Journal of Clinical and Translational Hepatology **2026** vol. 14(1) | 31–38
DOI: 10.14218/JCTH.2025.00645

Original Article

Serum Interleukin-8 for Differentiating Invasive Pulmonary Aspergillosis from Bacterial Pneumonia in Patients with HBV-Associated Acute-on-Chronic Liver Failure

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Received: November 25, 2025 | **Revised:** December 16, 2025 | **Accepted:** December 21, 2025 | **Published online:** December 26, 2025

Abstract

Background and Aims: Infections are frequent and lethal complications of acute-on-chronic liver failure (ACLF). Reliable biomarkers to distinguish fungal from bacterial infections remain limited. Given the central role of immune dysfunction in ACLF, we aimed to evaluate the diagnostic value of serum cytokines in differentiating invasive pulmonary aspergillosis (IPA) from bacterial pneumonia (BP) in HBV-associated ACLF. ***Methods:*** This retrospective case-control study enrolled ACLF patients admitted to the Tongji Hospital, between 2018 and 2022. Patients were categorized into IPA, BP, and non-infection groups. The BP and non-infection groups were propensity score-matched to the IPA cases. Serum cytokines levels (IL-1β, sIL-2R, IL-6, IL-8, IL-10, TNF-α) and clinical data were collected, with the diagnostic performance of these cytokines as biomarkers assessed via ROC curves. ***Results:*** A total of 32 IPA, 96 BP, and 96 non-infection patients were enrolled, with balanced baseline characteristics. Compared with the non-infection group, the IPA group had higher sIL-2R (1,606.00 vs. 1,211.50 U/mL, P = 0.019) and IL-6 (69.03 vs. 15.98 pg/mL, P < 0.001) levels, but lower IL-8 levels (62.20 vs. 132.00 pg/mL, P = 0.025). The BP group showed elevated sIL-2R (1,792.00 U/mL), IL-6 (49.42 pg/mL), IL-10 (13.40 pg/mL) levels compared to the non-infection group (all P < 0.001). Also, IL-8 was lower in the IPA group than in the BP group (62.20 vs. 176.00 pg/mL, P < 0.001) and its assessment could best distinguish IPA from BP (AUC = 0.743, cut-off = 76.60 pg/mL; sensitivity = 66.7%, specificity = 82.1%). ***Conclusions:*** Serum IL-8 exhibited superior diagnostic value for IPA in patients with HBV-ACLF and could effectively discriminate *Aspergillus* infections from bacterial infections.

Citation of this article: Huang L, Feng Y, Wang W, Liu W, Liu Y, Chen L, *et al*. Serum Interleukin-8 for Differentiating Invasive Pulmonary Aspergillosis from Bacterial Pneumonia in Patients with HBV-Associated Acute-on-Chronic Liver Failure. J Clin Transl Hepatol 2026;14(1):31–38. doi: 10.14218/JCTH.2025.00645.

Introduction

Hepatitis B virus (HBV) infection remains a major global health challenge. In particular, HBV-related acute-on-chronic liver failure (HBV-ACLF) represents a life-threatening clinical syndrome associated with high short-term mortality. It is characterized by a rapid deterioration in liver function, and it especially affects populations in the Asia-Pacific and African regions, where short-term mortality rates can range from 50% to 60%.[1] Such infection not only triggers the progression of chronic liver disease into ACLF, but also exacerbates the condition, leading to higher mortality rates.[2,3]

Liver failure severely affects the immune system, leading to a dysregulation of both innate and adaptive immune responses, accompanied by a pronounced systemic inflammatory response and immunosuppression.[4] In liver failure, neutrophil and macrophage responses are decreased, the proportion of natural killer cells is reduced, and the functions of B cells and T cells are impaired, alongside an excessive secretion of inflammatory factors, resulting in what is often termed a "cytokine storm".[4] Patients with ACLF enter a state of multifactorial immunocompromise, rendering them more susceptible to infections.[5] Although less prevalent than bacterial infections, fungal infections have been increasingly recognized as key contributors to severe ACLF, and are associated with higher rates of intensive care unit admissions and disproportionately high mortality rates.[6–8] Among such cases, invasive pulmonary aspergillosis (IPA) poses a particularly grave threat, with reported mortality rates up to 81.80% in patients with ACLF.[9] Despite its relatively low incidence, with prevalence estimates ranging from 1.85% to 9.88% in general ACLF patients,[8,10] the clinical burden of IPA is substantial. Previous studies have identified several risk factors for IPA among patients with ACLF, such as an advanced age, hepatic encephalopathy,

Keywords: Interleukin 8; Invasive pulmonary aspergillosis; Bacterial pneumonia; Differential diagnosis; Acute-on-chronic liver failure.

