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Lymphopenic community-acquired pneumonia is associated with a dysregulated immune response and increased severity and mortality

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SUMMARY

Objectives: Lymphopenic (<724 lymphocytes/μL) community-acquired pneumonia (L-CAP) is an immunophenotype with an increased risk of mortality. We aimed to characterize the L-CAP immunophenotype though lymphocyte subsets and the inflammatory response and its relationship with severity at presentation and outcome.
Methods: Prospective study of 217 immunocompetent patients hospitalized for CAP. Lymphocyte subsets ($CD4^+$, $CD8^+$, $CD19^+$, and natural killer [NK] cells) and inflammatory cytokines were analyzed on days 1 and 4, and immunoglobulin subclasses were analyzed on day 1 in a nested group.
Results: 39% of patients showed L-CAP, with decreased levels of all lymphocyte subsets with a partial recovery of $CD4^+$ and $CD8^+$ cells by day 4. L-CAP patients exhibited higher initial severity and systemic levels of interleukin (IL)-8, IL-10, granulocyte colony-stimulating factor, and monocyte chemoattractant protein-1. Initial IgG2 levels were lower in patients with <724 lymphocytes/μL and positively correlated with ALC, $CD4^+$, and $CD19^+$ cell counts. Low $CD4^+$ counts (<129 cells/μL) also independently predicted 30-day mortality after adjusting for age, gender, and the CURB-65 score.
Conclusions: L-CAP is characterized by $CD4^+$ depletion, a higher inflammatory response, and low IgG2 levels that correlated with greater severity at presentation and worse prognosis. L-CAP is an immunophenotype useful for rapidly recognizing severity.
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Background

Community-acquired pneumonia (CAP) is the leading infectious cause of sepsis and death globally with a mortality rate of 4%–14% among hospitalized patients.[1] Published data indicate that at least, one-third of patients hospitalized for CAP present with severe sepsis,[2,3] and that the number of failing organs determines the outcome.[4]

The immunopathogenesis of pneumonia is a very complex area that still remains rather unknown. In the host response against microorganisms, eradication requires an intricate lung physiological processes and recruitment immune effector from blood.[5,6] In CAP, the host response has been evaluated from different perspectives, but analyses to date have mainly focused on innate immunity and the inflammatory response.[7–9] Adaptive immunity has traditionally been neglected as an actor in CAP, with research in this field being focusing on humoral immunity and only rarely on cell-mediated immunity.[10,11] The quantitative variations in lymphocyte subsets,

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as well as their relationship with the inflammatory response or cytokine expression and humoral immunity, remain poorly understood. Nevertheless, in patients with severe acute respiratory distress syndrome (ARDS) and septic shock, lymphopenia was found linked with the severity of lung damage.[12,13]

Recently, lymphopenia (724 lymphocytes/μL) at CAP diagnosis was identified as an independent risk factor for 30-day mortality.[14] However, our knowledge of lymphocyte subsets and their relationship with systemic inflammation remains poorly understood in patients hospitalized with CAP and negative human immunodeficiency virus (HIV). A more complete immunoprofiling,[15] by evaluating host's adaptive immunity, could represent an opportunity to improve comprehension of pathogenesis in CAP specially in severity assessment and prognosis prediction.

We aimed to characterize lymphopenic and non-lymphopenic CAP immunophenotypes by analyzing the lymphocyte subsets and their association with systemic cytokine pattern on days 1 and 4, in relationship with severity presentation and outcome. An additional aim was to study immunoglobulin (Ig) levels in a subgroup of patients. Our final purpose was to provide more insights for recognizing immunological endotypes of CAP, useful for a more accurate severity assessment and prognosis.

Patients and methods

Study design and patient selection

This was a prospective cohort study conducted in a large tertiary-care hospital in Spain (Hospital Universitario y Politécnico La Fe, Valencia). The local ethics committee approved the study (code: 2011/0859) and patients or their next of kin provided written informed consent.

