Combined Plasma Elevation of CRP, Intestinal-Type Fatty Acid-Binding Protein (I-FABP), and sCD14 Identify Older Patients at High Risk for Health Care-Associated Infections

Elena Paillaud, Sylvie Bastuji-Garin, Anne Plonquet, Emile Foucat, Bénédicte Fournier, Emmanuelle Boutin, Aurélie Le Thuaut, Yves Levy, Sophie Hue

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Paillaud E.[1,2,], Bastuji-Garin S.[2,3,4,], Plonquet A.[5] ,Foucat E.[6,7,], Fournier B.[8], Boutin E.[2,4], Le Thuaut A.[2,4], Levy Y. [6,7,9], Hue S.[5,6,7,]

[1]Assistance Publique-Hôpitaux de Paris (AP-HP), Groupe Hospitalier Henri-Mondor, Département de médecine interne et gériatrie, F 94010 Creteil, France

[2] Université Paris Est (UPEC), DHU A-TVB, IMRB, CEpiA (Clinical Epidemiology and Ageing) Unit EA 7376, F 94010 Creteil, France

[3]Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Henri-Mondor, Service de Santé Publique, F 94010 Creteil, France

[4]Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Henri-Mondor, Unité de Recherche Clinique (URC Mondor), F 94010 Creteil, France

[5]Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Henri-Mondor, Service d'immunologie biologique, F 94010 Creteil, France

[6] INSERM U955, team 16, IMRB Créteil, F 94010 Creteil, France

[7]Vaccine Research Institute (VRI), Université Paris Est Créteil, Faculté de Médecine, F 94010 Creteil, France

[8]Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Henri-Mondor, Service de biochimie, F 94010 Creteil, France

[9]Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Henri-Mondor, Service d'immunologie clinique, F 94010 Creteil, France;

E. Paillaud and S. Bastuji-Garin contributed equally to this manuscript.

Running title: Risk Biomarkers for Nosocomial Infection

Corresponding author:

Prof. Sophie Hüe, CHU Henri Mondor, 94010 Créteil, FRANCE

Phone: +33 149 812 298

Fax: +33 149 812 897

E-mail: sophie.hue@ aphp.fr

Abstract

Background: We hypothesized that low-grade inflammation was driven by microbial translocation and associated with an increased risk of healthcare-associated infections (HAIs).

Methods: We included 121 patients aged 75 years or over in this prospective cohort study. High-sensitivity C-reactive protein (hs-CRP), I-FABP, and sCD14 -- as markers for low-grade inflammation, intestinal epithelial barrier integrity, and monocyte activation, respectively -- were measured at admission.

Results: HAIs occurred during hospitalization in 62 (51%) patients. Elevated hs-CRP (≥6.02 mg/L, i.e., the median) was associated with a significantly higher HAI risk when I-FABP was in the highest quartile (odds ratio [OR], 4; 95% confidence interval [95%CI], 1.39-11.49; *P*=0.010). In patients with hs-CRP elevation and highest-quartile I-FABP, sCD14 elevation (≥0.65 pg/mL, i.e., the median) was associated with an 11-fold higher HAI risk (OR, 10.8; 95%CI, 2.28-51.1; *P*=0.003). Multivariate analyses adjusted for invasive procedures and comorbidities did not change the associations linking the three markers to the HAI risk.

Conclusion: Increased levels of hs-CRP, I-FABP, and sCD14 may reflect loss of intestinal epithelial barrier integrity with microbial translocation leading to monocyte activation and low-grade inflammation. In our cohort, these markers identified patients at high risk for HAIs.

Keywords: Nosocomial infections. Inflammaging. Microbial translocation. Prediction.

INTRODUCTION

Healthcare-associated infections (HAIs) are a major public-health concern as they are both common and associated with high morbidity and mortality rates that generate a heavy healthcare cost burden (1)(2). Among HAIs, 54% affect individuals aged 65 years or over (3). Risk factors for HAIs vary across infection sites, healthcare settings, and patient age groups. We previously reported that strong risk factors for HAI among patients aged 70 years or over included invasive procedures and comorbidities assessed using the Cumulative Illness Rating Scale for Geriatrics (CIRS-G)(4). In elderly individuals, the increased susceptibility to severe infections and decreased efficacy of vaccinations may reflect the immunological aging process known as immunosenescence, which involves nearly all immune-system components (5)(6).

