Plasma Ferritin as Marker of Macrophage Activation-Like Syndrome in Critically Ill Patients With Community-Acquired Pneumonia

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OBJECTIVES: Plasma ferritin levels above 4,420 ng/mL have been proposed as a diagnostic marker for macrophage activation-like syndrome in sepsis and used for selection of sepsis patients for anti-inflammatory therapy. We here sought to determine the frequency, presentation, outcome, and host response aberrations of macrophage activation-like syndrome, as defined by admission ferritin levels above 4,420 ng/mL, in critically ill patients with community-acquired pneumonia.

DESIGN: A prospective observational cohort study.

SETTING: ICUs in two tertiary hospitals in the Netherlands.

PATIENTS: One hundred fifty-three patients admitted with community-acquired pneumonia.

MEASUREMENTS AND MAIN RESULTS: Patients were stratified in community-acquired pneumonia–macrophage activation-like syndrome ($n = 15$; 9.8%) and community-acquired pneumonia–control groups ($n = 138$; 90.2%) based on an admission plasma ferritin level above or below 4,420 ng/mL, respectively. Community-acquired pneumonia–macrophage activation-like syndrome patients presented with a higher disease severity and had a higher ICU mortality (46.7% vs 12.3% in community-acquired pneumonia–controls; $p = 0.002$). Twenty-three plasma biomarkers indicative of dysregulation of key host response pathways implicated in sepsis pathogenesis (systemic inflammation, cytokine responses, endothelial cell activation, and barrier function, coagulation activation) were more disturbed in community-acquired pneumonia–macrophage activation-like syndrome patients. Hematologic malignancies were overrepresented in community-acquired pneumonia–macrophage activation-like syndrome patients (33.3% vs 5.1% in community-acquired pneumonia–controls; $p = 0.001$). In a subgroup analysis excluding patients with hematologic malignancies ($n = 141$), differences in mortality were not present anymore, but the exaggerated host response abnormalities in community-acquired pneumonia–macrophage activation-like syndrome patients remained.

CONCLUSIONS: Macrophage activation-like syndrome in critically ill patients with community-acquired pneumonia occurs more often in patients with hematologic malignancies and is associated with deregulation of multiple host response pathways.

KEY WORDS: biomarker; ferritin; lymphohistiocytosis; macrophage activation syndrome; pneumonia; sepsis

Acute lower respiratory tract infections are the fourth most common cause of death and the world's leading infectious killer (1). Community-acquired pneumonia (CAP) is the third most common cause of hospitalization for persons above 65 years old and the most common cause of sepsis (2–4). Sepsis is defined as a severe syndrome with dysregulation of the immune system resulting in organ failure (5, 6).

Macrophage activation syndrome (MAS) is a non-familial form of hemophagocytic lymphohistiocytosis (HLH) (7, 8). MAS (also named secondary HLH) is a life-threatening syndrome of excessive immune activation which can rapidly progress into organ dysfunction with pancytopenia, tissue hemophagocytosis, hepatobiliary dysfunction, disseminated intravascular coagulation, and dysfunction of the CNS as main features (8, 9). MAS can complicate the disease course in patients suffering from severe infections and hematologic malignancies (8–11). Knowledge about the pathogenesis of MAS is mostly derived from mouse studies supported by clinical investigations in children with familial HLH and adults with autoimmune conditions, showing that overactivation and reduced apoptosis of macrophages are responsible for the release of high amounts of pro-inflammatory mediators such as interleukin (IL)–1-β, IL-6, IL-18, interferon-γ, and ferritin (9, 12–14). Macrophages are an important source of serum ferritin, which may account for the association between MAS and very high ferritin levels (15).

The Hellenic Sepsis Study Group recently described the presence of a so-called "macrophage activation-like syndrome" (MALS) in 3–4% of critically ill sepsis patients, defined by a positive hemophagocytosis syndrome score and/or the copresence of hepatobiliary dysfunction and disseminated intravascular coagulation (14). MALS appeared to be an independent predictor of early mortality. The authors proposed the use of ferritin as a biomarker of MALS when plasma levels are above 4,420 ng/mL (97% specificity; 98% negative predictive value) (14). The presence of MALS may be of therapeutic relevance in sepsis considering the recent retrospective reanalysis of a phase 3 clinical trial evaluating the effect of recombinant IL-1 receptor antagonist (IL-1RA, anakinra), showing that although the overall sepsis population did not benefit from IL-1RA administration, in patients with concurrent hepatobiliary dysfunction and disseminated intravascular coagulation (considered to reflect MALS), treatment with IL-1RA was associated with significant improvement in the 28-day survival rate (16). This finding has led to a clinical trial in which sepsis patients were stratified to recombinant IL-1RA treatment based on plasma ferritin levels above 4,420 ng/mL (A Trial of Validation and Restoration of Immune Dysfunction in Severe Infections and Sepsis [PROVIDE]; ClinicalTrials.gov identifier: NCT03332225), the results of which await publication. Furthermore, the same cut off ferritin level will be used to assign sepsis patients to IL-1RA treatment in a larger trial funded by the European Commission (ImmunoSep; https://cordis.europa.eu/project/id/847422).