We included patients with CAP who were admitted to hospital. CAP was diagnosed as the presence of a new compatible infiltrate on chest x-ray plus acute respiratory symptoms and/or signs (temperature ≥37.8 °C, chills, chest pain, cough, expectoration, dyspnea). Exclusion criteria were age <18 years old, hospital admission for ≥48 h in the preceding 15 days, nursing-home patients, immunosuppression that could alter immune condition and/or increase the risk of mortality, life expectancy less than 3 months, patients in whom pneumonia was a terminal event and/or patients for whom a prior decision had been made to limit therapeutic effort. Regarding immunosuppression, the following were excluded: oncology, hematology, and HIV positive patients; transplant recipients; patients who had received chemotherapy or radiation therapy during the previous 6 months; and treatment with ≥20 mg/day of prednisone or equivalent for > 14 days.

Data collection and microbiological studies

We collected data on demographic characteristics, comorbidities (diabetes mellitus, chronic obstructive pulmonary disease, asthma, and heart, liver, neurological, and renal diseases) and concomitant treatment. The severity of illness at presentation was assessed using the CURB-65 (confusion, urea nitrogen, respiratory rate, blood pressure, age ≥65 years) score, and the Sequential Organ Failure Assessment (SOFA) score.

The following microbiological tests were carried out: urinary antigens for *Legionella pneumophila* and *Streptococcus pneumoniae*, sputum Gram stain (if <10 epithelial cells and > 25 leukocytes per field; magnification x 100) and culture. We also performed paired blood cultures and paired serological studies for *Chlamydophila pneumoniae, Mycoplasma pneumoniae, Coxiella burnetti,* and *Legionella pneumophila*. Nasopharyngeal swabs were taken to detect viral nucleic acids based on clinical suspicion.

Definitions

L-CAP was considered if absolute lymphocyte count (ALC) was <724 cells/μL as it has been reported in a previous publication an independent risk factor for CAP mortality.[14] As lymphopenia in usual clinical practice is considered as an ALC of <1000 cells/μL – the current lower limit of normality for the complete blood count (CBC) –,[16] analyses related to inflammatory cytokines and Ig were also performed with threshold <1000 cells/μL.

As outcomes, we evaluated treatment failure, length of stay and any mortality at 30 days after admission. Treatment failure was considered as persistence/reappearance of fever and symptoms or hemodynamic instability, the appearance (PaO2 <60 mmHg or saturation <90% with a FiO2 of 0.21) or impairment of respiratory failure, radiographic progression, or new infectious foci after 72 h of antimicrobial treatment as previously defined or clinical deterioration within 72 h of treatment, produced by one or more of the following causes: hemodynamic instability, appearance or impairment of respiratory failure, need of mechanical ventilation or radiographic progression.[17]

Lymphocyte subset, cytokine and immunoglobulin analysis

Samples of peripheral blood were obtained in anticoagulated ethylenediamine tetra-acetic acid tubes for lymphocyte subset, cytokine, and Ig analysis from a venipuncture during the first morning after hospital admission. In a nested group of 64 patients, a blood sample was also drawn on day 4 for analysis of the lymphocyte subsets. Samples for cytokine and Ig analysis were centrifuged for 15 min at 3000 revolutions per minute, and the supernatant/plasma was stored at −80°C until analysis.

Samples for lymphocyte subsets were analyzed using FACSCanto-II cytometers (Becton Dickinson, San Jose, CA). The following human lymphocyte subsets were determined (expressed as cells/μL): $CD4^+$ cells, $CD8^+$ cells, $CD19^+$ cells, and natural killer (NK) cells ($CD16/56^+$). The details of the flow cytometry procedure are provided in Supplementary File 1.

Cytokine levels in the plasma were evaluated using the Biorad© 17 plex assay (Hercules, CA, USA), according to the manufacturer's instructions. This system allows for quantitative measurement, reported in pg/mL, of tumor necrosis factor-alpha (TNF-α), interleukin (IL)−1 beta (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1 beta (MIP-1β).

Levels of IgG subclasses, IgA, and IgM in plasma were measured in a small group ($N = 44$ patients) using a multiplex Immunoglobulin Isotyping kit purchased from Biorad© on the same Luminex platform. Results are expressed in mg/dL.