Two main hypotheses are currently put forward to explain immunosenescence. One of them ascribes a key role to changes in T-cell subset counts related in part to repeated antigenic stimuli (7)(8). The immune risk phenotype (IRP)(9) reflecting these alterations includes inversion of the CD4/CD8 ratio and expansion of $CD8^+$ $CD28^-$ T cells with positive cytomegalovirus serology (10). In a previous study, lung infections were the only HAIs whose risk was increased among patients with the IRP (11). The second hypothesis stems from the high prevalence in elderly people of low-grade chronic inflammation, known as inflammaging (12). Chronic inflammation is common in many diseases (13)(14)(15) and may be driven by microbial translocation through the gut (16). Penetration of commensal microorganisms from the gut lumen into the gut wall is particularly common in older individuals (17) and constitutes the first step of microbial translocation. The gut immune system normally eliminates the organisms from the gut wall. If this mechanism fails, the organisms enter the systemic circulation, where they induce a chronic low-grade systemic

inflammatory response (18). This is the second step of microbial translocation. The plasma level of intestinal-type fatty acid-binding protein (I-FABP) increases with epithelial-cell layer apoptosis (19),which promotes the first step. Endotoxin (lipopolysaccharide, LPS) found in the membrane of Gram-negative bacteria binds to several proteins including CD14, which also exists in soluble form (sCD14). Monocytes shed sCD14, which binds LPS in a complex with LPS-binding protein and therefore serves as a marker of LPS-induced monocyte or macrophage activation (20). Thus, sCD14 serves as a marker for the second step of microbial translocation.

We hypothesized that age-related alterations in the intestinal epithelium and intestinal-wall innate immune responses promoted low-grade inflammation, a condition associated with an increased risk of HAIs. Our objective here was to test this hypothesis by assaying two markers of low-grade inflammation (high sensitivity C-reactive protein [hs-CRP] and IL-6), a marker of epithelial-cell apoptosis (I-FABP), and a marker of monocyte-macrophage activation by translocated microbial products (sCD14). These markers were measured at admission of older patients to a geriatric rehabilitation unit. Associations linking them to future HAIs were sought.

METHODS

Study design

We used data from a previously described (4) prospective cohort study conducted between July 2006 and November 2008 in a teaching hospital (1300 beds) in the Paris area, France. The cohort comprised 252 consecutive Caucasians aged 75 years or over who were referred to a geriatric rehabilitation unit by acute medical or surgical units during the study period. Inclusion criteria were medically stable status at admission; need for long-term care and rehabilitation; and absence of terminal disease (e.g., uncontrolled malignancy or severe dementia), fever, infection, cancer, or known immunological dysfunction. Patients treated with corticosteroids or immunosuppressants and those who stayed less than 48 hours in the rehabilitation unit were not eligible. Patients were followed up until discharge from the rehabilitation unit or up to 3 months after inclusion. The 121 patients for whom baseline serum samples were still available were included in the present study. The study was approved by the Ile-de-France IX ethics committee in Paris, France (#SCR06010). Written informed consent was obtained from each patient before inclusion in the cohort.

Assessment of hospital-acquired infections

As previously described (4), HAI was defined as a well-documented infection that was neither present nor incubating at admission and that met the Centers for Disease Control definition of nosocomial infection (21). The procedure for ascertaining HAIs is described in the Supplementary Material. HAIs were diagnosed by consensus between two geriatricians. Once a week, these two geriatricians visited each study patient and reviewed the medical records with the attending physician and nurses. The diagnosis was based on a combination of clinical findings (fever, pulmonary rales or dullness, dyspnea, cough, purulent sputum,

dysuria, urgency, suprapubic tenderness, clinical evidence of sepsis, and/or purulent drainage from a surgical incision), laboratory test results (blood and urine cultures, isolation of a pathogen from other specimens, and antigen- or antibody-detection tests), and findings from imaging studies (e.g., X-rays and computed tomography). Only HAIs requiring antibiotic therapy were taken into account; asymptomatic urinary tract infections were not counted.

Data collection

Technicians blinded to patient status assayed hs-CRP, IL-6, I-FABP, and sCD14 in baseline serum samples. Serum hs-CRP was assayed by immunoturbidimetry using a Cobas C501 analyzer (Roche Diagnostics, Mannheim, Germany) and IL-6, I-FABP, and sCD14 using specific commercially available ELISAs (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. For each patient, the two main known risk factors for HAIs -- comorbidities and invasive procedures -- were collected routinely on a standardized form. Comorbidities were detected at admission by completing the Cumulative Illness Rating Scale-Geriatric (CIRS-G) during an interview with the patient. Invasive procedures included insertion of an intravenous, indwelling urinary, or intermittent urinary catheter; nasogastric tube insertion; and endoscopy (upper gastrointestinal tract endoscopy, colonoscopy, or bronchoscopy). For each patient, invasive procedures were recorded until HAI occurrence or patient discharge.

