MAP patients. PLR (p=0.863), MPV (p=0.076), and LMR (p=0.883) were not significantly different in MSAP and SAP patients. Compared with survivors of AP, non-survivors were older (p=0.001) and had higher WBC (p=0.001), CRP (p<0.001), amylase (p=0.010), RDW (p<0.001), MPV (p=0.007), and NLR (p<0.001). Conversely, lymphocyte count (p<0.001), PLT (p=0.001), albumin (p<0.001), LMR (p<0.001), and PNI (p<0.001) were lower in non-survivors than in survivors.

Discrimination thresholds

ROC curves were constructed to evaluate the ability of each marker to predict mortality in AP (Fig. 1). Table 3 shows the AUCs and optimal cut-off values. An area of 1.0 indicates perfect discrimination, 0.90 to 1.0 is excellent, 0.80 to <0.90 is good, 0.70 to <0.80 is fair, and <0.70 is poor.[20 21] Thus, the ability of NLR to predict

mortality (AUC=0.823, p<0.001) was good; those of PNI (AUC=0.781, p<0.001), CRP (AUC=0.762, p<0.001), RDW (AUC=0.742, p<0.001), and LMR (AUC=0.710, p<0.001) were fair; and that of MPV (AUC=0.645, p=0.007) was poor. PLR was not able to predict mortality in AP (p=0.059). For NLR, the optimal cut-off value for mortality prediction was 16.69, with a specificity of 83.9%, sensitivity of 74.4%, positive likelihood ratio (+LR) of 3.28, and negative likelihood ratio (−LR) of 0.22.

Survival analysis

Kaplan−Meier survival curves demonstrate the relationships between inflammation-based prognostic markers and OS of patients with AP (Fig. 2A−G). Elevated NLR (p<0.001), CRP (p<0.001), RDW (p<0.001), MPV (p=0.002), and PLR (p=0.006) were associated with increased probability of death. Conversely, decreased PNI (p<0.001) and LMR (p<0.001) were associated with decreased OS.

Univariate analysis and Cox regression revealed that age (p=0.001), NLR (p<0.001), PNI (p<0.001), CRP (p<0.001), RDW (p<0.001), LMR (p=0.001), and MPV (p=0.010) were associated with AP mortality (Table 4). The factors significant in univariate analysis were evaluated by multivariate Cox regression. Age (HR=1.043, 95% CI: 1.013–1.073, p=0.005), NLR (HR=1.029, 95% CI: 1.000–1.058, p=0.048), PNI (HR=0.927, 95% CI: 0.868–0.990, p=0.025), CRP (HR=1.006, 95% CI: 1.002–1.009, p=0.001), and RDW (HR=1.354, 95% CI: 1.165–1.573, p<0.001) were independently associated with OS (Table 4).

DISCUSSION

AP is an inflammatory disease, with mortality arising mainly from organ failure or infected pancreatic necrosis.[4] Our study estimated the prognostic value of various

inflammation-based prognostic markers for predicting mortality of AP. The ability of the NLR to predict mortality was good, while those of PNI, CRP, RDW, and LMR were fair, and that of MPV was poor. PLR could not predict mortality. Cox regression revealed that age, NLR, PNI, CRP, and RDW were independently associated with OS.

In AP, inflammation propagates and promotes tissue destruction via activation of a cascade of inflammatory cytokines, proteolytic enzymes, and oxygen free radicals.[19 22] Neutrophils, lymphocytes, and monocytes are the three main types of WBCs. Neutrophils play a key role in development of local tissue destruction and systemic complications of SAP.[23] Depletion of neutrophils has been associated with improved prognosis of AP via attenuation of intrapancreatic trypsin activation, abolishment of acinar cell necrosis, and prevention of lung injury.[23] The percentage of immature neutrophilic granulocytes might be used clinically as a simple early predictor of an adverse outcome in SAP.[24] Additionally, recent studies revealed that the extent of lymphopenia was also associated with disease severity.[25-27] Lymphopenia has been reported to have independent prognostic value for some diseases,[19 26-29] including AP. Takeyama *et al.* found that impairment of cellular immunity caused by peripheral lymphocyte apoptosis was linked to subsequent development of infectious complications in AP.[28] Monocytes produce various cytokines and inflammatory mediators that further amplify inflammatory cell recruitment into the pancreas, as well as distant organs such as the lungs.[30] Similar to neutrophils, a protective effect was also found by depleting macrophages in a mouse model of cerulein-induced AP.[31] Theoretically, NLR and LMR, which combine two opposing parameters, should be more accurate than either parameter alone. In fact, we found that NLR had the greatest prognostic value of all the factors we evaluated. Despite this, it is important to apply it with caution in clinical settings. Broad-spectrum antibiotics with good

tissue penetration, which are essential medicines in the treatment of SAP, can affect WBC by reducing inflammation. Thus, the prognostic value of NLR in AP is uncertain if the effect of antibiotic treatment is not taken into account.[32] For this reason, the neutrophil and lymphocyte counts used this study were from the first CBCs, conducted during the emergency visit. We confirmed that more than half of the enrolled patients were untreated at that time; consequently, our results are most likely applicable to untreated patients. Unlike for NLR, a significant decrease of LMR was observed in SAP and MSAP compared with MAP patients, but the decreases observed in patients with MSAP and SAP showed no statistically significant differences. In addition, the predictive ability of LMR was only fair, and was not independently associated with OS in AP.

Serum albumin is a negative acute phase response reactant, and it reflects the body's nutritional status. Albumin <25 g/l was an independent prognostic factor related to the poor prognosis of AP.[33] The variation of albumin within 24 h has been identified as a risk factor for poor prognosis of critically ill patients in the early stages of SAP.[34] The PNI, which includes serum albumin and lymphocyte count, is an independent predictor of poor overall survival in patients with hepatocellular carcinoma.[35] To the best of our knowledge, few studies have reported the application of PNI for predicting mortality of AP, but we found that it was an independent prognostic factor and had the second largest AUC of the factors we investigated.

Numerous studies have reported RDW as a strong, independent prognostic factor in various diseases and conditions, such as cardiovascular diseases, rheumatoid arthritis, cancer, and critical illnesses.[18 36-38] Our results are consistent with the study by Yao J *et al.*[18], who reported a significant association between RDW and mortality of patients with AP. We also found the predictive ability of NLR, PNI, and CRP to be superior to

that of RDW, even though all four markers were independently associated with OS. The mechanisms underlying the association between RDW and mortality in AP remain unclear. The obvious metabolic abnormalities in non-survivors of AP, including inflammation, oxidative stress, poor nutritional status and persistent organ failure, lead to deregulation of red blood cell homeostasis involving both impaired erythropoiesis and abnormal red blood cell survival.[38] RDW reflects these impairments in homeostasis, but only further research can confirm this speculation.

Vascular thrombosis and systemic hypercoagulability are two complications of AP. As expected, PLT is involved in these complications, but the pathophysiological mechanism is not clear. An increase in MPV indicates platelet activation and has been associated with thrombotic diseases, but the relationship between MPV and AP is a topic of controversy. Two studies reported that MPV was significantly higher in patients with AP at admission than in healthy controls ($p<0.05$[18] and $p=0.005$[39]), and that MPV was significantly higher in patients with SAP than in those with MAP ($p<0.05$).[18] In contrast, Beyazit *et al.* found that MPV decreased in AP ($p<0.001$).[40] Interestingly, we found that MPV was significantly higher in patients with SAP and MSAP than in those with MAP ($p<0.05$) and there was no significant difference in MPV in SAP and MSAP. MPV has poor ability to discriminate survival status in AP (AUC=0.645, $p=0.007$), and it was not an independent prognostic factor related to mortality ($p=0.389$). However, MPV's limited predictive value does not reduce the importance of MPV determination in laboratory evaluation of AP.[41 42] MPV reportedly increases over time in samples collected in tubes with EDTA anticoagulant, and the increase was found proportional to the interval between sample collection and laboratory analysis.[42] Therefore, for reliable MPV determination, it is recommended that the time between sample collection and laboratory analyses be less than 2 h.[42] In

our study, MPV was determined in blood samples drawn during emergency admission, and turnaround time was less than 30 min; thus, we believe our results are reliable. Unfortunately, MPV of healthy controls was measured 2 h after venipuncture, and we decided not to include those results in this study. PLR also related to PLT but it was not an independent predictor of OS. Both platelet and lymphocyte counts decreased in SAP patients and in non-survivors of AP, so PLR did not have significant predictive value.

The prognostic markers evaluated in this study are direct or combined markers of systemic inflammation that are based on routine, inexpensive, and readily available laboratory tests. To the best of our knowledge, this is the first study to compare the prognostic value of these markers for predicting mortality in patients with AP. Some potential limitations of the current study should be noted. This was a retrospective, single-centre study; a larger, prospective study is needed to validate these results. Additionally, we only described the association of each of the predictors with mortality of AP; the underlying mechanisms need to be investigated.

In conclusion, we found that age, NLR, PNI, CRP, and RDW were independently associated with OS of AP. NLR was found to be the strongest predictor of mortality.

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Contributors

R.G. and Y.L. designed the experiments. R.G. and Y.L. contributed to the data collection. Y.Z. conducted the data analysis. Y.L., R.G and L.F. wrote the manuscript. All the authors reviewed the manuscript.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval This study was approved by the ethics committee of the First Affiliated Hospital of Zhejiang University School of Medicine, China

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

REFERENCES

1. Banks PA, Bollen TL, Dervenis C, *et al.* Classification of acute pancreatitis--2012: revision The diagnosis of acute pancreatitis requires two of the following three features: and definitions by international consensus. *Gut* 2013;62:102-11.
2. Maheshwari R, Subramanian RM. Severe Acute Pancreatitis and Necrotizing Pancreatitis. *Crit Care Clin* 2016;32:279-90.
3. Bugiantella W, Rondelli F, Boni M, *et al.* Necrotizing pancreatitis: A review of the interventions. *Int J Surg* 2016;28(Suppl 1):S163-71.
4. Petrov MS, Shanbhag S, Chakraborty M, *et al.* Organ failure and infection of pancreatic necrosis as determinants of mortality in patients with acute pancreatitis. *Gastroenterology* 2010;139:813-20.
5. Dellinger EP, Forsmark CE, Layer P, *et al.* Determinant-based classification of acute pancreatitis severity: an international multidisciplinary consultation. *Ann Surg* 2012;256:875-80.
6. Papachristou GI, Muddana V, Yadav D, *et al.* Comparison of BISAP, Ranson's, APACHE-II, and CTSI scores in predicting organ failure, complications, and mortality in acute pancreatitis. *Am J Gastroenterol* 2010;105:435-41; quiz 42.
7. Mounzer R, Langmead CJ, Wu BU, *et al.* Comparison of existing clinical scoring systems to predict persistent organ failure in patients with acute pancreatitis. *Gastroenterology* 2012;142:1476-82; quiz e15-6.
8. Bezmarevic M, Mirkovic D, Soldatovic I, *et al.* Correlation between procalcitonin and intra-abdominal pressure and their role in prediction of the severity of acute pancreatitis. *Pancreatology* 2012;12:337-43.
9. Yang CJ, Chen J, Phillips AR, *et al.* Predictors of severe and critical acute pancreatitis: a systematic review. *Dig Liver Dis* 2014;46:446-51.
10. Zampieri FG, Ranzani OT, Sabatoski V, *et al.* An increase in mean platelet volume after admission is associated with higher mortality in critically ill patients. *Ann Intensive Care* 2014;4:20.
11. Hunziker S, Celi LA, Lee J, *et al.* Red cell distribution width improves the simplified acute physiology score for risk prediction in unselected critically ill patients. *Crit Care* 2012;16:R89.
12. Kinoshita A, Onoda H, Imai N, *et al.* Comparison of the prognostic value of inflammation-based prognostic scores in patients with hepatocellular carcinoma. *Br J Cancer* 2012;107:988-93.
13. Chen L, Lou Y, Chen Y, *et al.* Prognostic value of the neutrophil-to-lymphocyte ratio in patients with acute-on-chronic liver failure. *Int J Clin Pract* 2014;68:1034-40.
14. Smith RA, Bosonnet L, Raraty M, *et al.* Preoperative platelet-lymphocyte ratio is an independent significant prognostic marker in resected pancreatic ductal adenocarcinoma. *Am J Surg* 2009;197:466-72.
15. Proctor MJ, Morrison DS, Talwar D, *et al.* An inflammation-based prognostic score (mGPS) predicts cancer survival independent of tumour site: a Glasgow Inflammation Outcome Study. *Br J Cancer* 2011;104:726-34.
16. Proctor MJ, Morrison DS, Talwar D, *et al.* A comparison of inflammation-based prognostic scores in patients with cancer. A Glasgow Inflammation Outcome Study. *Eur J Cancer* 2011;47:2633-41.
17. Teng JJ, Zhang J, Zhang TY, *et al.* Prognostic value of peripheral blood lymphocyte-to-monocyte ratio in patients with solid tumors: a meta-analysis. *Onco Targets Ther* 2016;9:37-47.
18. Yao J, Lv G. Association between red cell distribution width and acute pancreatitis: a cross-sectional study. *BMJ Open* 2014;4:e004721.

19. Suppiah A, Malde D, Arab T, *et al.* The prognostic value of the neutrophil-lymphocyte ratio (NLR) in acute pancreatitis: identification of an optimal NLR. J Gastrointest Surg 2013;17(4):675-81.
20. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;39:561-77.
21. Altman DG, Bland JM. Diagnostic tests 3: receiver operating characteristic plots. *BMJ* 1994;309:188.
22. Felderbauer P, Muller C, Bulut K, *et al.* Pathophysiology and treatment of acute pancreatitis: new therapeutic targets--a ray of hope? *Basic Clin Pharmacol Toxicol* 2005;97:342-50.
23. Xue J, Sharma V, Habtezion A. Immune cells and immune-based therapy in pancreatitis. *Immunol Res* 2014;58:378-86.
24. Zhu L, Chen G, Xia Q, *et al.* Use of band cell percentage as an early predictor of death and ICU admission in severe acute pancreatitis. *Hepatogastroenterology* 2010;57:1543-8.
25. Zahorec R. Ratio of neutrophil to lymphocyte counts--rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratisl Lek Listy* 2001;102:5-14.
26. de Jager CP, van Wijk PT, Mathoera RB, *et al.* Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. *Crit Care* 2010;14:R192.
27. Le Tulzo Y, Pangault C, Gacouin A, *et al.* Early circulating lymphocyte apoptosis in human septic shock is associated with poor outcome. *Shock* 2002;18:487-94.
28. Takeyama Y, Takas K, Ueda T, *et al.* Peripheral lymphocyte reduction in severe acute pancreatitis is caused by apoptotic cell death. *J Gastrointest Surg* 2000;4:379-87.
29. Pezzilli R, Billi P, Beltrandi E, *et al.* Circulating lymphocyte subsets in human acute pancreatitis. *Pancreas* 1995;11:95-100.
30. McKay C, Imrie CW, Baxter JN. Mononuclear phagocyte activation and acute pancreatitis. *Scand J Gastroenterol Suppl* 1996;219:32-6.
31. Saeki K, Kanai T, Nakano M, *et al.* CCL2-induced migration and SOCS3-mediated activation of macrophages are involved in cerulein-induced pancreatitis in mice. *Gastroenterology* 2012;142:1010-20 e9.
32. Binnetoglu E, Akbal E, Gunes F, *et al.* The prognostic value of neutrophil-lymphocyte ratio in acute pancreatitis is controversial. *J Gastrointest Surg* 2014;18:885.
33. Gonzalvez-Gasch A, de Casasola GG, Martin RB, *et al.* A simple prognostic score for risk assessment in patients with acute pancreatitis. *Eur J Intern Med* 2009;20:e43-8.
34. Chen Y, Zhang ZW, Wang B, *et al.* Relationship between early serum albumin variation and prognosis in patients with severe acute pancreatitis treated in ICU. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2013;44:237-41.
35. Pinato DJ, North BV, Sharma R. A novel, externally validated inflammation-based prognostic algorithm in hepatocellular carcinoma: the prognostic nutritional index (PNI). *Br J Cancer* 2012;106:1439-45.
36. Lappegard J, Ellingsen TS, Vik A, *et al.* Red cell distribution width and carotid atherosclerosis progression. The Tromso Study. *Thromb Haemost* 2015;113:649-54.

37. Bekler A, Tenekecioglu E, Erbag G, *et al.* Relationship between red cell distribution width and long-term mortality in patients with non-ST elevation acute coronary syndrome. *Anatol J Cardiol* 2015;15:634-9.
38. Salvagno GL, Sanchis-Gomar F, Picanza A, *et al.* Red blood cell distribution width: A simple parameter with multiple clinical applications. *Crit Rev Clin Lab Sci* 2015;52:86-105.
39. Akbal E, Demirci S, Kocak E, *et al.* Alterations of platelet function and coagulation parameters during acute pancreatitis. *Blood Coagul Fibrinolysis* 2013;24:243-6.
40. Beyazit Y, Sayilir A, Torun S, *et al.* Mean platelet volume as an indicator of disease severity in patients with acute pancreatitis. *Clin Res Hepatol Gastroenterol* 2012;36:162-8.
41. Varol E, Ozaydin M. Mean platelet volume in patients with acute pancreatitis: insight from methodological aspect. *Blood Coagul Fibrinolysis* 2014;25:196-7.
42. Lance MD, van Oerle R, Henskens YM, *et al.* Do we need time adjusted mean platelet volume measurements? *Lab Hematol* 2010;16:28-31.

Table 1 Demographics and laboratory findings in patients with acute pancreatitis and in healthy participants

Variable	1. Healthy controls	2. MAP	3. MSAP	4. SAP	1 *vs.* 2	2 *vs.* 3	3 *vs.* 4
Age (years)	49.92 ± 12.78	51.43 ± 16.00	48.47 ± 13.28	50.69 ± 14.61	/	/	
Sex (M/F)	104/83	108/89	41/35	49/37	/	/	/
Aetiology (1/2/3/4)	/	102/24/21/50	39/12/10/15	40/13/12/21	/	/	/
WBC ($\times10^9$/l)	5.6 (2.5–11.5)	11.5 (3.1–32.0)	14.1 (4.5–36.8)	16.05 (5.9–38.4)	<0.001[b]	<0.001[b]	0.278[b]
Lymphocyte ($\times10^9$/l)	1.90 (1–4.5)	1.1 (0.2–9.4)	1.0 (0.2–2.6)	0.80 (0.2–2.9)	<0.001[b]	0.004[b]	0.089[b]
PLT ($\times10^9$/l)	217 (99–375)	202 (21–502)	193 (58–548)	163 (27–540)	0.014[b]	0.376[b]	0.046[b]
Alb (g/l)	46.62 ± 2.95	38.29 ± 5.07	34.38 ± 6.39	29.99 ± 5.35	<0.001[a]	<0.001[a]	<0.001[a]
CRP (mg/l)	/	53.9 (0.7–386)	133.6 (3.2–436.5)	196.1 (27.1–426.7)	/	<0.001[b]	<0.001[b]
Amy (U/l)	/	398 (13–5191)	222 (27–3845)	581 (16–2377)	/	0.083[b]	0.056[b]
RDW (%)	13.0 (11.8–20.3)	12.8 (11.4–19.2)	13.0 (11.3–16.3)	13.7 (11.7–23.6)	0.013[b]	0.013[b]	0.014[b]
MPV	/	10.52 ± 1.21	10.92 ± 1.32	11.30 ± 1.34	/	0.017[a]	0.076[a]
NLR	1.59 (0.73–8.18)	8.46 (1.33–55)	14.60 (1.73–60)	19.65 (3.57–53.67)	<0.001[b]	<0.001[b]	0.02[b]
PLR	110.8 (47.9–303.6)	182 (21.4–990)	201.4 (46.5–931.5)	214.3 (21.2–1073.3)	<0.001[b]	0.026[b]	0.863[b]
LMR	4.83 (1.59–14.12)	1.88 (0.28–13.33)	1.03 (0.29–5.33)	1.14 (0.22–6.32)	<0.001[b]	<0.001[b]	0.883[b]
PNI	56.74 ± 4.26	44.53 ± 6.63	39.36 ± 6.71	34.55 ± 6.02	<0.001[a]	<0.001[a]	<0.001[a]
AP mortality	/	0	0	31	/	/	/

MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis; Aetiology (1/2/3/4), 1, 2, 3, and 4 represent gallstone, alcohol, hypertriglyceridemia, and other aetiologies, respectively. WBC, white blood cell count; PLT, platelet count; Alb, albumin; CRP, C-reactive protein; Amy, amylase; RDW, red cell distribution width; MPV, mean platelet volume; NLR, neutrophil lymphocyte ratio; PLR, platelet lymphocyte ratio; LMR, lymphocyte monocyte ratio; PNI, prognostic nutritional index; AP, acute pancreatitis.
1 *vs.* 2 The significance of differences in demographics and laboratory findings between healthy individuals and MAP; 2 vs. 3 The significance of differences in demographics and laboratory findings between MAP and MSAP; 3 vs. 4 The significance of differences in demographics and laboratory findings between MSAP and SAP. [a] independent samples t-test; [b] Mann–Whitney U test.