Statistical analysis

Statistical analysis was performed using IBM SPSS Version 20.0 software (IBM Corp., Armonk, NY, USA). The data are reported as N (%) or as median (interquartile range [IQR]) for categorical and continuous variables respectively. Categorical variables were compared using the χ^2 test and continuous variables were analyzed using the Mann-Whitney *U* test. The Spearman test was performed to assess correlations between the lymphocyte count, cytokine levels, and Ig levels. *P*-values of <0.05 (two-tailed) were considered statistically significant. Logistic regression analysis was performed to predict mortality at 30 days (dependent variable). As independent variables we included age, sex, CURB-65 score, and the lymphocyte subsets ($CD4^+$, $CD8^+$, $CD19^+$, and NK cells).

Table 1
Baseline characteristics, severity, microbiology, treatment, and outcomes.

Characteristics N	≥1000 lymphocytes/μL 89	<1000 lymphocytes/μL 128	*P* Value	≥724 lymphocytes/μL 132	<724 lymphocytes/μL 85	*P* Value
Demographics and usual treatment						
Age, years	63 (45–71)	74 (61–82)	<0.001	66 (50–75)	75 (62–82)	<0.001
Male sex	48 (53.9)	87 (68)	0.036	78 (59.1)	57 (67.1)	0.237
Current smoking	23 (25.8)	29 (22.7)	0.589	35 (26.5)	17 (20)	0.272
Alcohol abuse	1 (1.1)	8 (6.2)	0.062	1 (0.8)	8 (9.4)	0.002
Pneumococcal vaccine	4 (4.5)	10 (7.8)	0.328	8 (6.1)	6 (7.1)	0.770
Influenza vaccine	23 (25.8)	51 (39.8)	0.032	37 (28)	37 (43.5)	0.019
Inhaled corticosteroids	18 (20.2)	34 (26.6)	0.282	24 (18.2)	28 (32.9)	0.013
Oral corticosteroids	3 (3.4)	3 (2.3)	0.650	3 (2.3)	3 (3.5)	0.582
Proton pump inhibitors	26 (29.2)	58 (45.3)	0.017	37 (28)	47 (55.3)	<0.001
Statins	26 (29.2)	50 (39.1)	0.135	43 (32.6)	33 (38.8)	0.346
Antiplatelet agents	13 (14.6)	30 (23.4)	0.108	20 (15.2)	23 (27.1)	0.032
Antibiotics in previous month	28 (31.5)	44 (34.4)	0.654	41 (31.1)	31 (36.6)	0.409
Comorbidity						
Diabetes mellitus	16 (18)	35 (27.3)	0.109	32 (24.2)	19 (22.4)	0.749
Heart disease	18 (20.2)	48 (37.5)	0.007	35 (26.5)	31 (36.5)	0.120
Renal failure	9 (10.1)	15 (11.7)	0.711	12 (9.1)	12 (14.1)	0.249
Liver disease	2 (2.2)	5 (3.9)	0.496	4 (3)	3 (3.5)	0.839
Neurologic disease	8 (9)	20 (15.6)	0.151	16 (12.1)	12 (14.1)	0.668
Respiratory disease	29 (32.6)	40 (31.2)	0.836	38 (28.8)	31 (36.5)	0.236
COPD	15 (16.9)	34 (26.8)	0.087	22 (16.8)	27 (31.8)	0.010
Initial severity						
Initial ICU admission	0 (0)	13 (10.2)	0.002	3 (2.3)	10 (11.8)	0.004
ATS/IDSA ICU admission criteria	2 (2.2)	28 (21.9)	<0.001	6 (4.5)	24 (28.2)	<0.001
Change in baseline SOFA score	1 (0–2)	2 (1–3)	<0.001	2 (1–2)	2 (1–3)	0.002
CURB-65 ≥2	33 (37.1)	78 (60.9)	0.001	57 (43.2)	54 (63.5)	0.003
Laboratory test						
C-Reactive Protein (mg/dL)	17.1 (7.8–30)	19.3 (9.0–30.7)	0.472	19 (8.9–30.1)	18.3 (9–30.3)	0.888
Fibrinogen, mg/dL	723 (615–895)	732 (638–858)	0.724	723 (624–883)	732 (629–857)	0.677
Procalcitonin, ng/mL	0.3 (0.1–1.8)	0.6 (0.1–2.4)	0.258	0.4 (0.1–2)	0.6 (0.1–2.1)	0.728
Neutrophil to lymphocyte ratio	8.47 (5.47–12.52)	15.68 (11.58–25.25)	<0.001	9.86 (5.95–14.63)	18.96 (13.48-30.28)	<0.001
Microbiology						
Known etiology	36 (40.4)	81 (63.3)	0.001	60 (45.5)	57 (67.1)	0.002
Streptococcus pneumoniae	15 (41.7)	34 (42)	0.975	26 (43.3)	23 (40.4)	0.744
IPD	5 (13.9)	8 (9.9)	0.524	9 (15)	4 (7)	0.170
Legionella pneumophila	1 (2.8)	9 (11.1)	0.137	3 (5)	7 (12)	0.159
Atypical bacteria	11 (30.6)	18 (22.2)	0.335	17 (28.3)	12 (21.1)	0.362
Virus	9 (25)	24 (29.6)	0.608	15 (25)	18 (31.6)	0.429
Polymicrobial etiology	6 (16.7)	19 (23.5)	0.408	12 (20)	13 (22.8)	0.711
Treatment during CAP episode						
Fluorquinolone monotherapy	52 (58.4)	65 (50.8)	0.266	70 (53)	47 (55.3)	0.744
Cephalosporin plus macrolide combination	30 (33.7)	53 (41.4)	0.251	53 (40.2)	30 (35.3)	0.472
Other antibiotic regimen	7 (7.9)	10 (7.8)	0.989	9 (6.8)	8 (9.4)	0.488
Systemic corticosteroids	10 (11.2)	28 (21.9)	0.043	20 (15.2)	18 (21.2)	0.254
Outcomes						
LOS, days	6 (4–8)	7 (5–9)	0.040	6 (5–8)	7 (5–9)	0.176
Treatment failure	6 (6.7)	21 (16.4)	0.034	11 (8.3)	16 (18.8)	0.022
Mortality at 30 days	1 (1.1)	11 (8.6)	0.018	2 (1.5)	10 (11.8)	0.001