Statistical analysis

Qualitative variables were described as number (%) and compared using the Chi²-test or Fisher exact test, as appropriate. Quantitative variables were described as median [25th-75th percentiles] and compared using the nonparametric Mann-Whitney test. We compared the groups with and without HAI regarding the following baseline characteristics:

comorbidities (CIRS-G score), invasive procedures, and levels of the four laboratory markers (hs-CRP, IL6, I-FABP, and sCD14) (Table 1). Univariate odds ratios (OR) were estimated with their 95% confidence intervals (95%CIs) using logistic regression models. Because of their skewed distribution, CIRS-G, hs-CRP, IL6, I-FABP, and sCD14 were log-transformed; the ORs and 95%CIs are given for a 1-standard deviation (SD) variation in the log-transformed values. Associations between these three biomarkers were assessed using the nonparametric Spearman's rank correlation (Rho). Pairwise analyses were performed to assess interactions and confounding by fitting multiplicative models. When a significant interaction was found, a composite variable was built. For this step, quantitative variable values were categorized as below or within the highest quartile (Q3; coded 0 and 1, respectively) or as below or above the median value (Q2; coded 0 and 1, respectively), as appropriate. Since both hs-CRP and IL-6 are inflammatory markers, we considered two separate models, one combining hs-CRP, I-FABP, and sCD14 and the other using IL-6 instead of hs-CRP. Then, we used multivariate modeling to assess whether these markers were associated with the HAI risk independently from comorbidities and invasive procedures.

A sensitivity analysis was performed among patients who stayed at least 5 days in the rehabilitation unit, under the hypothesis that the 48-hour stay required for the main analysis might be too short for HAIs to become symptomatic. Finally, we performed a second sensitivity analysis separating early-onset and late-onset HAIs, with 10 days as the cut-off, using multinomial logistic modeling (no HAI, early-onset HAI, and late-onset HAI).

All tests were two-sided, and *P* values ≤ 0.05 were considered significant. No adjustments for multiple comparisons were performed. Data were analyzed using STATA software SE12.0 (StataCorp, College Station, TX).

RESULTS

Baseline characteristics

Table 1 displays the baseline characteristics of the 121 patients. Their mean length of stay in the rehabilitation unit was 45 days (range, 3-91). At least one HAI was diagnosed in 62 patients. The most common sites of HAI were the respiratory tract (50%; 31/62) and urinary tract (38.7%; 24/62). The median time to HAI diagnosis after admission to the rehabilitation unit was 12 days (range, 2-62).

Comorbidities and invasive procedures were significantly associated with subsequent HAI, while baseline leukocyte count was not.

Associations linking baseline biomarker levels to subsequent hospital-acquired infection (HAI)

Baseline levels of hs-CRP, IL6 and s-CD14 were significantly associated with HAI occurrence ($P<0.05$), while only a trend for an association was observed for I-FABP (Table 1). As expected, levels of hs-CRP and IL-6 were strongly correlated (Rho=0.76, $P<0.001$); significant correlations were also demonstrated between s-CD14 and both hs-CRP (Rho=0.29, $P=0.003$) and IL-6 (Rho=0.24, $P=0.02$).

Identification of patients at high risk for healthcare-associated infections (HAIs)

In joint analyses, a significant third-order interaction was observed for serum hs-CRP, I-FABP, and sCD14 levels; as well as two-order interactions for each of the three pairs formed by these three variables. Therefore, we built composite variables combining hs-CRP (≥Q2 versus <Q2), I-FABP (≥Q3 versus <Q3), and sCD14 (≥Q2 versus <Q2). Because the HAI risk was lower in patients with hs-CRP values below the median value, irrespective of

their I-FABP and sCD14 levels, all these patients were pooled in the reference category. As shown in Table 2 (model 1), hs-CRP elevation was associated with a significantly higher HAI risk when the I-FABP level was in the highest quartile (OR, 4; 95%CI, 1.39-11.49; *P*=0.010).

We then considered both I-FABP and sCD14 levels (Table 2, model 2). The patients with high levels of all three markers had an 11-fold higher risk of HAI (OR, 10.8; 95%CI, 2.28-51.1; *P*=0.003). In contrast, combined hs-CRP and I-FABP elevation without sCD14 elevation was not associated with a significantly higher HAI risk.

When we substituted IL-6 for hs-CRP as the marker for low-grade inflammation, the results were unchanged (Table 2, model 2): IL-6 elevation (≥ median) was associated with a significantly higher HAI risk when the I-FABP level was in the highest quartile, and patients with high levels of all three markers had a 6-fold higher HAI risk.

We suggest the term "biomarker risk profile" to designate the combination of high hs-CRP or IL-6, I-FABP, and sCD14 levels.