Knowledge of the frequency of MALS in sepsis caused by CAP is limited. In addition, the association of MALS with deregulation of key host response pathways implicated in sepsis pathogenesis has not been studied in great detail. We here aimed to determine the incidence, presentation, outcome, and host response aberrations of MALS as measured by admission ferritin levels above 4,420 ng/mL in critically ill patients with sepsis due to CAP. To obtain insight in host response pathways involved in the pathophysiology of sepsis, we measured 23 biomarkers reflecting systemic inflammation, cytokine release, endothelial cell activation, and dysfunction and coagulation activation, selected based on literature (4, 6, 17–21).

METHODS

Study Population

This study was conducted as part of the "Molecular Diagnosis and Risk Stratification of Sepsis" (MARS) project, a prospective observational cohort study in the mixed ICUs of two tertiary teaching hospitals (Academic Medical Center [AMC] in Amsterdam and University Medical Center in Utrecht) in the Netherlands (ClinicalTrials.gov identifier NCT01905033) (17, 22). Both hospitals are accredited by the Joint Commission International and follow the Surviving Sepsis Guidelines for sepsis treatment. All consecutive patients above 18 years old admitted to the two ICUs with an expected length of stay longer than 24 hours were included via an opt-out method approved by the medical ethical committees (Institutional Review Board [IRB] number 10-056C). For the current analysis, patients with CAP with a "probable or definite" likelihood according to previously described criteria (17, 22), admitted to the ICUs between January 2011 and December 2013, and

diagnosed within 24 hours after ICU admission were included. Exclusion criteria were readmission, transfer from another ICU (except when on the same day of presentation to the first ICU), a diagnosis of pneumonia greater than 2 days prior to ICU admission, a clinical suspicion of aspiration pneumonia, a concurrent coinfection (i.e., a second primary infection in an extrapulmonary site), or a cardiac arrest. All variables used for in- and exclusion criteria were prospectively registered in the MARS database and used for the current patient selection by the research team. All CAP patients included in the current analysis had sepsis, defined as the presence of at least one general, inflammatory, hemodynamic, organ dysfunction, or tissue perfusion variable derived from the 2001 International Sepsis Definitions Conference (23). EDTA anticoagulated plasma was obtained at admission and stored within 4 hours at –80ºC. Normal biomarker values were obtained from 50 subjects without infection or other acute disease (mean age, 69.2 yr; SD, 8.6) included at the outpatient clinic of the AMC in Amsterdam (ClinicalTrials.gov Identifier: NCT02928367); sampling of these subjects was approved by the medical ethical committee of the AMC (IRB number 2016_171), and written informed consent was obtained from these individuals.

Clinical Variables

A modified Sequential Organ Failure Assessment (SOFA) score excluding the CNS component (24) and Acute Physiology Score (APS) (25) were calculated upon ICU admission. The need of noradrenalin for hypotension in a dose of 0.1 µg/kg/min during at least 50% of the ICU admission day was used to define shock (17). Acute respiratory distress syndrome (ARDS) and acute kidney failure (AKI) were defined using strict criteria (26, 27). Comorbidities were defined as described (17), and the Charlson comorbidity index (28) was calculated based hereon.

Assays

See **Supplemental Digital Content 1** (http://links. lww.com/CCM/G431).

Statistical Analysis and Stratification of CAP Patients According to Plasma Ferritin Level

See **Supplemental Digital Content 2** (http://links. lww.com/CCM/G432).

RESULTS

Clinical Presentation and Outcome of Patients Stratified According to Plasma Ferritin Levels

One hundred fifty-three patients with CAP were enrolled. Plasma ferritin concentrations measured upon admission to the ICU were significantly higher in patients (median, 275 ng/mL; interquartile range [IQR], 132–623 ng/mL) than in noninfected controls (median, 103 ng/mL; IQR, 54–186 ng/mL; $p < 0.001$). In 127 CAP patients (83%), ferritin was above the median plasma concentration in controls. Fifteen CAP patients (9.8 %) had plasma ferritin levels above the suggested MALS cut off of 4,420 ng/mL (median, 13,880 ng/mL; IQR, 9,830–44,720 ng/mL; range 6,289–54,043 ng/mL) (**Fig. 1A**); these patients are further referred to as "CAP-MALS," as opposed to those with ferritin levels below 4,420 ng/mL (CAP-controls). CAP-MALS and CAP-control patients were similar in terms of demographics and chronic comorbidities except for a higher prevalence of hematologic malignancies in the CAP-MALS group (**Table 1**). CAP-MALS patients had more severe disease at admission, as reflected by higher APS and SOFA scores, a more frequent presence of shock and AKI, and higher lactate concentrations. In agreement with MALS criteria (14), CAP-MALS patients had higher concentrations of liver enzymes. Causative pathogens did not differ between CAP-MALS and CAP-control patients (**Supplemental Table 1**, Supplemental Digital Content 3, http://links. lww.com/CCM/G433).