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Table 1. Diagnostic criteria for determining IPA in patients with ACLF

Proven IPA	Histopathologic, cytopathologic, or direct microscopic examination of tissue invasion by septate, acutely branching filamentous fungi and/or by a positive culture for *Aspergillus* in a normally sterile specimen
Probable IPA	(1) Host risk factor: ACLF
	(2) Clinical features: abnormal radiological imaging compatible with lung infection
	(3) Mycological criteria: direct test (cytology, direct microscopy or cultures) indicating the presence of *Aspergillus* species or galactomannan antigen detected in serum or BALF
Possible IPA	Presence of a host factor and classical radiological features (dense, well-circumscribed lesion(s) with or without a halo sign, air-crescent sign and cavity), but absence of mycological criteria

IPA, invasive pulmonary aspergillosis; ACLF, acute-on-chronic liver failure; BALF, bronchoalveolar lavage fluid.

and extended exposure to glucocorticoids or broad-spectrum antibiotics.[8,10,11] The high prevalence and incidence of IPA in such patients, combined with the extreme mortality, make IPA an increasingly important cause of death in this vulnerable population, yet it remains under-recognized.[9] An early initiation of aggressive systemic antifungal therapy is crucial for improving patient outcomes. However, traditional diagnostic approaches for fungal infections have notable limitations. Despite notable advances in recent years in the development of galactomannan (GM) tests, PCR technology, *Aspergillus*-specific lateral flow chromatography devices, and microbial next-generation sequencing (mNGS), their performance still varies, and there remains a need for additional ancillary tests to further enhance the detection of fungal infections in the lungs.

Cytokines are key mediators of host immune responses to fungal pathogens and have emerged as promising early biomarkers for IPA. In patients with hematological disorders and chronic obstructive pulmonary disease (COPD), interleukin (IL)-6, IL-8, and IL-10 have demonstrated strong predictive value for IPA.[12–15] It has been reported that *Aspergillus* species drive T-helper (Th)1 and Th17 responses,[16–18] leading to a rapid elevation of specific cytokines and chemokines that often precede elevations in conventional inflammatory markers, such as C-reactive protein (CRP) and procalcitonin (PCT). This early kinetic profile underscores the potential application of cytokine-based biomarkers for the timely detection of IPA.

Therefore, in this proof-of-concept study, we aimed to evaluate the diagnostic utility of serum cytokines (specifically, IL-1β, sIL-2R, IL-6, IL-8, IL-10, and TNF-α) as potential biomarkers for distinguishing IPA from bacterial infections in patients with HBV-ACLF, building on evidence that the responses of specific cytokines in fungal infections occur both early on and are pathogen-specific.

Methods

Study design

In this proof-of-concept study, we performed a retrospective, single-center matched case-control study on hospitalized patients with HBV-ACLF treated in the Department of Infectious Diseases, Tongji Hospital, Huazhong University of Science and Technology in Wuhan. The study period was between January 2018 and December 2022. Cases and controls were identified, with cases defined as those patients presenting with proven or probable IPA. Patients with "possible" IPA were excluded. Eligible patients were categorized into three groups based on their infection status at enrollment: IPA group, comprising patients with confirmed IPA according to established international criteria; BP group, comprising patients with confirmed BP without clinical, microbiological, or radiological evidence of IPA; and the non-infection group, comprising patients with no clinical or laboratory evidence of any active infection. The patients in the IPA group were enrolled consecutively. For the two other (control) groups, propensity score matching (PSM) was applied to select patients with bacterial pneumonia and those without infection, matched to IPA cases based on demographic and clinical covariates. Controls were individually matched with the cases based on previously determined factors that it was considered could potentially influence the outcome and cytokine levels: gender, similar age (+/- 5 years), identical underlying diseases, and the same treatment regimens. We recorded the patients' baseline information and CRP, PCT, and cytokine levels during hospitalization.

The study was approved by the Medical Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (Approval No. 2023S043) and adhered to the principles outlined in the Declaration of Helsinki. Written informed consent was waived due to the retrospective nature of the study.

Inclusion and exclusion criteria

ACLF was diagnosed based on the criteria established by the Chinese Group on the Study of Severe Hepatitis B (COSSH) in 2017.[1] Patients were excluded if they met any of the following criteria: (1) viral infections (not including HBV infection); (2) hepatocellular carcinoma or other malignancies; (3) other etiologies-induced hepatitis (non-alcoholic steatohepatitis, alcoholic hepatitis, hepatitis C, hepatitis D, autoimmune hepatitis, etc.); (4) liver transplantation; (5) pregnancy; (6) age <18 years old or >75 years old; or (7) any other type of immunodeficiency.