Association between the biomarker risk profile and the risk of healthcare-associated infections (HAIs)

We previously reported that comorbidities and invasive procedures were major risk factors for HAIs(11). Adjusting for these two factors in the multivariate analysis had little effect on our results: the biomarker risk profile using hs-CRP remained associated with a 10-fold higher HAI risk after adjustment for dependency according to CIRS-G criteria or for invasive procedures (Table 3, model 1). Likewise, the biomarker risk profile using IL-6 remained associated with a 5-fold higher HAI risk after adjustment for dependency according to CIRS-G criteria or for invasive procedures (Table 3, model 2).

Sensitivity analyses

Analyses performed among the 100 patients who stayed at least 5 days in the rehabilitation unit produced closely similar results as described in the supplementary eTable 1. The biomarker risk profile with hs-CRP was associated with a 13-fold higher risk of HAI and remained associated with the HAI risk after adjustment for dependency according to CIRS-G criteria or for procedures.

The combination of three markers was significantly associated with both early-onset and late-onset HAIs. The ORs were twice as high for early infections compared to late infections, but the differences were not significant (P= 0.23 and P=0.19 in the models adjusted for invasive procedures and CIRS-G, respectively (supplementary eTable 2).

DISCUSSION

We assessed whether biomarkers for inflammation associated with microbial translocation predicted the risk of HAI. In patients aged 75 years or over and admitted to a geriatric rehabilitation unit, concomitant elevations in the levels of three biomarkers – hs-CRP, I-FABP, and sCD14 – were associated with a 11-fold higher risk of HAI. Adjustment on two known risk factors, comorbidities and invasive procedures did not change the associations linking the three markers to the HAI risk. Using another inflammatory marker, IL-6, we obtained closely similar results, suggesting that this biomarker risk profile may help to identify patients at high risk for HAI.

Immunosenescence is a multifactorial process of which one component is low-level inflammation, known as inflammaging. Our study supports microbial translocation as a contributor to the increased HAI risk observed in older individuals. Thus, patients with high levels of hs-CRP or IL-6 and of I-FABP exhibited a 4-fold higher risk of HAI. Microbial translocation plays a key role in driving persistent immune activation, as shown in HIV-infected patients (16). Aging may induce intestinal-barrier disruption comparable to that caused by the HIV. Thus, aging epithelial cells have mitochondrial mutations that affect their progeny in the mucosa (22), and aging is associated with remodeling of the tight junctions between epithelial cells (17). The local control of microorganisms that cross the intestinal barrier may also be compromised by many age-related immunological alterations such as impaired chemotaxis and phagocytosis, altered expression of pattern recognition receptors (PRR), activation of these receptors by endogenous ligands associated with cellular damage, and aberrant signaling events downstream of PRR activation resulting in cytokine secretion (12).

High plasma sCD14 was one of the three components of the biomarker risk profile identified in our study. Plasma sCD14 levels reflect monocyte activation. LPS, found in the membrane of Gram-negative bacteria, is a potent monocyte activator that binds to CD14 and induces the shedding of sCD14. In previous studies, sCD14 levels were associated with a high risk of future clinical cardiovascular disease in older adults (23) and a high risk of mortality in gram-negative septic shock (24). In another study, LPS-binding protein, another biomarker of microbial translocation, was associated with physical function in healthy older adults, while sCD14 was associated with several inflammatory markers but not with physical function (25).

In our study, I-FABP elevation consistent with intestinal barrier disruption was associated with the HAI risk only when sCD14 was also elevated, indicating monocyte activation, i.e., penetration of intestinal microorganisms and/or their products into the systemic circulation. Monocytes are crucial sentinels for controlling the passage of intestinal microorganisms through the intestinal barrier. Chronic exposure to systemic low levels of pro-inflammatory cytokines modulates phagocytic mononuclear activity, thereby affecting pathogen clearance. Importantly, in patients with high hs-CRP and I-FABP levels but low sCD14 levels, the HAI risk was similar to that in patients with normal hs-CRP levels. This finding suggests that intestinal barrier disruption may lead to microbial translocation only if the local immune system is deficient. Secretory IgA antibody on mucosal surfaces plays a pivotal role in controlling the microbiota (26). Deficient IgA production has been reported in elderly individuals (27). The combination of intestinal barrier disruption and impaired intestinal wall immunity may generate chronic low-level systemic inflammation, which may in turn affect immune response regulation, thus increasing the HAI risk. It is tempting to speculate that local deficiency of the gut immune system may affect other mucosal sites such as the respiratory and urinary tracts, leading to infections. Finally, in patients with high hs-

CRP but normal I-FABP levels, other mechanisms may be active, leading to different outcomes.

