GM-CSF and HMGB1 level were associated with the clinical characteristics and prognosis of childhood refractory mycoplasma pneumoniae pneumonia

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Research article

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Abstract

Background: To analyze the relationship between granulocyte-macrophage colony-stimulating factor (GM-CSF) and high mobility group box 1 (HMGB1) level in alveolar lavage fluid with the clinical characteristics and prognosis of children refractory mycoplasma pneumoniae pneumonia (MPP), so as to provide reliable targets for clinical diagnosis and treatment.

Methods: A total of 106 children diagnosed with MPP and prepared for bronchoalveolar lavage therapy were selected in this study, which were divided into 2 groups according to clinical diagnosis: those showing clinical and radiological deterioration despite appropriate antibiotic therapy for ≥7 days were classified into refractory MPP group (n=47), while the others were classified into non-refractory MPP group (n=59). The data of physical examination, treatment and outcome were collected. In addition, the GM-CSF and HMGB1 levels in alveolar lavage fluid during each bronchoalveolar lavage therapy were detected by ELISA kits.

Results: There was no significant difference in age, sex, course of fever, the highest temperature, WBC, L, PLT, ALT, AST, CK-MB, D-D, CK, IgG, IgA, IgM, C3, and C4 between refractory MMP group and non-refractory MMP group on admission (P□0.05). The levels of N, CRP, PCT, and LDH in refractory MPP group were higher than those in non-refractory MPP group, the difference had statistical significance (P□0.05). Both GM-CSF and HMGB1 levels were positively correlated with traditional indicators N, CRP, PCT and LDH (r=0.611-0.785, P<0.05). ROC analysis results showed that CRP, GM-CSF and HMGB1 had predictive value for refractory MPP attack (AUC=0.636, 0.657, 0.651, P<0.05). Logistic regression analysis results showed GM-CSF and HMGB1 were independent factors for refractory MPP (B□1.0, P<0.05). ROC analysis results showed that GM-CSF and HMGB1 at 2nd bronchoalveolar lavage therapy had predictive value for long hospital stay (>28 d) and poor prognosis of refractory MPP (AUC=0.782-0.825, P < 0.05).

Conclusion: The level of GM-CSF and HMGB1 in alveolar lavage fluid is closely related to the occurrence and development of refractory MPP, which can be used as an auxiliary indicator for clinical diagnosis and prognosis evaluation, and has certain guiding significance for its clinical treatment.

Backgrounds

Mycoplasma pneumoniae (MP) is one of the most common causes of community-acquired pneumonia (CAP) in children and young adults, with ability to cause local epidemics[1]. In general, Mycoplasma pneumoniae pneumonia (MPP) is mild and self-limiting, which could be rapidly improved by macrolide treatment. However, in recent years, the incidence of refractory MPP characterized by rapid development, long course of disease and severe pulmonary lesions has been increasing year by year[2]. In addition to improving the treatment methods, how to improve the early diagnosis rate and win the best treatment opportunity is also a focus of current researches[3]. Several mechanisms, including direct cytotoxicity, intracellular colonization, adsorption effect to respiratory epithelial cells, and causing immune dysfunction has been reported as so far, in which immune dysfunction, especially adaptive immune

dysfunction is considered to be the most important factor to promote the development of refractory MPP[4].

Despite being only a subsystem in the more basic and all-compassing system maintaining integrity, the adaptive immune system plays a key role in recognizing pathogens and distinguishing dead cells, thus clear harmful substances and block secondary damage[5]. It is reported that pathogen-associated molecular pattern molecules (PAMPs) and damage- associated molecular pattern molecules (DAMPs) are the most important adjuvants for adaptive immune system[6]. PAMPs has been identified galore, while there are only two types of typical DAMP as so far: high mobility Group Box 1 (HMGB1) protein and adenosine triphosphate proteins (ATP)[7]. HMGB1 is a kind of highly conserved encoded non-histone, nuclear DNA-binding protein widely found in eukaryotic cells. In addition to its classical role such as DNA recombination and cell differentiation, HMGB1 also sever as an inflammatory factor to participate in anti-pathogen activities.