Diagnostic criteria

IPA was classified according to a slightly modified version of the 2020 European Organization of Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) definitions, incorporating revised host criteria as shown in Table 1.[19,20] ACLF was added as a host factor because the existing criteria were developed primarily for patients with hematological malignancies or profound immunosuppression, and have not been validated in non-neutropenic populations at risk of IPA. Similar approaches have been applied in previous studies investigating the prevalence of IPA in non-neutropenic patients.[8,21–24]

Cytokines measurement

All the laboratory analyses were performed by the Clinical Laboratory Department of Tongji Hospital. Blood samples were collected in coagulation-promoting tubes containing an inert separation gel and centrifuged at 2,000 × g for 10

Table 2. Characteristics and clinical features of the patients with ACLF in the IPA, BP, and non-infection groups

	IPA group (N = 32)	BP group (N = 96)	Non-infection group (N = 96)	*P* value
Male, n (%)	26 (81.25%)	78 (81.25%)	78 (81.25%)	1.000
Age (years)	55.00 (41.25, 62.75)	51.50 (42.00, 61.00)	52.50 (39.00, 59.75)	0.526
Liver cirrhosis,n (%)	17 (53.13%)	58 (60.42%)	47 (48.96%)	0.277
Underlying diseases, n (%)				
Hypertension	4 (12.50%)	13 (13.54%)	14 (43.75%)	0.951
Diabetes	7 (21.88%)	20 (20.83%)	20 (20.83%)	0.991
Respiratory disease	3 (9.38%)	5 (5.21%)	4 (4.17%)	0.524
Chronic renal disease	2 (6.25%)	5 (5.21%)	3 (3.13%)	0.681
Smoking history	15 (46.88%)	38 (39.58%)	37 (38.54%)	0.698
Laboratory examination				
Total bilirubin, μmol/L	388.45 (315.80, 496.65)	338.80 (215.65, 435.88)	329.55 (248.18, 430.58)	0.072
INR	21.3 (1.89, 3.27)	2.21 (1.74, 2.77)	2.02 (1.68, 2.39)	0.074
Creatinine, μmol/L	77.00 (56.50, 115.25)	71.00 (55.00, 92.75)	67.00 (56.25, 81.00)	0.269
HBV DNA, $\log_{10}$ IU/mL	4.58 (2.78, 6.09)	4.20 (2.66, 6.04)	5.06 (4.05, 6.16)	0.051
CRP, mg/L	19.45 (13.25, 40.88)[b]	15.20 (9.80, 32.30)[b]	9.00 (5.80, 13.25)	<0.001
PCT, ng/mL	1.04 (0.44, 2.45)[b]	0.79 (0.47, 1.58)[b]	0.53 (0.33, 0.72)	<0.001

"a", $P < 0.05$, compared with the BP group; "b", $P < 0.05$, compared with the non-infection group. IPA, invasive pulmonary aspergillosis; ACLF, acute-on-chronic liver failure; BP, bacterial pneumonia; INR, international normalized ratio; CRP, C-reactive protein; PCT, procalcitonin.

minutes at 4 °C. Serum was separated immediately after centrifugation and processed for analysis. Cytokines levels, including IL-1β, sIL-2R, IL-6, IL-8, IL-10, and TNF-α, were quantified in the separated serum samples using a fully automated chemiluminescent immunoassay system (Immulite 1000, Siemens Healthineers, Germany) following the manufacturer's instructions.

Statistical analysis

All the data were analyzed with IBM SPSS statistical analysis software (version 26.0, Chicago, USA) and GraphPad Prism (version 8.0). A two-sided p-value of $P < 0.05$ was considered statistically significant. Categorical variables are reported as proportions, and continuous variables as medians plus the interquartile range (IQR). Comparisons between groups were performed using the chi-square test for categorical variables, and the Mann–Whitney U test or Kruskal–Wallis test for non-parametric continuous variables. *P* values were corrected for multiple comparisons. The diagnostic accuracy was assessed by receiver operating characteristic (ROC) curve analysis, with the area under the curve (AUC) calculated for each parameter. The optimal cut-off values were determined using the maximum Youden index. GraphPad Prism 8 software was utilized to generate distribution plots.

Results

Baseline characteristics of the study population

In total, 1,345 patients with HBV-ACLF were initially enrolled in this study. Among these, 77 (5.72%) were diagnosed with IPA; however, only 32 patients with IPA had complete serum cytokine profiles available at the time of diagnosis and were therefore included in the final analysis. The final study cohort comprised 224 patients, who were divided into the IPA group (N = 32), the BP group (N = 96), and the non-infection group (N = 96), as shown in Table 2. After PSM, the patients' baseline characteristics were well-balanced across the three groups, with no significant differences observed among the three groups in terms of their demographic characteristics (including sex and age distribution), or in the prevalence of liver cirrhosis or comorbidities (including hypertension, diabetes, respiratory diseases, chronic renal disease, and smoking history). Regarding the laboratory parameters reflecting disease severity, no significant differences were found among the three groups in terms of the levels of total bilirubin, international normalized ratio (INR), creatinine, or HBV DNA load. This successful matching was important as it minimizes the risk of confounding issues related to the baseline variables and strengthens the internal validity of the subsequent comparisons of the cytokine profiles and clinical outcomes. In terms of the inflammatory indicators, both the CRP (19.45 vs. 9.00 mg/L, $P < 0.001$) and PCT (1.04 vs. 0.53 ng/mL, $P < 0.001$) levels were found to be significantly elevated in the IPA group compared to in the non-infection group, and in the BP group compared to in the non-infection group (CRP: 15.20 vs. 9.00 mg/L, $P < 0.001$; and PCT: 0.79 vs. 0.53 ng/mL, $P < 0.001$). However, there were no significant differences observed in the CRP or PCT levels in the IPA and BP groups.

