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Lymphopenia is associated with broad host response aberrations in community-acquired pneumonia

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SUMMARY

Objectives: Lymphopenia at hospital admission occurs in over one-third of patients with community-acquired pneumonia (CAP), yet its clinical relevance and pathophysiological implications remain underexplored. We evaluated outcomes and immune features of patients with lymphopenic CAP (L-CAP), a previously described immunophenotype characterized by admission lymphocyte count < 0.724×10^9 cells/L.
Methods: Observational study in 149 patients admitted to a general ward for CAP. We measured 34 plasma biomarkers reflective of inflammation, endothelial cell responses, coagulation, and immune checkpoints. We characterized lymphocyte phenotypes in 29 patients using spectral flow cytometry.
Results: L-CAP occurred in 45 patients (30.2%) and was associated with prolonged time-to-clinical-stability (median 5 versus 3 days), also when we accounted for competing events for reaching clinical stability and adjusted for baseline covariates (subdistribution hazard ratio 0.63; 95% confidence interval 0.45–0.88). L-CAP patients demonstrated a proportional depletion of CD4 T follicular helper cells, CD4 T effector memory cells, naïve CD8 T cells and IgG+ B cells. Plasma biomarker analyses indicated increased activation of the cytokine network and the vascular endothelium in L-CAP.
Conclusions: L-CAP patients have a protracted clinical recovery course and a more broadly dysregulated host response. These findings highlight the prognostic and pathophysiological relevance of admission lymphopenia in patients with CAP.

Introduction

Community-acquired pneumonia (CAP) is the leading infectious cause of death in the world, responsible for more than 2.6 million deaths yearly.[1] In the United States, approximately 650 per 100,000 adults are hospitalized with CAP every year.[2] In-hospital mortality for CAP is estimated at 6.5%, and up to one-third of all patients hospitalized for CAP die within one year. Pneumonia is also the most common cause of sepsis—which is defined as life-threatening organ failure due to a dysregulated host response to infection.[3]

The host immune response in sepsis and pneumonia is complex and involves concurrent hyperinflammation and immunosuppression.[4–7] An important feature of sepsis-related immunosuppression is lymphopenia, i.e. low circulating numbers of one or more types of lymphocytes (T cells, B cells, and natural killer [NK] cells or other

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innate lymphoid cells [ILCs]). While exact mechanisms remain unknown, lymphopenia is hypothesized to result from enhanced apoptosis, recruitment to lymphoid organs or sites of infection, and reduced lymphopoiesis.[7–9] Lymphopenia in sepsis has been associated with poor clinical outcomes, including increased risk for secondary infections,[10] intensive care unit (ICU) admissions,[11,12] and mortality.[10,12,13] A key role of lymphocytes in sepsis outcomes is further substantiated by the promising results of novel immunostimulatory therapies such as interleukin (IL)-7, and antibodies against programmed cell death (PD)-1 and PD-ligand (L)1, that promote the survival of lymphocytes in animal models, human *ex vivo* studies and early clinical trials[7].

Although lymphopenia at hospital admission has been reported in up to 39% of patients with CAP,[14,15] the clinical and pathophysiological relevance of low lymphocyte counts in CAP is underexplored, particularly in non-critically ill patients without sepsis. Bermejo-Martin et al. identified and validated an immunophenotype of CAP patients the authors called lymphopenic CAP (L-CAP)—defined as CAP with a lymphocyte value below 0.724×10^9 cells/L—and linked this phenotype to increased 30-day mortality.[14] In a follow-up study, patients with L-CAP exhibited $CD4^+$ T cell depletion, low IgG2 levels, and increased levels of IL-8, granulocyte colony-stimulating factor (G-CSF), monocyte-chemoattractant protein-1 and IL-10.[15] In other studies, lymphopenia has also been linked to an increased risk of mortality in pneumococcal pneumonia,[16] primary care pneumonia,[17] COVID-19,[18] and ICU-acquired pneumonia.[19]

In this study, we aimed to ascertain whether L-CAP in hospitalized, yet non-critically ill, patients is associated with poor clinical outcomes, alterations in circulating lymphocyte phenotypes and aberrant host response biomarkers indicative of pneumonia pathophysiology. Further characterization of this proposed immunophenotype in patients with relatively mild disease will improve our understanding of the clinical and pathophysiological relevance of lymphopenia in CAP.

Materials and methods

Patients

Adult patients 18 years of age or older were recruited as part of the ELDER-BIOME study (clinicaltrials.gov identifier NCT02928367) or the OPTIMACT study (Dutch Trial Register identifier NTR6163) from October 2016 until March 2020 (prior to the first wave of COVID-19 in the Netherlands). Both studies were approved by the medical ethical committee of the Amsterdam University Medical Centers. Other participating sites included the BovenIJ Hospital, Flevo Hospital and the Spaarne Gasthuis. Trained research physicians conducted the screening of patients. Written informed consent was obtained from all participants or their legal representatives before enrollment. A more extensive description of these cohorts can be found in prior publications from our group.[4,20,21]

Patients were eligible for enrollment if admitted to a general hospital ward with a clinical suspicion of an acute infection of the respiratory tract, defined as at least one respiratory symptom (new cough or sputum production, chest pain, dyspnea, tachypnea, abnormal lung examination, or respiratory failure), one systemic symptom (documented fever or hypothermia, leukocytosis or leukopenia) and an evident new or progressive infiltrate, consolidation or pleural effusion on chest X-ray or computed tomography scan. Exclusion criteria were hospital admission for > 48 h in the previous 2 weeks, residence in a long-term care facility, and suspicion of an aspiration pneumonia. Patients were also excluded if lymphocyte counts were not measured upon presentation to the emergency department, or if patients suffered an immune deficiency likely to affect lymphocyte counts. The latter was defined as exposure to chemotherapy in the last 6 months; current diagnosis of hematological malignancy; human immunodeficiency virus infection with a CD4+ T cell count below 200/mm^3; the use of prednisone ≥30 mg for ≥14 days, or other immunosuppressive drugs linked to lymphopenia; and organ- or bone marrow transplant with ongoing immune suppression.

