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ORIGINAL RESEARCH

Predicting Healthcare-Associated Infection in Patients with Pneumonia via QuantiFERON®-Monitoring

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Objective: A functional immune system is essential for recovery from pneumonia; hence, measuring and monitoring immune-status indicators is clinically important. This study aimed to determine whether QuantiFERON monitoring (QMF) could predict healthcare-associated infection (HCAI) according to the immune-status of patients with pneumonia.

Methods: Prospective, observational, single-center study, patients ≥19 years hospitalized for pneumonia between October 2020 and November 2021. QFM was performed at hospital admission (D1) and seven days after (D2). Data from 90 patients in the D1 QFM group were analyzed, which was further divided into the non-healthcare-associated infection (non-HCAI, n = 41, 45.6%) and HCAI (n = 49, 54.4%) groups.

Results: The D1 and D2 QFM levels were both significantly higher in the non-HCAI group than in the HCAI group (D1 hCAI vs non-HCAI: 4.40 vs 5.75 IU/mL, D2 hCAI vs non-HCAI: 4.38 vs 6.10 IU/mL). Analysis of the change in D1 and D2 QFM levels by each group showed that D2 QFM levels increased over D1 QFM levels in the non-HCAI group (5.75 vs 6.10 IU/mL), while D2 QFM levels decreased over D1 QFM levels in the HCAI group (4.40 vs 4.38 IU/mL). D1 QFM was consistently negatively correlated with TNF-α and CRP. The integrated analysis of D1 QFM and CCI and D1 QFM and CURB-65 had fair to predict the occurrence of HCAI.

Conclusion: QFM can be used to predict the immune-status of patients in the context of healthcare-associated infections. These findings provide important insights into the current understanding of pneumonia treatment and recovery.

Keywords: QuantiFERON monitoring, pneumonia, immune status, prospective study, biomarker

Introduction

Healthcare-associated infections (HCAIs) are reported to be an important issue closely related to in-hospital mortality.[1,2] The main factors that affect the occurrence of HCAI include the length of hospital stay, the length of stay in the intensive care unit (ICU), age, body mass index (BMI), diabetic status, and indwell variable catheter.[3–5] The most common infection among HCAIs is pneumonia, which is associated with a significantly worse prognosis for patients and a high mortality rate.[6] This occurs especially in the elderly and is a major cause of death.[7–10]

The severity and incidence of HCAI are affected by various factors including age, comorbidities, length of hospitalization, and host immunity. Among these, host immune status plays an important role in the recovery of patients and overcoming the disease.

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Given the association between immune status and secondary infections, particularly HCAIs, accurate indicators of immune status are needed. However, no such reliable objective indicator exists, and few methods can simultaneously measure adaptive and innate immunity in relation to immune status.

QuantiFERON monitoring (QFM) can assess both adaptive and innate immune responses to infection by measuring interferon-gamma (IFN-γ) levels after external stimulation, enabling the evaluation of non-specific cellular responses. Assessment of innate immunity through NK cell, monocyte and macrophage activity and adaptive immunity through T cell, B cell analysis and cytokine profiling.[11,12] When utilizing QuantiFERON, the primary analyte IFN-γ, along with several related inflammatory cytokines, are simultaneously analyzed to investigate the relationships between immune status and inflammatory response components. IFN-γ is an immune cytokine secreted by T cells, macrophages, mucosal epithelial cells, and natural killer (NK) cells in response to foreign antigens, and it plays a role in inflammatory responses.[13] Therefore, these factors reflect the host's immune function. However, limited studies have examined the relationship between immune status and HCAI occurrence. In addition, previous studies using QFM have suggested a higher incidence of infection in patients with relatively high levels of IFN-γ after transplantation.[14,15] In addition, a previous study investigated whether a novel, widely available immune biomarker measuring IFN-γ could predict events after transplantation.[15] However, these studies have primarily focused on organ transplant recipients, with limited investigations conducted in patients experiencing severe infection-related immune responses. Notably, there is a paucity of research concerning the impact of immune status on the prognoses of patients who succumb to HCAI due to immunocompromise.[16]

This study aimed to investigate differences in immune status between pneumonia patients who develop HCAI and those who do not. We also aimed to analyse changes in immune status in pneumonia patients to determine the association between changes in immune status and the development of HCAI. Based on these data, we confirmed the usefulness of QFM to predict the recovery of patients' immunity, assess their susceptibility to infection, and investigate its correlation with clinical outcomes. We also examined existing validated inflammatory and immune-related biomarkers to validate the capabilities of the QFM. The results of this study may contribute to the search for markers to help identify patients at high risk for future infections at an early stage.