Table 2 Demographics and laboratory findings in survivors and non-survivors of acute pancreatitis

Variable	Survivors	Non-survivors	p-value
Age (years)	49.84 ± 14.88	58.90 ± 15.60	0.001[a]
Sex (M/F)	179/149	19/12	0.472[b]
Aetiology (1/2/3/4)	163/43/40/82	18/6/3/4	0.346[b]
WBC ($\times 10^9$/l)	12.85 (3.1–38.4)	18.5 (6.5–29.3)	0.001[c]
Lymphocytes ($\times 10^9$/l)	1.08 (0.17–9.40)	0.60 (0.30–1.60)	<0.001[c]
PLT ($\times 10^9$/l)	197 (21–548)	159 (27–376)	0.001[c]
Alb (g/l)	35.95 ± 6.30	30.44 ± 5.54	<0.001[a]
CRP (mg/l)	98.6 (0.7–436.5)	239.2 (27.1–398.2)	<0.001[c]
Amy (U/l)	343.5 (13–5191)	909 (16–2377)	0.010[c]
RDW (%)	13 (11.3–19.2)	13.8 (12.6–23.6)	<0.001[c]
MPV [16]	10.6 (8.0–14.7)	11.5 (8.9–13.8)	0.007[c]
NLR	10.47 (1.33–60.0)	25.0 (8.67–53.67)	<0.001[c]
PLR	194.1 (21.2–1073.3)	228.3 (45.0–736.67)	0.059[c]
PNI	41.71 ± 7.50	34.00 ± 6.35	<0.001[a]
LMR	1.51 (0.22–13.33)	1.13 (0.24–2.26)	<0.001[c]

Aetiology (1/2/3/4), 1, 2, 3, and 4 represent gallstone, alcohol, hypertriglyceridemia, and other aetiology, respectively. WBC, white blood cell count; PLT, platelet count; Alb, albumin; CRP, C-reactive protein; Amy, amylase; RDW, red cell distribution width; MPV, mean platelet volume; NLR, neutrophil lymphocyte ratio; PLR, platelet lymphocyte ratio; PNI, prognostic nutritional index; LMR, lymphocyte monocyte ratio. [a] independent samples *t*-test; [b] χ^2 test; [c] Mann–Whitney U test..

Table 3 Discriminatory ability of inflammation-based prognostic markers

Index	AUC(95% CI)	p-value	Cut-off	Sen%	Spe%	+LR	−LR	PPV%	NPV%
NLR	0.823(0.758–0.889)	<0.001	>16.69	83.9	74.4	3.28	0.22	23.7	98.0
PNI	0.781(0.704–0.858)	<0.001	<33.55	58.1	86.3	4.24	0.49	28.6	95.6
CRP	0.762(0.680–0.844)	<0.001	>162.25	74.2	69.8	2.46	0.37	18.9	96.7
RDW	0.742(0.667–0.817)	<0.001	>12.95	90.3	49.7	1.80	0.20	14.5	98.2
LMR	0.710(0.627–0.794)	<0.001	<1.44	80.6	52.7	1.70	0.37	13.9	96.6
MPV	0.645(0.548–0.743)	0.007	>11.15	61.3	66.8	1.85	0.58	14.9	94.8
PLR	0.603(0.494–0.711)	0.059	>264.5	48.4	74.1	1.88	0.70	15.0	93.8

NLR, neutrophil lymphocyte ratio; PNI, prognostic nutritional index; CRP, C-reactive protein; RDW, red cell distribution width; LMR, lymphocyte monocyte ratio; MPV, mean platelet volume; PLR, platelet lymphocyte ratio; AUC, area under the receiver operating characteristic curve; CI, confidence interval; Sen, sensitivity; Spe, specificity; +LR, positive likelihood ratio; −LR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.

Table 4 Prognostic factors of overall survival in patients with AP by univariate and multivariate analyses

Factors	Univariate analysis	Multivariate analysis	
	p value	p value	Hazard ratio(95%CI)
Age	0.001	0.005	1.043(1.013–1.073)
NLR	<0.001	0.048	1.029(1.000–1.058)
PNI	<0.001	0.025	0.927(0.868–0.990)
CRP	<0.001	0.001	1.006(1.002–1.009)
RDW	<0.001	<0.001	1.354(1.165–1.573)
LMR	0.001	0.588	
MPV	0.010	0.389	
PLR	0.123	/	

AP, acute pancreatitis; NLR, neutrophil lymphocyte ratio; PNI, prognostic nutritional index; CRP, C-reactive protein; RDW, red cell distribution width; LMR, lymphocyte monocyte ratio; MPV, mean platelet volume; PLR, platelet lymphocyte ratio; CI, confidence interval.

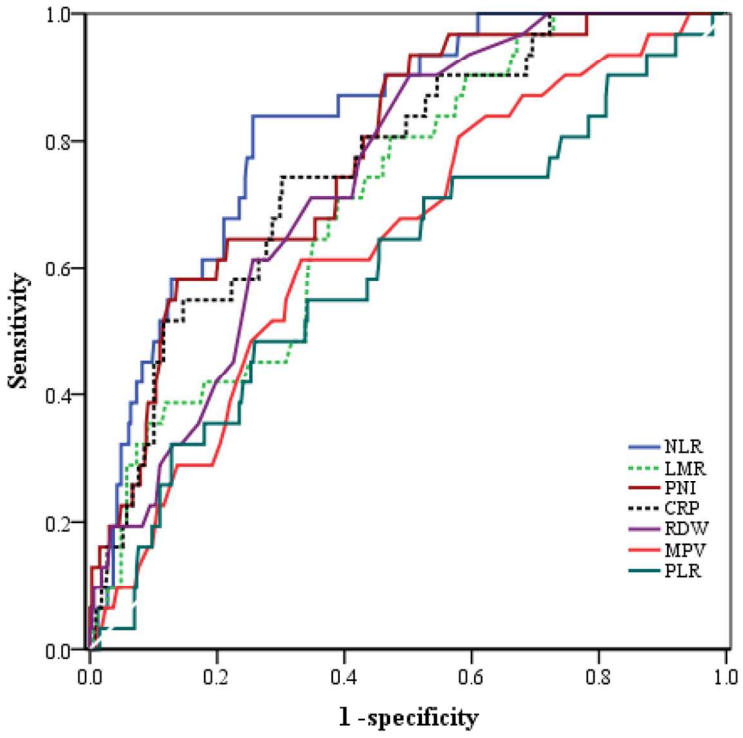
Figure Legends

Fig. 1. Area under receiver operating characteristics curves for mortality prediction by inflammation-based prognostic markers

NLR, neutrophil lymphocyte ratio; PNI, prognostic nutritional index; CRP, C-reactive protein; RDW, red cell distribution width; LMR, lymphocyte monocyte ratio; MPV, mean platelet volume; PLR, platelet lymphocyte ratio.

Fig. 2. Relationship between inflammation-based prognostic markers and overall survival in patients with acute pancreatitis

NLR, neutrophil lymphocyte ratio; PNI, prognostic nutritional index; CRP, C-reactive protein; RDW, red cell distribution width; LMR, lymphocyte monocyte ratio; MPV, mean platelet volume; PLR, platelet lymphocyte ratio. **A, B, C, D, E, F, and G** show the relationship between NLR, PNI, CRP, RDW, LMR, MPV, and PLR and overall survival in patients with acute pancreatitis, respectively.



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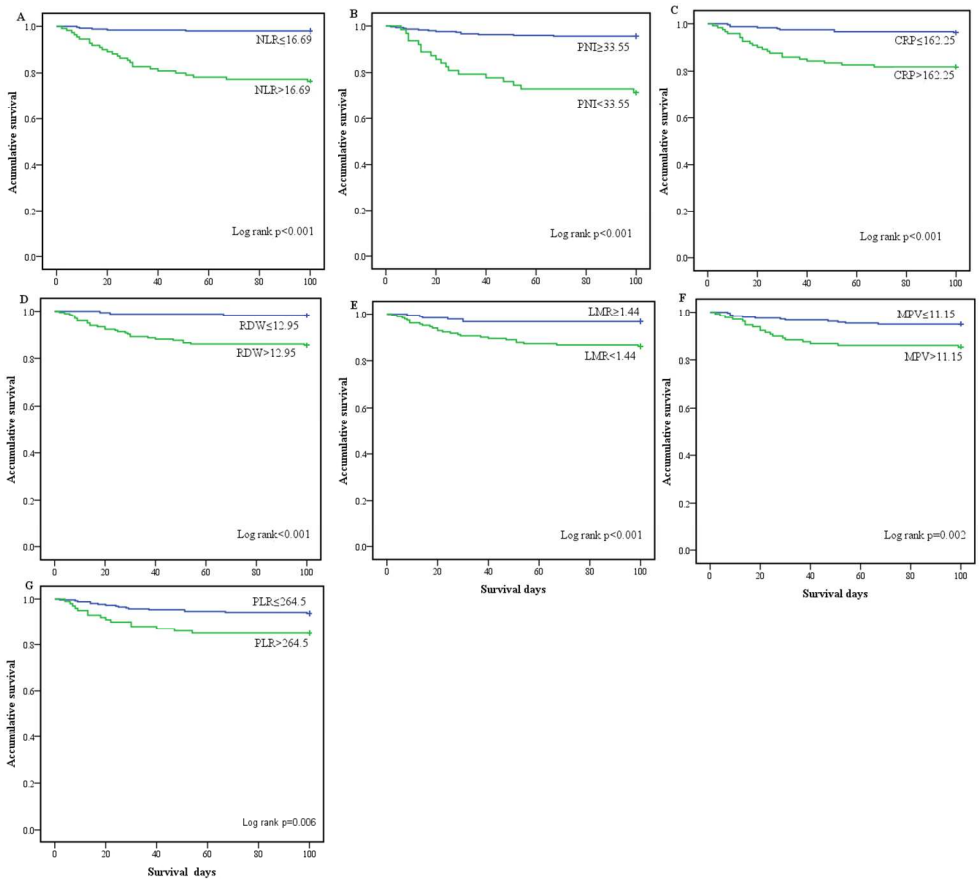

363x339mm (96 x 96 DPI)

TRIPOD Checklist: Prediction Model Development

Section/Topic	Item	Checklist Item	Page
Title and abstract			
Title	1	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	2
Introduction			
Background and objectives	3a	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	3,4
	3b	Specify the objectives, including whether the study describes the development or validation of the model or both.	4
Methods			
Source of data	4a	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	5
	4b	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	5
Participants	5a	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	5
	5b	Describe eligibility criteria for participants.	5
	5c	Give details of treatments received, if relevant.	Not relevant
Outcome	6a	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	5
	6b	Report any actions to blind assessment of the outcome to be predicted.	5
Predictors	7a	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	6
	7b	Report any actions to blind assessment of predictors for the outcome and other predictors.	6
Sample size	8	Explain how the study size was arrived at.	5
Missing data	9	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	7,8
,8Statistical analysis methods	10a	Describe how predictors were handled in the analyses.	6,7
	10b	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	6,7
	10d	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	6,7
Risk groups	11	Provide details on how risk groups were created, if done.	6,7
Results			
Participants	13a	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	7,8
	13b	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	7,8,19, 20
Model development	14a	Specify the number of participants and outcome events in each analysis.	7,8,19 ,20
	14b	If done, report the unadjusted association between each candidate predictor and outcome.	9
Model specification	15a	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	9
	15b	Explain how to the use the prediction model.	9
Model performance	16	Report performance measures (with CIs) for the prediction model.	8,9
Discussion			
Limitations	18	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	13
Interpretation	19b	Give an overall interpretation of the results, considering objectives, limitations, and results from similar studies, and other relevant evidence.	10-13
Implications	20	Discuss the potential clinical use of the model and implications for future research.	13
Other information			
Supplementary information	21	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	Not relevant
Funding	22	Give the source of funding and the role of the funders for the present study.	14

We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

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Comparison of the prognostic values of inflammation markers in patients with acute pancreatitis: a retrospective cohort study

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Comparison of the prognostic values of inflammation markers in patients with acute pancreatitis: a retrospective cohort study

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ABSTRACT

Objectives: Inflammation-based prognostic markers (neutrophil-lymphocyte ratio [NLR], prognostic nutritional index [PNI], red cell distribution width [RDW], and lymphocyte-monocyte ratio [LMR]) are associated with overall survival in some diseases. This study assessed their prognostic value in mortality and severity in acute pancreatitis (AP).

Design: A retrospective cohort study.

Setting: Patients with AP were recruited from the emergency department at our hospital.

Participants: A total of 359 AP patients (31non-survivors) were enrolled.

Primary and secondary outcome measures: Mortality and severity of AP were the primary and secondary outcome measures, respectively. Biochemistry and haematology results of the first test after admission were collected. Independent relationships between severe AP (SAP) and markers were assessed using multivariate logistic regression models. Mortality prediction ability was evaluated using receiver operating characteristic (ROC) curves. Overall survival was evaluated using the Kaplan–Meier method, with differences compared using the log-rank test. Independent relationships between mortality and each predictor were estimated using Cox proportional hazard models.

Results: Compared with survivors of AP, non-survivors had higher RDW ($p<0.001$), higher NLR ($p<0.001$), lower LMR ($p<0.001$), and lower PNI ($p<0.001$) at baseline. C-reactive protein (CRP) [odd ratio (OR)=8.251, $p<0.001$], RDW (OR=2.533, $p=0.003$), and PNI (OR=7.753, $p<0.001$) were independently associated with the occurrence of SAP. For predicting mortality, NLR had the largest area under the ROC curve (0.804, $p<0.001$), with a 16.64 cut-off value, 82.4% sensitivity, and 75.0%

specificity. RDW was a reliable marker for excluding death owing to its lowest negative likelihood ratio (0.11). NLR [hazard ratio (HR) =4.726, p=0.004], CRP (HR=3.503, p=0.003), RDW (HR=3.139, p=0.013), and PNI (HR=2.641, p=0.011) were independently associated with mortality of AP.

Conclusions: NLR was the most powerful marker of overall survival in this patient series.

Strengths and limitations of this study

- Compared with survivors of acute pancreatitis (AP), non-survivors had higher red cell distribution width (RDW) and neutrophil-lymphocyte ratio (NLR), and lower lymphocyte-monocyte ratio (LMR) and prognostic nutritional index (PNI) at baseline.
- NLR exhibited a higher area under the receiver operating characteristic curve for the prediction of mortality compared with other markers.
- RDW was suitable as a reliable marker to exclude death.
- NLR, PNI, C-reactive protein, and RDW were independently associated with overall survival of AP.
- This was a retrospective cohort analysis.

INTRODUCTION

Acute pancreatitis (AP) is rapid-onset inflammation of the pancreas that varies in severity from a self-limiting mild illness to rapidly progressive multiple organ failure. Statistics suggest that 10–20% of patients with AP develop severe acute pancreatitis (SAP),[1] which usually has an unfavourable disease progression and is associated with a poor prognosis.[2 3] Prediction of disease severity can guide the management of patients with AP and improve the outcome. Organ failure and infected pancreatic necrosis are common causes of mortality in such patients,[4] and a new international multidisciplinary classification of SAP incorporates both events as determinants of severity.[5] The predictive values of various markers, such as Acute Physiology and Chronic Health Evaluation II (APACHE II) and Bedside Index of Severity in Acute Pancreatitis scores, C-reactive protein (CRP), and procalcitonin, have been previously assessed.[6-8] A systematic review concluded it was justifiable to use blood urea nitrogen after 48 h of hospital admission for predicting persistent organ failure.[9] In clinical studies, most studies have focused on disease severity, and only a few have directly investigated the relationship between predictors and mortality of AP. Furthermore, no reliable predictor of persistent organ failure within 48 h of admission has been identified.[9]

There is increasing evidence that the presence of a systemic inflammatory response is associated with poor survival in patients with various aetiologies, including malignancy.[10-17] Many direct or combined markers of systemic inflammation are based on routine, inexpensive, and readily available laboratory tests. Red cell distribution width (RDW),[11] neutrophil-lymphocyte ratio (NLR), prognostic nutritional index (PNI),[12] and lymphocyte-monocyte ratio (LMR)[17] have been used to predict the prognosis of disease. RDW was found to be an independent marker of

short- and long-term prognosis in intensive care units.[11] NLR at admission served as an independent predictor of 3-month mortality rates in acute-on-chronic liver failure patients.[13] Increased pre-treatment LMR was associated with a significantly more favourable prognosis in patients with solid tumours.[17] Despite this evidence, very few studies have focused on the direct relationship between inflammation-based prognostic markers and mortality of AP. A cross-sectional study found a significant association between RDW and mortality in patients with AP.[18] Another study investigated the prognostic value of NLR in AP and determined an optimal ratio for prediction of severity.[19]

To the best of our knowledge, the current study is the first to simultaneously compare the prognostic value of these inflammation-based prognostic markers (NLR, PNI, CRP, RDW, and LMR) of mortality in patients with AP.

MATERIALS AND METHODS

Participants

This retrospective cohort analysis consecutively enrolled a series of patients with AP who were admitted to the emergency department at our hospital between 1 July 2013 and 18 August 2015. A diagnosis of AP required two of three features: (1) prolonged abdominal pain characteristic of AP, (2) threefold elevation of serum amylase and/or lipase levels above the normal range, and (3) characteristic findings of AP on abdominal ultrasonography and/or computed tomography scan.[1] Mild acute pancreatitis (MAP) was defined as absence of organ failure and an absence of local or systemic complications.[1] Moderately severe acute pancreatitis (MSAP) was defined as no evidence of persistent organ failure, but the presence of local or systemic complications and/or organ failure that resolved within 48 h. SAP was defined as

persistent organ failure (>48 h).[1] Patients with recurrent pancreatitis were enrolled only at first admission. Patients with traumatic pancreatitis, autoimmunepancreatitis, diabetes mellitus, tumour, or liver failure were excluded.

The prognostic information we focused on included overall survival and the severity of the disease. All enrolled patients were followed up for 100 days or until death. All clinical data were retrieved from medical records. For AP patients, 100 days of prognostic information (survival or non-survival) was obtained by checking medical records or by contacting the patients' family members.

Ethics statement

Each participant provided written informed consent after being provided with an explanation of the study by phone, letter, or e-mail. The Ethics Committee of The First Affiliated Hospital of Zhejiang University College of Medicine approved the consent procedure and experiment periods. The study was conducted in accordance with the ethical principles contained within the Declaration of Helsinki.

Demographic information and laboratory analysis

Demographic information, including age, sex, aetiology, and complication, was collected from medical records. Pre-treatment laboratory data, including complete blood counts, serum CRP, albumin, and amylase were obtained during the emergency visit. An XE-2100 haematology autoanalyzer (Sysmex Corp., Kobe, Japan), a Hitachi 7600 chemistry analyser (Hitachi High-Technologies, Tokyo, Japan), and Roche reagents (Roche Diagnostics, Indianapolis, IN, USA) were used in the laboratory.

We assessed the prognostic value of general inflammation-based prognostic markers (NLR, CRP, RDW, PNI, and LMR) for predicting the mortality of AP.

Additionally, their ability to predict the severity of AP (SAP or not SAP) was assessed. NLR and LMR were ratios of two types of blood cell. PNI = albumin (g/L) + 5 × total lymphocyte count (10^9/L).

Statistical analysis

Variables were expressed as mean±SD, median (range) or categorical data as percentages, if appropriate. The differences between the two groups were assessed with an independent sample *t* test, the Mann-Whitney U test or χ^2 test as appropriate. Multiple comparisons were performed by one-way analysis of variance or Kruskal-Wallis H tests, as appropriate. Multivariate logistic regression analyses were used to assess whether these inflammation markers were independent factors for predicting SAP in patients with AP by unadjusted and adjusted models successively. The AP patients were randomly divided into estimation and validation cohorts by random number generators. The accuracy of each marker to predict mortality was assessed using the receiver operating characteristic curve (ROC). The combination models were developed by binary logistic regression analyses. Overall survival curves were calculated using the Kaplan–Meier method, and differences in survival rates were compared using the log-rank test. Univariate and multivariate Cox proportional hazard models were used to estimate the significance and independence of the relationship of each marker and mortality. The variables with p-value <0.1 in univariate analysis were included in a multivariate Cox proportional hazard regression model. A p-value <0.05 was considered statistically significant. Statistical analyses were performed with SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

A total of 359 AP patients (197 MAP, 76 MSAP, and 86 SAP) were enrolled in the study. The predefined probability of type I error was 0.05 (α=0.05), and the sample size was large enough to guarantee 0.90 of test power (β=0.1). Forty-five patients were excluded from the analysis, including those with traumatic pancreatitis (n=1), autoimmune pancreatitis (n=5), diabetes mellitus (n=7), tumour (n=7), liver failure (n=2), or incomplete medical records or who were lost to follow-up (n=23). Tables 1 and 2 show the baseline characteristics of the patients. There were no significant differences in age (p=0.352), aetiology (p=0.875), or sex (p=0.919) among the three groups (MAP, MSAP, and SAP). As the illness worsened, CRP, RDW, and NLR gradually increased, but PNI decreased (all p<0.05; Table 1). LMR decreased significantly (p<0.001) in MSAP compared with MAP patients, but there was no significant difference between MSAP and SAP patients (p=0.883).

Compared with survivors of AP, non-survivors were older (p=0.001) and had higher CRP (p<0.001), amylase (p=0.010), RDW (p<0.001), and NLR (p<0.001). Conversely, lymphocyte count (p<0.001), platelet (p=0.001), albumin (p<0.001), LMR (p<0.001), and PNI (p<0.001) were lower in non-survivors than in survivors (Table 2).

The relationship between markers and severity of AP

The multivariate logistic regression models revealed that high CRP [>110 *vs.* ≤110mg/L, adjusted odd ratio (OR)=8.251, 95%CI: 3.897-17.468, p<0.001], RDW (>13.0 *vs.* ≤13.0%, adjusted OR= 2.533, 95%CI: 1.365-4.702, p=0.003) and low PNI(<41.1 *vs.* ≥41.1, adjusted OR=7.753, 95%CI: 3.400-17.680, p<0.001) were independent factors for predicting SAP in patients with AP (Table 3).

The markers' power for predicting 100 days mortality

The enrolled 359 patients with AP were randomly grouped into two cohorts: the estimation cohort (n=181) and the validation cohort (n=178). No significant difference was observed between the estimation and the validation cohorts in all characteristics (Supplementary Table S1). ROC curves of the estimation cohort were constructed to evaluate the ability of each marker to predict 100 days mortality in AP. Table 4 shows the area under the receiver operating characteristic curves (AUCs) and optimal cut-off values. The ability of NLR to predict mortality (AUC=0.804, p<0.001) was good; those of PNI (AUC=0.769, p<0.001), CRP (AUC=0.774, p<0.001), RDW (AUC=0.769, p<0.001), and LMR (AUC=0.744, p<0.001) were fair. The NLR had the largest AUC, and RDW and PNI had the highest sensitivity and specificity, respectively. Therefore, these three markers were selected for combination. The AUCs of NLR+PNI, NLR+RDW, and PNI+RDW were 0.825(95%CI: 0.761–0.877); 0.854(95%CI: 0.794–0.902), and 0.806(95%CI: 0.741–0.861), respectively (Fig. 1). There were no significant differences in AUCs of combined index and NLR (p=0.699; p=0.167; p=0.975, respectively).