Expression levels of cytokines in the IPA, BP, and non-infection groups

As shown in Table 3, no significant differences were observed in the IL-1β or TNF-α levels among the three groups, whereas the sIL-2R, IL-6, IL-8, and IL-10 levels were significantly different among the groups (Fig. 1). In particular, compared to the non-infection group, the IPA group exhibited significantly higher levels of sIL-2R (1,606.00 vs. 1,211.50 U/mL, $P = 0.019$) and IL-6 (69.03 vs. 15.98 pg/mL, $P < 0.001$), but significantly lower IL-8 levels (62.20 vs. 132.00 pg/mL, $P = 0.025$). The BP group also showed significantly elevated

Table 3. Serum cytokine expressions of the patients in the IPA, BP, and non-infection groups

	IPA group (N = 32)	BP group (N = 96)	Non-infection group (N = 96)	*P* value
IL-1ß, pg/mL	14.60 (6.10, 29.30)	12.10 (5.40, 21.20)	14.25 (7.18, 27.00)	0.532
sIL-2R, U/mL	1,606.00 (1,119.00, 2,312.00)[b]	1,792.00 (1,126.00, 2,244.00)[b]	1,211.50 (823.75, 1,553.50)	<0.001
IL-6, pg/mL	69.03 (35.11, 251.03)[b]	49.42 (25.25, 89.44)[b]	15.98 (11.34, 27.27)	<0.001
IL-8, pg/mL	62.20 (51.90, 130.00)[ab]	176.00 (82.00, 421.30)	132.00 (65.45, 290.75)	0.001
IL-10, pg/mL	8.60 (6.40, 18.40)	13.40 (7.10, 21.10)[b]	7.41 (5.00, 13.05)	<0.001
TNF-α, pg/mL	23.70 (13.80, 40.80)	21.60 (15.50, 33.50)	18.10 (13.60, 25.23)	0.141

"a", $P < 0.05$, compared with the BP group; "b", $P < 0.05$, compared with the non-infection group. IPA, invasive pulmonary aspergillosis; BP, bacterial pneumonia.

sIL-2R (1,792.00 vs. 1,211.50 U/mL, $P < 0.001$), IL-6 (49.42 vs. 15.98 pg/mL, $P < 0.001$), and IL-10 (13.40 vs. 7.41 pg/mL, $P < 0.001$) levels compared to the non-infection group. Compared to the BP group, the IPA group had significantly lower IL-8 levels (62.20 vs. 176.00 pg/mL, $P < 0.001$), but no significant differences in sIL-2R, IL-6, or IL-10 levels. Notably, IL-8 levels were markedly lower in the IPA group compared to in both the non-infection group (62.20 vs. 132.00 pg/mL, $P = 0.025$) and the BP group (62.20 vs. 176.00 pg/mL, $P < 0.001$). This distinct IL-8 profile, showing a reduction in IPA in contrast to an elevated level in BP, represents a key clinical discriminatory feature and may have important diagnostic implications for differentiating fungal from bacterial infections in this vulnerable population.

Performances of cytokines in differentiating IPA cases from non-infection cases

ROC curve analysis was conducted to determine the optimal cytokine cut-off values and the diagnostic performance for differentiating IPA cases from non-infection cases (Table 4). The results demonstrated that IL-6 offered the highest diagnostic accuracy (AUC = 0.854, cut-off = 22.85 pg/mL, $P < 0.001$), with a sensitivity of 96.90% and specificity of 72.90%. The ROC curve for IL-6 is depicted in Figure 2A.