Granulocyte macrophage colony stimulating factor (GM-CSF) was first detected in Lung tissue from mice cultured in vitro after LPS stimulation. Recent years, a few studies focused on GM-CSF expression in children with MPP, and found GM-CSF played an important role in the inflammatory response conducted by neutrophil in acute and severe MPP[8]. Liu Y, et al. [9] found the GM-CSF level of MPP Children was associated to the duration of fever and might be a good marker for prognosis. It was reported that the bronchial epithelium could produce GM-CSF in the stimulation of MP, which serve as a proinflammatory cytokines to recruit and activates neutrophils. Meanwhile, GM-CSF could also modulate oxidative stress activity through a priming phenomenon of human neutrophil respiratory burst. Otherwise, we noticed that both HMGB1 and GM-CSF has important effect on toll like receptor 2 -the main receptor to identify mycoplasma. In this study, we analyzed their relationship with patient clinical characteristics and prognosis, as a result, we found both GM-CSF and HMGB1 have prognostic value in determining disease status of refractory MPP.

Methods

Study population

A total of 106 children (including 47 refractory MMP) diagnosed with MPP and prepared for bronchoalveolar lavage therapy registered by the Affiliated Hospital of Changchun University of Traditional Chinese Medicine from July 2014 to November 2015 were selected for this study. All children had been diagnosed with the disease in accordance with the Guidelines for the management of community-acquired pneumonia in children. The refractory MMP cases were defined as those showing clinical and radiological deterioration despite appropriate antibiotic therapy for ≥ 7 days[10]. Children with congenital heart diseases, hereditary metabolic diseases, neurological disorders, bronchopulmonary dysplasia, and immunodeficiency were excluded. This research was conducted in compliance with the principles of the Declaration of Helsinki and approved by the ethics committee of Affiliated Hospital of

Changchun University of Traditional Chinese Medicine. Written informed consent was provided by all enrolled individuals.

Clinical data collection

Clinical parameters before and during the treatment were collected from patient medical records, including age, sex, course of fever, the highest temperature, white blood cell count (WBC), lymphocyte count (L), neutrophil count (N), blood platelet count (PLT), C-reaction protein (CRP), procalcitonin (PCT), D-dimer (D-D), aspartate aminotransferase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase isoenzyme (CK-MB), immunoglobulin (IgA, IgM and IgG), complement (C3 and C4), and multi detector computed tomography (MSCT) data.

Treatment methods

All children received a Bronchoalveolar lavage therapy defined by Guidelines for the management of community-acquired pneumonia in children consistent with the individual condition of each patient. In this study, the children in non-refractory MMP group accepted bronchoalveolar lavage therapy only once, while those in refractory MMP group accepted 2 to 4 times according to individual condition. The lavage fluids we used was prepared with 50 ml saline, 80,000u gentamicin, and 5 mg dexamethasone. Cases with localized pulmonary lesions was douched in the suspicious bronchopulmonary segment, while cases with diffuse interstitial pulmonary lesions was douched in the right middle lobe or left lingulaoflung. In addition, all children were given supportive treatment such as sensitive antibiotic, antitussive, anticonvulsant, sputum aspiration, parenteral nutrition, etc.

Quantification of GM-CSF and HMGB1 level in bronchoalveolar lavage fluid (BALF)

BALF was extracted after bronchoalveolar lavage therapy, and the cells in the BALF samples was separated by a centrifuge at 200 g for 10 min at 4 ℃, then stored at -80 ℃. The levels of HMGB1 and GM-CSF in those cells were quantified by ELISA kits purchased from Shanghai Meilian Biotechnology Co. Ltd (Shanghai, China) in a week. The experimental procedure is strictly carried out according to the instructions, in a word, each standard or sample was placed into the appropriate wells of 96-well plates, and incubated with biotinylated detection antibody adequately. Then, HRP-streptavidin solution was added and incubated, each plate was again inverted and washed prior to the addition of 100 μl of ELISA Colorimetric TMB Reagent. The absorbance was measured using a Universal Microplate Spectrophotometer at 450 nm.

Prognostic assessment methods

Most children in non-refractory MMP group got good prognosis and discharged from hospital in 7 to 15 days, so we mainly observed the effect of refractory MMP group. The hospital stay time and pulmonary function on discharge were assessed. Those with respiratory dysfunction such as pulmonary fibrosis, unilateral hyperlucent lung syndrome and asthma., etc. were classified into poor prognosis.

Statistical analysis

Statistical analyses were performed using SPSS software (version 22.0). All normally distributed quantitative variables are expressed as means ± SD, the remainder were expressed as median (IQR) values. ANOVA and LSD-t tests were performed to compare differences among groups for normally distributed variables, and Mann-Whitney U tests were performed for variables not normally distributed. Categorical data are expressed as percentages, and chi-square tests were performed to compare differences between groups. Kruskal–Wallis H test was performed for comparisons between groups with ranked data. In addition, ROC curve analysis was used to ascertain the predicted value of BALF HMGB1 and GM-CSF for the occurrence of refractory MMP and poor prognosis of refractory MMP.

