

Procalcitonin and C-reactive Protein Perform Better Than Neutrophil-Lymphocyte Count Ratio on Evaluation of Hospital Acquired Pneumonia

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Research article

Keywords: Hospital acquired pneumonia, Neutrophil-lymphocyte count ratio, Procalcitonin, C-reactive protein, Composite biomarker

Posted Date: January 10th, 2020

DOI: <https://doi.org/10.21203/rs.2.20487/v1>

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Abstract

Background: Early diagnosis and severity evaluation are key factors to achieve improved outcomes of hospital acquired pneumonia (HAP). We are constantly in search of more sensitive and specific biomarkers to improve timely diagnosis and survival.

Methods: 593 cases of adult patients were enrolled into this retrospective cohort study to determine neutrophil-lymphocyte count ratio (NLCR), procalcitonin (PCT), C-reactive protein (CRP), serum lactate level and APACHE (Acute Physiology and Chronic Health Evaluation) II score at the admission of ICU. Patients were divided into 2 groups according to diagnosis: non-infection and HAP. Discriminant analysis was applied to which marker or what composition of markers performed better regarding to the diagnostic value and severity evaluation. The diagnostic value of each individual biomarker was assessed by construction of receiver operating characteristic (ROC) curves, calculation of the area under each ROC curves (AUROC). Multivariate analysis was also applied to detect most appropriate prognostic factors.

Results: Remarkable differences were observed on NLCR, PCT, CRP and APACHE II scores between non-infection and HAP group. Regarding to discriminant ability of severe infection, the AUROC of NLCR (0.56; 95%CI 0.52-0.61) was not comparable with any of other single markers such as PCT (0.63; 95% CI 0.59-0.68), CRP (0.60; 95% CI 0.54-0.67), or APACHE II score (0.68; 95% CI 0.64-0.73). Compared to the single biomarkers, APACHE II score presented higher discriminant ability with greater AUROC. Besides, AUROC of the composite biomarker PCT-CRP-NLCR (0.66; 95% CI 0.61-0.70) was significantly greater than any of the single biomarkers, and its discriminant ability was comparable to APACHE II score.

Conclusions: NLCR is not comparable to other single biomarkers such as PCT, CRP, or APACHE II score regarding to diagnosis or to severity evaluation of HAP. Composite biomarkers can prompt early diagnosis and severity evaluation with improved accessibility, especially the composition of PCT-CRP-NLCR.

Background

Hospital-acquired pneumonia (HAP) is characterized by pneumonia acquired during hospitalization in patients without invasive mechanical ventilation. HAP is a frequent event for critical patients and remains the leading cause of death among hospital-acquired infections [1]. This life-threatening condition in intensive care unit (ICU) may require mechanical ventilation, associate with prolonged hospital stay and high mortality. Certain clinical and laboratory parameters were applied to facilitate diagnosis, to evaluate severity, to guide antibiotic administration and to predict prognosis of HAP. However, most of them has been proved to be moderately valuable on its severity evaluation and outcome prediction.

Procalcitonin (PCT) is a useful serum marker in prediction, diagnosis and severity evaluation of bacterial infections in critically ill patients [2]. It has been shown to be associated with the severity of inflammation and prognosis during sepsis and septic shock [3, 4]. Some large studies in the emergency department

have demonstrated low PCT values were associated with low risks of death in patients with community acquired pneumonia (CAP) [5]. The significance of PCT is emphasized that its pattern of levels may be able to guide antimicrobial therapy in patients with various infections, such as community acquired lower respiratory tract infection[6], ventilation acquired pneumonia [7], blood stream infection [8] and abdominal infection [9].

C-reactive protein, a highly conserved plasma protein, is a homopentameric acute-phase inflammatory protein, which was initially discovered in 1930 by Tillet and Francis. C-reactive protein exhibits elevated expression during inflammatory conditions such as rheumatoid arthritis, some cardiovascular diseases, and infection. As an acute phase protein, the plasma concentration of CRP deviates by at least 25% during inflammatory disorders [10]. As a traditional biomarker, CRP still plays great role at identifying and evaluating bacterial infections [11]. Although been proved to be predictive on severity of pneumonia, only very few investigations with poor quality compared its potential with NLCR at evaluation of inflammation [12].

Numerous studies have evaluated the diagnostic and evaluative performance of neutrophil lymphocyte count ration (NLCR) on various clinical conditions such as sepsis [13], septic shock [14], bacteremia [15, 16], renal, lung and colorectal carcinomas and intracranial tumors [17]. Besides, NLCR has been proved to be able to predict severity and outcome of CAP with higher prognostic accuracy as compared with traditional infection markers in the emergency department [12].