Material and Methods

Patient Enrollment and Study Design

This prospective observational study conducted at Pusan National University Yangsan Hospital involved patients hospitalized for pneumonia between October 2020 and November 2021 based on the following criteria: (1) patients aged ≥19 years, (2) hospitalized for pneumonia, and (3) provided informed consent to participate in the study. Patient treatment and follow-up were conducted by the attending physician following standard care protocols. QFM was performed twice: at hospital admission (D1, QuantiFERON baseline) and on the 7th day after the first collection (D2, 2nd QuantiFERON). Known inflammatory markers such as tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), interleukin 6 (IL-6), and interleukin 1 beta (IL-1ß) were analyzed for comparison with QFM data. Patients were identified and enrolled by an experienced researcher, ensuring that written informed consent was obtained. The requirement for informed consent was waived, due to the retrospective nature of the design and a standard-of-care observational study with no intervention. The procedures were followed in accordance with the ethical standards on human experimentation by the responsible committee of the hospital and the Declaration of Helsinki (revised in 2013), and was approved by the Institutional Review Board (IRB No.: 2021–01-07).

Blood Collection

Blood collection was conducted during regular working hours following acquisition of patient consent. A blood sample was obtained alongside a routine blood test, as per standard medical procedures. For QFM, an additional 1 cc of blood was collected. Within 1 hour of collection, the blood was transported to the Department of Laboratory Medicine, where both the receiving researcher and the transporter performed a simultaneous double-check.

The various inflammatory markers (TNF-α, CRP, IL-6, and IL-1β) were tested concurrently. Serum levels of IL-1β and TNF-α were determined using enzyme immunoassays, specifically the Human IL-1β Pre-Coated enzyme-linked immunosorbent assay (ELISA) kit (BioGems, Westlake Village, CA, USA) and the Human TNF-α Pre-Coated ELISA kit BGK01375 (BioGems). Serum IL-6 levels were measured using the Elecsys IL-6 kit (Roche Diagnostics, Mannheim, Germany) on the electrochemiluminescence-based COBAS e802 (Roche Diagnostics) instrument. Lactate dehydrogenase (LDH) activity was measured to examine its correlation with the inflammatory markers.

QFM Processing

The test tubes utilized in the experiment were obtained from QIAGEN (QFM, Qiagen, Germantown, MD, USA). Within 8 hours of collection, 1 mL of collected blood was transferred to a dedicated QuantiFERON tube. Subsequently, QFM LyoSpheres (stored at 4 °C) containing anti-CD3 (a T cell stimulant) and R848 (a toll-like receptor 7/8 ligand) were added to the QFM tube. The tubes were thoroughly shaken until the pellet was completely dissolved and then incubated for 16–24 h at 37 °C. The subsequent processing and classification of samples depended on the availability of a centrifuge. 1) If no centrifuge was available, the tubes were stored at 4–27 °C for up to 3 days after incubation. 2) If a centrifuge was available, centrifugation was performed at 2300 g (2000–3000 g, KUBOTA-4000) for 15 minutes at an approximate temperature of 20 °C. After centrifugation, QFM was either interpolated at 2–8 °C for 28 days as is or the plasma was separated and stored at −70 °C for >28 days. The samples stored in this manner were analyzed collectively once a certain number of patients were accumulated and once the experimental wells were pooled, they were analyzed together.[17,18] IFN-γ (IU/mL) levels in the plasma were measured using a QFM ELISA kit (Qiagen Inc) in a Dynex DS2® automated ELISA system (Dynex, Chantilly, VA, USA).

Definition

HCAI

HCAIs, as defined by the Centers for Disease Control (CDC), are local or systemic conditions resulting from an adverse reaction to the presence of an infectious agent or its toxin. Furthermore, there should be no evidence that the infection was present or incubating at the time of admission to the acute care setting.[19] Among the many healthcare-associated infections, we conducted a comparative analysis that included only the three most common healthcare-associated infections to maintain consistency in the purpose of the paper and to find the meaning of the results. HCAIs were included in this study as cases with newly proven urinary tract infections (UTIs), ventilator-associated pneumonias (VAPs), and/or bloodstream infections (BSIs).

Pneumonia is characterized as an infection of the pulmonary parenchyma caused by various organisms, accompanied by compatible laboratory findings and symptoms such as cough (with or without sputum), fever, chills, and respiratory difficulty.[20] BSIs are infectious diseases defined by the presence of viable bacterial or fungal microorganisms in the bloodstream, later confirmed by the positivity of one or more blood cultures.[21] UTI refers to an infection of the urinary system.[22] VAPs are defined as pneumonia occurring in mechanically ventilated patients, exhibiting clinical features and signs associated with pneumonia. Additionally, clinical response refers to the persistence or worsening of signs and/or symptoms associated with pneumonia. VAP include a new and persistent (>48-h) or progressive radiographic infiltrate plus two of the following: temperature of >38°C or <36°C, blood leukocyte count of >10,000 cells/mL or <5,000 cells/mL, purulent tracheal secretions, and gas exchange degradation.

Sepsis

Sepsis-3 was described in the 2016 consensus definition as a pattern of life-threatening organ dysfunction caused by a dysregulated host response to infection (1,2). Septic shock was defined as persistent hypotension requiring vasopressors to maintain a mean arterial pressure (MAP) greater than 65 mm Hg and elevated serum lactate greater than 2 mmol/L, despite adequate fluid resuscitation (30 mL/kg of fluids, urine output > 0.5 mL/kg/h, CVP of 8–12 mm Hg) (1).