Clinical variables and primary clinical outcome

Vital signs and severity scores, including the pneumonia severity index (PSI),[22] CURB-65 score,[23] Modified Early Warning Score (MEWS)[24] and quick Sequential Organ Failure Assessment (qSOFA)[25] were registered upon presentation to the emergency ward. L-CAP was defined as CAP with a lymphocyte value below 0.724×10^9 cells/L, as previously described.[14] The time to clinical stability was calculated using modified Halm's criteria,[26] defined as a stabilization of vital signs for 24 h (temperature ≤ 37.2 °C; heart rate ≤100/min; systolic blood pressure > 90 mm Hg; respiratory rate ≤24/min; SO^2 ≥90% or PaO_2 ≥60 mmHg without supplemental oxygen). If patients were discharged from the hospital prior to meeting these criteria, they were considered clinically stable unless they were transferred to another care facility or died. Thus, the primary clinical outcome for this study was a composite endpoint of time to clinical stability *or* discharge. We refer to this outcome as 'time to clinical stability' for brevity.

Data collection and assays

All baseline and clinical variables were scored from electronic records. Ethylenediaminetetraacetic acid (EDTA) anticoagulated blood was obtained within 24 h of hospital admission. Biomarkers were measured using Luminex multiplex assays (R&D, USA) and cytometric bead array (CBA; BioLegend, USA), as described previously.[27] Spectral flow cytometry was performed on cryopreserved peripheral blood mononuclear cells from a subset of patients using 36-color spectral flow cytometry based on the Optimized Multicolor Immunofluorescence Panel (OMIP) 069,[28] as previously reported.[29] Lymphocyte phenotyping for this study was performed using manual gating on data from 29 patients from the previous study that met the inclusion criteria for the current study and had available absolute lymphocyte counts. Representative gating plots are depicted in Supplementary Fig. 1. Proportions of relevant cell phenotypes (expressed as proportion of live total CD45+ cells throughout the manuscript) and median fluorescence intensity (MFI) of selected surface markers were assessed using FlowJo version 10.6.2 (USA).

Statistical analyses

Continuous data were analyzed using a Welch's *t*-test when normally distributed, or a Wilcoxon rank-sum test when non-normally distributed; categorical data were analyzed using the Fisher's exact test. We fitted competing risk models to estimate the association between L-CAP and time to clinical stability, with mortality and transfer to another care facility as competing risks.[30] A competing risk analysis provides two measures of association: the cause-specific hazard ratio (HR) and the subdistribution HR. The cause-specific hazard ratio can be interpreted as the association between a covariate (such as the presence of L-CAP) and (the relative change in) the rate of the event of interest in subjects who have not experienced any event (i.e. clinical stability, death, or transfer); while the most relevant interpretation here of the subdistribution hazard ratio is the association between a covariate and the incidence of an event, while taking competing risks into account.[30–32] Cause-specific hazards were estimated using Cox regression. Subdistribution hazards were estimated with Fine-Gray models using the R package *cmprsk*.[33] Fine-Gray models allow for estimation of the cumulative hazard function, which can be visualized similar to a Kaplan-Meier

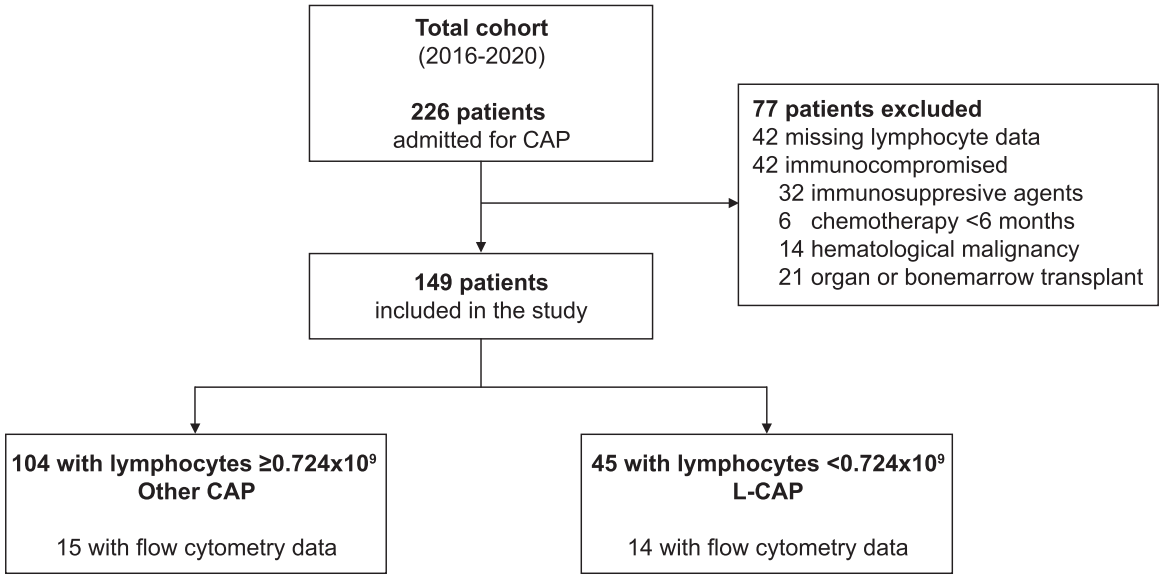

Fig. 1. Flow chart of patient selection. Patients could have more than one exclusion criterion and the total number of criteria listed therefore adds up to more than the total number of patients excluded. Abbreviations: (L-)CAP, (lymphopenic) community-acquired pneumonia.