Composite biomarkers have been applied to evaluate respiratory tract infections, however few studies have focused on their diagnostic accuracy and prognostic utility. Some findings showed greater value for composite biomarkers in discriminating severity of inflammation [18, 19, 20]. Our researches presented composite biomarkers of no greater diagnostic utility and of little use in clinical prediction [not published data].

As above described, different biomarkers have been applied to evaluate severity of inflammation. With respect to the diagnostic accuracy and predictive potency, divergence of opinion has been largely presented. Currently, controversies still exist that NLCR may be less suitable to detect the presence of sepsis in ICU patients [13]. Moreover, data about the comparison of NLCR and PCT in patients with HAP are very limited. Thus the intent of this study is to clarify whether NLCR presents advantages over conventional markers or whether composite biomarkers could be a better choice in regarding to diagnosis and evaluation of HAP.

Methods

Patients and study design

This retrospective study was conducted with data collected from Jan 2017 to June 2019 at the First Affiliated Hospital of Nanjing Medical University, a tertiary hospital with more than 2000 beds, in the southeast region of China. Patients ≥ 18 years admitted to the ICU in suspicion of HAP or non-infection

were consecutively enrolled into this study. All the physiological and pathophysiological data, laboratory and microbiological results and survival outcomes were recorded accordingly.

All medical records were retrospectively reviewed by two senior specialists in infectious disease and critical care medicine to determine whether the patients fulfilled HAP diagnosis and grouping criteria or without infection.

HAP was defined in patients who developed pneumonia after 48 h of not receiving invasive mechanical ventilation (iMV) [21, 22]. Clinical diagnosis of pneumonia was based on clinical criteria as suggested in the guidelines [21, 23, 24]: (1) new or progressive radiologic pulmonary infiltrate, (2) together with at least two of the following: temperature $> 38^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$, leukocytosis $> 12,000/\text{mm}^3$ or leukopenia $< 4000/\text{mm}^3$, or purulent respiratory secretions.

Inclusion criteria of the patients: (1) Adults: age of 18 to 89 years; (2) Admitted to ICU in the First Affiliated Hospital of Nanjing Medical University during the period from Jan 2017 to Jun 2019; (3) Diagnosed with HAP or non-infectious disease.

Exclusion criteria of the patients: (1) Hematological disease; (2) Chemotherapy; (3) Receiving glucocorticoids; (4) Receiving bone marrow stimulators.

Enrolled patients were designated into two groups according to the diagnosis: (1) non-infection group: patients have been ruled out of infection of any origin and organism.; (2) HAP group: patients have been assessed as applicable to the criteria of HAP.

Statistical analysis

All continuous variables were expressed as the median and interquartile range due to non-normal distribution. F test was used to compare variances of continuous variables between two groups, if variances were significantly different, unpaired t test with Welch's correction would be applied. In the case of variances being equal, Mann-Whitney U test would be applied. $p < 0.05$ was considered the difference to be significant.

Composite biomarkers were constructed using bivariate logistic regression analysis. Different compositions were consisted of PCT, CRP and NLCR. The most valuable composition was chosen as which presented the highest discriminant capability between groups. A comparison of the diagnostic accuracy of the biomarkers, alone and in combination, was made by receiver operating characteristics (ROC) curves analysis by calculating the area under the curves (AUROC). For comparison of AUROCs, Mann-Whitney U test for two correlated ROC curves was used. All tests were two-sided, and $p < 0.05$ was considered statistically significant. The statistical analysis and graph construction were performed using SPSS 23 and Stata 12 and Graphpad Prism 5.0.

Results

General characteristics

A total of 659 episodes of adult patients suspected with HAP or non-infection admitted to the ICU at the First Affiliated Hospital of Nanjing Medical University were enrolled. Of total enrolled population, 66 patients were excluded from the analysis of the data because of exclusion criteria (Figure 1). General characteristics of the overall population were displayed in Table 1.

Microorganisms profile

Positive cultures of the microbiological samples taken within 48 h of admission were reported in all episodes of HAP group patients. Of all positive cultures, 324 cases of microorganisms were isolated. For all analyzed cases, 237 isolates of bacterial infection were found in 237 episodes of patients, with 27 isolates of gram-positive organisms and 210 isolates of gram-negative organisms. Apart from these, 83 isolates of fungi and 3 isolates of viruses had been found. The detected microorganism profile has been shown in Table 2.

Co-morbid conditions

The incidence of cardiovascular co-morbid conditions on admission to the ICU was lower in patients of non-infection than in HAP group. On the other hand the incidence of malignancies was much higher in non-infection group. The co-morbid disease profile was presented in Table 3. Apart from this, the surgery operation incidence in non-infection group was much higher than that of HAP group, also described in Table 1.