Data are expressed as *n* (%) or median (interquartile range).
Abbreviations: L-CAP, lymphopenic community-acquired pneumonia; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; ATS/IDSA, American Thoracic Society/Infectious Diseases Society of America; SOFA, sequential organ failure assessment; CURB-65, Confusion, Urea, Respiratory rate, Blood pressure, Age ≥65; IPD, invasive pneumococcal disease; LOS, length of stay.

The best thresholds for prediction were calculated by selecting the medians of the lymphocyte subsets in non-survivors.

Results

Baseline characteristics

A total of 217 patients hospitalized with CAP were recruited, of whom 85 (39.2%) had L-CAP (128 patients (59%) had <1000 lymphocytes/μL) (Table 1). Patients with L-CAP or low ALC (<1000 lymphocytes/μL) were older and showed more comorbidities as well as a higher initial severity assessed by the need of initial Intensive Care Unit (ICU) admission, CURB-65 or SOFA score. A positive microbiology etiology was more frequent in patients with low ALC compared to those with normal ALC. In patients with microbiological diagnosis, there was no statistical difference in the number of lymphocyte cells between bacterial and viral / atypical pathogens. Finally, these patients experienced more frequently treatment failure, and showed a higher mortality at 30 days.

Lymphocyte subsets and initial severity

Lymphocyte subsets in patients according to L-CAP and ALC <1000 cells/μL are depicted in Fig. 1. Patients with low ALC (both L-CAP and those with <1000 cells/μL) showed lower counts of all lymphocyte subsets compared to those patients with > 1000 lymphocytes/μL ($P < 0.001$).