For NLR, the optimal cut-off value for mortality prediction was 16.64, with a sensitivity of 82.4% and specificity of 75.6%. RDW has the highest sensitivity (94.1%) and lowest negative likelihood ratio (0.11), so it was a reliable predictive index for excluding mortality in AP patients. PNI has the highest specificity (88.4%) and positive likelihood ratio (5.08), so it was most suitable for a confirmed index among the indexes assessed in this article.

In the validation cohort, the AUCs of NLR, CRP, RDW, PNI, and LMR were 0.851(95%CI: 0.790–0.900), 0.753(95%CI: 0.683–0.815), 0.708(95%CI:

0.635–0.773), 0.791(95%CI: 0.724–0.848), and 0.677(95%CI: 0.603–0.745), respectively. There were no significant differences in the AUCs of NLR, CRP, RDW, PNI, and LMR between the estimation and validation cohorts (p=0.477, p=0.809, p=0.437, p=0.782, and p=0.455, respectively).

Survival analysis

AP patients were stratified into groups by cut-off values. Kaplan−Meier survival curves demonstrate the relationships between inflammation-based prognostic markers and overall survival of patients with AP (Fig. 2A−E). Elevated NLR (p<0.001), CRP (p<0.001), and RDW (p<0.001) were associated with increased probability of death. Conversely, decreased PNI (p<0.001) and LMR (p=0.001) were associated with decreased overall survival.

According to the cut-off values of the factors, low NLR(≤16.64), low CRP(≤162.2mg/L), low RDW(≤13.0%), high PNI(≥33.1), and high LMR(≥1.40) were selected as references. Univariate analysis and Cox regression revealed that age (p<0.001), amylase (p=0.001), NLR (p<0.001), PNI (p<0.001), CRP (p<0.001), RDW (p<0.001), and LMR (p=0.002) were associated with AP mortality (Table 5). These factors were evaluated by multivariate Cox regression. Age (HR=4.039, 95% CI: 1.873-8.713, p<0.001), NLR (HR=4.726, 95% CI: 1.627-13.726, p=0.004), CRP(HR=3.503, 95% CI: 1.534-7.999, p=0.003), RDW(HR=3.139, 95% CI: 1.277-7.714, p=0.013), and PNI(HR=2.641, 95% CI: 1.248-5.590, p=0.011) were independently associated with mortality of AP (Table 5).

DISCUSSION

AP is an inflammatory disease, with mortality arising mainly from organ failure or infected pancreatic necrosis.[4] Our study estimated the prognostic value of various inflammation-based prognostic markers for predicting mortality of AP. According the classification of AUCs,[20 21] the ability of the NLR to predict mortality was good, while those of PNI, CRP, RDW, and LMR were fair. Cox regression analysis revealed that age, NLR, PNI, CRP, and RDW were independently associated with mortality of AP. Additionally, PNI, CRP, and RDW were independently associated with the occurrence of SAP in AP patients.

NLR, CRP, RDW, and PNI are inexpensive, convenient, and readily available in clinical settings. From examination of the AUCs, NLR had the best performance. With a NLR>16.64 at the time of admission, the risk of dying increased 3.726-fold compared with NLR≤16.64. RDW was the most reliable marker for excluding death in AP patients, owing to its lowest negative likelihood ratio (0.11). PNI has the highest specificity (88.4%) and positive likelihood ratio (5.08), so it was most suitable to be a confirmed index among the indexes assessed in this article. However, fluctuations in the NLR and CRP can be influenced sensitively by the use of antibiotics; therefore, NLR and CRP are not suitable for patients undergoing intensive use of antibiotics. Similarly, blood transfusion and parenteral nutrition may affect RDW and PNI, respectively, so the predictive value of RDW and PNI in these patients was discounted.

In AP, inflammation propagates and promotes tissue destruction via activation of a cascade of inflammatory cytokines, proteolytic enzymes, and oxygen free radicals.[19 22] Neutrophils, lymphocytes, and monocytes are the three main types of white blood cells (WBCs). Neutrophils play a key role in the development of local tissue destruction and systemic complications of SAP.[23] Depletion of neutrophils has been

associated with an improved prognosis of AP.[23] The percentage of immature neutrophilic granulocytes might be used clinically as a simple early predictor of an adverse outcome in SAP.[24] Additionally, recent studies revealed that the extent of lymphopenia was also associated with disease severity.[25-27] Lymphopenia has been reported to have independent prognostic value for some diseases,[19 26-29] including AP. Takeyama *et al.* found that impairment of cellular immunity caused by peripheral lymphocyte apoptosis was linked to the subsequent development of infectious complications in AP.[28] Monocytes produce various cytokines and inflammatory mediators that further amplify inflammatory cell recruitment into the pancreas as well as distant organs such as the lungs.[30] Similar to neutrophils, a protective effect was also found by depleting macrophages in a mouse model of AP.[31] Theoretically, NLR and LMR, which combine two opposing parameters, should be more accurate than either parameter alone. In fact, we found that NLR had the greatest prognostic value of all the factors we evaluated. Despite this, it is important to apply it with caution in clinical settings. Broad-spectrum antibiotics with good tissue penetration, which are essential medicines in the treatment of SAP, can affect WBC by reducing inflammation. Thus, the prognostic value of NLR in AP is uncertain if the effect of antibiotic treatment is not taken into account.[32] For this reason, the neutrophil and lymphocyte counts used in this study were from the first complete blood cell count, conducted during the emergency visit. We confirmed that the enrolled patients were untreated at that time; consequently, our results are most likely applicable to untreated patients. Unlike for NLR, the predictive ability of LMR was only fair, and was not independently associated with overall survival in AP.

Serum albumin is a negative acute phase response reactant, and it reflects the body's nutritional status. Albumin <25 g/L was an independent prognostic factor

related to a poor prognosis of AP.[33] Variation of albumin within 24 h has been identified as a risk factor for a poor prognosis of critically ill patients in the early stages of SAP.[34] The PNI, which includes serum albumin and lymphocyte count, is an independent predictor of poor overall survival in patients with hepatocellular carcinoma.[35] To the best of our knowledge, few studies have reported the application of PNI for predicting mortality of AP, but we found that it was an independent prognostic factor, and was suitable as a confirmed marker.

Numerous studies have reported RDW as a strong, independent prognostic factor in various diseases and conditions, such as cardiovascular diseases, rheumatoid arthritis, cancer, and critical illnesses.[18 36-38] Our results are consistent with the study by Yao J *et al.*,[18] who reported a significant association between RDW and mortality of patients with AP. Additionally, we found that RDW was most suitable as a reliable excluding marker among the markers we assessed. The mechanisms underlying the association between RDW and mortality in AP remain unclear. The obvious metabolic abnormalities in non-survivors of AP, including inflammation, oxidative stress, poor nutritional status, and persistent organ failure, lead to deregulation of red blood cell homeostasis involving both impaired erythropoiesis and abnormal red blood cell survival.[38] RDW reflects these impairments in homeostasis, but only further research can confirm this speculation.

The prognostic markers evaluated in this study are direct or combined markers of systemic inflammation that are based on routine, inexpensive, and readily available laboratory tests. To the best of our knowledge, this is the first study to compare the prognostic value of these markers for predicting mortality in patients with AP simultaneously. Additionally, suitable excluding and identifying markers were found.

Some potential limitations of the current study should be noted. This was a retrospective, single-centre study; a larger, prospective study is needed to validate these results. Second, only the first set of admission blood results were investigated. As factors change with time, they should be surveyed in the future because of the rapid-onset of inflammation. Third, the typical prediction models, such as APACHE II score, should be included in future research. Additionally, we only described the association of each of the predictors with mortality of AP; the underlying mechanisms need to be investigated.

In conclusion, we found that age, NLR, PNI, CRP, and RDW were independently associated with overall survival of AP. NLR had the best overall performance, RDW was suitable as a reliable marker to exclude death, and PNI was a good predictive marker for death. When applying these markers, any possible influence from therapy should be considered.

Abbreviations

AP, acute pancreatitis; AUC, area under the receiver operating characteristic curve; CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; LMR, lymphocyte-monocyte ratio; -LR, negative likelihood ratio; +LR, positive likelihood ratio; MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; NLR, neutrophil-lymphocyte ratio; OR, odd ratio; PNI, prognostic nutritional index; RDW, red cell distribution width; ROC, receiver operating characteristic curve; SAP, severe acute pancreatitis; WBC, white blood cell count.

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Contributors

R.G. and Y.L. designed the experiments. R.G. and Y.L. contributed to the data collection. Y.Z. conducted the data analysis. Y.L., R.G, and L.F. wrote the manuscript. All authors reviewed the manuscript.

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Competing interests

None declared.

Patient consent

Obtained.

Ethics approval

This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine, China

Provenance and peer review

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Data sharing statement

No additional data are available.

REFERENCES

1. Banks PA, Bollen TL, Dervenis C, *et al.* Classification of acute pancreatitis--2012: revision The diagnosis of acute pancreatitis requires two of the following three features: and definitions by international consensus. *Gut* 2013;62:102-11.
2. Maheshwari R, Subramanian RM. Severe Acute Pancreatitis and Necrotizing Pancreatitis. *Crit Care Clin* 2016;32:279-90.
3. Bugiantella W, Rondelli F, Boni M, *et al.* Necrotizing pancreatitis: A review of the interventions. *Int J Surg* 2016;28(Suppl 1):S163-71.
4. Petrov MS, Shanbhag S, Chakraborty M, *et al.* Organ failure and infection of pancreatic necrosis as determinants of mortality in patients with acute pancreatitis. *Gastroenterology* 2010;139:813-20.
5. Dellinger EP, Forsmark CE, Layer P, *et al.* Determinant-based classification of acute pancreatitis severity: an international multidisciplinary consultation. *Ann Surg* 2012;256:875-80.
6. Papachristou GI, Muddana V, Yadav D, *et al.* Comparison of BISAP, Ranson's, APACHE-II, and CTSI scores in predicting organ failure, complications, and mortality in acute pancreatitis. *Am J Gastroenterol* 2010;105:435-41; quiz 42.
7. Mounzer R, Langmead CJ, Wu BU, *et al.* Comparison of existing clinical scoring systems to predict persistent organ failure in patients with acute pancreatitis. *Gastroenterology* 2012;142:1476-82; quiz e15-6.
8. Bezmarevic M, Mirkovic D, Soldatovic I, *et al.* Correlation between procalcitonin and intra-abdominal pressure and their role in prediction of the severity of acute pancreatitis. *Pancreatology* 2012;12:337-43.
9. Yang CJ, Chen J, Phillips AR, *et al.* Predictors of severe and critical acute pancreatitis: a systematic review. *Dig Liver Dis* 2014;46:446-51.
10. Zampieri FG, Ranzani OT, Sabatoski V, *et al.* An increase in mean platelet volume after admission is associated with higher mortality in critically ill patients. *Ann Intensive Care* 2014;4:20.
11. Hunziker S, Celi LA, Lee J, *et al.* Red cell distribution width improves the simplified acute physiology score for risk prediction in unselected critically ill patients. *Crit Care* 2012;16:R89.

12. Kinoshita A, Onoda H, Imai N, *et al.* Comparison of the prognostic value of inflammation-based prognostic scores in patients with hepatocellular carcinoma. *Br J Cancer* 2012;107:988-93.
13. Chen L, Lou Y, Chen Y, *et al.* Prognostic value of the neutrophil-to-lymphocyte ratio in patients with acute-on-chronic liver failure. *Int J Clin Pract* 2014;68:1034-40.
14. Smith RA, Bosonnet L, Raraty M, *et al.* Preoperative platelet-lymphocyte ratio is an independent significant prognostic marker in resected pancreatic ductal adenocarcinoma. *Am J Surg* 2009;197:466-72.
15. Proctor MJ, Morrison DS, Talwar D, *et al.* An inflammation-based prognostic score (mGPS) predicts cancer survival independent of tumour site: a Glasgow Inflammation Outcome Study. *Br J Cancer* 2011;104:726-34.
16. Proctor MJ, Morrison DS, Talwar D, *et al.* A comparison of inflammation-based prognostic scores in patients with cancer. A Glasgow Inflammation Outcome Study. *Eur J Cancer* 2011;47:2633-41.
17. Teng JJ, Zhang J, Zhang TY, *et al.* Prognostic value of peripheral blood lymphocyte-to-monocyte ratio in patients with solid tumors: a meta-analysis. *Onco Targets Ther* 2016;9:37-47.
18. Yao J, Lv G. Association between red cell distribution width and acute pancreatitis: a cross-sectional study. *BMJ Open* 2014;4:e004721.
19. Suppiah A, Malde D, Arab T, *et al.* The prognostic value of the neutrophil-lymphocyte ratio (NLR) in acute pancreatitis: identification of an optimal NLR. J Gastrointest Surg 2013;17(4):675-81.
20. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;39:561-77.
21. Altman DG, Bland JM. Diagnostic tests 3: receiver operating characteristic plots. *BMJ* 1994;309:188.
22. Felderbauer P, Muller C, Bulut K, *et al.* Pathophysiology and treatment of acute pancreatitis: new therapeutic targets--a ray of hope? *Basic Clin Pharmacol Toxicol* 2005;97:342-50.
23. Xue J, Sharma V, Habtezion A. Immune cells and immune-based therapy in pancreatitis. *Immunol Res* 2014;58:378-86.
24. Zhu L, Chen G, Xia Q, *et al.* Use of band cell percentage as an early predictor of death and ICU admission in severe acute pancreatitis. *Hepatogastroenterology* 2010;57:1543-8.
25. Zahorec R. Ratio of neutrophil to lymphocyte counts--rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratisl Lek Listy* 2001;102:5-14.
26. de Jager CP, van Wijk PT, Mathoera RB, *et al.* Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. *Crit Care* 2010;14:R192.
27. Le Tulzo Y, Pangault C, Gacouin A, *et al.* Early circulating lymphocyte apoptosis in human septic shock is associated with poor outcome. *Shock* 2002;18:487-94.
28. Takeyama Y, Takas K, Ueda T, *et al.* Peripheral lymphocyte reduction in severe acute pancreatitis is caused by apoptotic cell death. *J Gastrointest Surg* 2000;4:379-87.
29. Pezzilli R, Billi P, Beltrandi E, *et al.* Circulating lymphocyte subsets in human acute pancreatitis. *Pancreas* 1995;11:95-100.
30. McKay C, Imrie CW, Baxter JN. Mononuclear phagocyte activation and acute pancreatitis. *Scand J Gastroenterol Suppl* 1996;219:32-6.

31. Saeki K, Kanai T, Nakano M, *et al.* CCL2-induced migration and SOCS3-mediated activation of macrophages are involved in cerulein-induced pancreatitis in mice. *Gastroenterology* 2012;142:1010-20 e9.
32. Binnetoglu E, Akbal E, Gunes F, *et al.* The prognostic value of neutrophil-lymphocyte ratio in acute pancreatitis is controversial. *J Gastrointest Surg* 2014;18:885.
33. Gonzalvez-Gasch A, de Casasola GG, Martin RB, *et al.* A simple prognostic score for risk assessment in patients with acute pancreatitis. *Eur J Intern Med* 2009;20:e43-8.
34. Chen Y, Zhang ZW, Wang B, *et al.* Relationship between early serum albumin variation and prognosis in patients with severe acute pancreatitis treated in ICU. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2013;44:237-41.
35. Pinato DJ, North BV, Sharma R. A novel, externally validated inflammation-based prognostic algorithm in hepatocellular carcinoma: the prognostic nutritional index (PNI). *Br J Cancer* 2012;106:1439-45.
36. Lappegard J, Ellingsen TS, Vik A, *et al.* Red cell distribution width and carotid atherosclerosis progression. The Tromso Study. *Thromb Haemost* 2015;113:649-54.
37. Bekler A, Tenekecioglu E, Erbag G, *et al.* Relationship between red cell distribution width and long-term mortality in patients with non-ST elevation acute coronary syndrome. *Anatol J Cardiol* 2015;15:634-9.
38. Salvagno GL, Sanchis-Gomar F, Picanza A, *et al.* Red blood cell distribution width: A simple parameter with multiple clinical applications. *Crit Rev Clin Lab Sci* 2015;52:86-105.

Table 1 Demographics and laboratory findings in patients with acute pancreatitis

Variables	1. MAP(n=197)	2. MSAP(n=76)	3. SAP(n=86)	p value		
				all groups	1 *vs.* 2	2 *vs.* 3
Age (years)	51.43 ± 16.00	48.47 ± 13.28	50.69 ± 14.61	0.352	0.122	0.317
Male (%)	108(54.8%)	41(53.9%)	49(57.0%)	0.919	0.896	0.699
Aetiology (1/2/3/4)%	52%/12%/11%/25%	51%/16%/13%/20%	47%/15%/14%/24%	0.875	0.664	0.892
WBC ($\times10^9$/L)	11.5 (3.1–32.0)	14.1 (4.5–36.8)	16.05 (5.9–38.4)	<0.001	<0.001	0.278
Lymphocyte ($\times10^9$/L)	1.1 (0.2–9.4)	1.0 (0.2–2.6)	0.80 (0.2–2.9)	<0.001	0.004	0.089
Platelet ($\times10^9$/L)	202 (21–502)	193 (58–548)	163 (27–540)	0.004	0.376	0.046
Albumin (g/L)	38.29 ± 5.07	34.38 ± 6.39	29.99 ± 5.35	<0.001	<0.001	<0.001
CRP (mg/L)	53.9 (0.7–386)	133.6 (3.2–436.5)	196.1 (27.1–426.7)	<0.001	<0.001	<0.001
Amylase (U/L)	398 (13–5191)	222 (27–3845)	581 (16–2377)	0.141	0.083	0.056
RDW (%)	12.8 (11.4–19.2)	13.0 (11.3–16.3)	13.7 (11.7–23.6)	<0.001	0.013	0.014
NLR	8.46 (1.33–55)	14.60 (1.73–60)	19.65 (3.57–53.67)	<0.001	<0.001	0.020
LMR	1.88 (0.28–13.33)	1.03 (0.29–5.33)	1.14 (0.22–6.32)	<0.001	<0.001	0.883
PNI	44.53 ± 6.63	39.36 ± 6.71	34.55 ± 6.02	<0.001	<0.001	<0.001
Mortality (%)	0(0%)	0(0%)	31(36.0%)	<0.001	–	<0.001

Continuous variables are presented as mean±SD or median (range).

Aetiology (1/2/3/4)%, 1, 2, 3, and 4 represent gallstone, alcohol, hypertriglyceridemia, and other aetiologies, respectively.

1 *vs.* 2, MAP group *vs.* MSAP group; 2 *vs.* 3, MSAP group *vs.*SAP group.

MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis; WBC, white blood cell count; CRP, C-reactive protein; RDW, red cell distribution width; NLR, neutrophil-lymphocyte ratio; LMR, lymphocyte-monocyte ratio; PNI, prognostic nutritional index.

Table 2 Demographics and laboratory findings in survivors and non-survivors of acute pancreatitis

Variables	Survivors(n=328)	Non-survivors(n=31)	p value
Age (years)	49.84 ± 14.88	58.90 ± 15.60	0.001
Male (%)	179(54.6%)	19(61.3%)	0.472
Aetiology (1/2/3/4)%	50%/13%/12%/25%	58%/19%/10%/13%	0.346
WBC ($\times10^9$/L)	12.85 (3.1–38.4)	18.5 (6.5–29.3)	0.001
Lymphocytes ($\times10^9$/L)	1.08 (0.17–9.40)	0.60 (0.30–1.60)	<0.001
Platelet ($\times10^9$/L)	197 (21–548)	159 (27–376)	0.001
Albumin (g/L)	35.95 ± 6.30	30.44 ± 5.54	<0.001
CRP (mg/L)	98.6 (0.7–436.5)	239.2 (27.1–398.2)	<0.001
Amylase (U/L)	343.5 (13–5191)	909 (16–2377)	0.010
RDW (%)	13 (11.3–19.2)	13.8 (12.6–23.6)	<0.001
NLR	10.47 (1.33–60.0)	25.0 (8.67–53.67)	<0.001
PNI	41.71 ± 7.50	34.00 ± 6.35	<0.001
LMR	1.51 (0.22–13.33)	1.13 (0.24–2.26)	<0.001

Continuous variables are presented as mean±SD or median (range).

Aetiology (1/2/3/4)%, 1, 2, 3, and 4 represent gallstone, alcohol, hypertriglyceridemia, and other aetiologies, respectively.

WBC, white blood cell count; CRP, C-reactive protein; RDW, red cell distribution width; NLR, neutrophil-lymphocyte ratio; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio.

Table 3 Odds ratios of prognostic factors for predicting SAP in patients with AP

Factors	Model 1		Model 2		Model 3	
	Odds ratio (95%CI)	p value	Odds ratio (95%CI)	p value	Odds ratio (95%CI)	p value
NLR (>11.36 *vs.*≤11.36)	3.707(2.173-6.326)	<0.001	3.578 (2.082-6.149)	<0.001	1.463(0.711-3.010)	0.301
CRP (>110 *vs.*≤110mg/L)	9.867(5.116-19.030)	<0.001	12.609 (6.304-25.218)	<0.001	8.251(3.897-17.468)	<0.001
RDW (>13.0 *vs.*≤13.0%)	3.368(2.003-5.663)	<0.001	3.529 (2.076-5.998)	<0.001	2.533(1.365-4.702)	0.003
PNI (<41.1 *vs.* ≥41.1)	9.951(5.055-19.589)	<0.001	11.356 (5.665-22.766)	<0.001	7.753(3.400-17.680)	<0.001
LMR (<1.43 *vs.* ≥1.43)	2.564(1.539-4.271)	<0.001	2.552 (1.524-4.274)	<0.001	0.722(0.355-1.471)	0.370

Model1: unadjusted model.