Results

Clinical characteristics of children in refractory MPP group and non-refractory MPP group before treatment

The clinical characteristics of children before treatment were shown in Table 1. There was no significant difference in age, sex, course of fever, the highest temperature, WBC, L, PLT, ALT, AST, CK-MB, D-D, CK, IgG, IgA, IgM, C3, and C4 between refractory MPP group and non-refractory MPP group. The levels of N, CRP, PCT, and LDH in refractory MPP group were all higher than those in non-refractory MPP group, the difference between groups had statistical significance (P□0.05).

Table 1

The basic data compare between children with and without refractory MMP.

Clinical characteristics	Refractory MMP group (n = 47)	Non-refractory MMP group (n = 59)	χ^2/t	P
Age (IQR, months)	35 (24, 66)	31 (24, 59)	-1.314	0.185
Male [n(%)]	23 (48.9%)	32 (54.2%)	0.294	0.587
Course of fever (IQR, d)	8 (4, 12)	4 (3, 6)	-1.217	0.224
The highest temperature ≥ 40 °C [n (%)]	11 (23.4%)	9 (15.3%)	1.135	0.287
WBC (IQR, 10^9/L)	9.22 (4.35, 25.25)	8.65 (5.01, 23.52)	-0.757	0.449
N (IQR, 10^9/L)	5.55 (2.15, 13.25)	4.82 (2.22, 11.12)	-2.070	0.038
L (IQR, 10^9/L)	3.02 (1.84, 9.25)	3.37 (2.01, 10.36)	-1.283	0.199
PLT (IQR, 10^9/L)	322.5 (85.3, 488.2)	313.2 (102.3, 451.3)	-1.141	0.254
CRP (IQR, mg/L)	25.34 (15.52, 52.31)	11.54 (6.33, 43.62)	-2.563	0.010
PCT (IQR, μg/L)	6.53 (1.52, 28.62)	7.15(1.44, 22.63)	-2.178	0.029
ALT (IQR, U/L)	22.13 (18.25, 84.6)	25.24 (15.25, 75.62)	-1.512	0.131
AST (IQR, U/L)	34.35 (29.38, 44.25)	36.25 (30.24, 42.15)	-0.998	0.318
LDH (IQR, U/L)	322.33 (125.32, 382.63)	302.52 (116.85, 335.21)	2.308	0.023
D-D (mean ± SD, mg/L)	3.36 ± 0.83	3.12 ± 0.91	1.402	0.164
CK (mean ± SD, U/L)	201.35 ± 46.52	185.63 ± 40.36	1.861	0.066
CK-MB (mean ± SD, U/L)	12.25 (6.35, 18.35)	8.22 (6.02, 16.21)	-0.016	0.987
IgG (mean ± SD, g/L)	9.15 ± 2.35	9.28 ± 2.52	0.272	0.786
IgA (mean ± SD, g/L)	0.81 ± 0.21	0.88 ± 0.19	1.798	0.075
IgM (mean ± SD, g/L)	1.35 ± 0.26	1.46 ± 0.31	1.947	0.054
C3 (mean ± SD, g/L)	1.26 ± 0.25	1.32 ± 0.28	1.149	0.253

WBC: white blood cell count; PLT: blood platelet count; L: lymphocyte count, N: neutrophil count; CRP: C-reactive protein; PCT: procalcitonin; D-D: D -dimer; AST: aspartate aminotransferase; ALT: alanine transaminase; LDH: lactate dehydrogenase; CK: creatine kinase; CK-MB: creatine kinase isoenzyme; IgA: A type immunoglobulin; IgM: M type immunoglobulin; IgG: G type immunoglobulin; C3: complement 3; C4: complement 4; BALF: bronchoalveolar lavage fluid; GM-CSF: granulocyte-macrophage colony-stimulating factor; HMGB1: high mobility group box 1.

Clinical characteristics	Refractory MMP group (n = 47)	Non-refractory MMP group (n = 59)	χ^2/t	P
C4 (mean ± SD, g/L)	0.32 ± 0.08	0.35 ± 0.10	1.673	0.097

WBC: white blood cell count; PLT: blood platelet count; L: lymphocyte count, N: neutrophil count; CRP: C-reactive protein; PCT: procalcitonin; D-D: D -dimer; AST: aspartate aminotransferase; ALT: alanine transaminase; LDH: lactate dehydrogenase; CK: creatine kinase; CK-MB: creatine kinase isoenzyme; IgA: A type immunoglobulin; IgM: M type immunoglobulin; IgG: G type immunoglobulin; C3: complement 3; C4: complement 4; BALF: bronchoalveolar lavage fluid; GM-CSF: granulocyte-macrophage colony-stimulating factor; HMGB1: high mobility group box 1.