If a patient in the study had any specimen reported as positive for culture, the principal investigator immediately confirmed whether it was HCAI.

Clinical Data Collection

Clinical data on patient outcomes from admission to discharge were collected retrospectively from medical records. The parameters collected included sex, age, weight, height, diagnostic code, septic shock status, use of steroids, causes of infection during ICU and hospital stay (respiratory tract, abdomen, urinary, soft tissue, blood, other), types of infection (viral, bacterial, fungal), identified microorganisms, ICU death, hospital death, occurrence of re-infection, duration of mechanical ventilation, length of hospital survival, length of ICU stay, length of hospital stay, and ICU readmission.

Statistical Analysis

Data are presented as mean ± standard deviation. Differences between groups were evaluated using Student's *t*-tests. Pearson's correlation coefficients were used to calculate correlations among variables, and Kaplan–Meier plots were used to assess survival times between groups. Univariate Cox regression was employed to determine related factors and their odds ratios. For the paired sample, the p-value is the Wilcoxon signed-rank test of a non-parametric method rather than a *t*-test because the comparison is to the median. The significance of differences was tested using Log rank tests. The predictive value of HCAIs for mortality was assessed using a receiver operating characteristic (ROC) curve. Statistical significance was defined as $P<0.05$. All statistical analyses were performed using IBM SPSS Statistics Software for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA).

Results

Baseline Characteristics of the Enrolled Patients

In total, 121 patients hospitalized for pneumonia were enrolled. Among them, 31 patients were excluded due to incomplete sampling. The remaining 90 patients were included in the D1 QFM analysis (Figure 1). Patients were divided into the HCAI (49 patients, 54.4%) and non-HCAI (41 patients, 45.6%) groups. The patients' baseline characteristics according to HCAI are shown in Table 1.

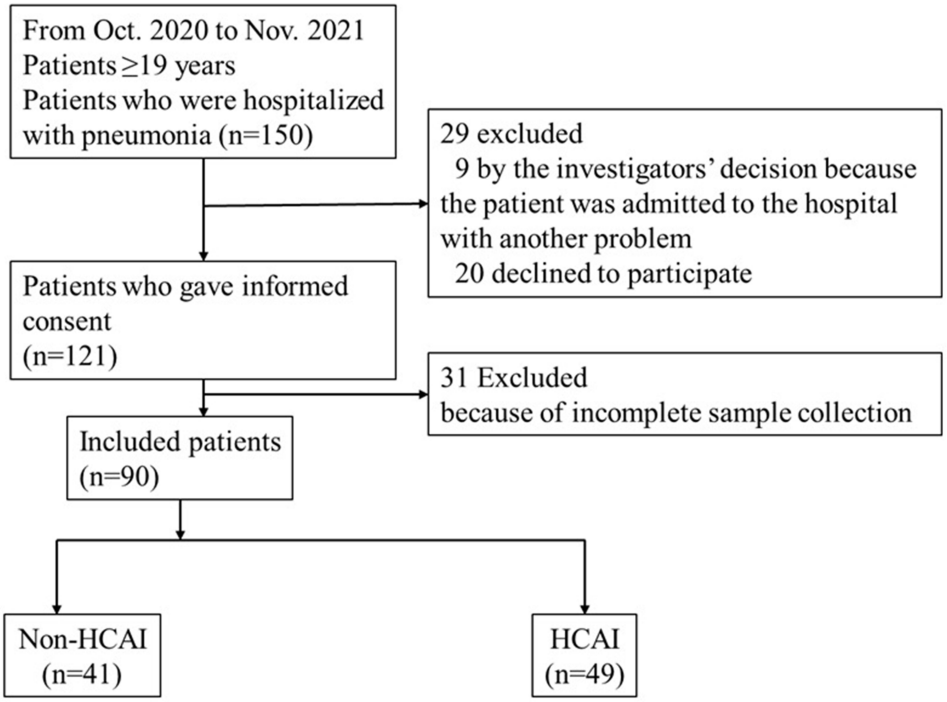

Figure 1 Flowchart illustrating the enrollment process for this study. Patients with pneumonia were enrolled and categorized based on their healthcare-associated infection status.