We used two strategies to test the robustness of our findings. First, we repeated the analysis after replacing hs-CRP with IL6, an inflammatory marker associated with inflammaging in the general population(28). Patients with high levels of IL-6, I-FABP, and sCD14 had a 6-fold higher HAI risk. Furthermore, the biomarker risk profile with IL-6 remained associated with a 5-fold higher HAI risk after adjustment for dependency or invasive procedures. These results support a link between microbial translocation and low-grade inflammation. Second, we confined the analysis to patients who stayed at least 5 days in the rehabilitation unit. The 48-hour stay required for the main analysis may be too short for HAIs to become symptomatic in patients coming from acute care units. Again, the results were essentially unchanged, with a 13-fold higher HAI risk among patients with high levels of all three markers. Moreover, mean time to HAI diagnosis was 12 days. These data suggest that elevation of the three markers at admission was linked to low-grade chronic inflammation. We are not aware of previous reports that a biomarker risk profile is strongly associated with HAIs in patients 75 years or older. Whether routine assessment of this profile followed by intensified prevention and monitoring if positive diminishes the morbidity and mortality rates associated with HAIs deserves investigation.

Our study has several limitations. First, the single-center design, may have led to recruitment bias, thereby compromising external validity. Second, the sample size was limited, and our hypothesis requires further investigation in a large independent sample. Third, our study of sCD14 production provides only indirect evidence that increased gut permeability resulted in bacterial translocation. Furthermore, sCD14 production in a given patient can vary as a result of changes in liver function (29) and of direct effects of inflammatory factors such as IL-6 and IL-1β. However, sCD14 is an easily measured,

endogenous biomarker for the host's response to microbial products that has been widely used, for several reasons: LPS is challenging to assay, LPS clearance involves a variety of mechanisms, and genetic factors governing monocyte responsiveness to LPS complicate the interpretation of LPS levels(18). Direct evidence of increased gut permeability resulting in bacterial translocation could be obtained by measuring blood levels of peptidoglycan or unmethylated CpG-containing DNA. Fourth, we were unable to assess changes in these markers during the rehabilitation unit stay, as we had a single blood sample, obtained at admission. Although associations with the markers appeared stronger for early than for late infections, the difference was not statistically significant. Additional longitudinal studies with repeated assays of cytokines, intestinal epithelial barrier dysfunction, and endotoxins over time are required.

CONCLUSION

This study identified a biomarker risk profile strongly associated with HAIs in patients 75 years or older who were admitted to a rehabilitation unit. The profile involves three biomarkers, hs-CRP or IL-6 for systemic inflammation, I-FABP for intestinal-barrier disruption, and sCD14 for systemic monocyte activation.

Our findings support a link between microbial translocation and the low-grade inflammation seen in some older patients with HAI and identify a combination of biomarkers as a risk factor for HIA. More research is needed to elucidate the role for low-grade inflammation and microbial translocation in HAIs. However, from a clinical stand-point, our findings suggest that the three biomarkers may help identify those individuals most at risk for HAI.

Conflicts of interest

The authors declared no conflicts of interest.

Funding

This work was supported by the 2006 *Soins Courants* grant #SCR06010 from the French Ministry of Health, *Direction de la Recherche Clinique et du Développement (Assistance Publique-Hôpitaux de Paris*, AP-HP).

Acknowledgments

We thank the study patients for their willingness to participate in this research project and A Wolfe, MD, for editing the manuscript. We are grateful to the Mondor Immunotoring Platform from the Vaccine Research Institute (labex ANR-10-LABX-77) for technical assistance.

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Table 1: Baseline characteristics of the overall study population (N=121) and of the subgroups with and without healthcare-associated infections (univariate analyses)

	Overall (N=121)	Healthcare-associated infection		Univariate analysis	
		No 59 (48.8%)	Yes 62 (51.2%)	OR (95%CI)	*P* value[a]
General characteristics					
Age, median (Q1-Q3), years	84 (81-90)	84 (80-90)	85.5 (81-89)	1.01 (0.95-1.08)	0.77
Female gender, N (%)	91 (75.2)	45 (76.3)	46 (74.2)	0.89 (0.39-2.04)	0.79
Invasive procedures, N (%)	38 (31.4)	9 (15.3)	29 (46.8)	4.88 (2.05-11.62)	<0.001
CIRS-G, median (Q1-Q3)	11 [10-14]	10 [9-13]	13 [10-15]	1.84 (1.20-2.82)	0.005
Biological parameters					
White blood cells ($x10^6$/L)	6.9 (5.6-8.3)	6.5 (5.6-8.0)	7.2 (5.7-8.6)	1.11 (0.83-1.48)	0.18
hs-CRP (mg/L), median (Q1-Q3)	6.43 (2.11-13)	6.02 (1.51-11)	6.59 (4.23-13.43)	1.54 (1.02-2.33) [b]	0.047
IL6 (pg/mL), median (Q1-Q3)	5.70 (3.14-9.86)	4.99 (2.67-9.46)	6.30 (4.34-10.27)	1.47 (1.02-2.12) [b]	0.04
I-FABP (pg/mL), median (Q1-Q3) (n=2)	1428 (951-2455)	1413 (938-2004)	1510 (1037-3057)	1.50 (1.00-2.24) [b]	0.06
sCD14 (µg/mL), median (Q1-Q3) (n=9)	0.68 (0.60-0.77)	0.65 (0.58-0.75)	0.71 (0.61-0.81)	1.55 (1.11-2.16) [b]	0.02