The relationship between BALF HMGB1 and GM-CSF with clinical characteristics of MPP

The levels of BALF HMGB1 and GM-CSF in refractory MPP group were all higher than those in non-refractory MPP group, the difference between groups had statistical significance (P□0.05). Both GM-CSF and HMGB1 levels were positively correlated with traditional indicators N, CRP, PCT and LDH (r = 0.611–0.785, P < 0.05). Their scatter plots and associated lines were shown in Fig. 1.

The relationship between BALF HMGB1 and GM-CSF with refractory MMP attack

We analyzed the diagnostic efficiency of N, CRP, PCT, LDH, HMGB1, and GM-CSF for refractory MPP. ROC analysis results (Fig. 2 and Table 2) showed that CRP, GM-CSF and HMGB1 had predictive value for refractory MPP attack (AUC = 0.636, 0.657, 0.651, P < 0.05). Logistic regression analysis results (Table 3) showed that GM-CSF and HMGB1 were independent influencing factors for refractory MPP (OR > 1.0, P < 0.05).

Table 2

ROC analysis results for each index to predict the occurrence of refractory MMP.

Indexes	Area	Sig.	95% C.I.		OOP	sensitivity	specificity
			Lower	Upper			
N	0.508	0.891	0.395	0.620	3.395	0.660	0.390
CRP	0.636	0.016	0.529	0.744	17.330	0.745	0.610
PCT	0.523	0.684	0.411	0.635	24.980	0.298	0.780
LDH	0.559	0.297	0.448	0.670	413.090	0.468	0.695
HMGB1	0.657	0.006	0.552	0.762	6.760	0.532	0.712
GM-CSF	0.651	0.008	0.539	0.763	6.745	0.468	0.949

N: neutrophil count; CRP: C-reactive protein; PCT: procalcitonin; LDH: lactate dehydrogenase; HMGB1: high mobility group box 1; GM-CSF: granulocyte-macrophage colony-stimulating factor; OPP: optimum operating point

Table 3

Logistic regression analysis results for the occurrence of refractory MMP.

Indexes	B	S.E.	Wald	Sig.	Exp(B)	95% C.I.for EXP(B)	
						Lower	Upper
HMGB1	0.664	0.250	7.057	0.008	1.943	1.190	3.173
GM-CSF	0.755	0.303	6.217	0.013	2.128	1.175	3.854
N	-0.772	0.284	7.380	0.007	0.462	0.265	0.806
CRP	0.069	0.048	2.076	0.150	1.071	0.976	1.176
PCT	-0.095	0.087	1.209	0.271	0.909	0.767	1.077
LDH	-0.131	0.137	0.914	0.339	0.878	0.671	1.147
Constant	-0.554	0.664	0.696	0.404	0.575	--	--

N: neutrophil count; CRP: C-reactive protein; PCT: procalcitonin; LDH: lactate dehydrogenase; HMGB1: high mobility group box 1; GM-CSF: granulocyte-macrophage colony-stimulating factor; OPP: optimum operating point

The relationship between BALF HMGB1 and GM-CSF with prognosis of refractory MPP

The hospital stay time of refractory MPP group were 16 to 37 days. There were 7 cases of poor prognosis in all, including 1 pulmonary fibrosis, 1 cryptogenic organizing pneumonia, 1 unilateral hyperlucent lung syndrome, and 1 asthma. The BALF HMGB1 and GM-CSF level decreased with the treatment, and the levels at 2nd bronchoalveolar lavage therapy had predictive value for long hospital stay (> 28d) and poor prognosis of refractory MPP (Fig. 3).

Discussion

Serum specific antibody test is the most common clinical method for the diagnosis of MP infection, but the MP antibody usually appears a week after the attack of disease, and children' MP antibody appears even later because of their poor and not fully developed immune system function[11]. The delay in clinical diagnosis contributes greatly to disease progression and the attack of refractory MPP. With the progression of refractory MPP, some cases might develop into long-term respiratory dysfunction or life-threatening complications[12, 13]. More and more studies focused on finding new methods or new biochemical markers for early diagnosis of refractory MPP in recent years. For example, Zhang Y, et al. [2] compared the clinical data between 145 refractory MPP patients and 489 general MMP patients, and found CRP, LDH, and IL-6 might be the significant predictors of children refractory MPP. Liu T, et al. [14] claimed serum lactate dehydrogenase isoenzymes 4 plus 5 had better sensitivity compared to traditional index LDH for refractory MPP. Cheng S, et al. [15] develop a nomogram for predicting refractory MPP, in which high fever, albumin, and LDH served as important factors.