CAP-MALS patients demonstrated a higher ICU ($p = 0.002$) and 30-day mortality when compared with CAP-control patients ($p = 0.018$) (**Fig. 1B**). The difference between groups was especially prominent within the first 5 days (5 d mortality 33.3% in CAP-MALS vs 6.6% in CAP-control patients; $p = 0.003$). Also, 10-day mortality was higher in CAP-MALS group (33.3% in CAP-MALS vs 10.9% in CAP-control patients; $p = 0.041$). A multivariate logistic regression analysis showed that hematologic malignancy was independently associated with an increased risk for MALS (odds ratio, 6.74; 95% CI, 1.42–30.42; $p = 0.01$), reflecting an absolute risk increase from 7.1% (95% CI, 2.81–11.3%) in patients without hematologic malignancies to 41.7% (95% CI, 13.9–69.6%) in patients with hematologic malignancies (**Supplemental Table 2**, Supplemental Digital Content 4, http://links.lww.com/CCM/G434).
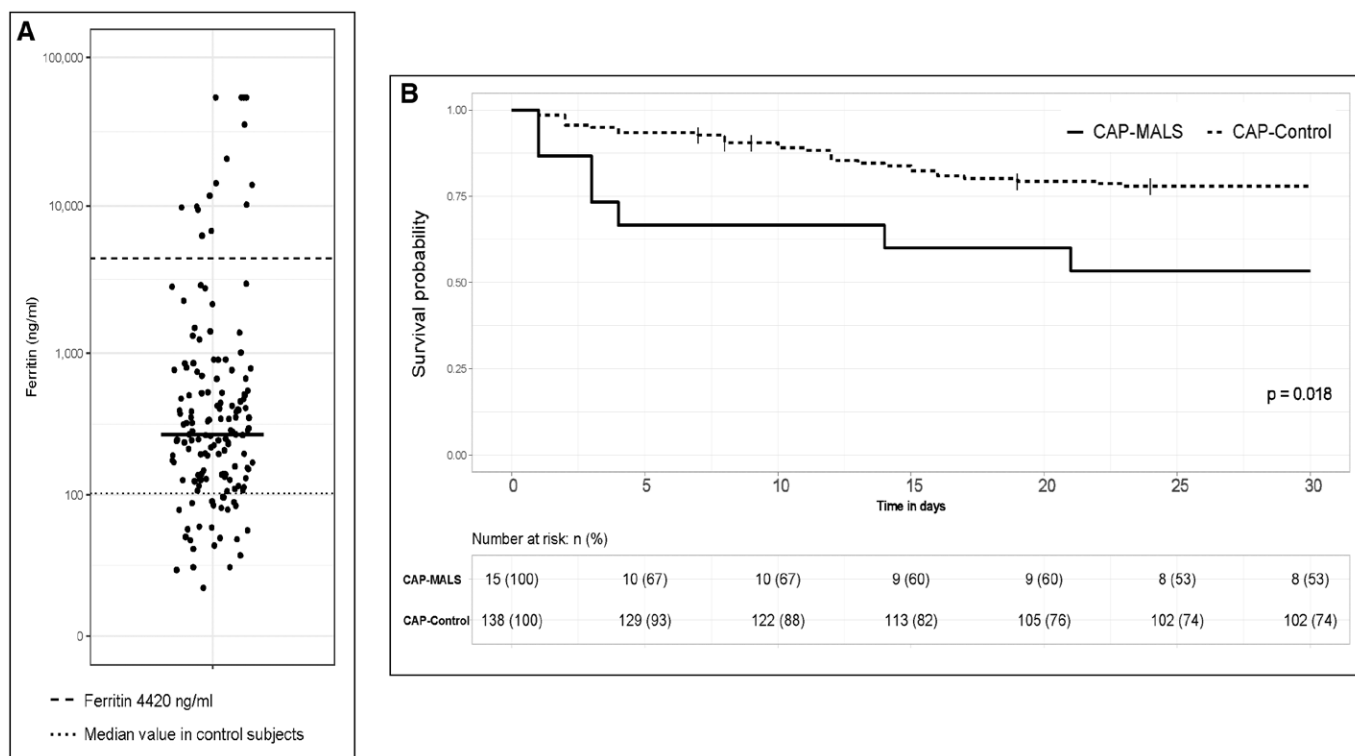
Figure 1. Plasma ferritin levels and survival of critically ill patients with community-acquired pneumonia with or without macrophage activation-like syndrome (MALS). **A**, Plasma ferritin concentrations in community-acquired pneumonia (CAP) patients at ICU admission (*n* = 153). Data are expressed as individual data points with *horizontal solid line* depicting the median. The *upper dotted line* shows the ferritin cut off value for MALS (4,420 ng/mL) (14); the *lower dotted line* indicates the median ferritin levels in noninfected control subjects (*n* = 50), which were significantly lower as compared to CAP patients (*p* < 0.001). **B**, Kaplan-Meier curves of CAP-control (*n* = 131) and CAP-MALS (*n* = 15) patients after ICU admission. *Ticks* represent the time a patient was censored. The number of patients at risk is shown below the *x*-axis.