Model2: adjusted for age, gender and amylase.

Model3: NLR was adjusted for age, gender, amylase, CRP, RDW, PNI, and LMR; CRP was adjusted for age, gender, amylase, NLR, RDW, PNI, and LMR; RDW was adjusted for age, gender, amylase, CRP, NLR, PNI, and LMR; PNI was adjusted for age, gender, amylase, NLR, CRP, RDW, and LMR; LMR was adjusted for age, gender, amylase, NLR, CRP, RDW, and PNI.

AP, acute pancreatitis; SAP, severe acute pancreatitis; NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; RDW, red cell distribution width; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio; CI, confidence interval.

Table 4 Discriminatory ability of inflammation-based markers for predicting mortality in AP patients

Index	AUC(95% CI)	p value	Cut-off	Sensitivity	Specificity	+LR	-LR
NLR	0.804(0.738-0.859)	<0.001	16.64	82.4%	75.6%	3.38	0.23
CRP	0.774(0.706-0.833)	<0.001	162.2mg/L	76.5%	73.8%	2.92	0.32
RDW	0.769(0.700-0.828)	<0.001	13.0%	94.1%	54.3%	2.06	0.11
PNI	0.769(0.701- 0.828)	<0.001	33.1	58.8%	88.4%	5.08	0.47
LMR	0.744(0.674-0.806)	<0.001	1.40	82.4%	57.3%	1.93	0.31

NLR, neutrophil-lymphocyte ratio; PNI, prognostic nutritional index; CRP, C-reactive protein; RDW, red cell distribution width; LMR, lymphocyte-monocyte ratio; AUC, area under the receiver operating characteristic curve; CI, confidence interval; +LR, positive likelihood ratio; -LR, negative likelihood ratio.

The p value is comparing the AUC with 0.5.

Table 5 Prognostic factors of overall survival in patients with acute pancreatitis by univariate and multivariate analyses

Factors	Univariate analysis		Multivariate analysis	
	Hazard ratio (95%CI)	p value	Hazard ratio (95%CI)	p value
Age (>63 *vs.* ≤63 years)	5.384(2.653-10.925)	<0.001	4.039(1.873-8.713)	<0.001
Gender (female *vs.* male)	0.767(0.372-1.579)	0.471		
Amylase (>618 *vs.*≤618U/L)	3.544(1.699-7.526)	0.001	2.173(0.965-4.891)	0.061
NLR(>16.64 *vs.* ≤16.64)	13.130(5.041-34.205)	<0.001	4.726(1.627-13.726)	0.004
CRP(>162.2 *vs.*≤162.2mg/L)	6.127(2.740-13.701)	<0.001	3.503(1.534-7.999)	0.003
RDW(>13.0 *vs.*≤13.0%)	4.929(2.022-12.017)	<0.001	3.139(1.277-7.714)	0.013
PNI(<33.1 *vs.* ≥33.1)	6.912(3.414-13.991)	<0.001	2.641(1.248-5.590)	0.011
LMR(<1.40 *vs.* ≥1.40)	3.797(1.636-8.813)	0.002	1.036(0.403-2.659)	0.942

NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; RDW, red cell distribution width; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio; CI, confidence interval.

Figure Legends

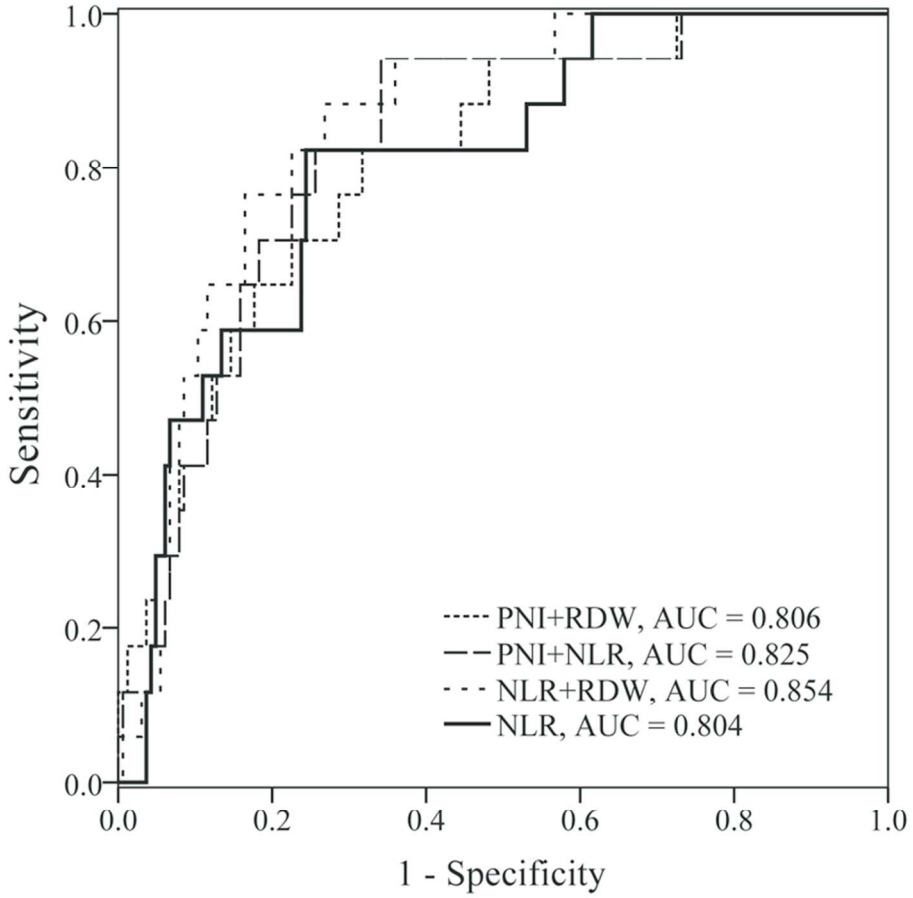
Fig. 1. ROC curves analysis for predicting mortality by NLR and combined markers in the estimation cohort.

ROC, receiver operating characteristic; AUC, area under the receiver operating characteristic curve; NLR, neutrophil-lymphocyte ratio; RDW, red cell distribution width; PNI, prognostic nutritional index.

Fig. 2. Relationship between inflammation-based prognostic markers and overall survival in patients with acute pancreatitis

A, B, C, D, and E show the relationship between NLR, CRP, RDW, PNI, and LMR, and overall survival in patients with acute pancreatitis, respectively.

NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; RDW, red cell distribution width; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio.



ROC, receiver operating characteristic; AUC, area under the receiver operating characteristic curve; NLR, neutrophil-lymphocyte ratio; RDW, red cell distribution width; PNI, prognostic nutritional index.

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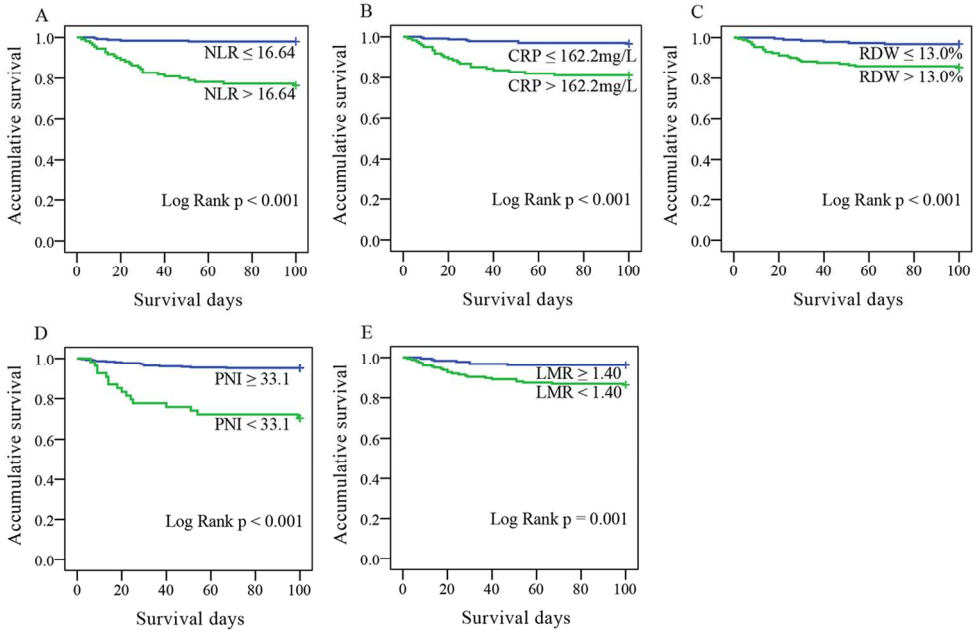

A, B, C, D, and E show the relationship between NLR, CRP, RDW, PNI, and LMR, and overall survival in patients with acute pancreatitis, respectively.
NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; RDW, red cell distribution width; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio.

170x115mm (300 x 300 DPI)

Supplementary Table S1 Demographics and laboratory findings in estimation and validation cohorts

Variable	All patients（n=359）	Training set(n=181)	Validation set(n =178)	p value
Age (years)	50.63±15.13	51.66 ±15.69	49.58 ±14.51	0.194
Male [N (%)]	198(55.2%)	96(53.0%)	102(57.3%)	0.417
Aetiology (1/2/3/4) %	54.3/9.7/12.0/24.0	55.2/12.7/12.2/19.9	53.4/6.7/11.8/28.1	0.118
WBC ($\times10^9$/L)	12.9(3.1-38.4)	13.3 (3.1–38.4)	12.9 (4.2–36.8)	0.942
Lymphocytes ($\times10^9$/L)	1.00 (0.17–9.40)	1.00 (0.20–9.40)	1.00 (0.17–4.80)	0.965
Platelet ($\times10^9$/L)	192 (21–548)	193 (27–502)	191.5 (21–548)	0.354
Albumin (g/L)	35.47 ±6.42	35.72 ±6.61	35.22 ±6.24	0.456
CRP (mg/L)	110 (0.7–436.5)	102.3 (0.8–436.5)	116.85 (0.7–419.4)	0.081
Amylase (U/L)	398 (13–5191)	501 (13–5191)	330 (16–4927)	0.238
RDW (%)	13.0(11.3-23.6)	13.1 (11.3–19.2)	13.0 (11.4–23.6)	0.421
NLR	11.36 (1.33–60.0)	11.50 (1.33–55.0)	11.18 (1.39–60.0)	0.786
PNI	41.05±7.72	41.22 ±7.74	40.87 ±7.71	0.670
LMR	1.43(0.22–13.33)	1.48 (0.24–13.33)	1.36 (0.22–10.00)	0.367
Mortality [N (%)]	31(8.6%)	17(9.4%)	14(7.9%)	0.607

Continuous variables are presented as mean±SD or median (range).

p value was training set versus validation set.

Aetiology (1/2/3/4)%, 1, 2, 3, and 4 represent gallstone, alcohol, hypertriglyceridemia, and other aetiologies, respectively.

WBC, white blood cell count; CRP, C-reactive protein; RDW, red cell distribution width; NLR, neutrophil-lymphocyte ratio; LMR, lymphocyte-monocyte ratio; PNI, prognostic nutritional index.

TRIPOD Checklist: Prediction Model Development

Section/Topic	Item	Checklist Item	Page
Title and abstract			
Title	1	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	2,3
Introduction			
Background and objectives	3a	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	4,5
	3b	Specify the objectives, including whether the study describes the development or validation of the model or both.	5
Methods			
Source of data	4a	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	5
	4b	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	5
Participants	5a	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	5,6
	5b	Describe eligibility criteria for participants.	5,6
	5c	Give details of treatments received, if relevant.	Not relevant
Outcome	6a	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	6
	6b	Report any actions to blind assessment of the outcome to be predicted.	6
Predictors	7a	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	6,7
	7b	Report any actions to blind assessment of predictors for the outcome and other predictors.	6,7
Sample size	8	Explain how the study size was arrived at.	8
Missing data	9	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	8
,8Statistical analysis methods	10a	Describe how predictors were handled in the analyses.	7
	10b	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	7
	10d	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	7
Risk groups	11	Provide details on how risk groups were created, if done.	7
Results			
Participants	13a	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	8
	13b	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	8, Table1 and 2
Model development	14a	Specify the number of participants and outcome events in each analysis.	8, Table1 and 2
	14b	If done, report the unadjusted association between each candidate predictor and outcome.	8,10, Table 3 and 5
Model specification	15a	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	8,10, Table 3 and 5
	15b	Explain how to the use the prediction model.	8,10
Model performance	16	Report performance measures (with CIs) for the prediction model.	8,10, Table3 and 5
Discussion			
Limitations	18	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	13
Interpretation	19b	Give an overall interpretation of the results, considering objectives, limitations, and results from similar studies, and other relevant evidence.	11-14
Implications	20	Discuss the potential clinical use of the model and implications for future research.	11,14
Other information			
Supplementary	21	Provide information about the availability of supplementary resources, such as	Yes

TRIPOD Checklist: Prediction Model Development

information		study protocol, Web calculator, and data sets.	
Funding	22	Give the source of funding and the role of the funders for the present study.	15

We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

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Comparison of the prognostic values of inflammation markers in patients with acute pancreatitis: a retrospective cohort study

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Comparison of the prognostic values of inflammation markers in patients with acute pancreatitis: a retrospective cohort study

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ABSTRACT

Objectives: Inflammation-based prognostic markers (neutrophil-lymphocyte ratio [NLR], prognostic nutritional index [PNI], red cell distribution width [RDW], and lymphocyte-monocyte ratio [LMR]) are associated with overall survival in some diseases. This study assessed their prognostic value in mortality and severity in acute pancreatitis (AP).

Design: A retrospective cohort study.

Setting: Patients with AP were recruited from the emergency department at our hospital.

Participants: A total of 359 AP patients (31 non-survivors) were enrolled.

Primary and secondary outcome measures: Mortality and severity of AP were the primary and secondary outcome measures, respectively. Biochemistry and haematology results of the first test after admission were collected. Independent relationships between severe AP (SAP) and markers were assessed using multivariate logistic regression models. Mortality prediction ability was evaluated using receiver operating characteristic (ROC) curves. Overall survival was evaluated using the Kaplan–Meier method, with differences compared using the log-rank test. Independent relationships between mortality and each predictor were estimated using Cox proportional hazard models.

Results: Compared with survivors of AP, non-survivors had higher RDW ($p<0.001$), higher NLR ($p<0.001$), lower LMR ($p<0.001$), and lower PNI ($p<0.001$) at baseline. C-reactive protein (CRP) [odd ratio (OR)=8.251, $p<0.001$], RDW (OR=2.533, $p=0.003$), and PNI (OR=7.753, $p<0.001$) were independently associated with the occurrence of SAP. For predicting mortality, NLR had the largest area under the ROC curve (0.804, $p<0.001$), with a 16.64 cut-off value, 82.4% sensitivity, and 75.0%

specificity. RDW was a reliable marker for excluding death owing to its lowest negative likelihood ratio (0.11). NLR (hazard ratio (HR) =4.726, p=0.004), CRP (HR=3.503, p=0.003), RDW (HR=3.139, p=0.013), and PNI (HR=2.641, p=0.011) were independently associated with mortality of AP.

Conclusions: NLR was the most powerful marker of overall survival in this patient series.

Strengths and limitations of this study

- Compared with survivors of acute pancreatitis (AP), non-survivors had higher red cell distribution width (RDW) and neutrophil-lymphocyte ratio (NLR), and lower lymphocyte-monocyte ratio (LMR) and prognostic nutritional index (PNI) at baseline.
- NLR exhibited a higher area under the receiver operating characteristic curve for the prediction of mortality compared with other markers.
- RDW was suitable as a reliable marker to exclude death.
- NLR, PNI, C-reactive protein, and RDW were independently associated with overall survival of AP.
- This was a retrospective cohort analysis.

INTRODUCTION

Acute pancreatitis (AP) is rapid-onset inflammation of the pancreas that varies in severity from a self-limiting mild illness to rapidly progressive multiple organ failure. Statistics suggest that 10–20% of patients with AP develop severe acute pancreatitis (SAP),[1] which usually has an unfavourable disease progression and is associated with a poor prognosis.[2 3] Prediction of disease severity can guide the management of patients with AP and improve the outcome. Organ failure and infected pancreatic necrosis are common causes of mortality in such patients,[4] and a new international multidisciplinary classification of SAP incorporates both events as determinants of severity.[5] The predictive values of various markers, such as Acute Physiology and Chronic Health Evaluation II (APACHE II) and Bedside Index of Severity in Acute Pancreatitis scores, C-reactive protein (CRP), and procalcitonin, have been previously assessed.[6-8] A systematic review concluded it was justifiable to use blood urea nitrogen after 48 h of hospital admission for predicting persistent organ failure.[9] In clinical studies, most studies have focused on disease severity, and only a few have directly investigated the relationship between predictors and mortality of AP. Furthermore, no reliable predictor of persistent organ failure within 48 h of admission has been identified.[9]

There is increasing evidence that the presence of a systemic inflammatory response is associated with poor survival in patients with various aetiologies, including malignancy.[10-17] Many direct or combined markers of systemic inflammation are based on routine, inexpensive, and readily available laboratory tests. Red cell distribution width (RDW),[10] neutrophil-lymphocyte ratio (NLR), prognostic nutritional index (PNI),[11] and lymphocyte-monocyte ratio (LMR)[12] have been used to

predict the prognosis of disease. RDW was found to be an independent marker of short- and long-term prognosis in intensive care units.[10] NLR at admission served as an independent predictor of 3-month mortality rates in acute-on-chronic liver failure patients.[13] Increased pre-treatment LMR was associated with a significantly more favourable prognosis in patients with solid tumours.[12] Despite this evidence, very few studies have focused on the direct relationship between inflammation-based prognostic markers and mortality of AP. A cross-sectional study found a significant association between RDW and mortality in patients with AP.[18] Another study investigated the prognostic value of NLR in AP and determined an optimal ratio for prediction of severity.[19]

To the best of our knowledge, the current study is the first to simultaneously compare the prognostic value of these inflammation-based prognostic markers (NLR, PNI, CRP, RDW, and LMR) of mortality in patients with AP.

MATERIALS AND METHODS

Participants

This retrospective cohort analysis consecutively enrolled a series of patients with AP who were admitted to the emergency department at our hospital between 1 July 2013 and 18 August 2015. A diagnosis of AP required two of three features: (1) prolonged abdominal pain characteristic of AP, (2) threefold elevation of serum amylase and/or lipase levels above the normal range, and (3) characteristic findings of AP on abdominal ultrasonography and/or computed tomography scan.[1] Mild acute pancreatitis (MAP) was defined as an absence of organ failure and an absence of local or systemic complications.[1] Moderately severe acute pancreatitis (MSAP) was defined as no evidence of persistent organ failure, but the presence of local or systemic

complications and/or organ failure that resolved within 48 h. SAP was defined as persistent organ failure (>48 h).[1] Patients with recurrent pancreatitis were enrolled only at first admission. Patients with traumatic pancreatitis, autoimmune pancreatitis, diabetes mellitus, tumour, or liver failure were excluded.

The prognostic information we focused on included overall survival and the severity of the disease. All enrolled patients were followed for 100 days or until death. All clinical data were retrieved from medical records. For AP patients, 100 days of prognostic information (survival or non-survival) was obtained by checking medical records or by contacting the patients' family members.

Ethics statement

Each participant provided written informed consent after being provided with an explanation of the study by phone, letter, or e-mail. The Ethics Committee of The First Affiliated Hospital of Zhejiang University College of Medicine approved the consent procedure and experiment periods. The study was conducted in accordance with the ethical principles contained within the Declaration of Helsinki.

Demographic information and laboratory analysis

Demographic information, including age, sex, aetiology, and complication, was collected from medical records. Pre-treatment laboratory data, including complete blood counts, serum CRP, albumin, and amylase were obtained during the emergency visit. An XE-2100 haematology autoanalyzer (Sysmex Corp., Kobe, Japan), a Hitachi 7600 chemistry analyser (Hitachi High-Technologies, Tokyo, Japan), and Roche reagents (Roche Diagnostics, Indianapolis, IN, USA) were used in the laboratory.

We assessed the prognostic value of general inflammation-based prognostic markers (NLR, CRP, RDW, PNI, and LMR) for predicting the mortality of AP. Additionally, their ability to predict the severity of AP (SAP or not SAP) was assessed. NLR and LMR were ratios of two types of blood cell. PNI = albumin (g/L) + 5 × total lymphocyte count (10^9/L).

Statistical analysis

Variables are expressed as mean ± SD or median (range) and categorical data as percentages, as appropriate. Differences between the two groups were assessed using an independent sample *t*-test, Mann–Whitney U test, or χ^2 test, as appropriate. Multiple comparisons were performed by one-way analysis of variance or Kruskal–Wallis H tests, as appropriate. The Bonferroni method was used to adjust for multiple comparisons. Multivariate logistic regression analyses were used to assess whether the inflammation markers were independent factors for predicting SAP in patients with AP by unadjusted and adjusted models successively. AP patients were randomly divided into estimation and validation cohorts by random number generators. The accuracy of each marker to predict mortality was assessed using receiver operating characteristic curves (ROC). The sensitivity, specificity, positive likelihood ratio (+LR), and negative likelihood ratio (–LR) were calculated. +LR represents the ratio of the true positive rate to the false positive rate. –LR represents the ratio of the false negative rate to the true negative rate. These two parameters, which are not influenced by prevalence rate, are stable and objective for assessing diagnostic value. Combination models were developed using binary logistic regression analyses. Overall survival curves were calculated using the Kaplan–Meier method, and differences in survival rates were compared using the log-rank test.