We measured BALF HMGB1 and GM-CSF in this study for two reasons: the BALF sample derived directly from the lower respiratory tract, which means a less chance of pollution, and a closer distance to the lesion. Several studies pointed out that BALF culture has higher pathogen detection rate than sputum culture[16]. We also considered the anti-pathogen activities of immune system after MP infection, HMGB1 is mainly released by mononuclear macrophages and dendritic cells and plays a role in the adaptive immune phase[17], while GM-CSF is mainly released by activated T cells and play role in the cellular immune phase. After released outside the cell, both of them plays a synergistic role with various pro-inflammatory factors and forms a positive feedback loop to amplify the inflammatory response. Besides BALF HMGB1 and GM-CSF, we found 4 traditional biochemical markers difference between refractory MPP and non-refractory MPP: N, CRP, PCT, LDH. We also found there were a good correlation between GM-CSF and HMGB1 levels with those traditional indicators N, CRP, PCT and LDH ($r = 0.611–0.785$, $P < 0.05$).

Serve as immune cells, neutrophils play an important role in anti-pathogens[18]. Increased neutrophils in peripheral blood and alveolar lavage fluid are important clinical features of mycoplasma pneumonia[19]. CRP and PCT are both inflammatory markers commonly used in clinic. A retrospective study involved 119 children with community-acquired pneumonia revealed that PCT on admission is correlated to inflammatory response degree, while CRP on admission is a predictor of lobar consolidation[20]. Neeser, et al.[21] found CRP/PCT ratio could provide reliable information to help discriminating MPP from streptococcus pneumoniae. Several studies claimed PCT concentrations in children hospitalized with

CAP had ability to distinguish typical bacteria (eg, Streptococcus pneumoniae and Staphylococcus aureus) and atypical bacteria (Mycoplasma pneumoniae and Chlamydophila pneumoniae) [22–24]. LDH occurs in the blood when tissues and organs are damaged, so it is used to be a biomarker to evaluate disease severity of MPP in clinic[25]. There was evidence that LDH is an easily accessible biological marker that has been associated with several pulmonary disorders[26, 27]. A prospective cohort study of 300 children with refractory pneumonia and 353 with general pneumonia showed serum LDH with a cutoff of 379 U/L could be used to predict refractory MPP at early stage of hospitalization [28]. In brief, the traditional markers N, CRP, PCT, and LDH have good predictive value for the occurrence and development of MPP, and the correlation between BALF HMGB1 and GM-CSF with them indicate a potential value of HMGB1and GM-CSF in MPP evaluation. After Logistic analysis and ROC analysis, we found CRP, GM-CSF and HMGB1 had predictive value for refractory MPP attack, and GM-CSF and HMGB1 were independent factors for refractory MPP. The results further confirmed their important diagnostic value.

In addition, we found the BALF HMGB1 and GM-CSF level decreased with the treatment. Considering that the number of cases decreased greatly at 3rd bronchoalveolar lavage therapy, we analyzed the prognostic evaluation effect of BALF HMGB1 and GM-CSF level in the first two treatments. The results showed the levels at 2nd bronchoalveolar lavage therapy had predictive value for long hospital stay (> 28d) and poor prognosis of refractory MPP. A few studies have showed HMGB1 might be a reliable biochemical marker for prognosis evaluation of MPP and community acquired pneumonia [29, 30], which consistent with our study. The clinical studies about BALF GM-CSF in MPP are limited quantity. Previous studies have shown that GM-CSF plays a vital role in neutrophil inflammation in M. A proper GM-CSF secretion is beneficial for lung protection, while increased GM-CSF in pulmonary could causes alveolar macrophage accumulation, which contribute to a lung parenchymal injury derived excessive immune inflammatory response[31]. This may be one of the reasons for GM-CSF level lead to poor prognosis.

Conclusions

In conclusion, the level of GM-CSF and HMGB1 in alveolar lavage fluid is closely related to the occurrence and development of refractory MPP, which can be used as an auxiliary indicator for clinical diagnosis and prognosis evaluation, and has certain guiding significance for its clinical treatment.