Table 1 Baseline Characteristics of the Enrolled Patients (N=90)

Variables	Characteristics	Total (n=90)	Non-HCAI (n=41)	HCAI (n=49)	*P*-value
Sex	Female	39 (43.3)	19 (46.3)	20 (40.8)	0.598
	Male	51 (56.7)	22 (53.7)	29 (59.2)	
Age, years		60.06±16.56	52.88±15.97	66.06±14.66	<0.001
	<65	52 (57.8)	32 (78.0)	20 (40.8)	<0.001
	≥65	38 (42.2)	9 (22.0)	29 (59.2)	
BMI, kg/m^2		23.21±5.76	24.92±5.26	21.79±5.82	0.011
HT	No	66 (73.3)	29 (70.7)	37 (75.5)	0.610
	Yes	24 (26.7)	12 (29.3)	12 (24.5)	
DM	No	66 (73.3)	30 (73.2)	36 (73.5)	0.975
	Yes	24 (26.7)	11 (26.8)	13 (26.5)	
CKD	No	82 (91.1)	39 (95.1)	43 (87.8)	0.221
	Yes	8 (8.9)	2 (4.9)	6 (12.2)	
Bacterial pneumonia	No	46 (51.1)	12 (29.3)	34 (69.4)	<0.001
	Yes	44 (48.9)	29 (70.7)	15 (30.6)	
Viral pneumonia	No	44 (48.9)	29 (70.7)	15 (30.6)	<0.001
	Yes	46 (51.1)	12 (29.3)	34 (69.4)	
VAP	No	65 (72.2)	36 (87.8)	29 (59.2)	0.003
	Yes	25 (27.8)	5 (12.2)	20 (40.8)	
CCI		3.07±2.60	2.07±2.05	3.90±2.73	0.001
CURB-65		1.47±1.20	1.05±1.05	1.82±1.22	0.002
Sepsis	No	67 (74.4)	36 (87.8)	31 (63.3)	0.008
	Yes	23 (25.6)	5 (12.2)	18 (36.7)	
Septic shock	No	79 (87.8)	39 (95.1)	40 (81.6)	0.052
	Yes	11 (12.2)	2 (4.9)	9 (18.4)	
ICU admission	No	24 (26.7)	16 (39.0)	8 (16.3)	0.015
	Yes	66 (73.3)	25 (61.0)	41 (83.7)	
Use of Steroid	No	35 (38.9)	16 (39.0)	19 (38.8)	0.981
	Yes	55 (61.1)	25 (61.0)	30 (61.2)	
Initial Laboratory findings					
WBC 10^3/μL		10.01±7.76	8.50±7.67	11.27±7.69	0.092
Neutrophil %		77.88±13.31	73.66±12.86	81.40±12.75	0.005
Lymphocyte 10^3/μL		13.87±9.54	16.45±9.37	11.72±9.23	0.018
Platelet 10^3/μL		199.98±120.63	202.04±89.20	198.27±142.64	0.879
Lactic acid mmol/L		1.84±1.67	1.66±1.31	2.00±1.93	0.350

Notes: Data are presented as number (percentages) or mean±standard deviation.
Abbreviations: HCAI, healthcare-associated infection; BMI, body mass index; HT, hypertension; DM, diabetes mellitus; CKD, chronic kidney disease; VAP, ventilator-associated pneumonias; CCI, Charlson comorbidity index; ICU, intensive care unit; WBC, white blood count.

There were significant differences in age (<65 or ≥65 years) and BMI between the groups (non-HCAI vs HCAI; age: 52.88±15.97 vs 66.6±14.66 years, P<0.001; <65 years: 78.0% vs 40.8%; ≥65 years: 22.0% vs 59.2%, P<0.001; BMI: 24.92±5.26 kg/m^2 vs 21.79±5.82 kg/m^2, P<0.001) and in the etiology of the pneumonia (non-HCAI vs HCAI; bacterial pneumonia: 70.7% vs 30.6%, viral pneumonia: 29.3% vs 69.4%, P<0.001; HCAP: 12.2% vs 40.8%, non-HCAP: 87.8% vs 59.2%, P=0.003). The severity, Charlson Comorbidity Index (CCI) value, CURB-65 score, non-sepsis rate, and ICU admission rate were significantly higher in the non-HCAI group than in the HCAI group (non-HCAI vs HCAI; CCI: 2.07 ±2.05 vs 3.90±2.73, P=0.001; CURB-65: 1.05±1.05 vs 1.82±1.22, P=0.002; non-sepsis: 87.8% vs 63.3%, P=0.008, ICU admission: 61.0% vs 83.7%, P=0.015). Regarding the initial laboratory findings, neutrophil levels were higher in the HCAI group than in the non-HCAI group (73.66±12.86% vs 81.40±12.75%, P=0.005). However, lymphocyte levels were

lower in the HCAI group (16.45±9.37 10^3/μL vs 11.72±9.23 10^3/μL, *P*=0.018). Sex, comorbidity, and steroid use were not significantly different between the groups.

QFM Measurements

D2 QFM was obtained from 70 patients 7 days after the initial collection. Patients who were discharged, deceased, or transferred to other hospitals within 7 days did not have their D2 QFM data collected. QFM was measured until D2 to assess treatment response, allowing sufficient time for any immune response to develop.