Univariate and multivariate Cox proportional hazard models were used to estimate the significance and independence of the relationship of each marker and mortality. The variables with a p-value <0.1 in univariate analysis were included in a multivariate Cox proportional hazard regression model. A p-value <0.05 was considered statistically significant. Statistical analyses were performed with SPSS ver. 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

A total of 359 AP patients (197 MAP, 76 MSAP, and 86 SAP) were enrolled in the study. The predefined probability of type I error was 0.05 (α=0.05), and the sample size was large enough to guarantee 0.90 of test power (β=0.1). Forty-five patients were excluded from the analysis, including those with traumatic pancreatitis (n=1), autoimmune pancreatitis (n=5), diabetes mellitus (n=7), tumour (n=7), liver failure (n=2), or incomplete medical records or who were lost to follow-up (n=23). Tables 1 and 2 show the baseline characteristics of the patients. There were no significant differences in age (p=0.352), aetiology (p=0.875), or sex (p=0.919) among the three groups (MAP, MSAP, and SAP). As the illness worsened, CRP, RDW, and NLR gradually increased, but PNI decreased (all p<0.05; Table 1). LMR decreased significantly (p<0.001) in MSAP compared with MAP patients, but there was no significant difference between MSAP and SAP patients (p=0.883).

Compared with survivors of AP, non-survivors were older (p=0.001) and had higher CRP (p<0.001), amylase (p=0.010), RDW (p<0.001), and NLR (p<0.001). Conversely, lymphocyte count (p<0.001), platelets (p=0.001), albumin (p<0.001),

LMR (p<0.001), and PNI (p<0.001) were lower in non-survivors than in survivors (Table 2).

The relationship between markers and severity of AP

The multivariate logistic regression models revealed that high CRP (>110 *vs.* ≤110 mg/L, adjusted odd ratio (OR)=8.251, 95%CI: 3.897–17.468, p<0.001), RDW (>13.0 *vs.* ≤13.0%, adjusted OR= 2.533, 95%CI: 1.365–4.702, p=0.003), and low PNI (<41.1 *vs.* ≥41.1, adjusted OR=7.753, 95%CI: 3.400–17.680, p<0.001) were independent factors for predicting SAP in patients with AP (Table 3).

The markers' power for predicting 100 days mortality

The enrolled 359 patients with AP were randomly grouped into two cohorts: the estimation cohort (n=181) and the validation cohort (n=178). No significant difference was observed between the estimation and the validation cohorts in all characteristics (Supplementary Table S1). ROC curves of the estimation cohort were constructed to evaluate the ability of each marker to predict 100 days mortality in AP. Table 4 shows the area under the receiver operating characteristic curves (AUC) and optimal cut-off values. The ability of NLR to predict mortality (AUC=0.804, p<0.001) was good; those of PNI (AUC=0.769, p<0.001), CRP (AUC=0.774, p<0.001), RDW (AUC=0.769, p<0.001), and LMR (AUC=0.744, p<0.001) were fair. The NLR had the largest AUC, and RDW and PNI had the highest sensitivity and specificity, respectively. Therefore, these three markers were selected for combination. The AUC for NLR+PNI, NLR+RDW, and PNI+RDW were 0.825 (95%CI: 0.761–0.877); 0.854 (95%CI: 0.794–0.902), and 0.806 (95%CI: 0.741–0.861), respectively (Fig. 1). There

were no significant differences in AUC for combined index and NLR (p=0.699; p=0.167; p=0.975, respectively).

For NLR, the optimal cut-off value for mortality prediction was 16.64, with a sensitivity of 82.4% and specificity of 75.6%. RDW had the highest sensitivity (94.1%) and lowest negative likelihood ratio (0.11), so it was a reliable predictive index for excluding mortality in AP patients. PNI had the highest specificity (88.4%) and positive likelihood ratio (5.08), so it was most suitable for use as a confirmed index among the indexes assessed.

In the validation cohort, AUC for NLR, CRP, RDW, PNI, and LMR were 0.851 (95%CI: 0.790–0.900), 0.753 (95%CI: 0.683–0.815), 0.708 (95%CI: 0.635–0.773), 0.791 (95%CI: 0.724–0.848), and 0.677 (95%CI: 0.603–0.745), respectively. There were no significant differences in AUC for NLR, CRP, RDW, PNI, and LMR between the estimation and validation cohorts (p=0.477, p=0.809, p=0.437, p=0.782, and p=0.455, respectively).

Survival analysis

AP patients were stratified into groups by cut-off values. Kaplan−Meier survival curves demonstrate the relationships between inflammation-based prognostic markers and overall survival of patients with AP (Fig. 2A−E). Elevated NLR (p<0.001), CRP (p<0.001), and RDW (p<0.001) were associated with increased probability of death. Conversely, decreased PNI (p<0.001) and LMR (p=0.001) were associated with decreased overall survival.

According to the cut-off values for the factors, low NLR (≤16.64), low CRP (≤162.2 mg/L), low RDW (≤13.0%), high PNI (>33.1), and high LMR (>1.40) were selected as references. Univariate analysis and Cox regression revealed that age

(p<0.001), amylase (p=0.001), NLR (p<0.001), PNI (p<0.001), CRP (p<0.001), RDW (p<0.001), and LMR (p=0.002) were associated with AP mortality (Table 5). These factors were evaluated using multivariate Cox regression. Age (HR=4.039, 95%CI: 1.873–8.713, p<0.001), NLR (HR=4.726, 95%CI: 1.627–13.726, p=0.004), CRP (HR=3.503, 95%CI: 1.534–7.999, p=0.003), RDW (HR=3.139, 95%CI: 1.277–7.714, p=0.013), and PNI (HR=2.641, 95%CI: 1.248–5.590, p=0.011) were independently associated with mortality of AP (Table 5).

DISCUSSION

AP is an inflammatory disease, with mortality arising mainly from organ failure or infected pancreatic necrosis.[4] Our study estimated the prognostic value of various inflammation-based prognostic markers for predicting mortality of AP. According to classifications of AUC,[20 21] the ability of the NLR to predict mortality was good, while those of PNI, CRP, RDW, and LMR were fair. Cox regression analysis revealed that age, NLR, PNI, CRP, and RDW were independently associated with mortality of AP. Additionally, PNI, CRP, and RDW were independently associated with the occurrence of SAP in AP patients.

NLR, CRP, RDW, and PNI are inexpensive, convenient, and readily available in clinical settings. From examination of AUC, NLR had the best performance. With a NLR >16.64 at the time of admission, the risk of dying increased 3.726-fold compared with NLR ≤16.64. RDW was the most reliable marker for excluding death in AP patients, owing to its lowest negative likelihood ratio (0.11). PNI had the highest specificity (88.4%) and positive likelihood ratio (5.08), so it was most suitable to be a confirmed index among the indexes assessed. However, fluctuations in the NLR and CRP can be influenced by the use of antibiotics; therefore, NLR and CRP

are not suitable for patients undergoing intensive use of antibiotics. Similarly, blood transfusion and parenteral nutrition may affect RDW and PNI, respectively, so the predictive value of RDW and PNI in these patients was discounted.

In AP, inflammation propagates and promotes tissue destruction via activation of a cascade of inflammatory cytokines, proteolytic enzymes, and oxygen free radicals.[19 22] Neutrophils, lymphocytes, and monocytes are the three main types of white blood cells (WBC). Neutrophils play a key role in the development of local tissue destruction and systemic complications of SAP.[23] Depletion of neutrophils has been associated with an improved prognosis of AP.[23] The percentage of immature neutrophilic granulocytes might be used clinically as a simple early predictor of an adverse outcome in SAP.[24] Additionally, recent studies revealed that the extent of lymphopenia was associated with disease severity.[25-27] Lymphopenia has been reported to have independent prognostic value for some diseases,[19 26-29] including AP. Takeyama *et al.* found that impairment of cellular immunity caused by peripheral lymphocyte apoptosis was linked to the subsequent development of infectious complications in AP.[28] Monocytes produce various cytokines and inflammatory mediators that further amplify inflammatory cell recruitment into the pancreas as well as distant organs such as the lungs.[30] Similar to neutrophils, a protective effect was also found by depleting macrophages in a mouse model of AP.[31] Theoretically, NLR and LMR, which combine two opposing parameters, should be more accurate than either parameter alone. We found that the NLR had the greatest prognostic value of all the factors we evaluated. It is, however, important to apply the NLR with caution in clinical settings. Broad-spectrum antibiotics with good tissue penetration, which are essential medicines in the treatment of SAP, can affect WBC by reducing inflammation. Thus, the prognostic value of NLR in AP is uncertain if the effect of

antibiotic treatment is not taken into account.[32] For this reason, the neutrophil and lymphocyte counts used in this study were from the first complete blood cell count, conducted during the emergency visit. We confirmed that the enrolled patients were untreated at that time; consequently, our results are most likely applicable to untreated patients. Unlike for the NLR, the predictive ability of the LMR was only fair, and was not independently associated with overall survival in AP.

Serum albumin is a negative acute phase response reactant, and reflects the body's nutritional status. Albumin <25 g/L was an independent prognostic factor related to a poor prognosis of AP.[33] Variation of albumin within 24 h has been identified as a risk factor for a poor prognosis of critically ill patients in the early stages of SAP.[34] The PNI, which includes serum albumin and lymphocyte count, is an independent predictor of poor overall survival in patients with hepatocellular carcinoma.[35] To the best of our knowledge, few studies have reported on the application of PNI for predicting mortality of AP, but we found that it was an independent prognostic factor, and was suitable as a confirmed marker.

Numerous studies have reported RDW as a strong independent prognostic factor in various diseases and conditions, such as cardiovascular diseases, rheumatoid arthritis, cancer, and critical illnesses.[18 36-38] Our results are consistent with the study by Yao J *et al.*,[18] who reported a significant association between RDW and mortality of patients with AP. Additionally, we found that RDW was most suitable as a reliable excluding marker among the markers we assessed. The mechanisms underlying the association between RDW and mortality in AP remain unclear. The obvious metabolic abnormalities in non-survivors of AP, including inflammation, oxidative stress, poor nutritional status, and persistent organ failure, lead to deregulation of red blood cell homeostasis involving both impaired erythropoiesis and abnormal red

blood cell survival.[38] RDW reflects these impairments in homeostasis, but only further research can confirm this speculation.

The prognostic markers evaluated in this study are direct or combined markers of systemic inflammation that are based on routine, inexpensive, and readily available laboratory tests. To the best of our knowledge, this is the first study to compare the prognostic value of these markers for predicting mortality in patients with AP simultaneously. Additionally, suitable excluding and identifying markers were found.

Some potential limitations of the study should be noted. Although we have taken special care to avoid sources of bias and confounding, some potential bias may still exist in this retrospective, single-centre study. Information available at the beginning of the study may have affected the selection of the study participants, although the medical records and laboratory data were collected separately by two people. The reasons for incomplete medical records or why patients were lost to follow-up (n=23) are not known. These patients were excluded from the analyses. As a result, a larger, prospective study is needed to validate the results. Second, only the first set of admission blood results were investigated. As factors change with time, they should be surveyed in the future because of the rapid onset of inflammation. Third, the typical prediction models, such as APACHE II score, should be included in future research. Fourth, for better validity, +LR should be near 10, and −LR should be 0.2. Unfortunately, no marker examined had perfect +LR and −LR simultaneously. However, these markers are still valuable based on their acceptable AUC. Finally, we only described the association of each of the predictors with mortality of AP; the underlying mechanisms need to be investigated.

In conclusion, we found that age, NLR, PNI, CRP, and RDW were independently associated with overall survival of AP. NLR had the best overall performance, RDW

was suitable as a reliable marker to exclude death, and PNI was a good predictive marker for death. When applying these markers, any possible influence from therapy should be considered.

Abbreviations

AP, acute pancreatitis; AUC, area under the receiver operating characteristic curve; CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; LMR, lymphocyte-monocyte ratio; −LR, negative likelihood ratio; +LR, positive likelihood ratio; MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; NLR, neutrophil-lymphocyte ratio; OR, odd ratio; PNI, prognostic nutritional index; RDW, red cell distribution width; ROC, receiver operating characteristic curve; SAP, severe acute pancreatitis; WBC, white blood cell count.

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Contributors

R.G. and Y.L. designed the experiments. R.G. and Y.L. contributed to the data collection. Y.Z. conducted the data analysis. Y.L., R.G, and L.F. wrote the manuscript. All authors reviewed the manuscript.

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Competing interests

None declared.

Patient consent

Obtained.

Ethics approval

This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine, China

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

Open access

REFERENCES

1. Banks PA, Bollen TL, Dervenis C, *et al.* Classification of acute pancreatitis--2012: revision The diagnosis of acute pancreatitis requires two of the following three features: and definitions by international consensus. *Gut* 2013;62:102-11.
2. Maheshwari R, Subramanian RM. Severe Acute Pancreatitis and Necrotizing Pancreatitis. *Crit Care Clin* 2016;32:279-90.
3. Bugiantella W, Rondelli F, Boni M, *et al.* Necrotizing pancreatitis: A review of the interventions. *Int J Surg* 2016;28(Suppl 1):S163-71.
4. Petrov MS, Shanbhag S, Chakraborty M, *et al.* Organ failure and infection of pancreatic necrosis as determinants of mortality in patients with acute pancreatitis. *Gastroenterology* 2010;139:813-20.
5. Dellinger EP, Forsmark CE, Layer P, *et al.* Determinant-based classification of acute pancreatitis severity: an international multidisciplinary consultation. *Ann Surg* 2012;256:875-80.
6. Papachristou GI, Muddana V, Yadav D, *et al.* Comparison of BISAP, Ranson's, APACHE-II, and CTSI scores in predicting organ failure, complications, and mortality in acute pancreatitis. *Am J Gastroenterol* 2010;105:435-41; quiz 42.
7. Mounzer R, Langmead CJ, Wu BU, *et al.* Comparison of existing clinical scoring systems to predict persistent organ failure in patients with acute pancreatitis. *Gastroenterology* 2012;142:1476-82; quiz e15-6.
8. Bezmarevic M, Mirkovic D, Soldatovic I, *et al.* Correlation between procalcitonin and intra-abdominal pressure and their role in prediction of the severity of acute pancreatitis. *Pancreatology* 2012;12:337-43.
9. Yang CJ, Chen J, Phillips AR, *et al.* Predictors of severe and critical acute pancreatitis: a systematic review. *Dig Liver Dis* 2014;46:446-51.
10. Hunziker S, Celi LA, Lee J, *et al.* Red cell distribution width improves the simplified acute physiology score for risk prediction in unselected critically ill patients. *Crit Care* 2012;16:R89.
11. Kinoshita A, Onoda H, Imai N, *et al.* Comparison of the prognostic value of inflammation-based prognostic scores in patients with hepatocellular carcinoma. *Br J Cancer* 2012;107:988-93.
12. Teng JJ, Zhang J, Zhang TY, *et al.* Prognostic value of peripheral blood lymphocyte-to-monocyte ratio in patients with solid tumors: a meta-analysis. *Onco Targets Ther* 2016;9:37-47.
13. Chen L, Lou Y, Chen Y, *et al.* Prognostic value of the neutrophil-to-lymphocyte ratio in patients with acute-on-chronic liver failure. *Int J Clin Pract* 2014;68:1034-40.
14. Smith RA, Bosonnet L, Raraty M, *et al.* Preoperative platelet-lymphocyte ratio is an independent significant prognostic marker in resected pancreatic ductal adenocarcinoma. *Am J Surg* 2009;197:466-72.
15. Proctor MJ, Morrison DS, Talwar D, *et al.* An inflammation-based prognostic score (mGPS) predicts cancer survival independent of tumour site: a Glasgow Inflammation Outcome Study. *Br J Cancer* 2011;104:726-34.
16. Proctor MJ, Morrison DS, Talwar D, *et al.* A comparison of inflammation-based prognostic scores in patients with cancer. A Glasgow Inflammation Outcome Study. *Eur J Cancer* 2011;47:2633-41.
17. Zampieri FG, Ranzani OT, Sabatoski V, *et al.* An increase in mean platelet volume after admission is associated with higher mortality in critically ill patients. *Ann Intensive Care* 2014;4:20.
18. Yao J, Lv G. Association between red cell distribution width and acute pancreatitis: a cross-sectional study. *BMJ Open* 2014;4:e004721.

19. Suppiah A, Malde D, Arab T, *et al.* The prognostic value of the neutrophil-lymphocyte ratio (NLR) in acute pancreatitis: identification of an optimal NLR. J Gastrointest Surg 2013;17(4):675-81.
20. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;39:561-77.
21. Altman DG, Bland JM. Diagnostic tests 3: receiver operating characteristic plots. *BMJ* 1994;309:188.
22. Felderbauer P, Muller C, Bulut K, *et al.* Pathophysiology and treatment of acute pancreatitis: new therapeutic targets--a ray of hope? *Basic Clin Pharmacol Toxicol* 2005;97:342-50.
23. Xue J, Sharma V, Habtezion A. Immune cells and immune-based therapy in pancreatitis. *Immunol Res* 2014;58:378-86.
24. Zhu L, Chen G, Xia Q, *et al.* Use of band cell percentage as an early predictor of death and ICU admission in severe acute pancreatitis. *Hepatogastroenterology* 2010;57:1543-8.
25. Zahorec R. Ratio of neutrophil to lymphocyte counts--rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratisl Lek Listy* 2001;102:5-14.
26. de Jager CP, van Wijk PT, Mathoera RB, *et al.* Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. *Crit Care* 2010;14:R192.
27. Le Tulzo Y, Pangault C, Gacouin A, *et al.* Early circulating lymphocyte apoptosis in human septic shock is associated with poor outcome. *Shock* 2002;18:487-94.
28. Takeyama Y, Takas K, Ueda T, *et al.* Peripheral lymphocyte reduction in severe acute pancreatitis is caused by apoptotic cell death. *J Gastrointest Surg* 2000;4:379-87.
29. Pezzilli R, Billi P, Beltrandi E, *et al.* Circulating lymphocyte subsets in human acute pancreatitis. *Pancreas* 1995;11:95-100.
30. McKay C, Imrie CW, Baxter JN. Mononuclear phagocyte activation and acute pancreatitis. *Scand J Gastroenterol Suppl* 1996;219:32-6.
31. Saeki K, Kanai T, Nakano M, *et al.* CCL2-induced migration and SOCS3-mediated activation of macrophages are involved in cerulein-induced pancreatitis in mice. *Gastroenterology* 2012;142:1010-20 e9.
32. Binnetoglu E, Akbal E, Gunes F, *et al.* The prognostic value of neutrophil-lymphocyte ratio in acute pancreatitis is controversial. *J Gastrointest Surg* 2014;18:885.
33. Gonzalvez-Gasch A, de Casasola GG, Martin RB, *et al.* A simple prognostic score for risk assessment in patients with acute pancreatitis. *Eur J Intern Med* 2009;20:e43-8.
34. Chen Y, Zhang ZW, Wang B, *et al.* Relationship between early serum albumin variation and prognosis in patients with severe acute pancreatitis treated in ICU. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2013;44:237-41.
35. Pinato DJ, North BV, Sharma R. A novel, externally validated inflammation-based prognostic algorithm in hepatocellular carcinoma: the prognostic nutritional index (PNI). *Br J Cancer* 2012;106:1439-45.
36. Lappegard J, Ellingsen TS, Vik A, *et al.* Red cell distribution width and carotid atherosclerosis progression. The Tromso Study. *Thromb Haemost* 2015;113:649-54.

37. Bekler A, Tenekecioglu E, Erbag G, *et al.* Relationship between red cell distribution width and long-term mortality in patients with non-ST elevation acute coronary syndrome. *Anatol J Cardiol* 2015;15:634-9.
38. Salvagno GL, Sanchis-Gomar F, Picanza A, *et al.* Red blood cell distribution width: A simple parameter with multiple clinical applications. *Crit Rev Clin Lab Sci* 2015;52:86-105.

Table 1 Demographics and laboratory findings in patients with acute pancreatitis

Variables	1. MAP(n=197)	2. MSAP(n=76)	3. SAP(n=86)	p value		
				all groups	1 *vs.* 2	2 *vs.* 3
Age (years)	51.43 ± 16.00	48.47 ± 13.28	50.69 ± 14.61	0.352	0.446	1.000
Male (%)	108(54.8%)	41(53.9%)	49(57.0%)	0.919	0.896	0.699
Aetiology (1/2/3/4)%	52%/12%/11%/25%	51%/16%/13%/20%	47%/15%/14%/24%	0.875	0.664	0.892
WBC ($\times10^9$/L)	11.5 (3.1–32.0)	14.1 (4.5–36.8)	16.05 (5.9–38.4)	<0.001	<0.001	0.278
Lymphocyte ($\times10^9$/L)	1.1 (0.2–9.4)	1.0 (0.2–2.6)	0.80 (0.2–2.9)	<0.001	0.004	0.089
Platelet ($\times10^9$/L)	202 (21–502)	193 (58–548)	163 (27–540)	0.004	0.376	0.046
Albumin (g/L)	38.29 ± 5.07	34.38 ± 6.39	29.99 ± 5.35	<0.001	<0.001	<0.001
CRP (mg/L)	53.9 (0.7–386)	133.6 (3.2–436.5)	196.1 (27.1–426.7)	<0.001	<0.001	<0.001
Amylase (U/L)	398 (13–5191)	222 (27–3845)	581 (16–2377)	0.141	0.083	0.056
RDW (%)	12.8 (11.4–19.2)	13.0 (11.3–16.3)	13.7 (11.7–23.6)	<0.001	0.013	0.014
NLR	8.46 (1.33–55)	14.60 (1.73–60)	19.65 (3.57–53.67)	<0.001	<0.001	0.020
LMR	1.88 (0.28–13.33)	1.03 (0.29–5.33)	1.14 (0.22–6.32)	<0.001	<0.001	0.883
PNI	44.53 ± 6.63	39.36 ± 6.71	34.55 ± 6.02	<0.001	<0.001	<0.001
Mortality (%)	0(0%)	0(0%)	31(36.0%)	<0.001	–	<0.001

Continuous variables are presented as mean ± SD or median (range).