survival curve. Additional description on competing risks can be found in the Supplementary Methods.

We expressed the differences in plasma biomarker concentrations as the Hedges' *g*—a commonly used effect size measure[34]– with 95% confidence intervals (CI) based on the 2.5th and 97.5th percentile of 2000 bootstrap replicates and calculated statistical significance for these comparisons using Welch's *t*-test. We performed a sensitivity analysis using a cut-off value of 1×10^9 cells/L (a commonly used cut-off for lymphopenia in routine clinical laboratories and clinical trials).[9] In another sensitivity analysis, we assessed the relationship between continuous lymphocyte counts and plasma biomarker levels by fitting linear regression models for each biomarker as the dependent variable and continuous lymphocyte count as the independent variable. We tested whether a linear or non-linear relationship best explained the data by comparing indices of fit (Akaike's information criterion and the F-test) between these models and models with a restricted cubic spline with five knots. All tests were two-sided and a *P*-value < 0.05 was considered statistically significant. Results were not adjusted for multiple testing due to the relatively low sample size. Where appropriate, continuous variables were log-transformed to better approximate normal distributions. Statistical analysis was performed in the R statistical framework (version 4.0.5 Vienna, Austria).

Results

Patient characteristics

Out of 226 consecutive patients hospitalized for CAP who consented to participate, 77 (34.1%) were excluded (Fig. 1)—42 (18.6%) patients due to missing admission lymphocyte counts. Patients in whom lymphocyte counts were not determined at admission were highly similar to patients with known lymphocyte counts with regard to clinical presentation and outcomes (with only a minor difference in body mass index [P = 0.030]; Supplementary Table 1), indicating that patients with available lymphocyte counts were representative of the total population. Of the 149 included patients, 45 (30.2%) had lymphocyte counts $< 0.724 \times 10^9$ and were thus classified as L-CAP. L-CAP patients were slightly older than other patients with CAP, had a lower body mass index, and had increased disease severity in two out of four disease severity scores (PSI and MEWS) (Table 1). Furthermore, L-CAP patients had significantly lower leukocyte counts and platelet counts.

Prolonged time to clinical stability in patients with L-CAP

Time to clinical stability was longer in patients with L-CAP (median 5 days, interquartile range [IQR] 2–9) than in patients with lymphocyte counts $\geq 0.724 \times 10^9$/L (median 3 days, IQR 2–6; $P = 0.004$). We used competing risk models to further evaluate the association between L-CAP and reaching clinical stability while taking competing risks mortality and hospital transfer into account. In these models, L-CAP remained associated with a longer time to clinical stability (cause-specific HR: 0.63; 95% CI, 0.44–0.91, subdistribution HR 0.62; 95% CI 0.45–0.85). These findings were robust when adjusting for age, sex, and disease severity (PSI; cause-specific HR 0.64; 95% CI, 0.44–0.94, subdistribution HR 0.63; 95% CI 0.45–0.88). Fig. 2 shows the cumulative incidence function for time to clinical stability estimated from the Fine-Gray models.

L-CAP is predominantly related to alterations in CD4 T cells

To explore whether L-CAP is associated with an altered composition and phenotype of circulating lymphoid cells, we performed spectral flow cytometry in a subset of 29 patients, of whom 14 (48.3%) had L-CAP (Supplementary Table 2). Patients included in this cohort were similar to other patients, with only lower incidences in diabetes mellitus and a lower baseline temperature (Supplementary table 3). Patients with L-CAP had proportionally fewer cells in several CD4 T cell subsets (particularly T follicular helper cells), naïve CD8 T cells, and IgG+ B cells, but a higher proportion of (non-NK cell) ILCs (Fig. 3A; proportions of all lymphoid cells in Supplementary Table 4). As T cells appeared predominantly affected, we quantified surface markers on CD4 and CD8 central and effector memory T (T_{CM} and T_{EM}) cells that could hint at possible mechanisms involved in lymphopenia in CAP. In patients with L-CAP, we found higher co-expression of human leukocyte antigen (HLA)-DR and CD38, and higher expression of programmed death 1 (PD-1) on CD4 T_{CM} cells, suggesting an activated and possibly exhausted CD4 T_{CM} cell phenotype (Fig. 3B-C).[35] We found no such difference on CD4 T_{EM} cells, CD8 T_{EM} or CD8 T_{CM} cells. Additionally, we observed no differences for any of the memory T cell subsets in the expression of CD95/Fas

Table 1
Baseline characteristics and outcomes of patients with L-CAP and other CAP.