Abbreviations: OR, odds ratio; 95%CI, 95% confidence interval; CIRS-G, Cumulative Illness Rating Scale for Geriatrics; N, number of patients; Q1-Q3, 25^{th}-75^{th} percentile; hs-CRP, high sensitivity C-reactive protein; (n=), number of patients with missing data

[a] *P* value obtained using the nonparametric Mann-Whitney test, Chi2 test, or Fisher's exact test, as appropriate

[b] Odds ratios were computed for an increase by 1 standard deviation in the log-transformed values.

Table 2: Risk of healthcare-associated infections according to the hs-CRP, I-FABP, and sCD14 serum levels (univariate analyses using logistic regression models)

		Model 1 [a]			*Model 2* [b]	
		OR 95%CI	***P* value**		**OR 95%CI**	***P* value**
Hs-CRP < Q2 (6.02 mg/L)[c] *N=55* *HAI: 25 (45.5%)*		1 (reference category)			1 (reference category)	
CRP ≥ Q2 *N=62* *HAI: 37(59.7%)*	**I-FABP < Q3 (2004 pg/mL)**[c] *N=36* *HAI: 17 (47.2%)*	0.89 [0.39-2.02]	0.78	**sCD14 < Q2 (0.65 µg/mL)**[c] *N=14* *HAI: 5 (35.7%)*	0.67 [0.20-2.25]	0.51
				sCD14 ≥ Q2 *N=22* *HAI: 11 (50.0%)*	1.44 [0.53-3.89]	0.47
	I-FABP ≥ Q3 *N=26* *HAI: 20 (76.9%)*	**4.00 [1.39-11.49]**	**0.01**	**sCD14 < Q2** *N=6* *HAI: 2 (33.3%)*	0.60 [0.12-3.55]	0.57
				sCD14 ≥ Q2 *N=20* *HAI: 18 (90 %)*	**10.80 [2.28-51.1]**[6]	**0.003**
Sensitivity analysis using IL6 instead of hs-CRP to assess low-grade inflammation						
IL6 < Q2 (4.99 pg/mL)[c] *N=52* *HAI: 22 (42.3%)*		1 (reference category)			1 (reference category)	
IL6 ≥ Q2 *N=63* *HAI: 38(60.3%)*	**I-FABP < Q3 (2004 pgm/L)**[c] *N=36* *HAI: 18 (50%)*	1.24 [0.55-2.80]	0.61	**sCD14 < Q2 (0.65 µg/mL)**[c] *N=15* *HAI: 6 (40%)*	0.91 [0.28-2.93]	0.87
				sCD14 ≥ Q2 *N=21* *HAI: 12 (57.1%)*	1.82 [0.65-5.06]	0.25
	I-FABP ≥ Q3 *N=27* *HAI: 20 (74.1%)*	**6.90 [1.40-10.82]**	**0.009**	**sCD14 < Q2** *N=5* *HAI: 2 (40%)*	0.91 [0.14-5.91]	0.92
				sCD14 ≥ Q2 *N=22* *HAI: 18 (81.8%)*	**6.14 [1.82-20.68]**	**0.003**

Abbreviations: hs-CRP, high sensitivity C-reactive protein; I-FABP, intestinal fatty acid-binding protein; sCD14, soluble CD14; OR, odds ratio; 95%CI, 95% confidence interval; N, number of patients; Q2, median value; Q3, 75th percentile

[a] Model 1 takes into account a combination of hs-CRP (IL6) and I-FABP levels coded as follows: 0 if hs-CRP (/)IL6) < Q2 whatever the I-FABP level; 1 if hs-CRP (/)IL6 ≥ Q2, and I-FAPB <Q3; and 2 if hs-CRP (IL6) ≥ Q2 and I-FABP ≥ Q3.

[b] Model 2 takes into account a combination of hs- CRP (IL6), I-FABP, and sCD14 levels coded as follows: 0 if hs- CRP (IL6) < Q2 whatever the I-FABP and sCD14 levels; 1 if hs- CRP (IL6) ≥ Q2, I-FAPB < Q3, and sCD14 < Q2; 2 if hs-CRP (IL6) ≥ Q2, I-FABP < Q3, and sCD14 ≥ Q2; 3 if hs- CRP (IL6) ≥ Q2, I-FABP ≥ Q3, and sCD14 < Q2; and 4 if hs- CRP (IL6) ≥ Q2, I-FABP ≥ Q3, and sCD14 ≥ Q2.