Diagnostic performance of the markers

Serum levels of various biomarkers and severity scores between different groups were compared to determine the discriminant capability. Our study found that PCT, CRP, NLCR, WBC, NE, APACHE II score and 28-day survival rate were all discriminant between two groups. However, serum lactate (LAC) did not present to be differential between these two groups. Compared to other markers, APACHE II score and CRP levels showed much greater differential potency, as the p value of t test at comparison of the two groups presented to be most significant (Figure 2).

In the ROC curve analysis, APACHE II score (AUC 0.68; 95% CI 0.61 - 0.74) showed the highest ability for discrimination of HAP compared to the single biomarkers. As single biomarkers, CRP (AUC 0.60; 95% CI 0.54 - 0.67) and PCT (AUC 0.58; 95% CI 0.52 - 0.65) presented to have greater potential to differentiate HAP with non-infection group than NLCR (AUC 0.56; 95% CI 0.52 - 0.61) and LAC (AUC 0.54; 95% CI 0.45 - 0.65) (Figure 3).

In regard to composite biomarkers, the three-marker composition of NLCR, PCT and CRP had the highest AUC (AUC 0.66; 95% CI 0.61 - 0.70), which was significantly higher than all other biomarker compositions (data not shown). Thus it is been selected to be the most valuable composition applied in this study,

which was almost comparable to the differential potency of APACHE II score, which presented the highest AUROC of all observed in HAP patients (Figure 3).

As analyzed above, NLCR, LAC, WBC counts and NE presented to be differential between two groups. However, LAC in verified HAP patients did not present to be differential to those in non-infective patients, not only based on the median (interquartile) analysis (Table 1 and Figure 2), but also according to the AUROC calculation (Figure 3).

Survival and mortality

The overall 28-day mortality rate of enrolled population was 25.3% ($n = 150$), with 30.7% ($n = 104$) in HAP group and 18.1% ($n = 46$) in non-infection group. With statistical calculation, the mortality rate in HAP group was significantly higher than that of non-infection group.

In statistical analysis of 28-day survival in HAP group, the APACHE II score, CRP, NLCR and NE was much elevated in the survival population (Figure 4). Meanwhile, the ROC analysis has displayed that APACHE II score and CRP had the greatest discriminant ability than NLCR or any other biomarkers (Figure 5).

Discussion

Over the past few years, a great many researches have been done to investigate the clinical value of various biomarkers in diagnosis, prognosis and stratification of pneumonia [25]. Many studies have focused on the significance of single biomarkers, meanwhile interests in multiple-biomarkers have increased, especially in severity evaluation of infective conditions, such as sepsis, septic shock and CAP. Contradicted conclusions have been presented due to inconsistent results drawn by small sample sized population [26,27]. In this present study, we have investigated the clinical value of NLCR, PCT, CRP and APACHE II score alone and in combination with a large population consisting of 593 episodes of adult patients.

In the present study, we found that patients with HAP had higher levels of PCT and CRP than patients with non-infection (Table 1 and Figure 2). These patients showed greater severity with higher APACHE II scores (Table 1 and Figure 2). This is in accordance with the data from Liu et al., who reported that PCT was associated with the severity of illness in patients with severe pneumonia and appears to be a prognostic marker of morbidity and mortality comparable to the APACHE II score [27]. The results from the present study suggest that APACHE II performed best in discriminating HAP with non-infection status as a single indicator (Figure 2,3). It has been proved to be a valuable marker to predict mortality in septic patients [28].

PCT and CRP, as traditional biomarkers, have been intensely investigated for the past few years and the outcomes have been demonstrated contradicting [27,29]. This may caused by small sample sized research population, which could undermine the whole reliability of the results [30].

In our present study, PCT demonstrated a relatively high specificity (Figure 2, 3) for differentiation of HAP with non-infection patients, which indicates that the patients with a higher level of PCT could be likely diagnosed HAP in combination with clinical history.

NLCR has been reported to be a indicator that correlated with the severity of series of diseases, and has been applied to predict prognosis of various clinical circumstances, ranging from colorectal cancer [17], glial tumor [31], sepsis and/or septic shock [32,18], to acute coronary syndrome [33].

Regarding to AUROC analysis, APACHE II score performed the best as a single biomarker to discriminate HAP with non-infection patients, and to predict 28-day mortality in HAP groups (Fig 3, 5). Meanwhile, PCT and CRP showed moderate AUROCs to discriminate HAP with non-infection patients, and to predict 28-day mortality in HAP groups (Fig 3, 5), but NLCR only presented to have very weak discriminant ability with very low AUROC in both scenarios above.