n	L-CAP (< 0.724 ×109 cells/L) (n = 45)	Other CAP (≥0.724 ×109 cells/L) (n = 104)	P-value
Demographics			
Age, years	77 [64,83]	72 [60,79]	**0.026**
Sex, male	30 (66.7)	57 (54.8)	0.24
Body mass index	24 [21,26]	26 [23,28]	**0.030**
Medical history			
COPD	19 (42.2)	32 (30.8)	0.24
Asthma	1 (2.2)	15 (14.4)	0.06
Myocardial infarction	10 (22.2)	15 (14.4)	0.35
Congestive heart failure	5 (11.1)	7 (6.7)	0.56
Diabetes mellitus (type 1 or 2)	9 (20.0)	28 (26.9)	0.49
Stroke	7 (15.6)	6 (5.8)	0.10
Chronic renal disease	5 (11.1)	8 (7.7)	0.72
Vital signs			
Respiratory rate, bpm	23 [18,26]	22 [18,28]	0.75
Temperature, °C	38.1 (1.3)	38.3 (1.1)	0.37
Heart rate, bpm	100 [86,110]	97 [85,105]	0.20
Oxygen saturation, %	94 [90,96]	94 [91,96]	0.53
Mean arterial pressure, mmHg	93 (17)	98 (17)	0.09
Disease severity scores			
Pneumonia Severity Index	4 [3,5]	4 [2,4]	**0.001**
CURB-65	2 [1,2]	2 [1,2]	0.25
Modified Early Warning Score	4 [3,5]	3 [2,4]	**0.005**
qSOFA	1 [0,1]	1 [0,1]	0.42
Laboratory tests			
Leukocytes x10^9/L	9.8 [7.2, 13.3]	12.8 [10.3, 16.9]	**0.002**
Lymphocytes x10^9/L	0.5 [0.4, 0.6]	1.2 [0.9, 1.8]	**< 0.001**
Neutrophils x10^9/L	8.4 [5.7, 11.8]	10.1 [7.5, 13.5]	**0.033**
Monocytes x10^9/L	0.7 [0.4, 0.8]	0.9 [0.6, 1.3]	**0.002**
Platelets x10^9/L	194 [154,284]	244 [179,303]	**0.046**
Disease outcomes			
Time to clinical stability, days	5 [2,9]	3 [2,6]	**0.004**
ICU admission	5 (11.1)	4 (3.8)	0.18
28-day mortality	3 (6.7)	7 (6.7)	> 0.99

Normally distributed continuous data are displayed as mean (standard deviation) and compared using Welch's t-test. Non-normally distributed continuous data are displayed as median [interquartile range] and compared using Wilcoxon's rank-sum test. Categorical data are displayed as count (percentage) and compared using Fisher's exact test. Abbreviations: bpm, breaths/beats per minute; CAP, community-acquired pneumonia; COPD, chronic obstructive pulmonary disease; CURB-65, confusion, blood urea nitrogen, respiratory rate, blood pressure, age 65 or older; L-CAP, lymphopenic community-acquired pneumonia; qSOFA, quick sequential organ failure assessment score.
Bold values are statistically significant ($P < 0.05$).

(indicative of susceptibility to FasL-mediated apoptosis) or CD57 (indicative of terminally differentiated T cells prone to apoptosis; Supplementary Fig. 2).[36] Taken together, these exploratory analyses suggest that L-CAP is predominantly related to alterations in proportions of T cell subsets and activated CD4 T_{CM} cells, but do not provide evidence for enhanced Fas-mediated apoptosis of circulating T cells as a possible mechanism.

L-CAP is associated with a dysregulated host response

To gain insight into the association between lymphopenia and the host response to pneumonia, we measured plasma concentrations of 34 biomarkers reflective of four pathophysiological domains relevant for the host response to infection (Fig. 4; Supplementary Table 5). In the domain systemic inflammation, the pro-inflammatory cytokines IL-6 and IL-8 were higher in patients with L-CAP, as well as the anti-inflammatory cytokines IL-1RA and IL-10. L-

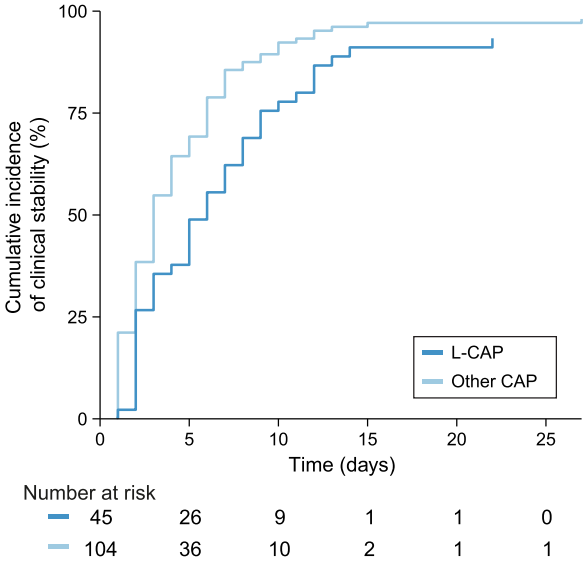

Fig. 2. Estimated cumulative incidence curves for clinical stability. Estimated cumulative incidence curves for clinical stability, with mortality and transfer as competing events, between L-CAP patients (< 0.724 ×109 lymphocytes/L) and other CAP patients (≥0.724 ×109 lymphocytes/L). If patients were discharged from the hospital prior to meeting criteria for clinical stability, they were considered clinically stable. In the L-CAP group (n = 45), two patients were transferred and one died before reaching clinical stability. In the other CAP group (n = 104), one patient was transferred and one died. Cumulative incidence curves for patients with one of these competing events (transfer or death) are not shown in the graph.