Aetiology (1/2/3/4)%, 1, 2, 3, and 4 represent gallstone, alcohol, hypertriglyceridemia, and other aetiologies, respectively.

1 *vs.* 2, MAP group *vs.* MSAP group; 2 *vs.* 3, MSAP group *vs.* SAP group.

MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis; WBC, white blood cell count; CRP, C-reactive protein; RDW, red cell distribution width; NLR, neutrophil-lymphocyte ratio; LMR, lymphocyte-monocyte ratio; PNI, prognostic nutritional index.

Table 2 Demographics and laboratory findings in survivors and non-survivors of acute pancreatitis

Variables	Survivors(n=328)	Non-survivors(n=31)	p value
Age (years)	49.84 ± 14.88	58.90 ± 15.60	0.001
Male (%)	179(54.6%)	19(61.3%)	0.472
Aetiology (1/2/3/4)%	50%/13%/12%/25%	58%/19%/10%/13%	0.346
WBC ($\times 10^9$/L)	12.85 (3.1–38.4)	18.5 (6.5–29.3)	0.001
Lymphocytes ($\times 10^9$/L)	1.08 (0.17–9.40)	0.60 (0.30–1.60)	<0.001
Platelet ($\times 10^9$/L)	197 (21–548)	159 (27–376)	0.001
Albumin (g/L)	35.95 ± 6.30	30.44 ± 5.54	<0.001
CRP (mg/L)	98.6 (0.7–436.5)	239.2 (27.1–398.2)	<0.001
Amylase (U/L)	343.5 (13–5191)	909 (16–2377)	0.010
RDW (%)	13 (11.3–19.2)	13.8 (12.6–23.6)	<0.001
NLR	10.47 (1.33–60.0)	25.0 (8.67–53.67)	<0.001
PNI	41.71 ± 7.50	34.00 ± 6.35	<0.001
LMR	1.51 (0.22–13.33)	1.13 (0.24–2.26)	<0.001

Continuous variables are presented as mean ± SD or median (range).

Aetiology (1/2/3/4)%, 1, 2, 3, and 4 represent gallstone, alcohol, hypertriglyceridemia, and other aetiologies, respectively.

WBC, white blood cell count; CRP, C-reactive protein; RDW, red cell distribution width; NLR, neutrophil-lymphocyte ratio; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio.

Table 3 Odds ratios of prognostic factors for predicting SAP in patients with AP

Factors	Model 1		Model 2		Model 3	
	Odds ratio (95%CI)	p value	Odds ratio (95%CI)	p value	Odds ratio (95%CI)	p value
NLR (>11.36 *vs.*≤11.36)	3.707(2.173-6.326)	<0.001	3.578 (2.082-6.149)	<0.001	1.463(0.711-3.010)	0.301
CRP (>110 *vs.*≤110mg/L)	9.867(5.116-19.030)	<0.001	12.609 (6.304-25.218)	<0.001	8.251(3.897-17.468)	<0.001
RDW (>13.0 *vs.*≤13.0%)	3.368(2.003-5.663)	<0.001	3.529 (2.076-5.998)	<0.001	2.533(1.365-4.702)	0.003
PNI (<41.1 *vs.* ≥41.1)	9.951(5.055-19.589)	<0.001	11.356 (5.665-22.766)	<0.001	7.753(3.400-17.680)	<0.001
LMR (<1.43 *vs.* ≥1.43)	2.564(1.539-4.271)	<0.001	2.552 (1.524-4.274)	<0.001	0.722(0.355-1.471)	0.370

Model 1: unadjusted model.

Model 2: adjusted for age, gender, and amylase.

Model 3: NLR was adjusted for age, gender, amylase, CRP, RDW, PNI, and LMR; CRP was adjusted for age, gender, amylase, NLR, RDW, PNI, and LMR; RDW was adjusted for age, gender, amylase, CRP, NLR, PNI, and LMR; PNI was adjusted for age, gender, amylase, NLR, CRP, RDW, and LMR; LMR was adjusted for age, gender, amylase, NLR, CRP, RDW, and PNI.

AP, acute pancreatitis; SAP, severe acute pancreatitis; NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; RDW, red cell distribution width; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio; CI, confidence interval.

Table 4 Discriminatory ability of inflammation-based markers for predicting mortality in AP patients

Index	AUC(95% CI)	p value[&]	Cut-off[#]	Sensitivity	Specificity	+LR	-LR
Training cohort							
NLR	0.804(0.738-0.859)	<0.001	16.64	82.4%	75.6%	3.38	0.23
CRP	0.774(0.706-0.833)	<0.001	162.2mg/L	76.5%	73.8%	2.92	0.32
RDW	0.769(0.700-0.828)	<0.001	13.0%	94.1%	54.3%	2.06	0.11
PNI	0.769(0.701- 0.828)	<0.001	33.1	58.8%	88.4%	5.08	0.47
LMR	0.744(0.674-0.806)	<0.001	1.40	82.4%	57.3%	1.93	0.31
Validation cohort							
NLR	0.851(0.790-0.900)	<0.001	16.64	85.7%	73.8%	3.27	0.19
CRP	0.753(0.683-0.815)	<0.001	162.2mg/L	71.4%	65.2%	2.06	0.44
RDW	0.708(0.635-0.773)	0.001	13.0%	85.7%	50.0%	1.71	0.29
PNI	0.791(0.724- 0.848)	<0.001	33.1	42.9%	88.4%	3.70	0.65
LMR	0.677(0.603-0.745)	0.015	1.40	78.6%	49.4%	1.55	0.43
Overall							
NLR	0.823(0.780-0.861)	<0.001	16.64	83.9%	74.4%	3.27	0.22
CRP	0.762(0.714-0.805)	<0.001	162.2mg/L	74.2%	69.8%	2.46	0.37
RDW	0.742(0.693-0.786)	<0.001	13.0%	90.3%	49.7%	1.80	0.19
PNI	0.781(0.734- 0.822)	<0.001	33.1	51.6%	88.4%	4.46	0.55
LMR	0.710(0.660-0.757)	<0.001	1.40	77.4%	54.0%	1.68	0.42

NLR, neutrophil-lymphocyte ratio; PNI, prognostic nutritional index; CRP, C-reactive protein; RDW, red cell distribution width; LMR, lymphocyte-monocyte ratio; AUC, area under the receiver operating characteristic curve; CI, confidence interval; +LR, positive likelihood ratio; −LR, negative likelihood ratio.

[&] The p-value is comparing the AUC with 0.5.

[#] The cut-off values were derived from a training cohort.

Table 5 Prognostic factors of overall survival in patients with acute pancreatitis by univariate and multivariate analyses

Factors	Univariate analysis		Multivariate analysis	
	Hazard ratio (95%CI)	p value	Hazard ratio (95%CI)	p value
Age (>63 *vs.* ≤63 years)	5.384(2.653-10.925)	<0.001	4.039(1.873-8.713)	<0.001
Gender (female *vs.* male)	0.767(0.372-1.579)	0.471		
Amylase (>618 *vs.*≤618U/L)	3.544(1.699-7.526)	0.001	2.173(0.965-4.891)	0.061
NLR(>16.64 *vs.* ≤16.64)	13.130(5.041-34.205)	<0.001	4.726(1.627-13.726)	0.004
CRP(>162.2 *vs.*≤162.2mg/L)	6.127(2.740-13.701)	<0.001	3.503(1.534-7.999)	0.003
RDW(>13.0 *vs.*≤13.0%)	4.929(2.022-12.017)	<0.001	3.139(1.277-7.714)	0.013
PNI(≤33.1 *vs.* >33.1)	6.912(3.414-13.991)	<0.001	2.641(1.248-5.590)	0.011
LMR(≤1.40 *vs.*>1.40)	3.797(1.636-8.813)	0.002	1.036(0.403-2.659)	0.942

NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; RDW, red cell distribution width; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio; CI, confidence interval.

Figure Legends

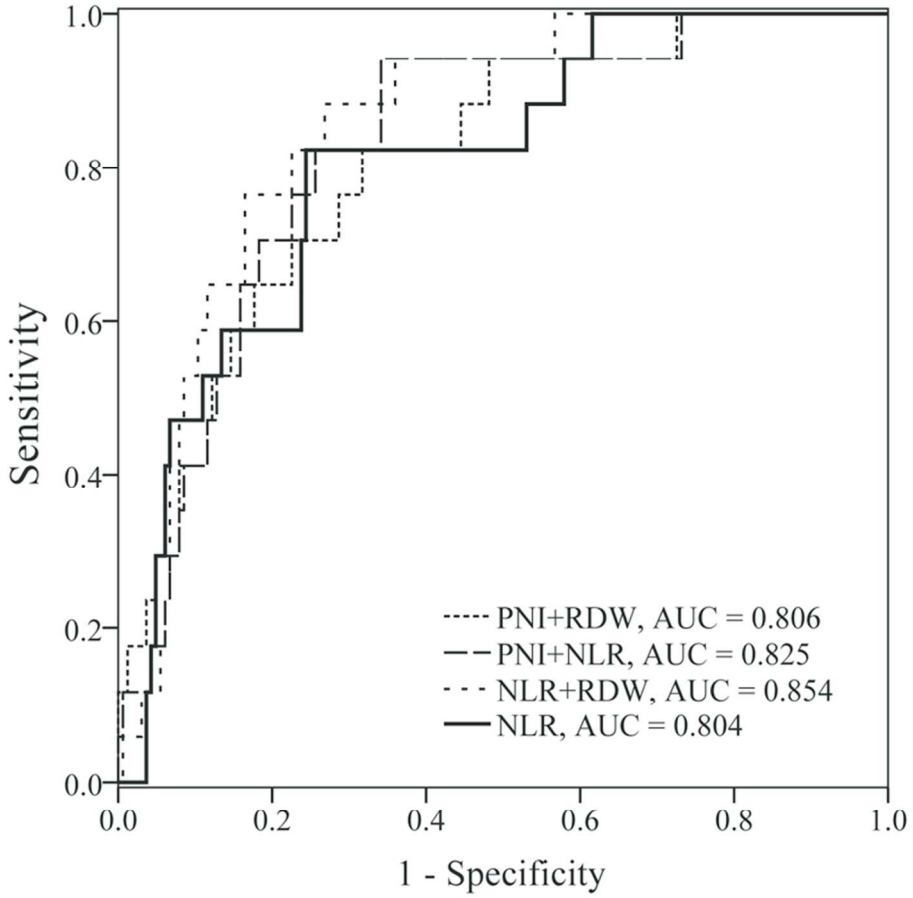
Fig. 1. ROC curves analysis for predicting mortality by NLR and combined markers in the estimation cohort.

ROC, receiver operating characteristic; NLR, neutrophil-lymphocyte ratio; RDW, red cell distribution width; PNI, prognostic nutritional index.

Fig. 2. Relationship between inflammation-based prognostic markers and overall survival in patients with acute pancreatitis

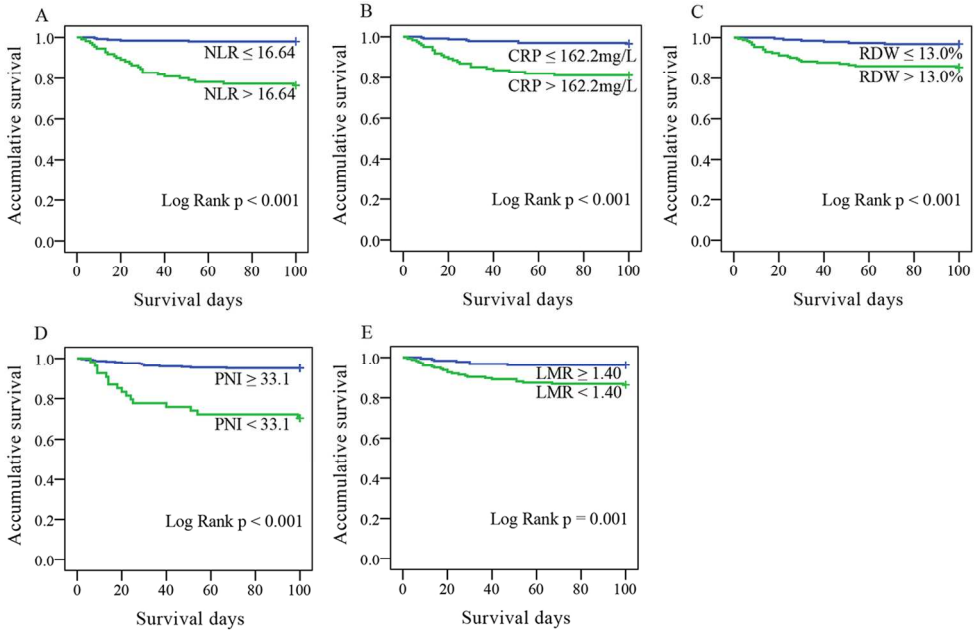
A, B, C, D, and E show the relationship between NLR, CRP, RDW, PNI, and LMR, and overall survival in patients with acute pancreatitis, respectively.

NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; RDW, red cell distribution width; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio.



ROC, receiver operating characteristic; AUC, area under the receiver operating characteristic curve; NLR, neutrophil-lymphocyte ratio; RDW, red cell distribution width; PNI, prognostic nutritional index.

75x72mm (300 x 300 DPI)



A, B, C, D, and E show the relationship between NLR, CRP, RDW, PNI, and LMR, and overall survival in patients with acute pancreatitis, respectively.
NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; RDW, red cell distribution width; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio.

170x115mm (300 x 300 DPI)

Supplementary Table S1 Demographics and laboratory findings in estimation and validation cohorts

Variable	All patients（n=359）	Training set(n=181)	Validation set(n =178)	p value
Age (years)	50.63±15.13	51.66 ±15.69	49.58 ±14.51	0.194
Male [N (%)]	198(55.2%)	96(53.0%)	102(57.3%)	0.417
Aetiology (1/2/3/4) %	54.3/9.7/12.0/24.0	55.2/12.7/12.2/19.9	53.4/6.7/11.8/28.1	0.118
WBC ($\times10^9$/L)	12.9(3.1-38.4)	13.3 (3.1–38.4)	12.9 (4.2–36.8)	0.942
Lymphocytes ($\times10^9$/L)	1.00 (0.17–9.40)	1.00 (0.20–9.40)	1.00 (0.17–4.80)	0.965
Platelet ($\times10^9$/L)	192 (21–548)	193 (27–502)	191.5 (21–548)	0.354
Albumin (g/L)	35.47 ±6.42	35.72 ±6.61	35.22 ±6.24	0.456
CRP (mg/L)	110 (0.7–436.5)	102.3 (0.8–436.5)	116.85 (0.7–419.4)	0.081
Amylase (U/L)	398 (13–5191)	501 (13–5191)	330 (16–4927)	0.238
RDW (%)	13.0(11.3-23.6)	13.1 (11.3–19.2)	13.0 (11.4–23.6)	0.421
NLR	11.36 (1.33–60.0)	11.50 (1.33–55.0)	11.18 (1.39–60.0)	0.786
PNI	41.05±7.72	41.22 ±7.74	40.87 ±7.71	0.670
LMR	1.43(0.22–13.33)	1.48 (0.24–13.33)	1.36 (0.22–10.00)	0.367
Mortality [N (%)]	31(8.6%)	17(9.4%)	14(7.9%)	0.607

Continuous variables are presented as mean±SD or median (range).

p value was training set versus validation set.

Aetiology (1/2/3/4)%, 1, 2, 3, and 4 represent gallstone, alcohol, hypertriglyceridemia, and other aetiologies, respectively.

WBC, white blood cell count; CRP, C-reactive protein; RDW, red cell distribution width; NLR, neutrophil-lymphocyte ratio; LMR, lymphocyte-monocyte ratio; PNI, prognostic nutritional index.

TRIPOD Checklist: Prediction Model Development

Section/Topic	Item	Checklist Item	Page
Title and abstract			
Title	1	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	3,4
Introduction			
Background and objectives	3a	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	5,6
	3b	Specify the objectives, including whether the study describes the development or validation of the model or both.	6
Methods			
Source of data	4a	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	6
	4b	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	6,7
Participants	5a	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	6,7
	5b	Describe eligibility criteria for participants.	6, 7
	5c	Give details of treatments received, if relevant.	Not relevant
Outcome	6a	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	7
	6b	Report any actions to blind assessment of the outcome to be predicted.	7
Predictors	7a	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	7,8
	7b	Report any actions to blind assessment of predictors for the outcome and other predictors.	7,8
Sample size	8	Explain how the study size was arrived at.	9
Missing data	9	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	9
,8Statistical analysis methods	10a	Describe how predictors were handled in the analyses.	8,9
	10b	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	8,9
	10d	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	8,9
Risk groups	11	Provide details on how risk groups were created, if done.	8,9
Results			
Participants	13a	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	9
	13b	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	9,10 Table1 and 2
Model development	14a	Specify the number of participants and outcome events in each analysis.	9 Table1 and 2
	14b	If done, report the unadjusted association between each candidate predictor and outcome.	10,11, 12, Table 3 and 5
Model specification	15a	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	10,11, Table 3 and 5
	15b	Explain how to the use the prediction model.	10,11
Model performance	16	Report performance measures (with CIs) for the prediction model.	10,11, Table3 and 5
Discussion			
Limitations	18	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	15
Interpretation	19b	Give an overall interpretation of the results, considering objectives, limitations, and results from similar studies, and other relevant evidence.	12-15
Implications	20	Discuss the potential clinical use of the model and implications for future research.	12,13, 15

TRIPOD Checklist: Prediction Model Development

Other information			
Supplementary information	21	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	Yes
Funding	22	Give the source of funding and the role of the funders for the present study.	16

We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

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Comparison of the prognostic values of inflammation markers in patients with acute pancreatitis: a retrospective cohort study

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Comparison of the prognostic values of inflammation markers in patients with acute pancreatitis: a retrospective cohort study

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ABSTRACT

Objectives: Inflammation-based prognostic markers (neutrophil-lymphocyte ratio [NLR], prognostic nutritional index [PNI], red cell distribution width [RDW], and lymphocyte-monocyte ratio [LMR]) are associated with overall survival in some diseases. This study assessed their prognostic value in mortality and severity in acute pancreatitis (AP).

Design: A retrospective cohort study.

Setting: Patients with AP were recruited from the emergency department at our hospital.

Participants: A total of 359 AP patients (31 non-survivors) were enrolled.

Primary and secondary outcome measures: Mortality and severity of AP were the primary and secondary outcome measures, respectively. Biochemistry and haematology results of the first test after admission were collected. Independent relationships between severe AP (SAP) and markers were assessed using multivariate logistic regression models. Mortality prediction ability was evaluated using receiver operating characteristic (ROC) curves. Overall survival was evaluated using the Kaplan–Meier method, with differences compared using the log-rank test. Independent relationships between mortality and each predictor were estimated using Cox proportional hazard models.

Results: Compared with survivors of AP, non-survivors had higher RDW ($p<0.001$), higher NLR ($p<0.001$), lower LMR ($p<0.001$), and lower PNI ($p<0.001$) at baseline. C-reactive protein (CRP) [odd ratio (OR)=8.251, $p<0.001$], RDW (OR=2.533, $p=0.003$), and PNI (OR=7.753, $p<0.001$) were independently associated with the occurrence of SAP. For predicting mortality, NLR had the largest area under the ROC curve (0.804, $p<0.001$), with a 16.64 cut-off value, 82.4% sensitivity, and 75.0%

specificity. RDW was a reliable marker for excluding death owing to its lowest negative likelihood ratio (0.11). NLR (hazard ratio (HR) =4.726, p=0.004), CRP (HR=3.503, p=0.003), RDW (HR=3.139, p=0.013), and PNI (HR=2.641, p=0.011) were independently associated with mortality of AP.

Conclusions: NLR was the most powerful marker of overall survival in this patient series.

Strengths and limitations of this study

- Compared with survivors of acute pancreatitis (AP), non-survivors had higher red cell distribution width (RDW) and neutrophil-lymphocyte ratio (NLR), and lower lymphocyte-monocyte ratio (LMR) and prognostic nutritional index (PNI) at baseline.
- NLR exhibited a higher area under the receiver operating characteristic curve for the prediction of mortality compared with other markers.
- RDW was suitable as a reliable marker to exclude death.
- NLR, PNI, C-reactive protein, and RDW were independently associated with overall survival of AP.
- This was a retrospective cohort analysis.

INTRODUCTION

Acute pancreatitis (AP) is rapid-onset inflammation of the pancreas that varies in severity from a self-limiting mild illness to rapidly progressive multiple organ failure. Statistics suggest that 10–20% of patients with AP develop severe acute pancreatitis (SAP),[1] which usually has an unfavourable disease progression and is associated with a poor prognosis.[2 3] Prediction of disease severity can guide the management of patients with AP and improve the outcome. Organ failure and infected pancreatic necrosis are common causes of mortality in such patients,[4] and a new international multidisciplinary classification of SAP incorporates both events as determinants of severity.[5] The predictive values of various markers, such as Acute Physiology and Chronic Health Evaluation II (APACHE II) and Bedside Index of Severity in Acute Pancreatitis scores, C-reactive protein (CRP), and procalcitonin, have been previously assessed.[6-8] A systematic review concluded it was justifiable to use blood urea nitrogen after 48 h of hospital admission for predicting persistent organ failure.[9] In clinical studies, most studies have focused on disease severity, and only a few have directly investigated the relationship between predictors and mortality of AP. Furthermore, no reliable predictor of persistent organ failure within 48 h of admission has been identified.[9]

There is increasing evidence that the presence of a systemic inflammatory response is associated with poor survival in patients with various aetiologies, including malignancy.[10-17] Many direct or combined markers of systemic inflammation are based on routine, inexpensive, and readily available laboratory tests. Red cell distribution width (RDW),[10] neutrophil-lymphocyte ratio (NLR), prognostic nutritional index (PNI),[11] and lymphocyte-monocyte ratio (LMR)[12] have been used to

predict the prognosis of disease. RDW was found to be an independent marker of short- and long-term prognosis in intensive care units.[10] NLR at admission served as an independent predictor of 3-month mortality rates in acute-on-chronic liver failure patients.[13] Increased pre-treatment LMR was associated with a significantly more favourable prognosis in patients with solid tumours.[12] Despite this evidence, very few studies have focused on the direct relationship between inflammation-based prognostic markers and mortality of AP. A cross-sectional study found a significant association between RDW and mortality in patients with AP.[18] Another study investigated the prognostic value of NLR in AP and determined an optimal ratio for prediction of severity.[19]

To the best of our knowledge, the current study is the first to simultaneously compare the prognostic value of these inflammation-based prognostic markers (NLR, PNI, CRP, RDW, and LMR) of mortality in patients with AP.