Across all patients (regardless of whether they developed HCAIs), the D1 and D2 QFM levels were 4.63 (3.86–4.63) IU/mL and 5.30 (4.10–5.30) IU/mL, respectively. Overall, there was a slight increase in the D2 QFM levels when compared with the D1 QFM levels. The D1 and D2 QFM levels were also analyzed according to HCAI status (Figure 2 and Table S1). The median D1 and D2 QFM levels were 5.75 IU/mL and 6.10 IU/mL for the non-HCAI group, respectively, and 4.40 IU/mL and 4.38 IU/mL, for the HCAI group, respectively. The D1 and D2 QFM levels were significantly higher in the non-HCAI group than in the HCAI group (D1: 5.75 IU/mL vs 4.40 IU/mL, *P*=0.001; D2: 6.10 IU/mL vs 4.38 IU/mL, *P*=0.043).

In summary, significant differences in the D1 and D2 QFM levels were observed between the HCAI and non-HCAI groups (D1 QFM, *P*=0.001; D2 QFM, *P*=0.043). To confirm the statistical significance, the variables were analyzed as non-parametric due to a large number of outliers within 50, and corresponding graphs were generated (excluding those with more than 50 outliers; Figure 2). Non-parametric tests were utilized as the data were not normally distributed.

Clinical Outcomes According to HCAI

The clinical outcomes related to HCAIs are presented in Table 2. There were no significant differences between the groups (non-HCAI vs HCAI) in terms of length of hospital stay, length of ICU stay, or ICU mortality (28.59±54.18 day vs 43.94 ±35.76 day, *P*=0.125; 14.52±18.90 day vs 21.73±23.13 day, *P*=0.173; and 7.3% vs 18.4%, *P*=0.125, respectively). However, the non-HCAI group required significantly lower durations of mechanical ventilation and had lower hospital mortality than the HCAI group (1.95±3.69 day vs 12.24±20.43 day, *P*=0.001 and 7.3% vs 22.4%, *P*=0.049, respectively).

Correlation of QFM with Inflammatory Markers

On the first day of hospitalization, various immune-related biomarkers, namely, IL-1β, TNF-α, CRP, and LDH, were measured, analyzed, and compared to D1 QFM levels. The measurements in the total group were as follows: D1 QFM: 11.57±42.18 IU/mL; D2 QFM: 15.69±29.51 IU/mL; IL-1β: 29.41±53.75 pg/mL; IL-6: 146.6±230.6 pg/mL; TNF-α: 70.64 ±46.34 pg/mL; CRP: 9.22±8.90 mg/dL; and LDH: 394.54±629.89 IU/L (Figure S1). Correlation analyses revealed significant differences in the TNF-α and CRP levels at D1 (TNF-α: R=−0.208, *P*=0.049; CRP: R=−0.347, *P*=0.001; Table 3).

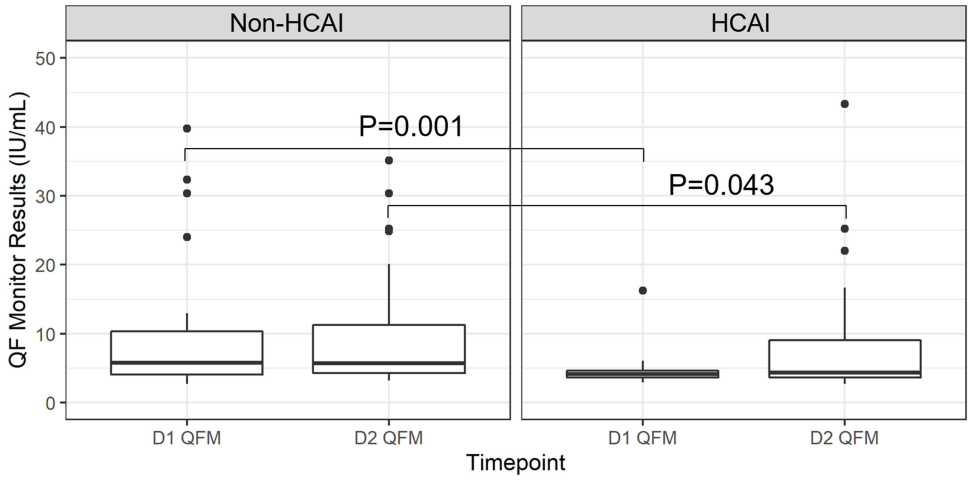

Figure 2 Correlation coefficients between healthcare-associated infection and the D1 and D2 QFM levels.

Table 2 Clinical Outcomes Based on Healthcare-Associated Infection Status (N=90)

	Total (n=90)	Non-HCAI (n=41)	HCAI (n=49)	*P*-value
Length of hospital stay (day)	36.94±45.48	28.59±54.18	43.94±35.76	0.125
Length of ICU stay (day)	19.00±21.76	14.52±18.90	21.73±23.13	0.173
Length of MV (day)	7.56±16.05	1.95±3.69	12.24±20.43	0.001
Hospital mortality	14 (15.6)	3 (7.3)	11 (22.4)	0.049
ICU mortality	12 (13.3)	3 (7.3)	9 (18.4)	0.125
Type of HCAI				<0.001
BSI			189(36.7)	
VAP			1 (2.0)	
UTI			25 (51.0)	

Notes: Data are presented as number (percentages) or mean±standard deviation.
Abbreviations: HCAI, healthcare-associated infection; ICU, intensive care unit; MV, mechanical ventilator; VAP, ventilator-associated pneumonia; UTI, urinary tract infection.