CAP patients also showed exaggerated endothelial responses, as reflected by higher plasma levels of the endothelial cell activation marker soluble vascular cell adhesion molecule (VCAM)-1 and angiopoietin-2, commonly associated with pro-inflammatory responses and increased endothelial permeability, respectively.[37–39] The angiopoietin-2:angiopoietin-1 ratio was higher in L-CAP patients, suggesting a more compromised endothelial barrier integrity in L-CAP.[38,39] There were no significant differences in markers indicative of coagulation activation such as soluble tissue factor or D-dimer. The plasma levels of biomarkers relating to checkpoint regulators did not differ between groups with the exception of soluble P-selectin glycoprotein ligand (PSGL)-1, which was significantly lower in L-CAP patients. Thus, plasma host response biomarkers suggest that L-CAP is associated with a more pronounced systemic inflammatory response, activation of the endothelium, and endothelial barrier dysfunction.

Sensitivity analyses

We performed a sensitivity analysis with a commonly used lymphocyte cut-off value of 1×10^9 cells/L to define lymphopenia.[9] Out of 149 patients, 76 (51%) were lymphopenic with this definition (Supplementary Table 6). Patients with lymphocyte counts $< 1 \times 10^9$/L, relative to other patients, had a lower body mass index, higher PSI and MEWS scores, and lower total leukocyte, monocyte and platelet counts. Time to clinical stability remained prolonged when lymphocyte counts were below 1×10^9/L, though this distinction was no longer statistically significant (median 5 days, IQR 2–8, versus median in other patients 3 days, IQR 2–6; $P = 0.08$). In unadjusted competing risk analyses, the subdistribution HR for lymphopenia was significant (0.72; 95% CI, 0.53–0.97) but not the cause-specific HR (0.79; 95% CI, 0.57–1.10), and both were not significant when adjusting for age, sex, and PSI (subdistribution HR 0.75, 95% CI 0.55–1.02, cause-specific HR 0.82; 95% CI, 0.58–1.15). Biomarkers reflective of endothelial cell activation and barrier dysfunction (VCAM-1, angiopoietin-2 and the angiopoietin-2:angiopoietin-1

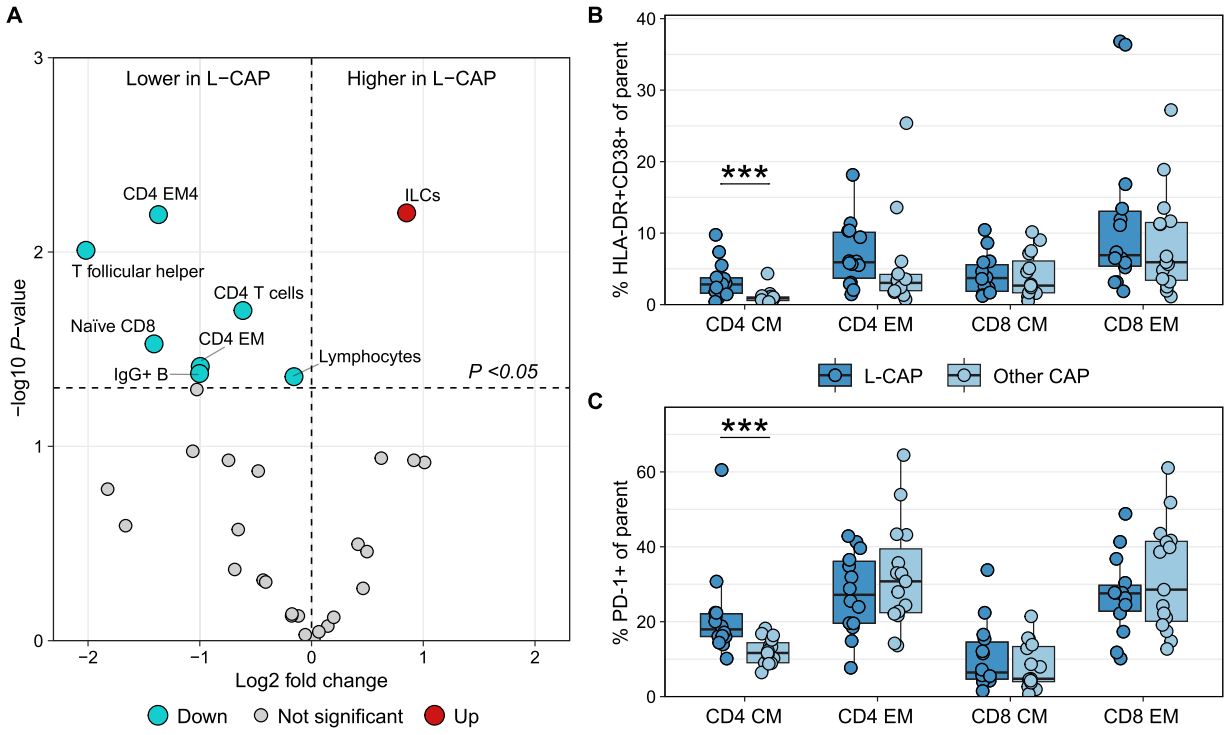

Fig. 3. Lymphocyte proportions and phenotypes. Lymphocytes were quantified and phenotyped in a subset of patients with L-CAP (n = 14) and other CAP (n = 15) using spectral flow cytometry. (A) Volcano plot depicting the fold change of log2-transformed frequency for each lymphocyte subset (as proportion of total cells per patient), and the corresponding *P*-value obtained using Welch's *t*-test. Analyses were exploratory and therefore not adjusted for multiple testing. (B) Proportion of cells within central memory (CM) and effector memory (EM) T cell subsets co-expressing human leukocyte antigen—DR isotype (HLA-DR) and CD38. (C) Proportion of cells within CM and EM T cell subsets expressing programmed death ligand 1 (PD-1). Data in panels B and C are shown as box & whiskers, with medians and individual data points. ***$P < 0.001$. Abbreviations: CM, central memory; EM, effector memory.