Abbreviations

Granulocyte-macrophage colony-stimulating factor GM-CSF

High mobility group box 1 HMGB1

Mycoplasma pneumoniae pneumonia MPP

Mycoplasma pneumoniae MP

Community-acquired pneumonia CAP

Damage- associated molecular pattern molecules DAMPs

Pathogen-associated molecular pattern molecules PAMPs

Adenosine triphosphate proteins ATP

White blood cell count WBC

Lymphocyte count L

Neutrophil count N

Blood platelet count PLT

C-reaction protein CRP

Procalcitonin PCT

D-dimer D-D

Aspartate aminotransferase AST

Alanine transaminase ALT

Lactate dehydrogenase LDH

Creatine kinase CK

Creatine kinase isoenzyme CK-MB

Immunoglobulin A IgA

Immunoglobulin M IgM

Immunoglobulin G IgG

Complement 3 C3

Complement 4 C4

Multi detector computed tomography MSCT

Declarations

Ethics approval and consent to participate

This research was conducted in compliance with the principles of the Declaration of Helsinki and approved by the ethics committee of Affiliated Hospital of Changchun University of Traditional Chinese Medicine. Written informed consent was provided by all enrolled individuals and the parent's patient has given their parental consent for this study.

Consent for publication

Approval.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

None of the authors of the present manuscript have a commercial or other association that might pose a conflict of interest.

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None.

Authors'contributions

LC and YW conceived the study. HL acquired data. HY performed the statistical analysis. LC drafted the manuscript. All authors read and approved the manuscript prior to submission.