CAP-MALS Patients Display Exaggerated Systemic Inflammation, Cytokine, Endothelial Cell, and Procoagulant Responses

To determine if there is an association of MALS with dysregulation of key host response pathways implicated in sepsis pathogenesis, we measured 23 host response plasma biomarkers, which were selected based on previous studies (4, 6, 17–21). CAP-MALS patients showed signs of enhanced systemic inflammation (**Fig. 2**) and exaggerated cytokine responses (**Supplemental Fig. 1**, Supplemental Digital Content 5, http://links. lww.com/CCM/G435; **legend**, http://links.lww.com/CCM/G442), as reflected by significantly higher plasma levels of C-reactive protein (CRP), pentraxin-3, IL-6, IL-8, IL-17, IL-23, IL-10, IL-1RA, and IL-27 relative to CAP-controls. Plasma IL-10/IL-6 and IL-10/IL-8 ratios tended to be lower in CAP-MALS patients, but the difference with CAP-control patients did not reach statistical significance (**Supplemental Fig. 2**, Supplemental Digital Content 6, http://links.lww.com/CCM/G436;

legend, http://links.lww.com/CCM/G442). CAP-MALS patients had strongly elevated plasma levels of soluble CD163, like ferritin considered to be a macrophage activation marker (9), as compared to CAP-controls. In addition, CAP-MALS patients had higher plasma concentrations of biomarkers indicative of endothelial activation (soluble vascular cell adhesion molecule [sVCAM]–1, endocan, fractalkine, thrombomodulin), a disturbed glycocalyx integrity (syndecan) (**Fig. 3**), and impaired endothelial barrier function (angiopoietin-2 and angiopoietin-2/1 ratio) (**Supplemental Fig. 3**, Supplemental Digital Content 7, http://links.lww.com/CCM/G437; legend, http://links.lww.com/CCM/G442). Furthermore, CAP-MALS was associated with enhanced activation of the coagulation system, as indicated by lower platelet counts, higher plasma levels of D-dimer, and longer activated partial thromboplastin time (aPTT) values; prothrombin time values were not different between CAP-MALS and CAP-control patients (**Fig. 4**).

TABLE 1.

Clinical Characteristics and Outcome of Community-Acquired Pneumonia Patients Stratified According to Plasma Ferritin Levels at Admission

| Clinical Characteristics and Outcome	CAP–Macrophage Activation-Like Syndrome Ferritin ≥ 4,420 ng/mL	CAP-Controls Ferritin < 4,420 ng/mL	p
Patients, n	15	138	
Demographics			
Age, yr, mean (SD)	58.4 (15.2)	60.9 (14.9)	0.53[b]
Sex, male, n (%)	10 (66.7)	84 (60.9)	0.87[a]
Ethnicity, Caucasian, n (%)	13 (86.7)	112 (81.2)	0.86[a]
Body mass index, median (IQR)	25.2 (23.5–28.1)	23.7 (21.3–26.7)	0.11
Chronic comorbidity			
Chronic obstructive pulmonary disease, n (%)	3 (20.0)	39 (28.3)	0.71[a]
Cardiovascular disease, n (%)	5 (33.3)	66 (47.8)	0.43[a]
Diabetes, n (%)	2 (13.3)	27 (19.6)	0.81[a]
Malignancy, n (%)	6 (40.0)	28 (20.3)	0.16[a]
Hematologic malignancy, n (%)	5 (33.3)	7 (5.1)	0.001[a]
Liver cirrhosis, n (%)	0 (0.0)	2 (1.4)	> 0.99[a]
Chronic renal disease, n (%)	1 (6.7)	19 (13.8)	0.71[a]
Immune suppression, n (%)	7 (46.7)	51 (37.0)	0.65[a]
Charlson comorbidity index, median (IQR)	4.0 (1.0–5.5)	3.00 (2.0–5.0)	0.80
Severity of disease at admission			
Acute Physiology Score, median (IQR)	73.00 (63.50–111.50)	60.00 (48.00–73.75)	0.03
Sequential Organ Failure Assessment score, median (IQR)	11 (8–15)	7 (4–8)	< 0.001
Shock, n (%)	12 (80.0)	62 (44.9)	0.02[a]
Acute respiratory distress syndrome, n (%)	9 (60.0)	48 (34.9)	0.10[a]
Acute kidney injury, n (%)	11 (73.3)	39 (28.3)	0.001[a]
Biological variables during the first 24 hr			
Alanine aminotransferase, U/L	52 (36–158)	27 (18–60)	0.017
Aspartate transaminase, U/L	87 (44–1,567)	43 (28–81)	0.028
Bilirubin, µmol/L	20 (12–32)	9 (5–15)	0.007
Creatinine, µmol/L	183 (96–259)	89 (61–144)	0.008
Lactate (maximum), mmol/L	4.8 (1.7–9.6)	2.3 (1.4–3.5)	0.07
Glucose, mmol/L	11.2 (9.0–12.2)	10.0 (8.0–12.9)	0.29
Albumin (min), g/L	23 (20–27)	28 (22–32)	0.16