ratio) remained elevated in the lymphopenic group. Among inflammation-related biomarkers, only IL-10 remained significantly higher (Supplementary Fig. 3). PSLG-1 was no longer significantly different between groups, but sCD25 was higher in patients with lymphocyte counts below 1×10^9/L.

When using linear regression models to analyse the relationship between biomarker levels and lymphocyte counts as a continuous variable (significant associations shown in Fig. 5; all associations shown in Supplementary Table 7), we found results similar to the primary analysis: biomarkers increased in L-CAP, with the addition of IL-18, showed a significant negative association with lymphocyte counts – i.e. the lower the lymphocyte counts, the higher the biomarker level—but PSGL-1 was no longer significant. Fitting models with restricted cubic splines did not improve indices of model fit when compared with basic linear regression models, indicating a linear relationship between lymphocyte count and the (significant) biomarkers. These analyses suggest that, while a decrease in lymphocyte count is linearly related to an increase in plasma biomarkers indicative of a dysregulated host response, the lymphocyte cut-off for L-CAP ($< 0.724 \times 10^9$ cells/L) is more suited for dichotomizing patients into subgroups with worse clinical outcomes and a more disturbed host response when compared with the commonly used cut-off of $< 1 \times 10^9$ cells/L.

Discussion

In this study, we aimed to characterize host immune aberrations associated with L-CAP, a proposed CAP immunophenotype defined by absolute lymphocyte counts $< 0.724 \times 10^9$ cells/L and previously linked to poor clinical outcomes.[14,15] In this study, we demonstrate that among patients admitted to a general ward, who were not immunocompromised prior to hospitalization, L-CAP is associated with several key clinical and immunological alterations. These include an extended time to achieve clinical stability, a proportional decrease in various lymphocyte populations such as CD4 T cell subsets, naïve CD8 T cells, and IgG+ B cells, as well as elevated plasma levels of biomarkers indicative of systemic inflammation and endothelial activation. These findings indicate that lymphopenia in non-critically ill patients hospitalized for CAP, is associated with a broadly altered host immune response at the level of circulating cellular phenotypes and plasma protein biomarkers, encompassing pathophysiological domains typically linked to hyperinflammation.

In our cohort, almost one-third of patients hospitalized for CAP had L-CAP, in line with previous studies reporting prevalence of L-CAP at hospital admission between 34% and 39%.[14,15] We found that L-CAP patients had a prolonged time clinical stability, an association that remained robust when adjusting for other potential predictors of poor outcome—such as the higher disease severity in L-CAP patients. The overall low to moderate severity of illness in our cohort precluded detection of differences in ICU admission or mortality rates, but the prolonged time to clinical stability is consistent with poor clinical outcomes of patients with lymphopenia in previous studies of CAP and/or sepsis, including length of hospital stay,[12] ICU admissions,[11,12] and mortality.[10–12,14,15,17]

We found a proportional decrease of CD4 T cell subsets, CD8 naïve T cells and IgG+ B cells in patients with L-CAP. Previous studies in L-CAP patients similarly found a reduction within the T cell compartment, primarily in CD4 T cells – which independently predicted mortality—and to a lesser extent CD8 T cells.[15] In sepsis, data similarly suggest that lymphopenia predominantly implies alterations in the T cell compartment.[8,9] Interestingly, this study found reduced proportion of IgG+ B-cells in L-CAP with a simultaneous depletion of follicular T helper cells, which may hint towards reduced antibody class switching. A reduction in total circulating IgG levels has also been reported previously and had been linked to increased mortality in patients with sepsis.[40]