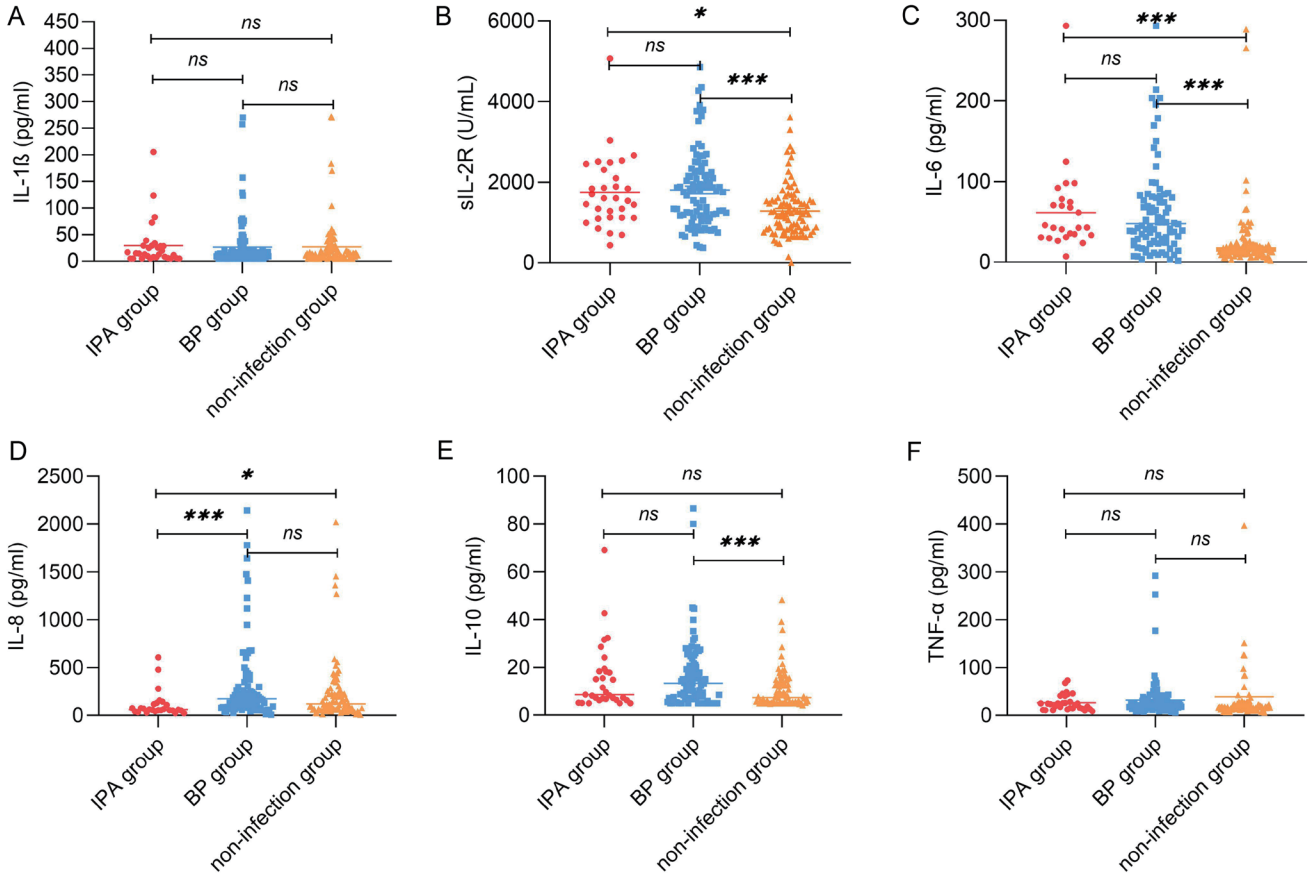

Fig. 1. Distribution plots of the levels of interleukin (IL)-1ß (A), sIL-2R (B), IL-6 (C), IL-8(D), IL-10(E) and TNF-α(F) in the IPA, BP, and non-infection groups. "*ns*" indicates no statistically significant difference. *indicates a significant difference, with $P < 0.05$; **indicates a significant difference, with $P < 0.01$; ***indicates a significant difference, with $P < 0.001$. IPA, invasive pulmonary aspergillosis; BP, bacterial pneumonia.

Table 4. Diagnostic efficiency of 6 cytokines in distinguishing IPA from non-infection patients with ACLF

	AUC	95%CI	*P* value	Cut-off value	Sensitivity	Specificity
IL-1ß (pg/mL)	0.517	(0.396, 0.638)	0.775	24.45	38.70%	73.40%
sIL-2R (U/mL)	0.670	(0.560, 0.779)	0.005	1,601.50	51.60%	77.70%
IL-6 (pg/mL)	0.854	(0.782, 0.926)	<0.001	22.85	96.90%	72.90%
IL-8 (pg/mL)	0.664	(0.549, 0.780)	0.009	74.70	63.00%	69.10%
IL-10 (pg/mL)	0.638	(0.528, 0.749)	0.021	6.25	83.90%	44.80%
TNF-α (pg/mL)	0.568	(0.448, 0.689)	0.255	23.40	51.60%	70.20%

IPA, invasive pulmonary aspergillosis; ACLF, acute-on-chronic liver failure.