[c]Cutpoint

Table 3. Risk of healthcare-associated infections according to the CRP, I-FABP and sCD14 serum levels adjusted for major risk factors for healthcare-associated infections (multivariate logistic regression analyses adjusted for either invasive procedure or CIRS-G)

	OR (95%CI)	*P* value
Model with invasive procedures [a]		
Invasive procedures [b]	4.70 (1.84-12.01)	0.001
Hs-CRP ≥ Q2, I-FABP ≥ Q3, and sCD14 ≥ Q2	9.62 (2.04-45.4)	0.004
Model with CIRS-G [a]		
CIRS-G	1.14 (1.01-1.28)	0.029
Hs-CRP ≥ Q2, I-FABP ≥ Q3, and sCD14 ≥ Q2	9.45 (2.05-43.69)	0.004
Models using IL6 instead of hs-CRP to assess low-grade inflammation		
Model with invasive procedures [a]		
Invasive procedures [b]	4.83 (1.90-12.27)	0.001
IL6 ≥ Q2, I-FABP ≥ Q3, and sCD14 ≥ Q2	5.0 (1.50-16.64)	0.009
Model with CIRS-G [a]		
CIRS-G	1.15 (1.02-1.29)	0.024
IL6 ≥ Q2, I-FABP ≥ Q3, and sCD14 ≥ Q2	4.68 (1.44-15.7)	.01

Abbreviations: OR, odds ratio; 95%CI, 95% confidence interval; hs-CRP, high-sensitivity C-reactive protein; CIRS-G, Cumulative Illness Rating Scale for Geriatrics; Q2, median value; Q3, 75th percentile

[a] For these models, instead of using the previous combinations of hs-CRP (IL6), I-FABP, and sCD14 levels (hs- CRP (IL6) < Q2 whatever the I-FABP and sCD14 levels; hs- CRP (IL6) ≥ Q2, I-FAPB <Q3, and sCD14 <Q2; hs- CRP (IL6) ≥ Q2, I-FABP < Q3, and sCD14 ≥ Q2; hs- CRP (IL6) ≥ Q2, I-FABP ≥ Q3, and sCD14 < Q2; and hs- CRP (IL6) ≥ Q2, I-FABP ≥ Q3, and sCD14 ≥ Q2), we compared hs-CRP (IL6) ≥ Q2, I-FAPB ≥ Q3, and sCD14 ≥ Q2 *versus* all other combinations pooled.

[b] Invasive procedures recorded for each patient until HAI occurred or the patient was discharged from the rehabilitation unit included intravenous catheter, indwelling urinary catheter, intermittent urinary catheter, gastrointestinal tract endoscopy, nasogastric tube, colonoscopy, and bronchoscopy.

Supplementary eTable 1. Risk of healthcare-associated infections according to the hs-CRP, I-FABP, and sCD14 serum levels among the 100 patients who stayed at least five days in the rehabilitation unit (univariate and multivariate analyses using logistic regression models)

Univariate analyses

		Model 1[a]			*Model 2*[b]	
		OR 95%CI	***P* value**		**OR 95%CI**	***P* value**
Hs-CRP < Q2 (4.8 mg/L) *N=40* *HAI: 15 (37.5%)*		1 (reference category)			1 (reference category)	
CRP ≥ Q2 *N=57* *HAI: 34(59.6%)*	**I-FABP < Q3 (2106 pg/mL)** *N=32* *HAI: 15 (46.9%)*	1.40 [0.56-3.53]	0.47	**sCD14 < Q2 (0.65 µg/mL)** *N=11* *HAI: 5 (35.7%)*	0.95 [0.24-3.81]	0.95
				sCD14 ≥ Q2 *N=21* *HAI: 11 (52.4%)*	1.83 [0.63-5.34]	0.27
	I-FABP ≥ Q3 *N=25* *HAI: 19 (76.0%)*	**5.28 [1.72-16.16]**	**0.004**	**sCD14 < Q2** *N=7* *HAI: 3 (42.9%)*	1.25 [0.25-6.37]	0.5779
				sCD14 ≥ Q2 *N=18* *HAI: 16 (88.9 %)*	**13.33 [2.68-66.26]**	**0.002**