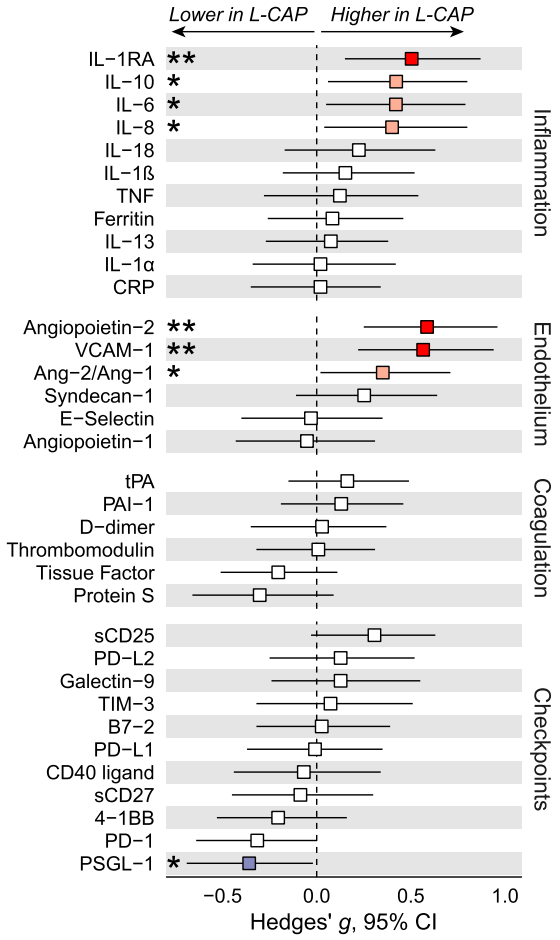

Fig. 4. Biomarker levels in L-CAP patients versus other CAP patients. The X-axis depicts effect sizes (Hedges' g). Confidence intervals are based on the 2.5th and 97.5th percentiles of 2000 bootstrap replicates. Statistical significance was calculated using Welch's *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$. The color of the squares corresponds to the biomarkers that are significantly higher (red) or significantly lower (blue) in L-CAP ($< 0.724 \times 10^9$ lymphocytes/L) versus other CAP ($\geq 0.724 \times 10^9$ lymphocytes/L). A darker shade of the squares corresponds to a higher significance level. Abbreviations: Ang, angiopoietin; IL, interleukin; CRP, C-reactive protein; PAI-1, plasminogen activator inhibitor-1; PD-1, programmed cell death protein 1; PD-L, programmed death-ligand; PSGL-1, P-selectin glycoprotein ligand-1; RA, receptor antagonist; TIM-3, T-cell immunoglobulin and mucin domain 3; TNF, tumor necrosis factor; tPA, tissue plasminogen activator; VCAM, vascular cell adhesion protein.

The exact mechanisms for lymphopenia in CAP are unknown. Suggested pathways include increased migration to site of infection or lymphoid organs, reduced lymphopoiesis, and induction of apoptosis.[8,9] Studies in both patients and mice with sepsis have demonstrated evidence for a role of both extrinsic—mediated through Fas and other surface receptors—and intrinsic pathways of apoptosis.[8] In an exploratory analysis of Fas/CD95 and CD57 expression on circulating T cells, we did not find evidence for increased propensity towards extrinsic apoptosis as a potential driver of lymphopenia, although apoptosis may occur via other mechanisms or outside of the blood compartment. Enhanced co-expression of HLA-DR and CD38 and enhanced expression of PD-1 on circulating CD4 T_{CM} cells may point towards increased T cell activation. While speculative, it is conceivable that we did not find differences in activation status of circulating T_{EM} cells because these cells have migrated into inflammatory tissues after activation. This hypothesis may be supported by the stark elevations of plasma VCAM-1 and angiopoietin-2 in patients with L-CAP, indicative of endothelial cell activation and compromised endothelial barrier integrity, respectively. VCAM-1, when expressed on the surface of endothelial cells, is a major regulator of leukocyte transendothelial migration to inflammatory tissues via the integrin α4β1.[37] Thus, while a causal interpretation is not possible with these data, our findings are most consistent with the hypothesis that activation and migration into (inflammatory) tissues acts as a driver of lymphopenia in patients with CAP of mild to moderate severity. Notably, L-CAP patients also showed decreased neutrophil, monocyte and platelet counts, which suggests that more global bone marrow suppressive mechanisms are simultaneously at play.

While lymphopenia is considered a quintessential indicator of immunosuppression,[10] plasma biomarker analyses suggested enhanced rather than reduced inflammatory reactions in patients with L-CAP, as reflected by elevated proinflammatory cytokine levels (IL-6 and IL-8), and increased markers for endothelial cell activation (VCAM-1) and endothelial dysfunction (angiopoietin-2 and angiopoietin-2/1 ratio). Activation of the cytokine network in L-CAP was further illustrated by elevated plasma levels of the anti-inflammatory mediators IL-1RA and IL-10. In agreement, a previous study reported elevations of plasma IL-8 and IL-10 in L-CAP.[15] In earlier work by our group, we similarly demonstrated that a different proxy for immunosuppression—a reduced capacity of blood leukocytes to produce TNF upon *ex vivo* stimulation with lipopolysaccharide – was associated with higher plasma levels of biomarkers indicative of systemic inflammation in CAP patients.[4] Collectively, these data support the current understanding of the host response during sepsis, in which hyperinflammation and immunosuppression occur simultaneously, and further endorse the notion that these seemingly opposite immune alterations are already present in patients with relatively mild disease, such as those with CAP studied here.[4–7]

Strengths of this study include the detailed characterization of the L-CAP clinical and immunological phenotype (including high-resolution cell surface marker phenotyping), the robustness of the clinical results to adjustment for competing events and other potential predictors; and the sensitivity analyses. Most knowledge of the association of infection-induced lymphopenia with clinical outcomes and host responses is derived from studies in critically ill patients[8,9]; we here show the relevance of lymphopenia in CAP patients hospitalized to a general ward, with relatively mild disease. Limitations include that we did not adjust for multiple testing due to the relatively low sample size, and results should therefore be considered hypothesis-generating. Non-NK cell ILCs were gated through exclusion of membership to other cell populations; our panel did not allow for further characterization of known subsets of ILCs. Finally, L-CAP patients were slightly older than other CAP patients and this may subtly affect the frequencies of certain cell subsets such as naïve T cells.[41]

This study highlights the value of lymphopenia as an indicator of poor prognosis and as a sign of more profound immune dysregulation in CAP patients admitted to a general hospital ward. Future research could investigate whether lymphocyte counts at admission – an inexpensive and routinely available laboratory test—should be taken into consideration by physicians when deciding on the intensity of vital sign monitoring and therapeutic interventions (e.g. oral or intravenous antibiotics). Absolute lymphocyte counts have been shown to add independent prognostic information when used in conjunction with prediction models commonly applied in clinical practice, such as the PSI- and CURB-65-scores,[15,16] and future studies should examine whether integrating admission lymphocyte counts improves these models.