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Figures

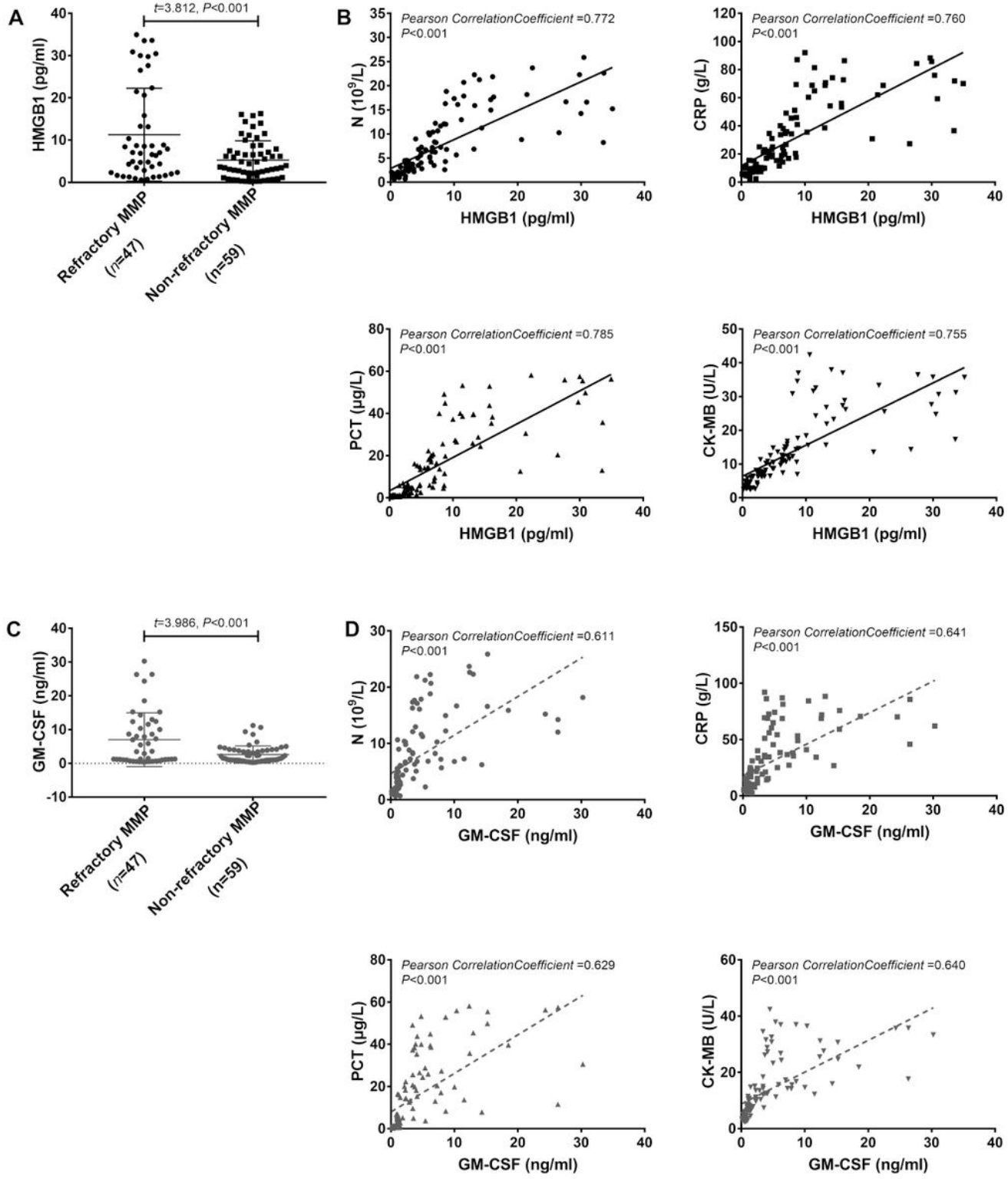

Figure 1

The scatter plots and associated lines of BALF HMGB1 and GM-CSF with N, CRP, PCT, and LDH. A：BALF HMGB1 levels compare between refractory MPP and non-refractory MPP. B: The correlation lines between BALF HMGB1 with N, CRP, PCT, LDH. C: BALF GM-CSF levels compare between refractory MPP and non-refractory MPP. D: The correlation lines between BALF GM-CSF with N, CRP, PCT, LDH.

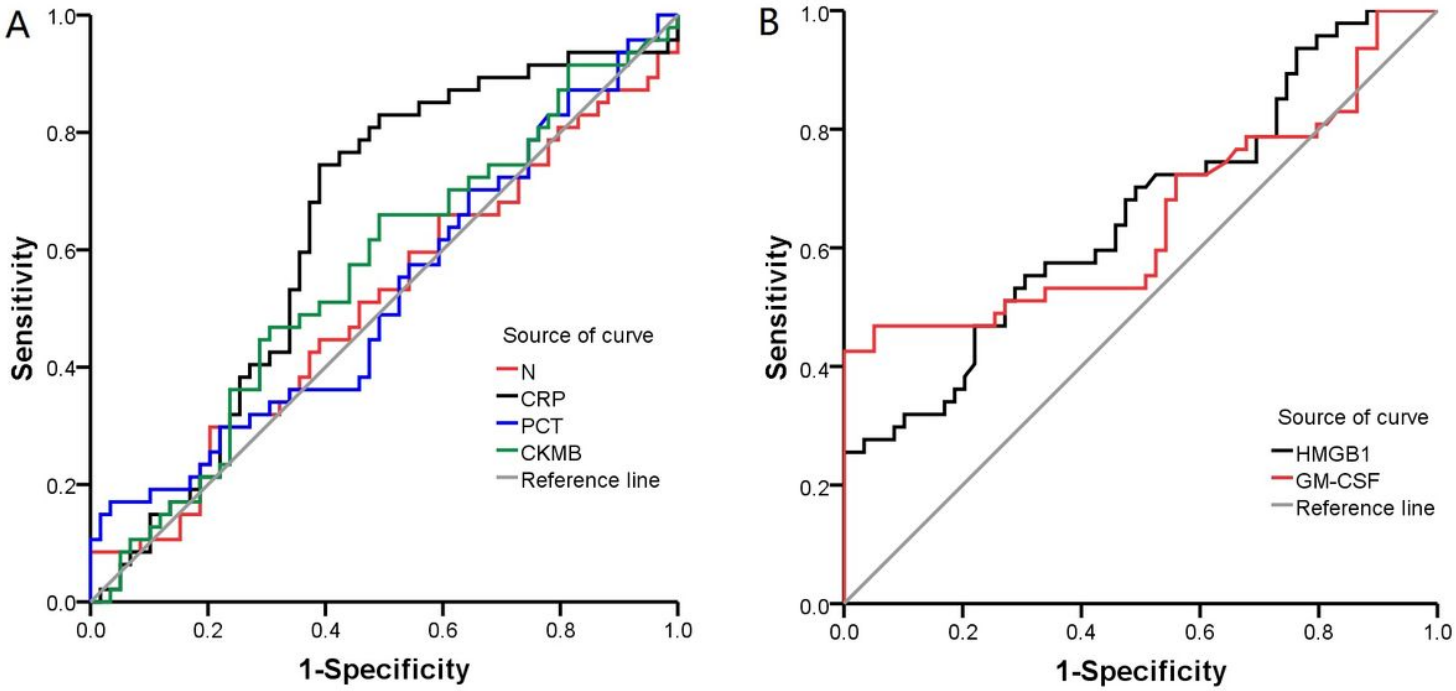

Figure 2

ROC analyze results for N, CRP, PCT, LDH, BALF HMGB1 and GM-CSF to predict refractory MPP attack. A ROC analyze for traditional indexes N, CRP, PCT, and LDH. B: ROC analyze for BALF HMGB1 and GM-CSF.