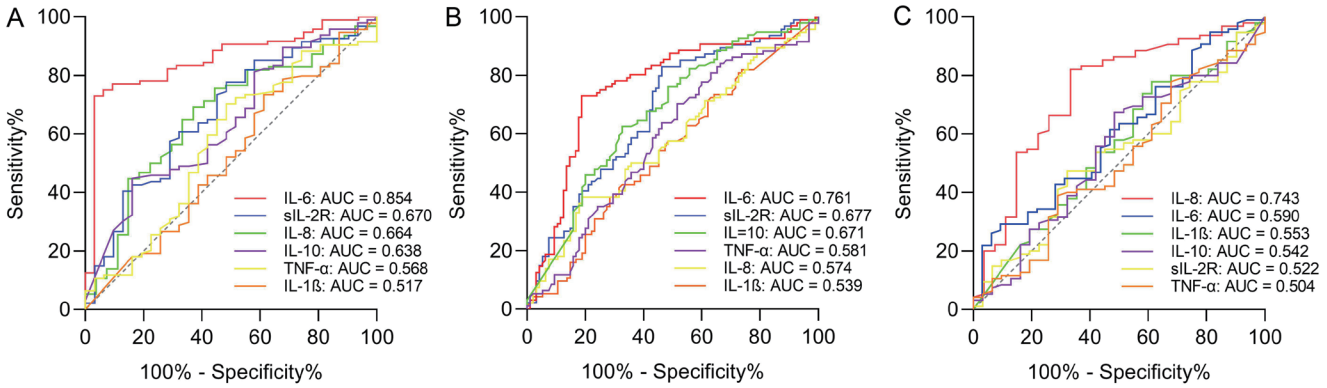

Fig. 2. Diagnostic performance of serum cytokines for differentiating the infectious status of patients with HBV-ACLF. (A) Area under the receiver operating characteristic (ROC) curves for 6 cytokines in differentiating ACLF patients with IPA from non-infection; (B) ROC curve for 6 cytokines in differentiating ACLF patients with BP from non-infection; (C) ROC curve for 6 cytokines in differentiating ACLF patients with IPA from bacterial pneumonia. IPA, invasive pulmonary aspergillosis; ACLF, acute-on-chronic liver failure; BP, bacterial pneumonia.

Performance of cytokines in differentiating BP cases from non-infection cases

ROC curve analysis was also performed to differentiate BP from non-infection cases (Table 5). The results demonstrate that IL-6 offered superior diagnostic performance (AUC = 0.761, cut-off = 22.38 pg/mL, $P < 0.001$), with a sensitivity of 81.3% and specificity of 72.9%. The ROC curve for IL-6 is depicted in Figure 2B.

Performance of cytokines in differentiating IPA cases from BP cases

To distinguish IPA from BP, ROC curve analysis was employed to determine the optimal cut-off values for various cytokines and assess their performance (Table 6). Among the cytokines assessed, IL-8 demonstrated the highest discriminatory performance, with an AUC of 0.743 (cut-off = 76.60 pg/mL, $P < 0.001$), yielding a sensitivity of 66.7% and specificity of 82.1%. These findings support our initial hypothesis that serum cytokines may serve as effective biomarkers for distinguishing IPA from BP in HBV-ACLF patients, with IL-8 emerging as the most promising candidate biomarker for such differentiation. The ROC curve for IL-8 is presented in Figure 2C.

Discussion

In recent years, the incidence and detection of IPA have risen significantly due to the widespread use of glucocorticoids, increase in invasive procedures, and advancements in mNGS technology, resulting in heightened clinical awareness about IPA. IPA usually exhibits nonspecific and insidious symptoms, making diagnosis challenging. Currently, there are limited diagnostic methods available for IPA, and there remains an

Table 5. Diagnostic efficiency of 6 cytokines in distinguishing BP from non-infection patients with ACLF

	AUC	95%CI	*P* value	Cut-off value	Sensitivity	Specificity
IL-1ß (pg/mL)	0.539	(0.457, 0.622)	0.350	16.55	67.40%	42.60%
sIL-2R (U/mL)	0.677	(0.600, 0.753)	0.003	1,737	53.70%	83.00%
IL-6 (pg/mL)	0.761	(0.690, 0.832)	<0.001	22.38	81.30%	72.90%
IL-8 (pg/mL)	0.574	(0.492, 0.656)	0.079	78.30	81.10%	38.30%
IL-10 (pg/mL)	0.671	(0.594, 0.747)	<0.001	8.65	67.40%	62.50%
TNF-α (pg/mL)	0.581	(0.499, 0.663)	0.055	23.25	48.40%	70.20%

ACLF, acute-on-chronic liver failure; BP, bacterial pneumonia.

Table 6. Diagnostic efficiency of 6 cytokines in distinguishing IPA from BP in patients with ACLF

	AUC	95%CI	*P* value	Cut-off value	Sensitivity	Specificity
IL-1ß (pg/mL)	0.553	(0.436, 0.671)	0.372	24.75	38.70%	77.90%
sIL-2R (U/mL)	0.522	(0.408, 0.636)	0.711	1,861.00	67.70%	47.40%
IL-6 (pg/mL)	0.590	(0.476, 0.704)	0.134	23.35	96.90%	21.90%
IL-8 (pg/mL)	0.743	(0.633, 0.854)	<0.001	76.60	66.70%	82.10%
IL-10 (pg/mL)	0.542	(0.424, 0.660)	0.482	8.65	51.60%	67.40%
TNF-α (pg/mL)	0.504	(0.386, 0.622)	0.948	14.95	32.30%	77.90%