MATERIALS AND METHODS

Participants

This retrospective cohort analysis consecutively enrolled a series of patients with AP who were admitted to the emergency department at our hospital between 1 July 2013 and 18 August 2015. A diagnosis of AP required two of three features: (1) prolonged abdominal pain characteristic of AP, (2) threefold elevation of serum amylase and/or lipase levels above the normal range, and (3) characteristic findings of AP on abdominal ultrasonography and/or computed tomography scan.[1] Mild acute pancreatitis (MAP) was defined as an absence of organ failure and an absence of local or systemic complications.[1] Moderately severe acute pancreatitis (MSAP) was defined as no evidence of persistent organ failure, but the presence of local or systemic

complications and/or organ failure that resolved within 48 h. SAP was defined as persistent organ failure (>48 h).[1] Patients with recurrent pancreatitis were enrolled only at first admission. Patients with traumatic pancreatitis, autoimmune pancreatitis, diabetes mellitus, tumour, or liver failure were excluded.

The prognostic information we focused on included overall survival and the severity of the disease. All enrolled patients were followed for 100 days or until death. All clinical data were retrieved from medical records. For AP patients, 100 days of prognostic information (survival or non-survival) was obtained by checking medical records or by contacting the patients' family members.

Ethics statement

Each participant provided written informed consent after being provided with an explanation of the study by phone, letter, or e-mail. The Ethics Committee of The First Affiliated Hospital of Zhejiang University College of Medicine approved the consent procedure and experiment periods. The study was conducted in accordance with the ethical principles contained within the Declaration of Helsinki.

Demographic information and laboratory analysis

Demographic information, including age, sex, aetiology, and complication, was collected from medical records. Pre-treatment laboratory data, including complete blood counts, serum CRP, albumin, and amylase were obtained during the emergency visit. An XE-2100 haematology autoanalyzer (Sysmex Corp., Kobe, Japan), a Hitachi 7600 chemistry analyser (Hitachi High-Technologies, Tokyo, Japan), and Roche reagents (Roche Diagnostics, Indianapolis, IN, USA) were used in the laboratory.

We assessed the prognostic value of general inflammation-based prognostic markers (NLR, CRP, RDW, PNI, and LMR) for predicting the mortality of AP. Additionally, their ability to predict the severity of AP (SAP or not SAP) was assessed. NLR and LMR were ratios of two types of blood cell. PNI = albumin (g/L) + 5 × total lymphocyte count (10^9/L).

Statistical analysis

Variables are expressed as mean ± SD or median (range) and categorical data as percentages, as appropriate. Differences between the two groups were assessed using an independent sample *t*-test, Mann–Whitney U test, or χ^2 test, as appropriate. Multiple comparisons were performed by one-way analysis of variance or Kruskal–Wallis H tests, as appropriate. The Bonferroni method was used to adjust for multiple comparisons. Multivariate logistic regression analyses were used to assess whether the inflammation markers were independent factors for predicting SAP in patients with AP by unadjusted and adjusted models successively. AP patients were randomly divided into estimation and validation cohorts by random number generators. The accuracy of each marker to predict mortality was assessed using receiver operating characteristic curves (ROC). The sensitivity, specificity, positive likelihood ratio (+LR), and negative likelihood ratio (–LR) were calculated. +LR represents the ratio of the true positive rate to the false positive rate. –LR represents the ratio of the false negative rate to the true negative rate. These two parameters, which are not influenced by prevalence rate, are stable and objective for assessing diagnostic value. Combination models were developed using binary logistic regression analyses. Overall survival curves were calculated using the Kaplan–Meier method, and differences in survival rates were compared using the log-rank test.

Univariate and multivariate Cox proportional hazard models were used to estimate the significance and independence of the relationship of each marker and mortality. The variables with a p-value <0.1 in univariate analysis were included in a multivariate Cox proportional hazard regression model. A p-value <0.05 was considered statistically significant. Statistical analyses were performed with SPSS ver. 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

A total of 359 AP patients (197 MAP, 76 MSAP, and 86 SAP) were enrolled in the study. The predefined probability of type I error was 0.05 (α=0.05), and the sample size was large enough to guarantee 0.90 of test power (β=0.1). Forty-five patients were excluded from the analysis, including those with traumatic pancreatitis (n=1), autoimmune pancreatitis (n=5), diabetes mellitus (n=7), tumour (n=7), liver failure (n=2), or incomplete medical records or who were lost to follow-up (n=23). Tables 1 and 2 show the baseline characteristics of the patients. There were no significant differences in age (p=0.352), aetiology (p=0.875), or sex (p=0.919) among the three groups (MAP, MSAP, and SAP). As the illness worsened, CRP, RDW, and NLR gradually increased, but PNI decreased (all p<0.05; Table 1). LMR decreased significantly (p<0.001) in MSAP compared with MAP patients, but there was no significant difference between MSAP and SAP patients (p=0.883).

Compared with survivors of AP, non-survivors were older (p=0.001) and had higher CRP (p<0.001), amylase (p=0.010), RDW (p<0.001), and NLR (p<0.001). Conversely, lymphocyte count (p<0.001), platelets (p=0.001), albumin (p<0.001),

LMR (p<0.001), and PNI (p<0.001) were lower in non-survivors than in survivors (Table 2).

The relationship between markers and severity of AP

The multivariate logistic regression models revealed that high CRP (>110 *vs.* ≤110 mg/L, adjusted odd ratio (OR)=8.251, 95%CI: 3.897–17.468, p<0.001), RDW (>13.0 *vs.* ≤13.0%, adjusted OR= 2.533, 95%CI: 1.365–4.702, p=0.003), and low PNI (<41.1 *vs.* ≥41.1, adjusted OR=7.753, 95%CI: 3.400–17.680, p<0.001) were independent factors for predicting SAP in patients with AP (Table 3).

The markers' power for predicting 100 days mortality

The enrolled 359 patients with AP were randomly grouped into two cohorts: the estimation cohort (n=181) and the validation cohort (n=178). No significant difference was observed between the estimation and the validation cohorts in all characteristics (Supplementary Table S1). ROC curves of the estimation cohort were constructed to evaluate the ability of each marker to predict 100 days mortality in AP. Table 4 shows the area under the receiver operating characteristic curves (AUC) and optimal cut-off values. The ability of NLR to predict mortality (AUC=0.804, p<0.001) was good; those of PNI (AUC=0.769, p<0.001), CRP (AUC=0.774, p<0.001), RDW (AUC=0.769, p<0.001), and LMR (AUC=0.744, p<0.001) were fair. The NLR had the largest AUC, and RDW and PNI had the highest sensitivity and specificity, respectively. Therefore, these three markers were selected for combination. The AUC for NLR+PNI, NLR+RDW, and PNI+RDW were 0.825 (95%CI: 0.761–0.877); 0.854 (95%CI: 0.794–0.902), and 0.806 (95%CI: 0.741–0.861), respectively (Fig. 1). There

were no significant differences in AUC for combined index and NLR (p=0.699; p=0.167; p=0.975, respectively).

For NLR, the optimal cut-off value for mortality prediction was 16.64, with a sensitivity of 82.4% and specificity of 75.6%. RDW had the highest sensitivity (94.1%) and lowest negative likelihood ratio (0.11), so it was a reliable predictive index for excluding mortality in AP patients. PNI had the highest specificity (88.4%) and positive likelihood ratio (5.08), so it was most suitable for use as a confirmed index among the indexes assessed.

In the validation cohort, AUC for NLR, CRP, RDW, PNI, and LMR were 0.851 (95%CI: 0.790–0.900), 0.753 (95%CI: 0.683–0.815), 0.708 (95%CI: 0.635–0.773), 0.791 (95%CI: 0.724–0.848), and 0.677 (95%CI: 0.603–0.745), respectively. There were no significant differences in AUC for NLR, CRP, RDW, PNI, and LMR between the estimation and validation cohorts (p=0.477, p=0.809, p=0.437, p=0.782, and p=0.455, respectively).

Survival analysis

AP patients were stratified into groups by cut-off values. Kaplan−Meier survival curves demonstrate the relationships between inflammation-based prognostic markers and overall survival of patients with AP (Fig. 2A−E). Elevated NLR (p<0.001), CRP (p<0.001), and RDW (p<0.001) were associated with increased probability of death. Conversely, decreased PNI (p<0.001) and LMR (p=0.001) were associated with decreased overall survival.

According to the cut-off values for the factors, low NLR (≤16.64), low CRP (≤162.2 mg/L), low RDW (≤13.0%), high PNI (>33.1), and high LMR (>1.40) were selected as references. Univariate analysis and Cox regression revealed that age

(p<0.001), amylase (p=0.001), NLR (p<0.001), PNI (p<0.001), CRP (p<0.001), RDW (p<0.001), and LMR (p=0.002) were associated with AP mortality (Table 5). These factors were evaluated using multivariate Cox regression. Age (HR=4.039, 95%CI: 1.873–8.713, p<0.001), NLR (HR=4.726, 95%CI: 1.627–13.726, p=0.004), CRP (HR=3.503, 95%CI: 1.534–7.999, p=0.003), RDW (HR=3.139, 95%CI: 1.277–7.714, p=0.013), and PNI (HR=2.641, 95%CI: 1.248–5.590, p=0.011) were independently associated with mortality of AP (Table 5).

DISCUSSION

AP is an inflammatory disease, with mortality arising mainly from organ failure or infected pancreatic necrosis.[4] Our study estimated the prognostic value of various inflammation-based prognostic markers for predicting mortality of AP. According to classifications of AUC,[20 21] the ability of the NLR to predict mortality was good, while those of PNI, CRP, RDW, and LMR were fair. Cox regression analysis revealed that age, NLR, PNI, CRP, and RDW were independently associated with mortality of AP. Additionally, PNI, CRP, and RDW were independently associated with the occurrence of SAP in AP patients.

NLR, CRP, RDW, and PNI are inexpensive, convenient, and readily available in clinical settings. From examination of AUC, NLR had the best performance. With a NLR >16.64 at the time of admission, the risk of dying increased 3.726-fold compared with NLR ≤16.64. RDW was the most reliable marker for excluding death in AP patients, owing to its lowest negative likelihood ratio (0.11). PNI had the highest specificity (88.4%) and positive likelihood ratio (5.08), so it was most suitable to be a confirmed index among the indexes assessed. However, fluctuations in the NLR and CRP can be influenced by the use of antibiotics; therefore, NLR and CRP

are not suitable for patients undergoing intensive use of antibiotics. Similarly, blood transfusion and parenteral nutrition may affect RDW and PNI, respectively, so the predictive value of RDW and PNI in these patients was discounted.

In AP, inflammation propagates and promotes tissue destruction via activation of a cascade of inflammatory cytokines, proteolytic enzymes, and oxygen free radicals.[19 22] Neutrophils, lymphocytes, and monocytes are the three main types of white blood cells (WBC). Neutrophils play a key role in the development of local tissue destruction and systemic complications of SAP.[23] Depletion of neutrophils has been associated with an improved prognosis of AP.[23] The percentage of immature neutrophilic granulocytes might be used clinically as a simple early predictor of an adverse outcome in SAP.[24] Additionally, recent studies revealed that the extent of lymphopenia was associated with disease severity.[25-27] Lymphopenia has been reported to have independent prognostic value for some diseases,[19 26-29] including AP. Takeyama *et al.* found that impairment of cellular immunity caused by peripheral lymphocyte apoptosis was linked to the subsequent development of infectious complications in AP.[28] Monocytes produce various cytokines and inflammatory mediators that further amplify inflammatory cell recruitment into the pancreas as well as distant organs such as the lungs.[30] Similar to neutrophils, a protective effect was also found by depleting macrophages in a mouse model of AP.[31] Theoretically, NLR and LMR, which combine two opposing parameters, should be more accurate than either parameter alone. We found that the NLR had the greatest prognostic value of all the factors we evaluated. It is, however, important to apply the NLR with caution in clinical settings. Broad-spectrum antibiotics with good tissue penetration, which are essential medicines in the treatment of SAP, can affect WBC by reducing inflammation. Thus, the prognostic value of NLR in AP is uncertain if the effect of

antibiotic treatment is not taken into account.[32] For this reason, the neutrophil and lymphocyte counts used in this study were from the first complete blood cell count, conducted during the emergency visit. We confirmed that the enrolled patients were untreated at that time; consequently, our results are most likely applicable to untreated patients. Unlike for the NLR, the predictive ability of the LMR was only fair, and was not independently associated with overall survival in AP.

Serum albumin is a negative acute phase response reactant, and reflects the body's nutritional status. Albumin <25 g/L was an independent prognostic factor related to a poor prognosis of AP.[33] Variation of albumin within 24 h has been identified as a risk factor for a poor prognosis of critically ill patients in the early stages of SAP.[34] The PNI, which includes serum albumin and lymphocyte count, is an independent predictor of poor overall survival in patients with hepatocellular carcinoma.[35] To the best of our knowledge, few studies have reported on the application of PNI for predicting mortality of AP, but we found that it was an independent prognostic factor, and was suitable as a confirmed marker.

Numerous studies have reported RDW as a strong independent prognostic factor in various diseases and conditions, such as cardiovascular diseases, rheumatoid arthritis, cancer, and critical illnesses.[18 36-38] Our results are consistent with the study by Yao J *et al.*,[18] who reported a significant association between RDW and mortality of patients with AP. Additionally, we found that RDW was most suitable as a reliable excluding marker among the markers we assessed. The mechanisms underlying the association between RDW and mortality in AP remain unclear. The obvious metabolic abnormalities in non-survivors of AP, including inflammation, oxidative stress, poor nutritional status, and persistent organ failure, lead to deregulation of red blood cell homeostasis involving both impaired erythropoiesis and abnormal red

blood cell survival.[38] RDW reflects these impairments in homeostasis, but only further research can confirm this speculation.

The prognostic markers evaluated in this study are direct or combined markers of systemic inflammation that are based on routine, inexpensive, and readily available laboratory tests. To the best of our knowledge, this is the first study to compare the prognostic value of these markers for predicting mortality in patients with AP simultaneously. Additionally, suitable excluding and identifying markers were found.

Some potential limitations of the study should be noted. Although we have taken special care to avoid sources of bias and confounding, some potential bias may still exist in this retrospective, single-centre study. Information available at the beginning of the study may have affected the selection of the study participants, although the medical records and laboratory data were collected separately by two people. The reasons for incomplete medical records or why patients were lost to follow-up (n=23) are not known. These patients were excluded from the analyses. As a result, a larger, prospective study is needed to validate the results. Second, only the first set of admission blood results were investigated. As factors change with time, they should be surveyed in the future because of the rapid onset of inflammation. Third, the typical prediction models, such as APACHE II score, should be included in future research. Fourth, for better validity, +LR should be near 10, and −LR should be 0.2. Unfortunately, no marker examined had perfect +LR and −LR simultaneously. However, these markers are still valuable based on their acceptable AUC. Finally, we only described the association of each of the predictors with mortality of AP; the underlying mechanisms need to be investigated.

In conclusion, we found that age, NLR, PNI, CRP, and RDW were independently associated with overall survival of AP. NLR had the best overall performance, RDW

was suitable as a reliable marker to exclude death, and PNI was a good predictive marker for death. When applying these markers, any possible influence from therapy should be considered.

Abbreviations

AP, acute pancreatitis; AUC, area under the receiver operating characteristic curve; CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; LMR, lymphocyte-monocyte ratio; −LR, negative likelihood ratio; +LR, positive likelihood ratio; MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; NLR, neutrophil-lymphocyte ratio; OR, odd ratio; PNI, prognostic nutritional index; RDW, red cell distribution width; ROC, receiver operating characteristic curve; SAP, severe acute pancreatitis; WBC, white blood cell count.

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Contributors

R.G. and Y.L. designed the experiments. R.G. and Y.L. contributed to the data collection. Y.Z. conducted the data analysis. Y.L., R.G, and L.F. wrote the manuscript. All authors reviewed the manuscript.

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Competing interests

None declared.

Patient consent

Obtained.

Ethics approval

This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine, China

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

Open access

REFERENCES

1. Banks PA, Bollen TL, Dervenis C, *et al.* Classification of acute pancreatitis--2012: revision The diagnosis of acute pancreatitis requires two of the following three features: and definitions by international consensus. *Gut* 2013;62:102-11.
2. Maheshwari R, Subramanian RM. Severe Acute Pancreatitis and Necrotizing Pancreatitis. *Crit Care Clin* 2016;32:279-90.
3. Bugiantella W, Rondelli F, Boni M, *et al.* Necrotizing pancreatitis: A review of the interventions. *Int J Surg* 2016;28(Suppl 1):S163-71.
4. Petrov MS, Shanbhag S, Chakraborty M, *et al.* Organ failure and infection of pancreatic necrosis as determinants of mortality in patients with acute pancreatitis. *Gastroenterology* 2010;139:813-20.
5. Dellinger EP, Forsmark CE, Layer P, *et al.* Determinant-based classification of acute pancreatitis severity: an international multidisciplinary consultation. *Ann Surg* 2012;256:875-80.
6. Papachristou GI, Muddana V, Yadav D, *et al.* Comparison of BISAP, Ranson's, APACHE-II, and CTSI scores in predicting organ failure, complications, and mortality in acute pancreatitis. *Am J Gastroenterol* 2010;105:435-41; quiz 42.
7. Mounzer R, Langmead CJ, Wu BU, *et al.* Comparison of existing clinical scoring systems to predict persistent organ failure in patients with acute pancreatitis. *Gastroenterology* 2012;142:1476-82; quiz e15-6.
8. Bezmarevic M, Mirkovic D, Soldatovic I, *et al.* Correlation between procalcitonin and intra-abdominal pressure and their role in prediction of the severity of acute pancreatitis. *Pancreatology* 2012;12:337-43.
9. Yang CJ, Chen J, Phillips AR, *et al.* Predictors of severe and critical acute pancreatitis: a systematic review. *Dig Liver Dis* 2014;46:446-51.
10. Hunziker S, Celi LA, Lee J, *et al.* Red cell distribution width improves the simplified acute physiology score for risk prediction in unselected critically ill patients. *Crit Care* 2012;16:R89.
11. Kinoshita A, Onoda H, Imai N, *et al.* Comparison of the prognostic value of inflammation-based prognostic scores in patients with hepatocellular carcinoma. *Br J Cancer* 2012;107:988-93.
12. Teng JJ, Zhang J, Zhang TY, *et al.* Prognostic value of peripheral blood lymphocyte-to-monocyte ratio in patients with solid tumors: a meta-analysis. *Onco Targets Ther* 2016;9:37-47.
13. Chen L, Lou Y, Chen Y, *et al.* Prognostic value of the neutrophil-to-lymphocyte ratio in patients with acute-on-chronic liver failure. *Int J Clin Pract* 2014;68:1034-40.
14. Smith RA, Bosonnet L, Raraty M, *et al.* Preoperative platelet-lymphocyte ratio is an independent significant prognostic marker in resected pancreatic ductal adenocarcinoma. *Am J Surg* 2009;197:466-72.
15. Proctor MJ, Morrison DS, Talwar D, *et al.* An inflammation-based prognostic score (mGPS) predicts cancer survival independent of tumour site: a Glasgow Inflammation Outcome Study. *Br J Cancer* 2011;104:726-34.
16. Proctor MJ, Morrison DS, Talwar D, *et al.* A comparison of inflammation-based prognostic scores in patients with cancer. A Glasgow Inflammation Outcome Study. *Eur J Cancer* 2011;47:2633-41.
17. Zampieri FG, Ranzani OT, Sabatoski V, *et al.* An increase in mean platelet volume after admission is associated with higher mortality in critically ill patients. *Ann Intensive Care* 2014;4:20.
18. Yao J, Lv G. Association between red cell distribution width and acute pancreatitis: a cross-sectional study. *BMJ Open* 2014;4:e004721.