Table 3 Correlation of D1 QuantiFERON Monitoring (QFM) With Inflammatory Markers (N=90)

	D1 QFM	IL-1ß	TNF-α	CRP	LDH
D1 QFM	1	−0.013 (0.902)	−0.208 (0.049)	−0.347 (0.001)	−0.093 (0.385)
IL-1ß		1	−0.021 (0.843)	−0.043 (0.687)	−0.066 (0.537)
TNF-α			1	0.192 (0.070)	−0.068 (0.527)
CRP				1	0.176 (0.098)

Abbreviations: QFM, QuantiFERON monitor; IL-1ß, interleukin 1 beta; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein; LDH, lactate dehydrogenase.

A subgroup analysis was conducted to validate the trends observed in D1 QFM and inflammatory markers (Table S2). Survival, sepsis, ICU admission, and CRP were significantly associated with D1 QFM levels. Furthermore, LDH was a significant marker in the survival group (D1 QFM: R=−0.334, *P*=0.003; LDH: R=−0.250, *P*=0.030). No biomarkers were associated with D1 QFM levels in the sepsis group. However, a significant association between CRP and D1 QFM, as well as LDH, was observed in the non-sepsis group (D1 QFM: R=−0.298, *P*=0.008; LDH: R=−0.250, *P*=0.027). D1 QFM levels and TNF-α were significantly related to CRP in the non-ICU admission group (D1 QFM: R=−0.450, *P*=0.027; TNF-α: R=0.447, *P*=0.029). Conversely, in the ICU admission group, CRP was solely correlated with the D1 QFM (CRP: R=−0.270, *P*=0.029). In all analyses, D1 QFM was consistently negatively correlated with TNF-α and CRP.

Logistic Regression Analysis for Risk Factors of HCAI

Univariate analyses revealed that age (OR: 1.058, 95% CI: 1.025–1.091, *p*<0.001), D1 QFM (OR: 0.952, 95% CI: 0.890–1.018, *p*=0.150), CRP (OR: 1.077, 95% CI: 1.017–1.140, *p*=0.011), neutrophil count (OR: 1.048, 95% CI: 1.012–1.084, *p*=0.008), and lymphocyte levels (OR: 0.947, 95% CI: 0.903–0.992, *p*=0.022) were associated with HCAI status. All of these variables were included in the multivariate analysis, which indicated that age (OR: 1.047, 95% CI: 1.005–1.090, *p*=0.026), D1 QFM (OR: 0.799, 95% CI: 0.644–0.991, *p*=0.041), and neutrophil count (OR: 1.062, 95% CI: 1.012–1.114, *p*=0.015) were associated with HCAI status (Table 4).

HCAI Prediction

ROC curves depicting the association of clinical factors with HCAI status are presented in Figure 3. Among the various clinical factors, age (area under the curve [AUC]: 0.73, 95% CI: 0.62–0.83, *P*<0.001, sensitivity: 0.76, specificity: 0.63) and length of hospital stay (AUC: 0.77, 95% CI: 0.67–0.87, *P*<0.001, sensitivity: 0.69, specificity: 0.80) had fair predictive power (Figure 3A and B). The results of the integrated analysis of significant clinical factors using D1 QFM showed that the combination of D1 QFM and CCI (AUC: 0.74, 95% CI: 0.64–0.85, *P*<0.001, sensitivity: 0.65,

Table 4 Logistic Regression Analysis for Risk Factors of Healthcare-Associated Infections (N=90)

Variables	Univariate OR 95% CI	P-value	Multivariate OR 95% CI	P-value
Age	1.058 (1.025, 1.091)	<0.001	1.047 (1.005, 1.090)	0.026
D1 QFM	0.952 (0.890, 1.018)	0.150	0.799 (0.644, 0.991)	0.041
IL-1ß	1.012 (0.964, 1.062)	0.627		
TNF-α	1.002 (0.993, 1.011)	0.719		
CRP	1.077 (1.017, 1.140)	0.011		
LDH	1.000 (0.999, 1.001)	0.870		
WBC	1.057 (0.988, 1.131)	0.107		
Neutrophil	1.048 (1.012, 1.084)	0.008	1.062 (1.012, 1.114)	0.015
Lymphocyte	0.947 (0.903,0.992)	0.022		
Platelet	1.000 (0.996, 1.003)	0.882		
Lactic acid	1.150 (0.846, 1.564)	0.373		

Abbreviations: QFM, QuantiFERON monitor; IL-1ß, interleukin 1 beta; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein; LDH, lactate dehydrogenase; WBC, white blood cell.

specificity: 0.80) and the combination of D1 QFM and CURB-65 (AUC: 0.72, 95% CI: 0.62–0.83, $P<0.001$, sensitivity: 0.59, specificity: 0.78) had fair to predict the occurrence of HCAI (Figure 3C and D).