(*Continued*)

TABLE 1. (*Continued*).
Clinical Characteristics and Outcome of Community-Acquired Pneumonia Patients Stratified According to Plasma Ferritin Levels at Admission

Clinical Characteristics and Outcome	CAP–Macrophage Activation-Like Syndrome Ferritin ≥ 4,420 ng/mL	CAP-Controls Ferritin < 4,420 ng/mL	p
Outcome			
ICU length of stay, d, median (IQR)	5 (3–14)	6 (3–12)	0.82
Hospital length of stay, d, median (IQR)	11 (4–24)	15 (8–27)	0.20
ICU mortality, n (%)	7 (46.7)	17 (12.3)	0.002[a]
Hospital mortality (n (%)	7 (46.7)	31 (22.5)	0.08[a]

CAP = community-acquired pneumonia, IQR = interquartile range.
Comparisons were done by Mann-Whitney *U* test, except for those indicated by [a](χ^2) or [b](Student *t* test).
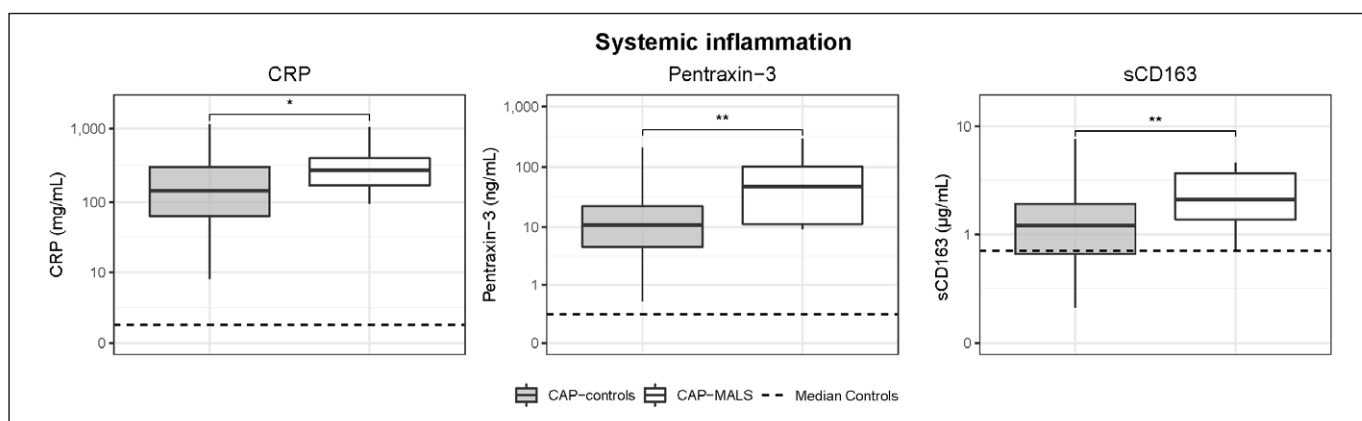
Figure 2. Host response plasma biomarkers reflecting systemic inflammation in community-acquired pneumonia (CAP)-control and CAP–macrophage activation-like syndrome (CAP-MALS) patients. Data are expressed as *box-and-whisker* diagrams with the *horizontal line* depicting the median, the *top* and *bottom* of the box representing the upper and lower quartiles, and *whiskers* extending to the farthest points that are not outliers (i.e., that are within 1.5 times the interquartile range of the highest and the lowest quartile, respectively). *Dotted lines* represent median values obtained in 50 noninfected control subjects. Values in patients were all different from those in control subjects. *Asterisks* indicate differences between CAP-control and CAP-MALS patients (Benjamini-Hochberg corrected, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). CRP = C-reactive protein, sCD = soluble cluster of differentiation.

Subgroup Analysis of Patients Without Hematologic Malignancy

Considering that hematologic malignancies were over-represented in the CAP-MALS group, we performed a subgroup analysis excluding the 12 patients (7.8%) suffering from hematologic malignancies (5 CAP-MALS and 7 CAP-control patients) (**Supplemental Table 3**, Supplemental Digital Content 8, http://links.lww.com/CCM/G438). Although in this subgroup, the severity of disease upon ICU admission was still higher in CAP-MALS patients, mortality did not differ between CAP-MALS and CAP-control patients, suggesting that the increased presence of hematologic malignancy was

an important factor herein in CAP-MALS patients. Importantly, however, the differences between CAP-MALS and CAP-control patients with regard to plasma biomarkers reflecting aberrations in key host response pathways largely remained in patients without hematologic malignancies (**Supplemental Table 4**, Supplemental Digital Content 9, http://links.lww.com/CCM/G439).