IPA, invasive pulmonary aspergillosis; ACLF, acute-on-chronic liver failure; BP, bacterial pneumonia.

urgent need for novel blood biomarkers to facilitate the early diagnosis of IPA and to elucidate the underlying pathophysiological processes involved. In this study, we investigated the levels of a range of cytokines in diverse HBV-ACLF patients and found that IL-6 levels were significantly elevated at the onset of IPA, while IL-8 levels were significantly decreased in patients with probable IPA compared to those with bacterial pulmonary infections or no infection. Furthermore, we found that IL-8 could effectively allow differentiating IPA from bacterial pulmonary infections, while IL-6 could allow distinguishing IPA from non-infected patients. These findings provide indirect but clinically relevant evidence to support the use of cytokines as biomarkers for the early recognition of IPA.

The HBV-ACLF population differs from other high-risk populations with comorbid fungal infections associated with long-term neutropenia or hormone use,[25,26] and the risk of fungal infections primarily arises from the characteristic immune dysfunctions in such patients, such as congenital immune paralysis, disturbances in immune tolerance, reduced innate and adaptive responses, and systemic inflammatory responses.[27,28] The germination of *Aspergillus* within the host triggers an innate immune response. The innate immune system—comprising the ciliated epithelium, alveolar macrophages, monocytes, and dendritic cells—acts as the primary defense against *Aspergillus* invasion.[29] *Aspergillus* spores are initially cleared through phagocytosis by alveolar macrophages. When alveolar macrophages become overwhelmed, free spores swell and are recognized by ciliated epithelial cells. This interaction induces the secretion of the chemokine IL-8, which facilitates the recruitment of neutrophils capable of eliminating the mycelium to the site of infection.[30–33] In addition, proteases released by *Aspergillus* enhance the production of IL-6 by airway epithelial cells, which is believed to play a key role in T cell recruitment.[34]

IL-8, an important chemokine that induces neutrophils, is typically elevated in fungal infections and has been proposed as a potential diagnostic biomarker for such infections. However, prior reported evidence has been largely derived from neutropenic or hormone-treated populations.[12,35,36] In contrast, hepatic failure profoundly disrupts immune homeostasis, leading to imbalanced innate and adaptive immune responses, the excessive release of inflammatory mediators, and systemic inflammatory response syndrome.[4,37] Elevated IL-8 levels have been reported in ACLF and it was suggested that they may independently predict a poor prognosis.[38,39] However, in our study, IL-8 levels were significantly lower in ACLF patients with IPA than in both our bacterial infection and non-infection groups. This discrepancy from previous findings warrants careful consideration. Several explanations may account for this discrepancy. First, the relatively low incidence of fungal infections in ACLF patients (2.5–10%) suggests that the inflammatory cytokine profiles observed in general ACLF populations may not reflect the distinct immunological characteristics of IPA patients.[10,40] Second, prolonged antibiotic exposure may suppress immune responses.[41] Third, the limitations inherent in this single-center retrospective study (e.g., small sample size, selection bias) require further investigation and the findings need validation in a larger prospective cohort. Most plausibly, however, the observed reduction in IL-8 reflects a profound impairment of innate immunity, often described as "immune paralysis", in ACLF. It has been reported that severe liver injury disrupts the cytokine network, impairing the function of alveolar macrophages and epithelial cells, which are primary sources of IL-8.[42] Since IL-8 is essential for neutrophil recruitment and *Aspergillus* conidial clearance,[43] its suppressed production likely reflects broader immune dysfunction, thereby contributing to the increased susceptibility to IPA and high mortality observed in this population. This immune paralysis mechanism is consistent with prior reports of sepsis-like immunosuppression in ACLF and provides the most compelling explanation for the unexpectedly low IL-8 levels observed in our study.

In ACLF patients in the present study, the IL-6 levels were not significantly different between the patients with *Aspergillus* infections and those with bacterial infections but were significantly elevated in both groups compared to the non-infected group. Although IL-6 could effectively distinguish patients with IPA from non-infected individuals, it failed to distinguish IPA from bacterial infection. A pivotal role of IL-6 in protective immunity against *Aspergillus* has been demonstrated in murine models.[44] Moreover, *Aspergillus fumigatus* proteases have been reported to upregulate gene transcription and promote IL-6 release from A549 pulmonary epithelial cells and primary airway epithelial cells.[34] It was also reported that in bacterial infections, IL-6 is elevated earlier than procalcitonin and thus it has been widely used as a biomarker to identify the development of bacterial infections.[45,46]