Supplemental QFM Results Regarding Survival, Viral, Sepsis, ICU Admission, and HCAI

The median QFM values at D1 and D2 were 4.65 IU/mL and 5.50 IU/mL, respectively, in the survival group, and 4.43 IU/mL and 3.70 IU/mL, respectively, in the non-survival group. In the survival group, the QFM D2 levels increased when compared with the QFM D1 levels, whereas the opposite trend was observed in the non-survival group. However, there was no significant difference in QFM D2 levels ($P=0.060$) between the survival and non-survival groups.

The median QFM levels at D1 and D2 were 4.90 IU/mL and 7.73 IU/mL, respectively, in the non-viral group, and 4.53 IU/mL and 4.65 IU/mL, respectively, in the viral group. In both groups, QFM D2 levels increased when compared with the QFM D1 levels. Notably, the non-viral group showed a significantly higher increase in QFM D2 levels than the viral group. However, there was no significant difference in QFM D2 levels ($P=0.065$) between groups.

The median QFM values at D1 and D2 were 4.90 IU/mL and 5.33 IU/mL, respectively, in the non-sepsis group, and 3.70 IU/mL and 4.30 IU/mL, respectively, in the sepsis group. QFM D2 levels slightly increased in both groups and overall. A significant difference in QFM D1 values was observed between the sepsis and non-sepsis groups ($P=0.008$).

The median QFM values at D1 and D2 were 4.83 IU/mL and 5.30 IU/mL, respectively, in the non-ICU admission group, and 4.55 IU/mL and 5.35 IU/mL, respectively, in the ICU admission group. QFM D2 levels slightly increased in both groups.

The median QFM values at D1 and D2 were 5.75 IU/mL and 6.10 IU/mL, respectively, in the non- HCAI, and 4.40 IU/mL and 4.38 IU/mL, respectively, in HCAI. Significant differences were identified in both QFM D1 ($P=0.001$) and QFM D2 ($P=0.043$) levels between HCAI and non- HCAI (Table S3).

Discussion

QFM is a whole-blood immune function assay that measures plasma IFN-γ levels by stimulating innate and adaptive immune antigens.[23] It is a reliable monitoring technology that provides rapid results and objective measurements of immune function, and eliminates the need for lymphocyte isolation, washing, counting, dilution, or culturing as it utilizes whole blood. This technology is specifically designed for large-scale clinical testing and includes the standard materials and internal quality control materials necessary for clinical diagnosis, ensuring reproducibility.

Identifying individuals with reduced T cell responses to infection, particularly when combined with a dysfunctional immune system (eg, hyper- or hypo-immune state), can be valuable for predicting patients at a higher risk of severe disease. Moreover, it can aid in the development of personalized treatment strategies for patients with severe sepsis. In this study, QFM was utilized to quantify the immune status of patients against severe infection. The QuantiFERON assay from Qiagen was used to measure IFN-γ release and confirm humoral responses, demonstrating its utility in evaluating cellular immunity.