Correlations between the ALC and the lymphocyte subsets were analyzed. On day 1, there was a strong correlation between ALC and the T lymphocytes $CD4^+$ (rho 0.91) and $CD8^+$ (rho, 0.81) ($P < 0.001$) (Fig. 2). ALC also correlated moderately with $CD19^+$ (rho 0.57) and NK cells (rho 0.57) ($P < 0.001$). Similar results were

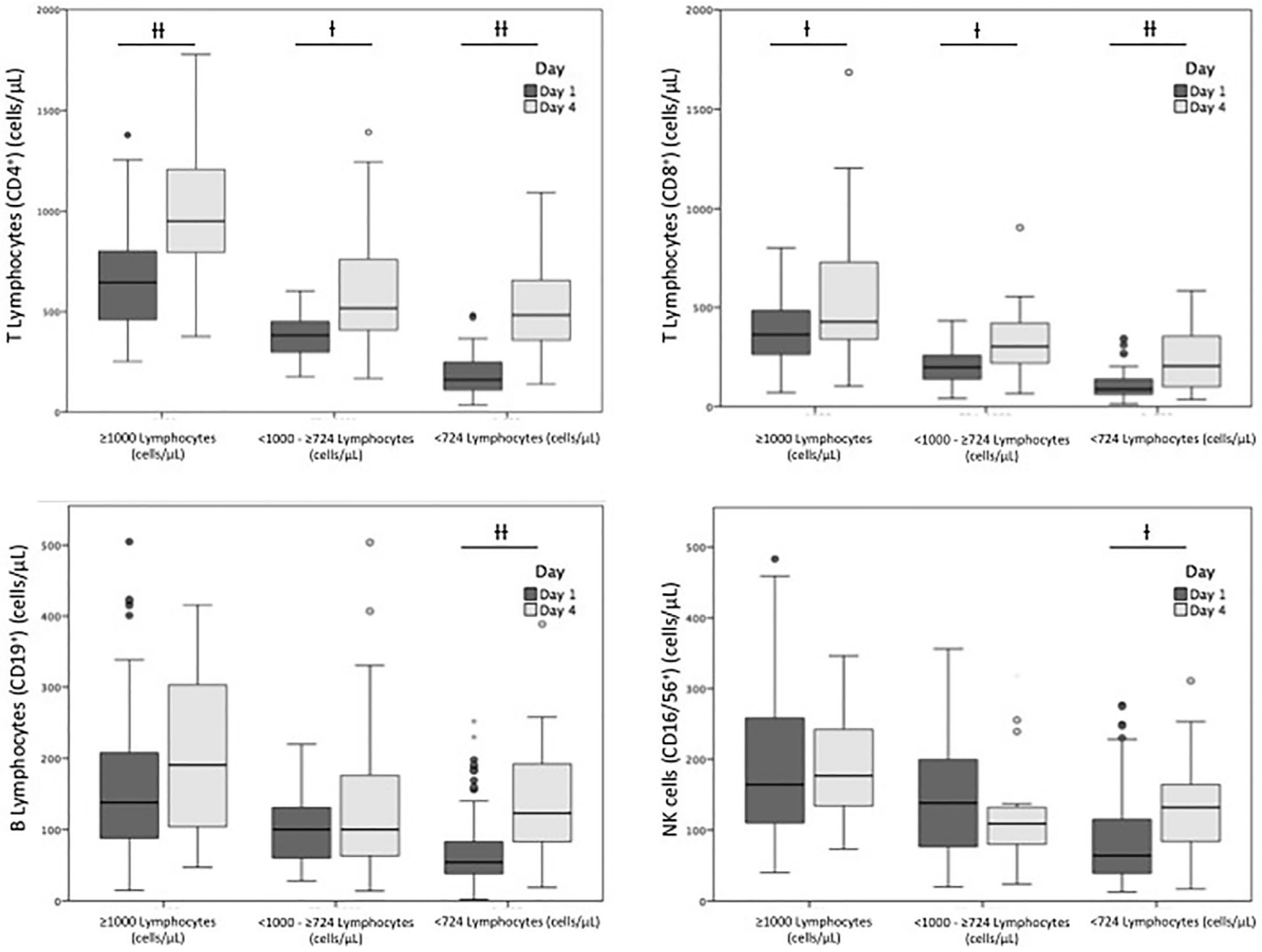

Fig. 1. Lymphocyte subsets regarding absolute lymphocyte count. I: $P < 0.05$; II: $P < 0.001$.