Many factors that affect the diagnostic performance of a biomarker may contribute to bring controversies, thereby may make it difficult and less viable to compare results from different studies.

In the current study, we employed different methods to combine several biomarkers into one variable. The diagnostic ability of PCT-CRP-NLCR composition were proved to be more valuable than all other compositions. Thus we reported only this combination instead of others. Composite biomarker PCT-CRP-NLCR has been proved to be as efficient as APACHE II score, and could be presented as a reasonable predictor of 28-day mortality, indicating it might also be employed for risk stratification purposes. With AUROC analysis, this 3-marker composition has been proved to be as valuable as APACHE II score, with almost effortless access, which could be widely applied to HAP patients for evaluation purposes. This composite biomarkers has presented significantly higher AUROCs than all the other single biomarkers, suggesting that joint interpretation of multiple biomarkers could be ever more valuable in evaluation of HAP. There are certain drawbacks of multiple-biomarker evaluation, including high costs and low patients compliance.

With respect to 28-day survival analysis, the survival rate of HAP group was much lower than that of non-infection group. Meanwhile, nearly all the biomarkers in non-survivors presented to be significantly elevated, which correlated with the changing tendency of severity score APACHE II (Figure 4, 5).

These data agree with previous observations that PCT in pneumonia patients correlated with the risk of death independent of the clinical risk assessment [34,35]. Besides, PCT was also proved to be capable of identifying unfavorable outcome in CAP and VAP (ventilator acquired pneumonia) patients in ICU [36,37,38]. While in clinical scenarios, PCT is more frequently used to guide antibiotic treatments.

Limitations of the study

Several limitations of this study render our concerns. First, patients with antibiotic treatment were not excluded from this study, therefore, false negative results may be generated leading to underestimation of the severity. Second, the clinical diagnosis of HAP may be lack of accuracy in many cases, where there

were no consistent changes on chest imaging, or there might be false negative results of microbiological sampling in patients receiving broad-spectrum antibiotics for a clinical diagnosis of HAP, or there might be positive results of microorganisms to be diagnosed as HAP, only in fact due to certain inflammation status combined with hospital acquired bacterial colonization instead of bacterail infection.

Conclusion

In patients with HAP, APACHE II score as a classical severity evaluation, presented great value to evaluate severity and predict prognosis of the disease. As conventional biomarkers, both CRP and PCT were associated with severity of the disease, and could be good prognostic markers for morbidity and mortality in HAP patients. NLCR, as a recently re-explored biomarker, presents no advantage over conventional markers on severity evaluation and prognosis prediction of HAP. Procalcitonin performs better than NLCR on severity evaluation of HAP. Mutiple-biomarker composition could be better choice for the purpose of disease diagnosis, severity evaluation, treatments guidance and prognosis prediction for HAP patients, as it presented with easy access, simple interpretation and comparable quality with the classical but both time and effort-consuming APACHE II score.

Declarations

Ethics approval and consent to participate

We have received ethical approval from the institutional review boards (IRBs) at the First Affiliated Hospital of Nanjing Medical University. Since this study does not contain protected health information and all data were anonymously used, a waiver of the requirement for informed consent was approved by the IRBs. Individual patients consent was not obtained since all data used in this study were acquired retrospectively from the laboratory information system without any additional sampling or laboratory analysis. □

Consent for publication

Yes. We the authors all give consent for publication.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was financed by National Natural Science Foundation of China, No.81770287.

Authors' contributions

NZ retrieved the data according to the background of non-infection and the hospital acquired pneumonia. YH analyzed and interpreted the data, constructed the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The statistical analysis of this study received help from Dr. Qiulun Lu from Nanjing Medical University, Nanjing, China.

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Abbreviations

APACHE II: Acute Physiology and Chronic Health Evaluation II; AUROC: Area under the ROC curve; CAP: community acquired pneumonia; CI: confidence interval; CRP: C-reactive protein; HAP: hospital acquired pneumonia; iMV: invasive mechanical ventilation; IRB: institutional review boards; LAC: lactate; NLCR: neutrophil-lymphocyte count ratio; PCT: procalcitonin; ROC: receiver operating characteristic.