Multivariate analyses adjusted for either invasive procedure or CIRS-G

	OR (95%CI)	***P* value**
Model with invasive procedure [c]		
At least 1 invasive procedure [d]	6.78 (2.32-19.84)	<0.001
Hs-CRP ≥ Q2, I-FABP ≥ Q3, and sCD14 ≥ Q2	11.27 (2.29-55.45)	0.003
Model with CIRS-G[c]		
CIRS-G	1.14 (1.00-1.30)	0.047
Hs-CRP ≥ Q2, I-FABP ≥ Q3, and sCD14 ≥ Q2	9.82 (2.08-46.44)	0.004

Abbreviations: hs-CRP, high sensitivity C-reactive protein; I-FABP, intestinal fatty acid-binding protein; sCD14, soluble CD14; OR, odds ratio; 95%CI, 95% confidence interval; N, number of patients; Q2, median value; Q3, 75th percentile; CIRS-G, Cumulative Illness Rating Scale for Geriatrics

[a] Model 1 takes into account a combination of hs-CRP (IL6) and I-FABP levels coded as follows: 0 if hs-CRP (/)IL6) < Q2 whatever the I-FABP level; 1 if hs-CRP (/)IL6 ≥ Q2 and I-FAPB <Q3; and 2 if hs-CRP (IL6) ≥ Q2 and I-FABP ≥Q3.

[b] Model 2 takes into account a combination of hs- CRP (IL6), I-FABP, and sCD14 levels coded as follows: 0 if hs- CRP (IL6) < Q2 whatever the I-FABP and sCD14 levels; 1 if hs- CRP (IL6) ≥ Q2, I-FAPB <Q3, and sCD14 <Q2; 2 if hs- CRP (IL6) ≥ Q2, I-FABP <Q3, and sCD14 ≥Q2; 3 if hs- CRP (IL6) ≥ Q2, I-FABP ≥Q3, and sCD14 <Q2; and 4 if hs- CRP (IL6) ≥ Q2, I-FABP ≥Q3, and sCD14 ≥Q2.

[c] For these models, instead of using the previous combinations of hs-CRP (IL6), I-FABP, and sCD14 levels, we compared hs- CRP (IL6) ≥ Q2, I-FAPB ≥ Q3, and sCD14 ≥ Q2 *versus* all other combinations pooled.

[d] Invasive procedures recorded for each patient until HAI occurred or the patient was discharged from the rehabilitation unit included intravenous catheter, indwelling urinary catheter, intermittent urinary catheter, gastrointestinal tract endoscopy, nasogastric tube, colonoscopy, and bronchoscopy.

Supplementary eTable 2. Risk of early- and late-onset healthcare-associated infections according to the hs-CRP, I-FABP, and sCD14 serum levels (multivariate analyses using multinomial logistic regression models)

	Early-onset HAIs (≤ 10 days) (N=27)		Late-onset HAIs (>10 days) (N=35)	
	OR (95%CI)[a]	*P* value	OR (95%CI)[a]	*P* value
Model with invasive procedure				
At least 1 invasive procedure [b]	4.83 (1.58-14.83)	0.006	4.62 [1.66-12.84]	0.003
Hs-CRP ≥ Q2, I-FABP ≥ Q3, and sCD14 ≥ Q2 [c]	13.84 (2.66-72.00)[d]	0.002	7.00 [1.34-36.55][d]	0.021
Model with CIRS-G				
CIRS-G	1.09 [0.94-1.27]	0.233	1.17 [1.03-1.34]	0.019
Hs-CRP ≥ Q2, I-FABP ≥ Q3, and sCD14 ≥ Q2 [e]	14.02 [2.76-71.13][f]	0.001	6.55 [1.27-33.77][f]	0.025

Abbreviations: HAIs, healthcare-associated infections; hs-CRP, high-sensitivity C-reactive protein; I-FABP, intestinal fatty acid-binding protein; sCD14, soluble CD14; OR, odds ratio; 95%CI, 95% confidence interval; N, number of patients; Q2, median value; Q3, 75th percentile; CIRS-G, Cumulative Illness Rating Scale for Geriatrics

[a]The "no infection" group was the reference category

[b] Invasive procedures recorded for each patient until HAI occurred or the patient was discharged from the rehabilitation unit included intravenous catheter, indwelling urinary catheter, intermittent urinary catheter, gastrointestinal tract endoscopy, nasogastric tube, colonoscopy, and bronchoscopy.

The odds ratios associated with the combination of the three markers, hs- CRP ≥ Q2 (6.02 mg/L), I-FAPB ≥ Q3 (2004 pg/L), and sCD14 ≥ Q2 (0.65 pg/mL) versus all other combinations pooled were adjusted for [c]invasive procedure or [e]Cumulative Illness Rating Scale for Geriatrics

The odds ratios for early-onset HAIs did not differ from those for late-onset HAIs ([d]*P* = 0.23, and [f]*P* = 0.19)