Analyses Using Other Ferritin Cut Off Levels in Patients Without Malignancies

In several ongoing trials involving patients with coronavirus disease 2019, different ferritin cut off levels have been (are) used to identify those with
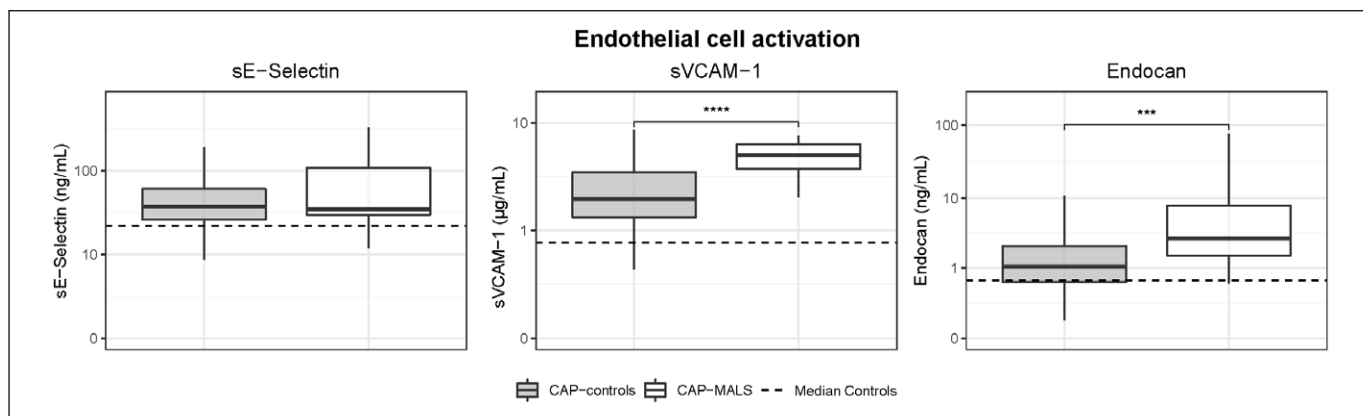
Figure 3. Host response biomarkers reflecting endothelial cell activation in community-acquired pneumonia (CAP)-control and CAP–macrophage activation-like syndrome (CAP-MALS) patients. Data are expressed as *box-and-whisker* diagrams with the *horizontal line* depicting the median, the *top* and *bottom* of the box representing the upper and lower quartiles, and *whiskers* extending to the farthest points that are not outliers (i.e., that are within 1.5 times the interquartile range of the highest and the lowest quartile, respectively). *Dotted lines* represent median values obtained in 50 noninfected control subjects. Values in patients were all different from those in control subjects. *Asterisks* indicate differences between CAP-control and CAP-MALS patients (Benjamini-Hochberg corrected, $^*p <$ 0.05, $^{**}p < 0.01$, $^{***}p < 0.001$). s = soluble, sVCAM = soluble vascular cell adhesion molecule.
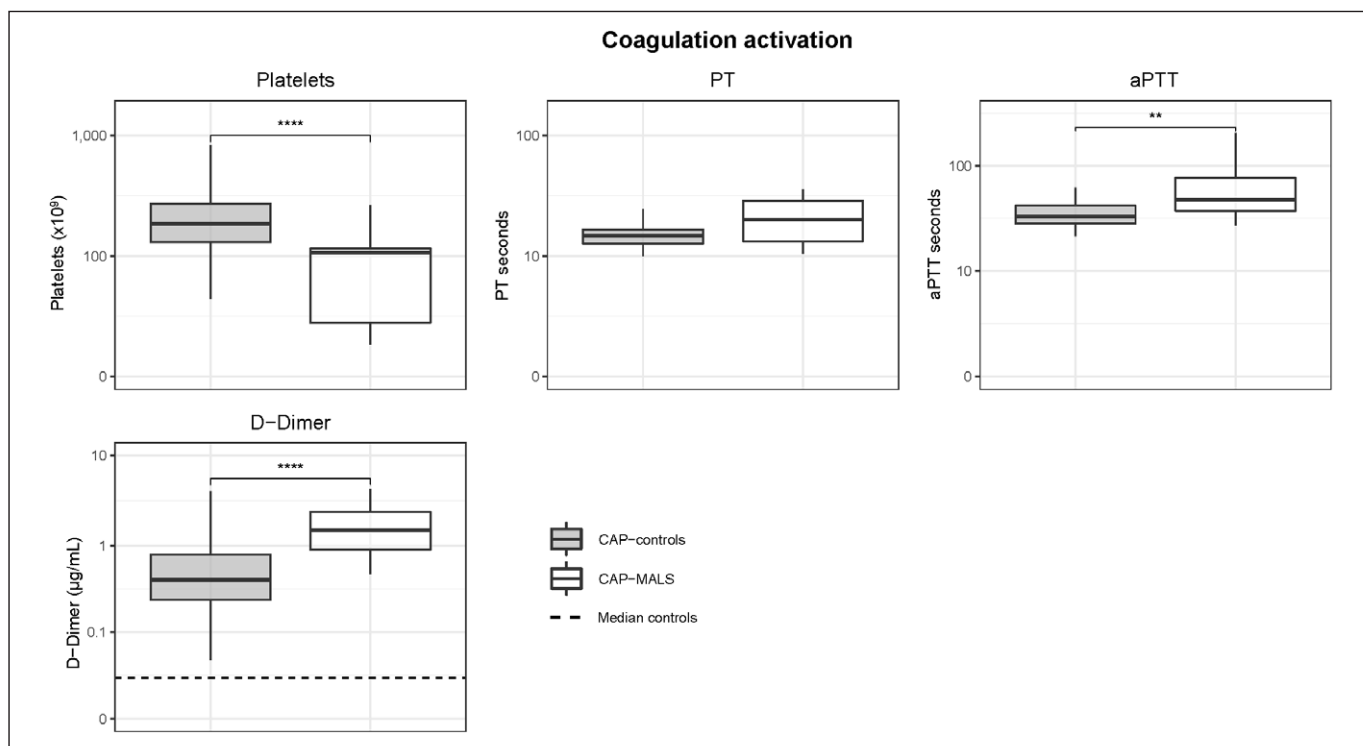
Figure 4. Host response biomarkers reflecting coagulation activation in community-acquired pneumonia (CAP)-control and CAP–macrophage activation-like syndrome (CAP-MALS) patients. Data are expressed as box-and-whisker diagrams with the *horizontal line* depicting the median, the *top* and *bottom* of the box representing the upper and lower quartiles, and *whiskers* extending to the farthest points that are not outliers (i.e., that are within 1.5 times the interquartile range of the highest and the lowest quartile, respectively). *Dotted lines* represent median values obtained in 50 noninfected control subjects. Values in patients were all different from those in control subjects. *Asterisks* indicate differences between CAP-control and CAP-MALS patients (Benjamini-Hochberg corrected, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). aPTT = activated partial thromboplastin time, PT = prothrombin time.