19. Suppiah A, Malde D, Arab T, *et al.* The prognostic value of the neutrophil-lymphocyte ratio (NLR) in acute pancreatitis: identification of an optimal NLR. J Gastrointest Surg 2013;17(4):675-81.
20. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;39:561-77.
21. Altman DG, Bland JM. Diagnostic tests 3: receiver operating characteristic plots. *BMJ* 1994;309:188.
22. Felderbauer P, Muller C, Bulut K, *et al.* Pathophysiology and treatment of acute pancreatitis: new therapeutic targets--a ray of hope? *Basic Clin Pharmacol Toxicol* 2005;97:342-50.
23. Xue J, Sharma V, Habtezion A. Immune cells and immune-based therapy in pancreatitis. *Immunol Res* 2014;58:378-86.
24. Zhu L, Chen G, Xia Q, *et al.* Use of band cell percentage as an early predictor of death and ICU admission in severe acute pancreatitis. *Hepatogastroenterology* 2010;57:1543-8.
25. Zahorec R. Ratio of neutrophil to lymphocyte counts--rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratisl Lek Listy* 2001;102:5-14.
26. de Jager CP, van Wijk PT, Mathoera RB, *et al.* Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. *Crit Care* 2010;14:R192.
27. Le Tulzo Y, Pangault C, Gacouin A, *et al.* Early circulating lymphocyte apoptosis in human septic shock is associated with poor outcome. *Shock* 2002;18:487-94.
28. Takeyama Y, Takas K, Ueda T, *et al.* Peripheral lymphocyte reduction in severe acute pancreatitis is caused by apoptotic cell death. *J Gastrointest Surg* 2000;4:379-87.
29. Pezzilli R, Billi P, Beltrandi E, *et al.* Circulating lymphocyte subsets in human acute pancreatitis. *Pancreas* 1995;11:95-100.
30. McKay C, Imrie CW, Baxter JN. Mononuclear phagocyte activation and acute pancreatitis. *Scand J Gastroenterol Suppl* 1996;219:32-6.
31. Saeki K, Kanai T, Nakano M, *et al.* CCL2-induced migration and SOCS3-mediated activation of macrophages are involved in cerulein-induced pancreatitis in mice. *Gastroenterology* 2012;142:1010-20 e9.
32. Binnetoglu E, Akbal E, Gunes F, *et al.* The prognostic value of neutrophil-lymphocyte ratio in acute pancreatitis is controversial. *J Gastrointest Surg* 2014;18:885.
33. Gonzalvez-Gasch A, de Casasola GG, Martin RB, *et al.* A simple prognostic score for risk assessment in patients with acute pancreatitis. *Eur J Intern Med* 2009;20:e43-8.
34. Chen Y, Zhang ZW, Wang B, *et al.* Relationship between early serum albumin variation and prognosis in patients with severe acute pancreatitis treated in ICU. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2013;44:237-41.
35. Pinato DJ, North BV, Sharma R. A novel, externally validated inflammation-based prognostic algorithm in hepatocellular carcinoma: the prognostic nutritional index (PNI). *Br J Cancer* 2012;106:1439-45.
36. Lappegard J, Ellingsen TS, Vik A, *et al.* Red cell distribution width and carotid atherosclerosis progression. The Tromso Study. *Thromb Haemost* 2015;113:649-54.

37. Bekler A, Tenekecioglu E, Erbag G, *et al.* Relationship between red cell distribution width and long-term mortality in patients with non-ST elevation acute coronary syndrome. *Anatol J Cardiol* 2015;15:634-9.
38. Salvagno GL, Sanchis-Gomar F, Picanza A, *et al.* Red blood cell distribution width: A simple parameter with multiple clinical applications. *Crit Rev Clin Lab Sci* 2015;52:86-105.

Table 1 Demographics and laboratory findings in patients with acute pancreatitis

Variables	1. MAP(n=197)	2. MSAP(n=76)	3. SAP(n=86)	p value		
				all groups	1 *vs.* 2	2 *vs.* 3
Age (years)	51.43 ± 16.00	48.47 ± 13.28	50.69 ± 14.61	0.352	0.446	1.000
Male (%)	108(54.8%)	41(53.9%)	49(57.0%)	0.919	0.896	0.699
Aetiology (1/2/3/4)%	52%/12%/11%/25%	51%/16%/13%/20%	47%/15%/14%/24%	0.875	0.664	0.892
WBC ($\times10^9$/L)	11.5 (3.1–32.0)	14.1 (4.5–36.8)	16.05 (5.9–38.4)	<0.001	<0.001	0.278
Lymphocyte ($\times10^9$/L)	1.1 (0.2–9.4)	1.0 (0.2–2.6)	0.80 (0.2–2.9)	<0.001	0.004	0.089
Platelet ($\times10^9$/L)	202 (21–502)	193 (58–548)	163 (27–540)	0.004	0.376	0.046
Albumin (g/L)	38.29 ± 5.07	34.38 ± 6.39	29.99 ± 5.35	<0.001	<0.001	<0.001
CRP (mg/L)	53.9 (0.7–386)	133.6 (3.2–436.5)	196.1 (27.1–426.7)	<0.001	<0.001	<0.001
Amylase (U/L)	398 (13–5191)	222 (27–3845)	581 (16–2377)	0.141	0.083	0.056
RDW (%)	12.8 (11.4–19.2)	13.0 (11.3–16.3)	13.7 (11.7–23.6)	<0.001	0.013	0.014
NLR	8.46 (1.33–55)	14.60 (1.73–60)	19.65 (3.57–53.67)	<0.001	<0.001	0.020
LMR	1.88 (0.28–13.33)	1.03 (0.29–5.33)	1.14 (0.22–6.32)	<0.001	<0.001	0.883
PNI	44.53 ± 6.63	39.36 ± 6.71	34.55 ± 6.02	<0.001	<0.001	<0.001
Mortality (%)	0(0%)	0(0%)	31(36.0%)	<0.001	–	<0.001

Continuous variables are presented as mean ± SD or median (range).

Aetiology (1/2/3/4)%, 1, 2, 3, and 4 represent gallstone, alcohol, hypertriglyceridemia, and other aetiologies, respectively.

1 *vs.* 2, MAP group *vs.* MSAP group; 2 *vs.* 3, MSAP group *vs.* SAP group.

MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis; WBC, white blood cell count; CRP, C-reactive protein; RDW, red cell distribution width; NLR, neutrophil-lymphocyte ratio; LMR, lymphocyte-monocyte ratio; PNI, prognostic nutritional index.

Table 2 Demographics and laboratory findings in survivors and non-survivors of acute pancreatitis

Variables	Survivors(n=328)	Non-survivors(n=31)	p value
Age (years)	49.84 ± 14.88	58.90 ± 15.60	0.001
Male (%)	179(54.6%)	19(61.3%)	0.472
Aetiology (1/2/3/4)%	50%/13%/12%/25%	58%/19%/10%/13%	0.346
WBC ($\times 10^9$/L)	12.85 (3.1–38.4)	18.5 (6.5–29.3)	0.001
Lymphocytes ($\times 10^9$/L)	1.08 (0.17–9.40)	0.60 (0.30–1.60)	<0.001
Platelet ($\times 10^9$/L)	197 (21–548)	159 (27–376)	0.001
Albumin (g/L)	35.95 ± 6.30	30.44 ± 5.54	<0.001
CRP (mg/L)	98.6 (0.7–436.5)	239.2 (27.1–398.2)	<0.001
Amylase (U/L)	343.5 (13–5191)	909 (16–2377)	0.010
RDW (%)	13 (11.3–19.2)	13.8 (12.6–23.6)	<0.001
NLR	10.47 (1.33–60.0)	25.0 (8.67–53.67)	<0.001
PNI	41.71 ± 7.50	34.00 ± 6.35	<0.001
LMR	1.51 (0.22–13.33)	1.13 (0.24–2.26)	<0.001

Continuous variables are presented as mean ± SD or median (range).

Aetiology (1/2/3/4)%, 1, 2, 3, and 4 represent gallstone, alcohol, hypertriglyceridemia, and other aetiologies, respectively.

WBC, white blood cell count; CRP, C-reactive protein; RDW, red cell distribution width; NLR, neutrophil-lymphocyte ratio; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio.

Table 3 Odds ratios of prognostic factors for predicting SAP in patients with AP

Factors	Model 1		Model 2		Model 3	
	Odds ratio (95%CI)	p value	Odds ratio (95%CI)	p value	Odds ratio (95%CI)	p value
NLR (>11.36 *vs.*≤11.36)	3.707(2.173-6.326)	<0.001	3.578 (2.082-6.149)	<0.001	1.463(0.711-3.010)	0.301
CRP (>110 *vs.*≤110mg/L)	9.867(5.116-19.030)	<0.001	12.609 (6.304-25.218)	<0.001	8.251(3.897-17.468)	<0.001
RDW (>13.0 *vs.*≤13.0%)	3.368(2.003-5.663)	<0.001	3.529 (2.076-5.998)	<0.001	2.533(1.365-4.702)	0.003
PNI (<41.1 *vs.* ≥41.1)	9.951(5.055-19.589)	<0.001	11.356 (5.665-22.766)	<0.001	7.753(3.400-17.680)	<0.001
LMR (<1.43 *vs.* ≥1.43)	2.564(1.539-4.271)	<0.001	2.552 (1.524-4.274)	<0.001	0.722(0.355-1.471)	0.370

Model 1: unadjusted model.

Model 2: adjusted for age, gender, and amylase.

Model 3: NLR was adjusted for age, gender, amylase, CRP, RDW, PNI, and LMR; CRP was adjusted for age, gender, amylase, NLR, RDW, PNI, and LMR; RDW was adjusted for age, gender, amylase, CRP, NLR, PNI, and LMR; PNI was adjusted for age, gender, amylase, NLR, CRP, RDW, and LMR; LMR was adjusted for age, gender, amylase, NLR, CRP, RDW, and PNI.

AP, acute pancreatitis; SAP, severe acute pancreatitis; NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; RDW, red cell distribution width; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio; CI, confidence interval.

Table 4 Discriminatory ability of inflammation-based markers for predicting mortality in AP patients

Index	AUC(95% CI)	p value[&]	Cut-off[#]	Sensitivity	Specificity	+LR	-LR
Training cohort							
NLR	0.804(0.738-0.859)	<0.001	16.64	82.4%	75.6%	3.38	0.23
CRP	0.774(0.706-0.833)	<0.001	162.2mg/L	76.5%	73.8%	2.92	0.32
RDW	0.769(0.700-0.828)	<0.001	13.0%	94.1%	54.3%	2.06	0.11
PNI	0.769(0.701- 0.828)	<0.001	33.1	58.8%	88.4%	5.08	0.47
LMR	0.744(0.674-0.806)	<0.001	1.40	82.4%	57.3%	1.93	0.31
Validation cohort							
NLR	0.851(0.790-0.900)	<0.001	16.64	85.7%	73.8%	3.27	0.19
CRP	0.753(0.683-0.815)	<0.001	162.2mg/L	71.4%	65.2%	2.06	0.44
RDW	0.708(0.635-0.773)	0.001	13.0%	85.7%	50.0%	1.71	0.29
PNI	0.791(0.724- 0.848)	<0.001	33.1	42.9%	88.4%	3.70	0.65
LMR	0.677(0.603-0.745)	0.015	1.40	78.6%	49.4%	1.55	0.43
Overall							
NLR	0.823(0.780-0.861)	<0.001	16.64	83.9%	74.4%	3.27	0.22
CRP	0.762(0.714-0.805)	<0.001	162.2mg/L	74.2%	69.8%	2.46	0.37
RDW	0.742(0.693-0.786)	<0.001	13.0%	90.3%	49.7%	1.80	0.19
PNI	0.781(0.734- 0.822)	<0.001	33.1	51.6%	88.4%	4.46	0.55
LMR	0.710(0.660-0.757)	<0.001	1.40	77.4%	54.0%	1.68	0.42

NLR, neutrophil-lymphocyte ratio; PNI, prognostic nutritional index; CRP, C-reactive protein; RDW, red cell distribution width; LMR, lymphocyte-monocyte ratio; AUC, area under the receiver operating characteristic curve; CI, confidence interval; +LR, positive likelihood ratio; −LR, negative likelihood ratio.

[&] The p-value is comparing the AUC with 0.5.

[#] The cut-off values were derived from a training cohort.

Table 5 Prognostic factors of overall survival in patients with acute pancreatitis by univariate and multivariate analyses

Factors	Univariate analysis		Multivariate analysis	
	Hazard ratio (95%CI)	p value	Hazard ratio (95%CI)	p value
Age (>63 *vs.* ≤63 years)	5.384(2.653-10.925)	<0.001	4.039(1.873-8.713)	<0.001
Gender (female *vs.* male)	0.767(0.372-1.579)	0.471		
Amylase (>618 *vs.*≤618U/L)	3.544(1.699-7.526)	0.001	2.173(0.965-4.891)	0.061
NLR(>16.64 *vs.* ≤16.64)	13.130(5.041-34.205)	<0.001	4.726(1.627-13.726)	0.004
CRP(>162.2 *vs.*≤162.2mg/L)	6.127(2.740-13.701)	<0.001	3.503(1.534-7.999)	0.003
RDW(>13.0 *vs.*≤13.0%)	4.929(2.022-12.017)	<0.001	3.139(1.277-7.714)	0.013
PNI(≤33.1 *vs.* >33.1)	6.912(3.414-13.991)	<0.001	2.641(1.248-5.590)	0.011
LMR(≤1.40 *vs.*>1.40)	3.797(1.636-8.813)	0.002	1.036(0.403-2.659)	0.942

NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; RDW, red cell distribution width; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio; CI, confidence interval.

Figure Legends

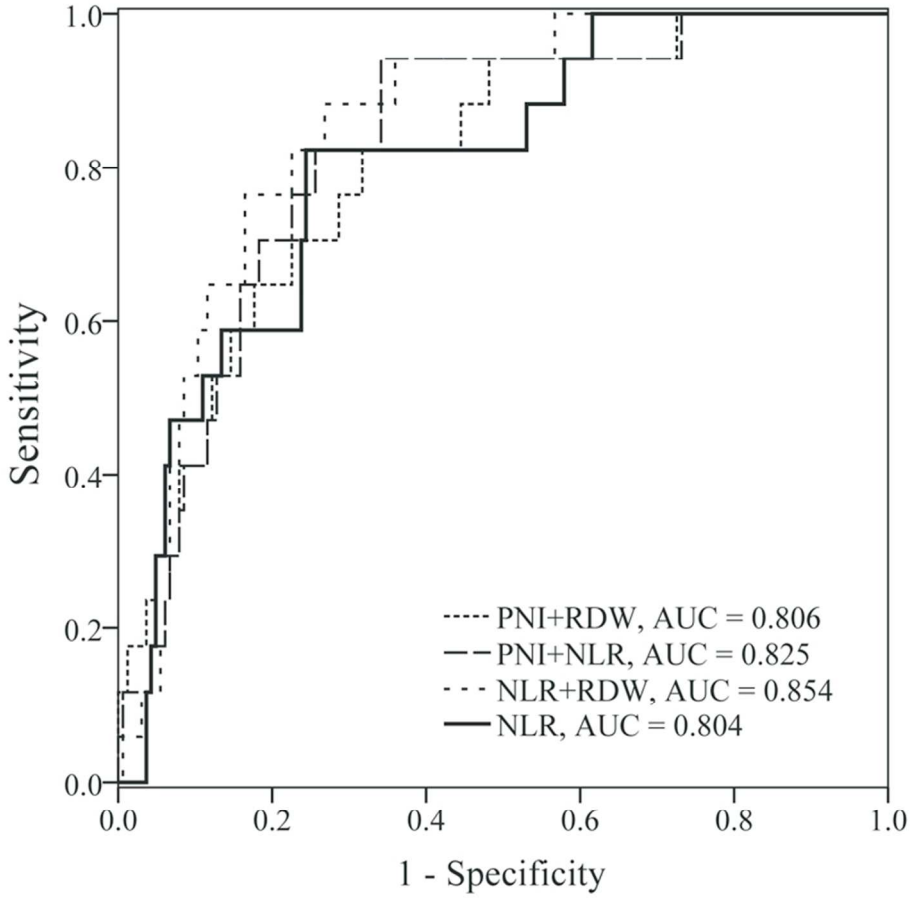
Fig. 1. ROC curves analysis for predicting mortality by NLR and combined markers in the estimation cohort.

ROC, receiver operating characteristic; NLR, neutrophil-lymphocyte ratio; RDW, red cell distribution width; PNI, prognostic nutritional index.

Fig. 2. Relationship between inflammation-based prognostic markers and overall survival in patients with acute pancreatitis

A, B, C, D, and E show the relationship between NLR, CRP, RDW, PNI, and LMR, and overall survival in patients with acute pancreatitis, respectively.

NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; RDW, red cell distribution width; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio.



ROC, receiver operating characteristic; AUC, area under the receiver operating characteristic curve; NLR, neutrophil-lymphocyte ratio; RDW, red cell distribution width; PNI, prognostic nutritional index.

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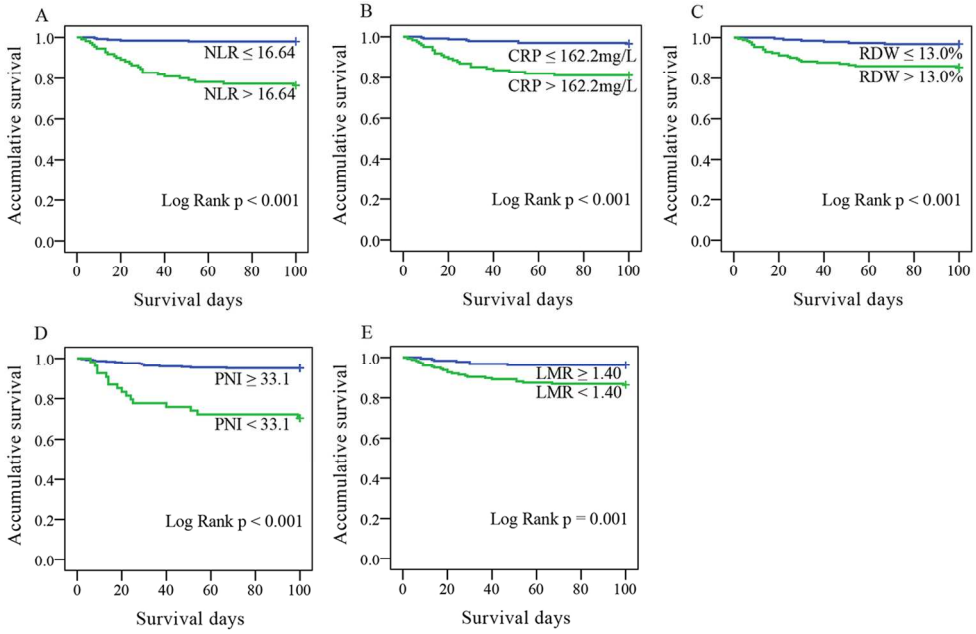

A, B, C, D, and E show the relationship between NLR, CRP, RDW, PNI, and LMR, and overall survival in patients with acute pancreatitis, respectively.
NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; RDW, red cell distribution width; PNI, prognostic nutritional index; LMR, lymphocyte-monocyte ratio.

170x115mm (300 x 300 DPI)

Supplementary Table S1 Demographics and laboratory findings in estimation and validation cohorts

Variable	All patients（n=359）	Training set(n=181)	Validation set(n =178)	p value
Age (years)	50.63±15.13	51.66 ±15.69	49.58 ±14.51	0.194
Male [N (%)]	198(55.2%)	96(53.0%)	102(57.3%)	0.417
Aetiology (1/2/3/4) %	54.3/9.7/12.0/24.0	55.2/12.7/12.2/19.9	53.4/6.7/11.8/28.1	0.118
WBC ($\times10^9$/L)	12.9(3.1-38.4)	13.3 (3.1–38.4)	12.9 (4.2–36.8)	0.942
Lymphocytes ($\times10^9$/L)	1.00 (0.17–9.40)	1.00 (0.20–9.40)	1.00 (0.17–4.80)	0.965
Platelet ($\times10^9$/L)	192 (21–548)	193 (27–502)	191.5 (21–548)	0.354
Albumin (g/L)	35.47 ±6.42	35.72 ±6.61	35.22 ±6.24	0.456
CRP (mg/L)	110 (0.7–436.5)	102.3 (0.8–436.5)	116.85 (0.7–419.4)	0.081
Amylase (U/L)	398 (13–5191)	501 (13–5191)	330 (16–4927)	0.238
RDW (%)	13.0(11.3-23.6)	13.1 (11.3–19.2)	13.0 (11.4–23.6)	0.421
NLR	11.36 (1.33–60.0)	11.50 (1.33–55.0)	11.18 (1.39–60.0)	0.786
PNI	41.05±7.72	41.22 ±7.74	40.87 ±7.71	0.670
LMR	1.43(0.22–13.33)	1.48 (0.24–13.33)	1.36 (0.22–10.00)	0.367
Mortality [N (%)]	31(8.6%)	17(9.4%)	14(7.9%)	0.607

Continuous variables are presented as mean±SD or median (range).

p value was training set versus validation set.

Aetiology (1/2/3/4)%, 1, 2, 3, and 4 represent gallstone, alcohol, hypertriglyceridemia, and other aetiologies, respectively.

WBC, white blood cell count; CRP, C-reactive protein; RDW, red cell distribution width; NLR, neutrophil-lymphocyte ratio; LMR, lymphocyte-monocyte ratio; PNI, prognostic nutritional index.

TRIPOD Checklist: Prediction Model Development

Section/Topic	Item	Checklist Item	Page
Title and abstract			
Title	1	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	3,4
Introduction			
Background and objectives	3a	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	5,6
	3b	Specify the objectives, including whether the study describes the development or validation of the model or both.	6
Methods			
Source of data	4a	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	6
	4b	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	6,7
Participants	5a	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	6,7
	5b	Describe eligibility criteria for participants.	6, 7
	5c	Give details of treatments received, if relevant.	Not relevant
Outcome	6a	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	7
	6b	Report any actions to blind assessment of the outcome to be predicted.	7
Predictors	7a	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	7,8
	7b	Report any actions to blind assessment of predictors for the outcome and other predictors.	7,8
Sample size	8	Explain how the study size was arrived at.	9
Missing data	9	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	9
,8Statistical analysis methods	10a	Describe how predictors were handled in the analyses.	8,9
	10b	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	8,9
	10d	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	8,9
Risk groups	11	Provide details on how risk groups were created, if done.	8,9
Results			
Participants	13a	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	9
	13b	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	9,10 Table1 and 2
Model development	14a	Specify the number of participants and outcome events in each analysis.	9 Table1 and 2
	14b	If done, report the unadjusted association between each candidate predictor and outcome.	10,11, 12, Table 3 and 5
Model specification	15a	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	10,11, Table 3 and 5
	15b	Explain how to the use the prediction model.	10,11
Model performance	16	Report performance measures (with CIs) for the prediction model.	10,11, Table3 and 5
Discussion			
Limitations	18	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	15
Interpretation	19b	Give an overall interpretation of the results, considering objectives, limitations, and results from similar studies, and other relevant evidence.	12-15
Implications	20	Discuss the potential clinical use of the model and implications for future research.	12,13, 15

TRIPOD Checklist: Prediction Model Development

Other information			
Supplementary information	21	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	Yes
Funding	22	Give the source of funding and the role of the funders for the present study.	16

We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.