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Tables

Table 1 Characteristics of the overall population

	Non-infection n = 254	HAP n = 339	P value
Age (years)	66.6 ± 17.1	71.0 ± 17.9 **	0.0028
Sex (M / F)	152 / 102	252 / 87 *	0.0378
WBC abnormalities, n, (%)	54 (21.3%)	130 (38.2%) *	0.0237
NE abnormalities, n, (%)	183 (72.0%)	254 (74.7%) *	0.0486
APACHE II score (mean ± sd)	17.7 ± 5.9	21.7 ± 6.1 ***	< 0.0001
NLCR	10.2 ± 9.8	13.0 ± 17.1 *	0.0127
PCT (ng/ml)	0.75 ± 3.25	4.60 ± 15.97 ***	< 0.0001
CRP (mg/ml)	45.8 ± 50.6	73.3 ± 79.6 ***	0.0003
Blood lactate (mmol/l)	1.6 ± 1.3	1.5 ± 0.8	0.7051
Surgery, n, (%)	100 (39.4%)	87 (25.6%) **	0.0012
28 days survival, n, (%)	205 (80.7%)	233 (68.5%) **	0.0013

Data presented as Mean ± Std. Deviation or number (percentage) of episodes. CRP, C-reactive protein; patients or mean ± standard deviation. *p < 0.05, **p < 0.01, ***p < 0.001 vs non-infection group.

Table 2. Microorganism profile for the patients in the study cohort

	28-day survival n = 235	Mortality n = 104	P value
Gram-positive isolates (n, %)	16 (6.8%)	11 (10.6%)	0.2772
S.Aureus	12 (5.1%)	10 (9.6%)	0.1505
MRSA	2 (0.9%)	0 (0)	1.0000
Streptococcus spp.	1 (0.4%)	0 (0)	1.0000
Enterococcus spp.	2 (0.9%)	1 (1.0%)	1.0000
Other	1 (0.4%)	0 (%)	1.0000
Gram-negative isolates (n, %)	158 (67.2%)	52 (50.0%)	0.0035
Acinetobacter baumannii	76 (32.3%)	37 (35.6%)	0.6175
Klebsiella spp.	59 (25.1%)	26 (25.0%)	1.0000
Pseudomonas spp.	50 (21.3%)	13 (12.5%)	0.0687
Enterobacter spp.	24 (10.2%)	6 (5.8%)	0.2177
S. maltophilia	16 (6.8%)	3 (2.9%)	0.2018
Other	9 (3.8%)	4 (3.8%)	1.0000
Fungi isolates (n, %)	55 (23.4%)	28 (26.9%)	0.4959
Candida albicans	28 (11.9%)	17 (16.3%)	0.2985
Candida glabrata	16 (6.8%)	2 (1.9%)	0.0702
Candida tropicalis	7 (3.0%)	4 (3.8%)	0.7423
Other	4 (1.7%)	4 (3.8%)	0.2556
Virus isolates (n, %)	2 (0.9%)	1 (1.0%)	1.0000
Tuberculosis isolates (n, %)	1 (0.4%)	0 (0)	1.0000

Data presented as number of isolates, not number of patients. S.Aureus, staphylococcus aureus; MRSA, methicillin-resistant staphylococcus aureus; S. maltophilia, Stenotrophomonas maltophilia; spp., species.

Table 3 Coexisting disease of the study population stratified by 28-day survival and mortality

	Non-infection n = 254		HAP n = 339	
	28d survival n = 208	Mortality n = 46	28d survival n = 235	Mortality n = 104
Diabetes mellitus	5 (2.4%)	0 (0)	0 (0)	0 (0)
Cardiovascular disease	22 (10.6%)	6 (13.0%)	7 (3.0%)	11 (10.6%)##
Hypertension	4 (1.9%)	1 (2.2%)	0 (0)	1 (1.0%)
Malignancies	50 (24.0%)	4 (8.7%)*	7 (3.0%)	3 (2.9%)
COPD	7 (3.4%)	3 (6.5%)	12 (5.1%)	5 (4.8%)
Liver cirrhosis	2 (1.0%)	0 (0)	0 (0)	0 (0)
Renal failure	1 (0.5%)	1 (2.2%)	7 (3.0%)	9 (8.7%)#

Data were expressed as number (percentage of current group);* p < 0.05 vs 28d survivals of non-infection group; #p < 0.05 vs 28d survivals of HAP group; ##p < 0.01 vs 28d survivals of HAP group; HAP, hospital acquired pneumonia; COPD, chronic obstructive pulmonary disease.