hyperinflammation, including 500 ng/mL (clinicaltrials.gov identifier NCT04324021 and NCT04443881 [29]) and 2,000 ng/mL (NCT04330638). We compared patients without hematologic malignancies based on these two ferritin cut offs (**Supplemental Table 5**, Supplemental Digital Content 10, http://links.lww.com/CCM/G440 and **Supplemental Table 6**, Supplemental Digital Content 11, http://links.lww.com/CCM/G441). At these cut offs, mortality did not differ between patients with high and low ferritin levels; however, many of the host response biomarker differences detected in the comparison CAP-MALS and CAP-controls remained, especially those related to endothelial cell function and D-dimer.

DISCUSSION

The failure of sepsis trials seeking to modulate the host response in order to improve outcomes has been ascribed at least in part to the strong heterogeneity of the patients enrolled (6, 30). Predictive enrichment of study populations has been proposed as an attractive strategy to reduce heterogeneity and increase the likelihood of a beneficial effect by a targeted intervention (31, 32). Support for this approach has (amongst others) been provided by a retrospective analysis of a sepsis trial testing IL-1RA, which showed a strong survival benefit in patients with MAS but not in those without this syndrome (16). The Hellenic Sepsis Study Group recently introduced the term MALS, based on a set of criteria that included clinical and laboratory variables (14). Patients were classified as having MALS in the presence of a so-called hemophagocytosis score above 151 or the presence of both hepatobiliary dysfunction and disseminated intravascular coagulation. This group subsequently evaluated and validated ferritin as a biomarker to identify MALS in sepsis patients (14). We here examined the incidence, clinical presentation, outcome, and host response aberrations of MALS in critically ill CAP patients, defined by the previously validated cut off ferritin level of 4,420 ng/mL (14). We report an incidence of 9.8% in CAP patients admitted to the ICU. CAP-MALS patients had more severe disease at admission, presented more often with shock and AKI, and demonstrated a higher mortality (particularly in the first 5 d after admission) when compared with CAP-control patients. After excluding patients with hematologic malignancies, mortality and the presence of shock did not differ between CAP-MALS and CAP-control patients.

We used ferritin at a cut off value of 4,420 ng/mL to stratify patients in CAP-MALS and CAP-controls. This cut off concentration was proposed considering its high specificity (97%) and negative predictive value (98%), which are required for use as a biomarker for selection of treatment with anti-inflammatory agents such as IL-1RA (14). Earlier studies in mixed ICU populations (i.e., not restricted to sepsis patients) reported optimal maximum serum ferritin levels for the diagnosis of MAS of 3,951 ng/mL (33) and 3,095 ng/mL (34) (i.e., in the same range as recommended for critically ill sepsis patients). We considered it of relevance to evaluate the clinical and pathophysiologic relevance of admission ferritin levels above 4,420 ng/mL since this cut off has been used to assign sepsis patients to IL-1RA treatment in the recently completed PROVIDE trial (NCT03332225; results pending) and will be used in a larger sepsis trial (ImmunoSep) funded by the European Commission (https://cordis.europa.eu/project/id/847422).