In conclusion, we demonstrate that L-CAP in non-critically ill patients is associated with a prolonged time to clinical stability, a more dysregulated host response, and alterations in the composition and phenotype of the lymphocyte compartment. These findings emphasize the clinical and pathophysiological relevance of lymphopenia in patients with CAP of modest disease severity.

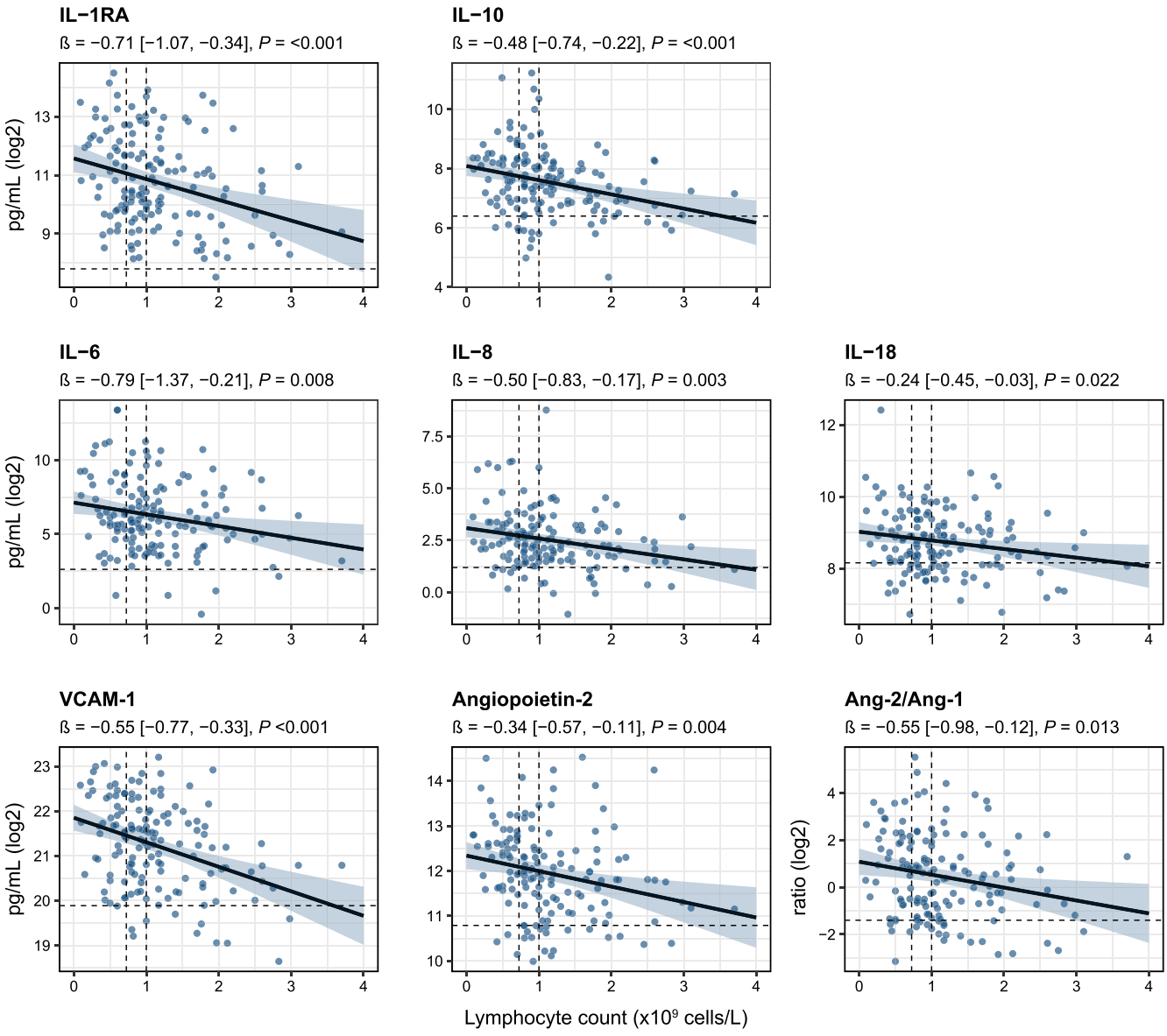

Fig. 5. Analysis of lymphocyte count as a continuous variable. Scatterplots for the log2-transformed concentrations (in pg/mL) or log2-transformed ratio of plasma biomarkers significantly associated with absolute lymphocyte counts based on linear regression models. The beta-coefficient for lymphocyte count, with corresponding 95% confidence interval and *P*-value, is displayed above each plot. The horizontal line indicates the median value in 28 noninfected control subjects. The vertical lines indicate the lymphocyte cut-offs for L-CAP (0.724×10^9 cells/L, left line) and the sensitivity analysis (1×10^9 cells/L, right line). Abbreviations: Ang, angiopoietin; IL, interleukin; VCAM, vascular cell adhesion protein.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106131.

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