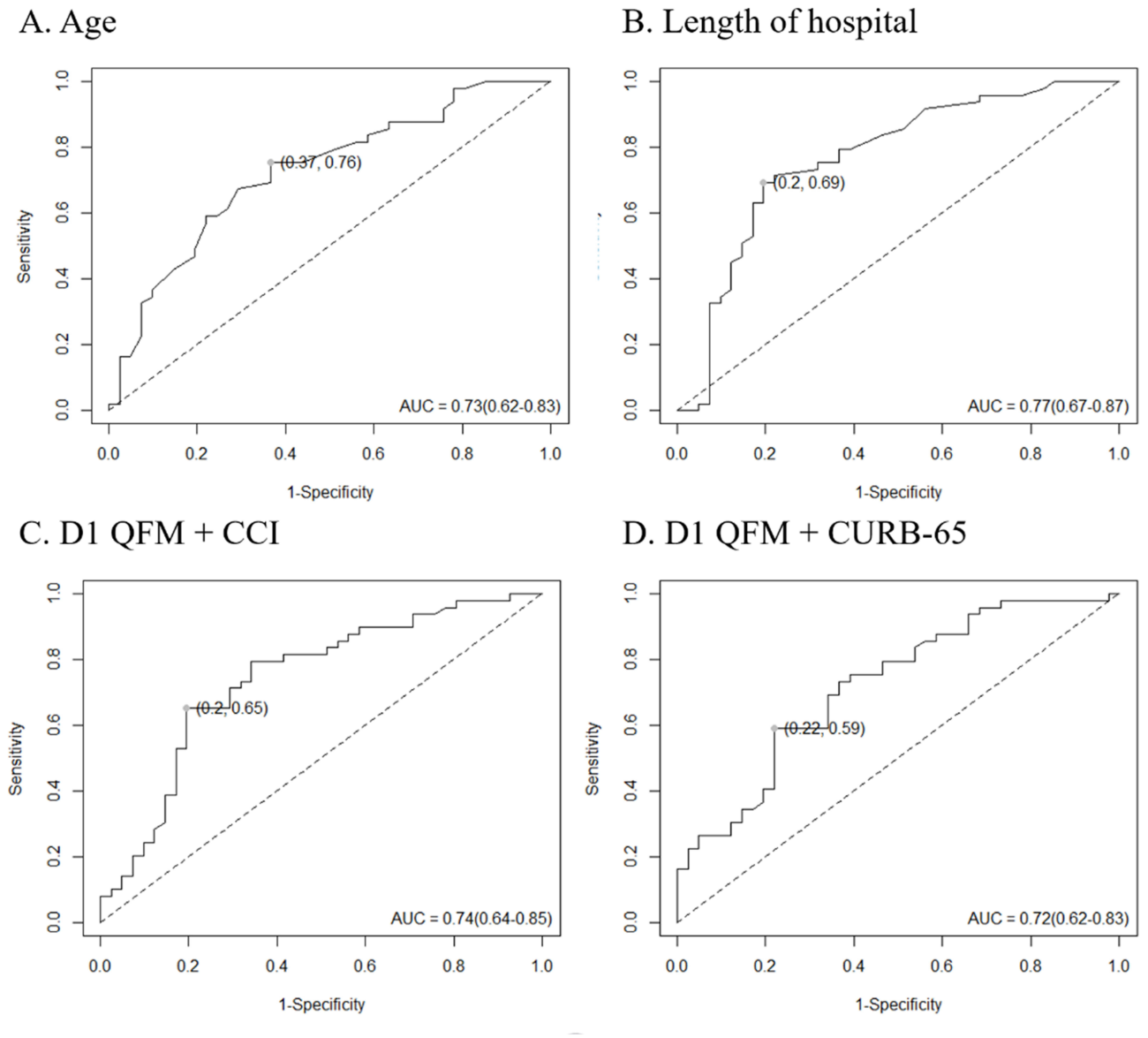

Figure 3 Receiver operating characteristic (ROC) curves demonstrating the association of D1 QFM and clinical factors with healthcare-associated infection status. (**A**) age (**B**) length of Hospital stay, (**C**) combination of D1 QFM and CCI, (**D**) combination of D1 QFM and CURB-65.

In the present study, HCAI group exhibited lower D1 QFM levels than the non-HCAI group, suggesting higher HCAI incidence in patients with lower QFM levels. QFM and immune-related inflammatory markers are important because they help us understand the relationship between inflammatory responses and validate the role of QFM. To this end, we determined how QFM correlates with traditional inflammatory markers. QFM was correlated with CRP and TNF-α levels in both the bacterial-infected and viral-infected groups, which are known inflammatory cytokines. The survival group had increased D2 QFM levels when compared with D1 QFM levels, whereas the opposite trend was observed in the non-survival group. The non-viral group had higher D2 QFM levels than D1 QFM levels, while no significant difference was observed in the viral group. Subgroup analysis of each relationship revealed a negative association between D1 QFM and CRP in the survival group. In the non-sepsis group, regardless of ICU admission status, D1 QFM was negatively associated with CRP. These findings validate the correlation between D1 QFM, CRP, and TNF-α levels. Additionally, the changes in D1 QFM levels between infected and non-infected patients were analyzed, and it was found that while single time-point D1 QFM levels were important, the trends observed were more relevant for assessing survival or recovery. Previous studies have also reported that QFM is associated with a patient's immune status, which correlates positively

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with traditional markers of infection.[14,15] In patients with pneumonia, elevated QFM levels may serve as a biomarker indicating immune recovery, whereas decreased QFM levels could be predictive of an increased risk of developing HCAI. Thus, QFM may serve as a novel biomarker related to HCAI.

This study has some limitations. It is a single-center study, and its statistical power is limited by the sample size and number of outcomes. Furthermore, as only patients with pneumonia were enrolled, further research involving other types of infections is necessary to generalize the study conclusions.

In conclusion, this study provides evidence supporting the potential role of QFM in assessing the immune status of patients with infectious diseases and demonstrates that QFM levels vary depending on the patient immune status and recovery. Specifically, higher QFM levels were independently associated with survival in patients with infections, increases in QFM levels were associated with recovery, and decreases were associated with poorer prognosis. QFM was strongly negatively correlated with TNF and CRP, which is consistent with clinical results. Thus, QFM holds potential as a novel biomarker for assessing the risk and occurrence of HCAIs. Therefore, the evaluation of a patient's immune status using QFM as a novel biomarker may play a significant role in predicting their prognosis during infections.

Data Sharing Statement

The datasets utilized and/or analyzed during the present study are obtainable from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The authors take full responsibility for all aspects of the work and have ensured that any questions regarding the accuracy or integrity of any part of the study have been thoroughly investigated and resolved. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki (2013 revision). Approval for the study was obtained from the Institutional Review Board (IRB No. 2021-01-07) of Pusan National University Yangsan Hospital (PNUYH). Individual consent for this retrospective analysis was waived, due to the retrospective nature of the design and a standard-of-care observational study with no intervention.

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Disclosure

The authors report no conflicts of interest in this work.

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