Figures

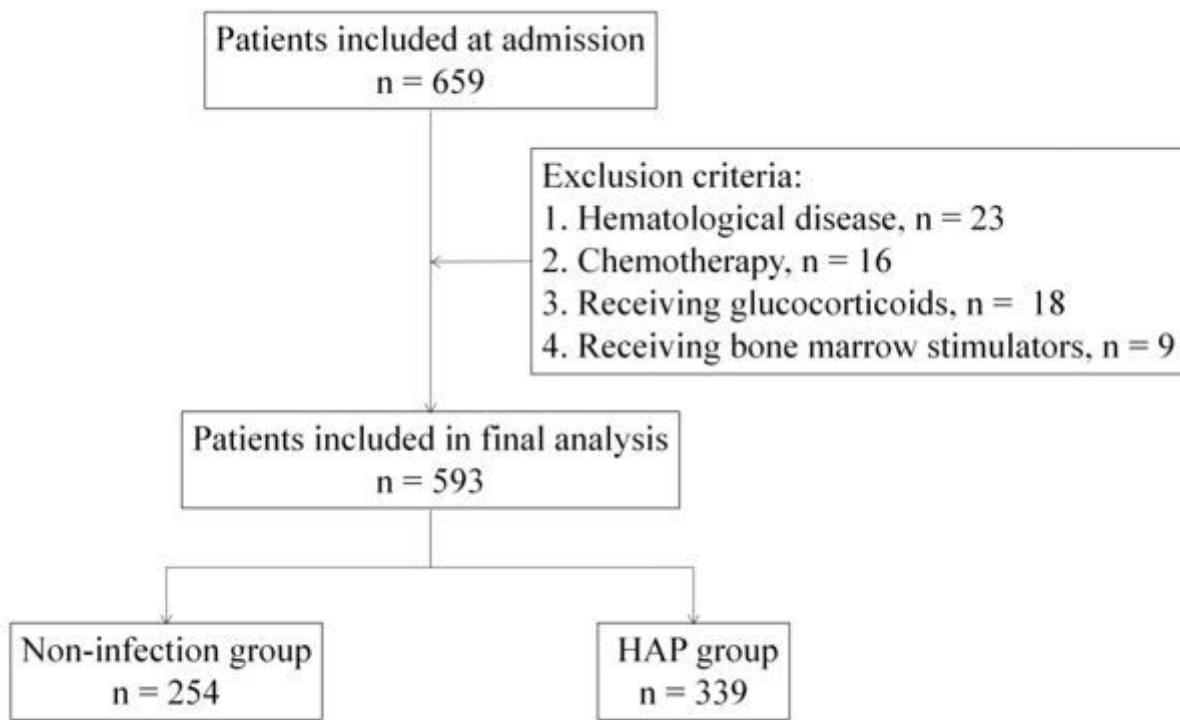


Figure 1

Enrollment flowchart

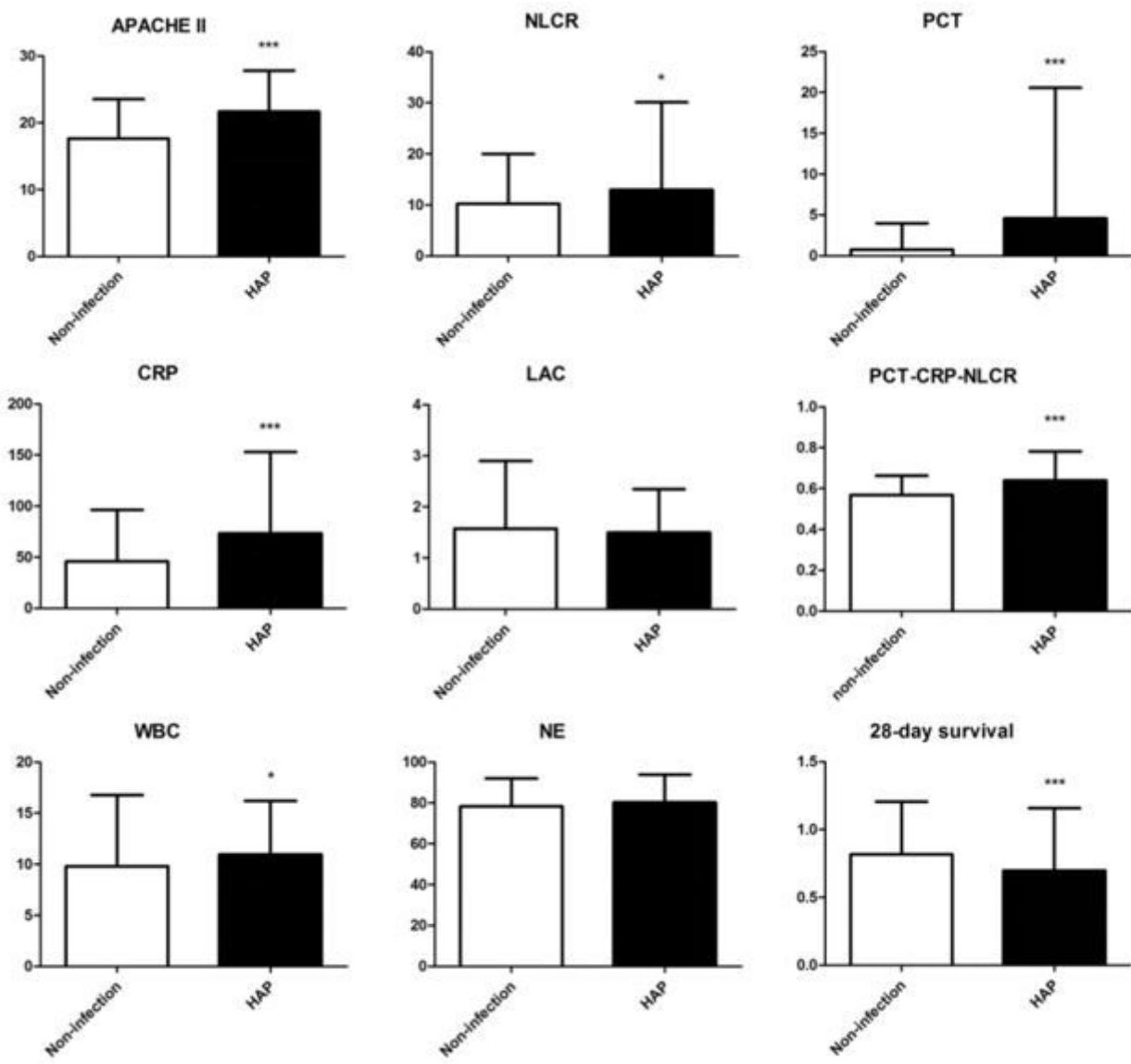
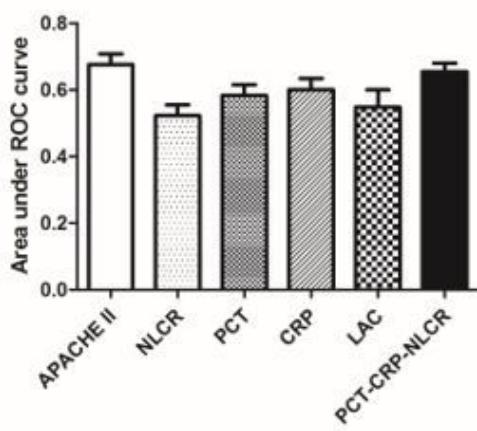
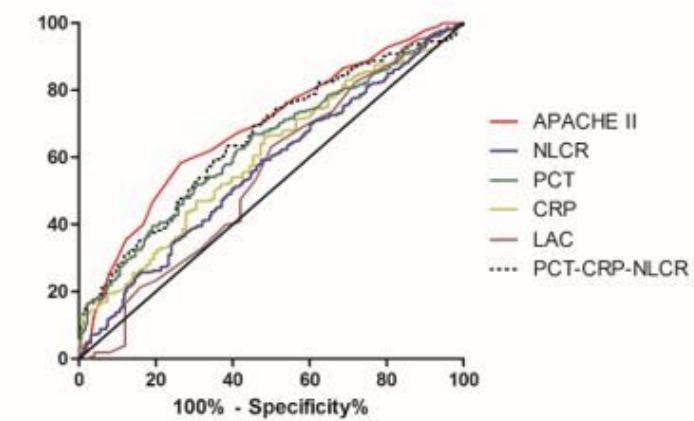


Figure 2

APACHE II score and all the biomarker levels of NLCR, PCT, CRP, LAC, WBC, NE and 28-day survival rate in non-infection and HAP group. * $p < 0.05$ vs non-infection group; ** $p < 0.01$ vs non-infection group; *** $p < 0.001$ vs non-infection group.



	APACHE II	NLCR	PCT	CRP	LAC	PCT-CRP-NLCR
AUROC	0.6757	0.522	0.584	0.6013	0.5483	0.6552
Std. Error	0.03236	0.03316	0.03149	0.03314	0.05172	0.02509
95% confidence interval	0.6123 to 0.7392	0.4570 to 0.5870	0.5223 to 0.6458	0.5363 to 0.6663	0.4469 to 0.6497	0.6060 to 0.7043
P value	<0.0001	0.509	0.01184	0.003364	0.3338	<0.0001

Figure 3

Receiver operator characteristic (ROC) curve for HAP discrimination with non-infection group, and area under the ROC (AUROC) for the biomarkers evaluated in this study.