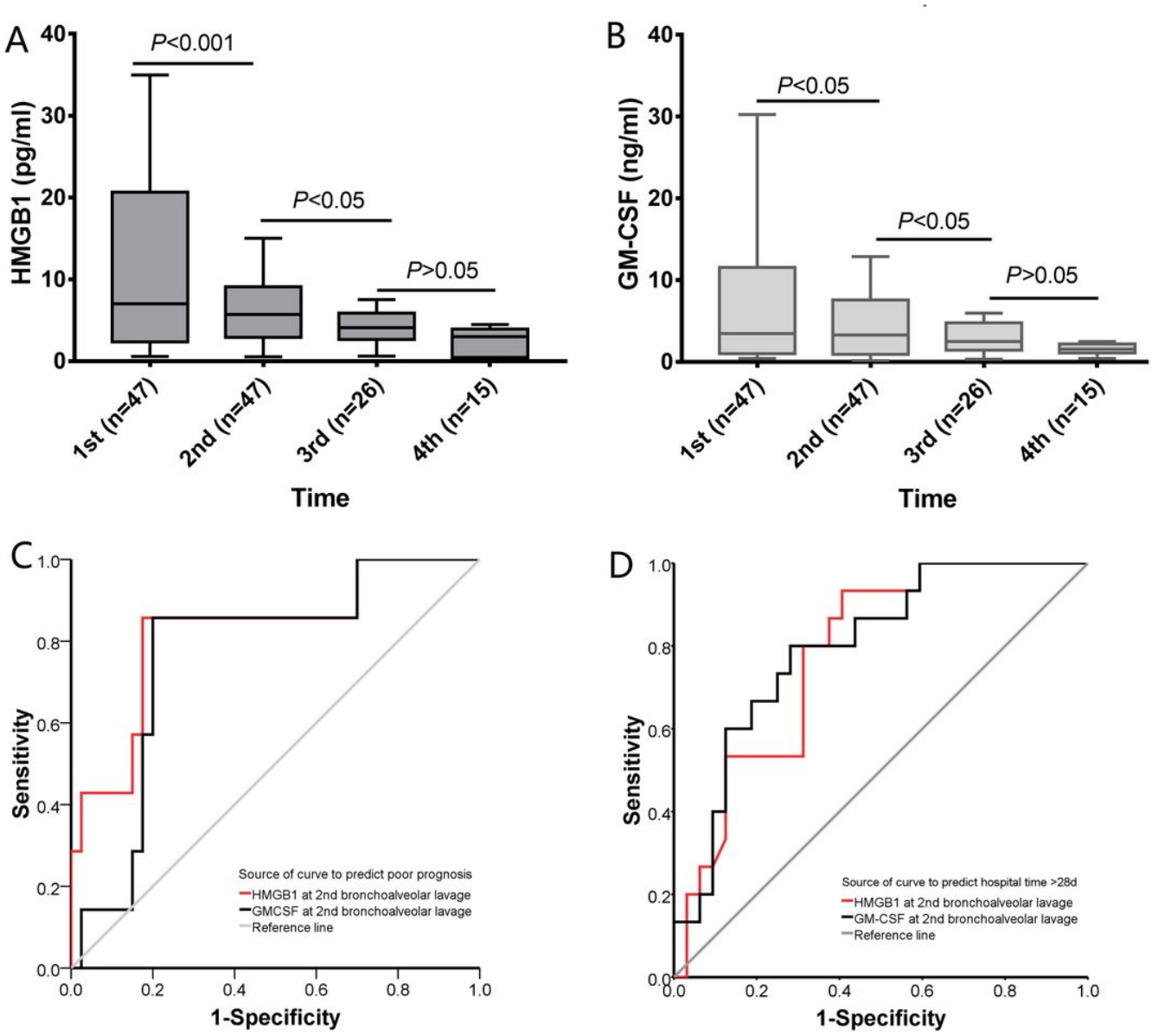

Figure 3

Changes of BALF HMGB1 and GM-CSF in treatment and their predictive value for prognosis. A BALF HMGB1 change trend in the bronchoalveolar lavage therapy. B: BALF GM-CSF change trend in the bronchoalveolar lavage therapy. C: ROC analyze results for BALF HMGB1 and GM-CSF at 2nd bronchoalveolar lavage therapy to predict poor prognosis; AUC=0.768, 0.825, P=0.025, 0.007. D: ROC analyze results for BALF HMGB1 and GM-CSF at 2nd bronchoalveolar lavage therapy to predict long hospital stay time; AUC=0.782, 0.798, P=0.002, 0.001.