The previously reported frequency of MALS defined as a ferritin level above 4,420 ng/mL in sepsis patients was 3.7–4.3% in a Greek test and validation cohort, respectively (14). We report a considerably higher incidence (9.8%) in CAP patients admitted to Dutch ICUs. Of note, however, the Greek study entailed patients with sepsis defined according to the 1992 consensus definition (suspected infection plus at least two systemic inflammatory response criteria [35] and admitted not only to ICUs but also general medicine and surgical wards [14]). More similar to the population reported here, in a third validation cohort from Swedish ICUs, the incidence of MALS was 15.6% in patients with severe sepsis (i.e., with organ failure) or septic shock (14). In the Greek cohort 28-day mortality rates in patients with MALS were 66.7% and 66% in the test and validation cohorts, respectively; in the Swedish cohort, 28-day mortality was 52.9% (14). In our CAP cohort, 28-day mortality in patients with MALS was 46.7%. Differences in the case-mix, including source of infection (18), might account for these differences.

We measured 23 host response biomarkers to obtain insight in the pathophysiologic implication of MALS defined by very high ferritin levels. MAS leads to excessive immune activation with overactive macrophages leading to a cytokine storm (8, 9, 12, 13). In

agreement, sepsis patients with ferritin levels above 4,420 ng/mL had elevated plasma levels of IL-6, IL-18, interferon-γ, and a decreased IL-10/tumor necrosis factor–α ratio (14). Similarly, we here demonstrate that CAP-MALS patients display an increased release of plasma markers of systemic inflammation and stronger cytokine responses relative to CAP-controls, as reflected by significantly higher levels of CRP, pentraxin 3, IL-6, IL-8, IL-17, IL-23, IL-10, IL-1RA, and IL-27. CAP-MALS patients also had significantly elevated levels of soluble cluster of differentiation-163, a marker for macrophage activation (9, 19). This study is the first to report that MALS in sepsis is associated with enhanced endothelial cell activation (as indicated by higher sVCAM-1, endocan and fractalkine levels), a more disturbed glycocalyx integrity (higher syndecan levels), and a reduced endothelial barrier function (higher angiopoietin-2 and angiopoietin 2/1 ratios). Endothelial cell dysfunction has received much attention as a potential target in the treatment of sepsis (20, 36, 37). The current data suggest that sepsis patients with MA(L)S may not only be likely to benefit more of IL-1RA, such as suggested previously (16), but also from vasculoprotective agents. In normal homeostasis, an intact endothelium acts as an anticoagulant surface, and a disturbed function facilitates procoagulant responses (21, 38). CAP-MALS patients showed stronger coagulation activation, as reflected by increased D-dimer levels, lower platelet counts, and longer aPTT values. Coagulation disorders are common in MAS (39, 40), and disseminated intravascular coagulation and thrombocytopenia have been associated with adverse outcome in MAS patients (41, 42). Together these results show that CAP-MALS patients present with a broad deregulation of multiple key host response pathways implicated in sepsis pathogenesis.

Earlier investigations have established that hematologic malignancies are a predisposing condition for developing MAS (7, 9, 13, 43). Hematologic malignancies were overrepresented in CAP-MALS patients and after excluding this subgroup, mortality did not differ between CAP-MALS and CAP-control patients. These data suggest that septic CAP patients suffering from hematologic malignancies are prone to develop MALS and are more at risk for early death. Importantly, in CAP-MALS patients without hematologic malignancies, the more disturbed host response remained. This was true for biomarkers reflecting systemic inflammation, cytokine release, endothelial cell activation and function, and coagulation activation, which may be of relevance for the use of ferritin as a biomarker for treatment selection in sepsis patients without malignancy. Hence, these data indicate that while coexisting hematologic malignancies likely are an important factor in mortality associated with MALS in CAP patients, the excessive activation of different host response pathways also occurs in the absence of this comorbidity.

Corticosteroids are the first-line of therapy for MAS to suppress the life-threatening inflammatory process, and IL-1RA treatment might work best when combined with corticosteroids (7, 8). Patients with ARDS can be stratified in hyperinflammatory and hypoinflammatory phenotypes based on a set of clinical and laboratory variables (44). It would be of interest to investigate ferritin levels as an additional parameter in this stratification.

CONCLUSIONS

The diagnosis of MALS in patients with sepsis may have relevance for selection of those who might benefit from anti-inflammatory therapy (16). Although MAS usually is diagnosed based on HLH-2004 criteria composed of a set of clinical and laboratory criteria (45), the use of ferritin as a biomarker would provide an advantage for clinical decision-making. The current data suggest that MALS, as measured by plasma ferritin levels above 4,420 ng/mL, occurs more often in CAP patients with hematologic malignancies and is associated with deregulation of several key host response pathways implicated in sepsis pathogenesis, entailing not only systemic inflammation but also endothelial dysfunction.

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