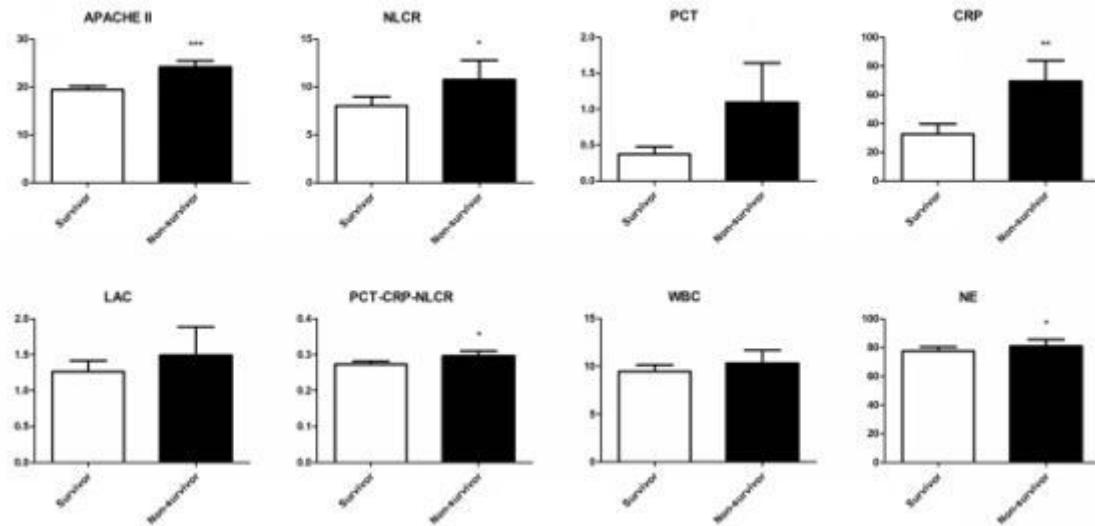
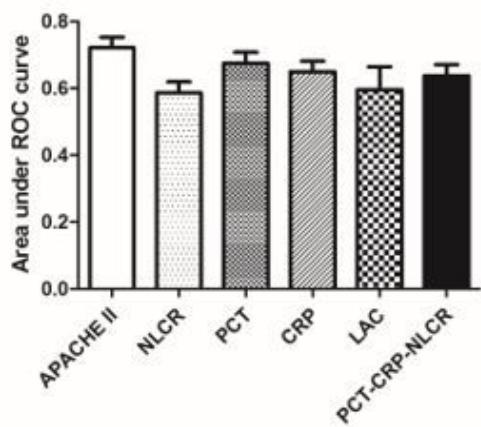
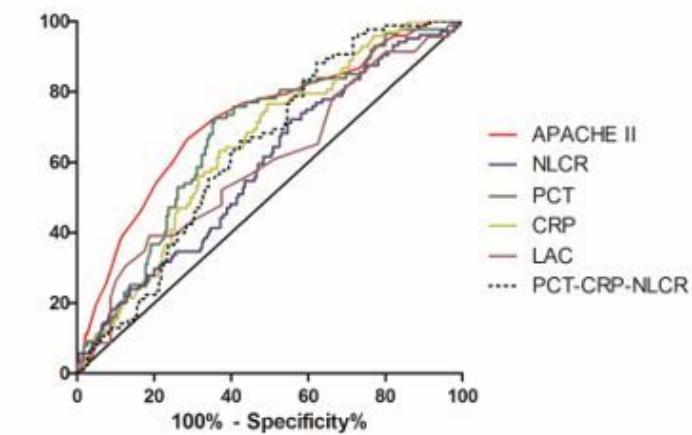


Figure 4

APACHE II score and all the biomarker levels of NLCR, PCT, CRP, LAC, WBC and NE in 28d survivals and non survivals of HAP group. * $p < 0.05$ vs 28d survivals; ** $p < 0.01$ vs 28d survivals; *** $p < 0.001$ vs 28d survivals.



	APACHE II	NLCR	PCT	CRP	LAC	PCT-CRP-NLCR
AUROC	0.7211	0.5863	0.6742	0.6494	0.5954	0.6379
Std. Error	0.0314	0.03296	0.03317	0.03151	0.06893	0.03269
95% confidence interval	0.6596 to 0.7827	0.5217 to 0.6509	0.6092 to 0.7392	0.5876 to 0.7112	0.4603 to 0.7305	0.5738 to 0.7020
P value	<0.0001	0.01135	<0.0001	<0.0001	0.1647	0.0002080

Figure 5

Receiver operator characteristic (ROC) curve for 28-day survival discrimination with non-survival in HAP group and area under ROC (AUROC) for the biomarkers evaluated in HAP group.