In contrast to our findings in HBV-related ACLF, studies in other clinical settings have demonstrated the stronger diagnostic performances of IL-6 and IL-8 for IPA. In patients with hematological malignancies, the levels of IL-6 and IL-8 in both serum and bronchoalveolar lavage fluid (BALF) were found to be significantly higher in those with probable/proven IPA compared to in controls without any evidence of IPA.[12] Furthermore, in hematological patients and stem-cell transplant recipients, the IL-8 level in BALF showed excellent diagnostic accuracy for IPA, with an AUC of 0.904, suggesting it could be used as an important biomarker for the diagnosis of IPA.[14] Similarly, in patients with COPD, serum IL-6 (AUC = 0.837) and IL-8 (AUC = 0.825) demonstrated moderate diagnostic performances for distinguishing IPA from the controls.[15] Taking together, although IL-6 has demonstrated good diagnostic performance in distinguishing fungal from bacterial infections in patients with hematologic disorders or respiratory diseases, its discriminatory value appears limited

in the HBV-ACLF population, likely due to differences in the underlying disease pathophysiology.[12,13,35]

Among the 32 patients in the IPA group, 28 patients underwent serum galactomannan testing, all of whom yielded positive results, while the remaining 4 patients were diagnosed based on positive sputum cultures for *Aspergillus* species. None of the patients underwent PCR or metagenomic next-generation sequencing testing. Consequently, this study did not allow a direct comparison of IL-8 with other established biomarkers for IPA. Notably, this is the first study to evaluate cytokine profiles as diagnostic biomarkers for IPA in patients with ACLF. To our knowledge, no previous studies have investigated cytokines beyond the six examined here in this specific population, thereby limiting the scope for direct comparative analyses.

In this retrospective case-control study, the observed incidence of IPA was 5.72%, which is consistent with previously reported ranges of 1.85–9.88%.[8,10] Notably, prior studies have indicated that the prevalence of IPA may be comparable or even higher in ACLF associated with other etiologies, particularly alcohol-related liver disease. For instance, in a cohort of 92 patients with severe alcoholic hepatitis, the prevalence of IPA was reported to be 11.7%,[22] and among 82 patients with predominantly alcohol-associated cirrhosis, the prevalence of IPA was 14%.[23] These findings suggest that while the incidence of IPA in HBV-ACLF patients in the present study was within the expected range, it can vary depending on the underlying etiology of ACLF, highlighting the need for an etiology-specific assessment of the fungal infection risk.

HBV infection represents the predominant etiology of ACLF in populations in China and the Asia-Pacific region, accounting for over 70% of cases in large cohort studies.[47] In contrast, ACLF in Western populations is primarily associated with alcohol-related liver disease or metabolic dysfunction-associated steatotic liver disease.[48] Accordingly, we restricted our analysis to patients with HBV-ACLF to enhance the cohort homogeneity and ensure the clinical relevance of our findings to this specific population. Furthermore, we believe that focusing on a single etiology minimized the risk of confounding issues due to etiological heterogeneity, thereby enabling a clearer assessment of the association between the cytokine profiles and IPA.

This study has several limitations to note. First, this was a single-center study with a small sample size of IPA cases, which may have led to data bias that could have affected the statistical power of the findings. Second, the retrospective design of this study limited our ability to do an accurate assessment of concurrent bacterial infections, particularly in patients with coexisting fungal infections. Third, only six cytokines were measured, thus limiting the scope of the immunological analysis. Finally, it is important to note that all the patients in our IPA group were classified as probable IPA cases rather than proven IPA cases, as coagulation disorders in patients with ACLF frequently preclude the collection of tissue samples required for definitive diagnosis. These limitations highlight the need for future large-scale, prospective longitudinal studies. Case-control analyses with stricter criteria are also needed to eliminate differences caused by variations in antibiotic treatments and the length of hospitalization. Additionally, monitoring dynamic changes in a broader range of cytokines may help identify more specific and clinically useful biomarkers for the early diagnosis of IPA.

Conclusion

In conclusion, we found that IL-8 levels were significantly lower in patients with IPA than in patients with bacterial pulmonary infections, while IL-6 levels were significantly higher in patients with IPA compared to in non-infection controls. The combination of assessing IL-6 and IL-8 levels demonstrated superior diagnostic performance in distinguishing IPA from bacterial pulmonary infections among patients with HBV-ACLF.

Funding

This study was supported by the National Key Research and Development Program of China (grant number 2023YFC2308405).

Conflict of interest

QN has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2024, The other authors have no conflict of interests related to this publication.

Author contributions

LH, YF and WW contributed equally as first authors. LZ had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: LZ, TC and QN. Acquisition, analysis, and interpretation of data: LH, YF, WW, WL, YL, LC, YN, TL, MS, YX, ZY, GC. Critical revision of the manuscript for important intellectual content: LZ, TC and QN. Statistical analysis: LH, YF, WW. Administrative, technical, and material support: TC and QN. Supervision: LZ, TC and QN. All authors have read and approved the final version of the manuscript.

Ethical statement

The study was approved by the Medical Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (Approval No. 2023S043) and adhered to the principles outlined in the Declaration of Helsinki. Written informed consent was waived due to the retrospective nature of the study.

Data sharing statement

The datasets generated and analyzed in the present study are available from the corresponding author upon reasonable request.

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