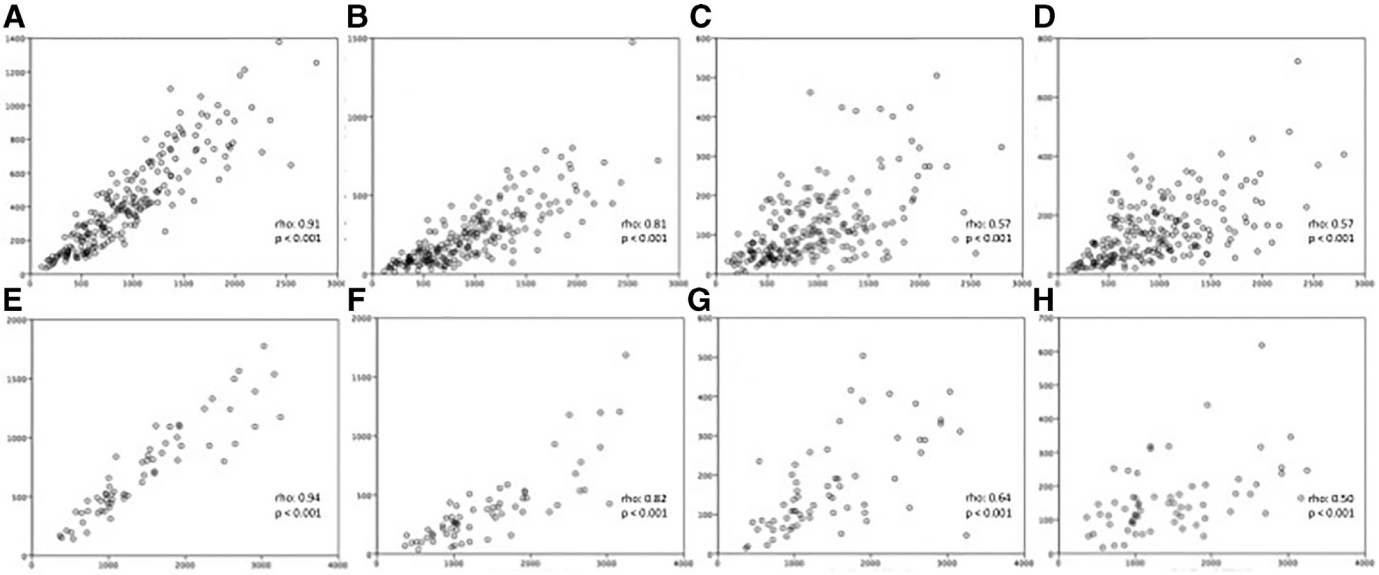

Fig. 2. Scatter plot showing correlations between the ALC and the lymphocyte subsets. Day 1: (A) ALC and $CD4^+$, (B) ALC and $CD8^+$, (C) ALC and $CD19^+$, (D) ALC and NK cells; Day 4: (E) ALC and $CD4^+$, (F) ALC and $CD8^+$, (G) ALC and $CD19^+$, (H) ALC and NK cells. Lymphocyte subsets are expressed in cells/µL. Abbreviations: ALC, absolute lymphocyte count; NK, natural killer.

Table 2
Lymphocyte subsets, cytokine, and immunoglobulin levels in patients with lymphopenic (<724 lymphocytes/μL) community-acquired pneumonia according to CURB-65 score.

	CURB-65 <2	CURB-65 ≥2	*P* Value
Lymphocyte subsets			
ALC	1020 (634–1422)	749 (489–1033)	<0.001
$CD4^+$	446 (278–699)	292 (163–487)	<0.001
$CD8^+$	259 (129–384)	149 (88–270)	0.001
$CD19^+$	115 (63–187)	73 (42–131)	<0.001
NK^+	127 (70–197)	115 (60–192)	0.202
Cytokines			
TNF-α	3.7 (3.3–3.9)	3.7 (1.9–25.6)	0.522
IL-1b	0.5 (0.4–0.5)	0.5 (0.5–1.4)	0.056
IL-2	0.9 (0.8–1)	0.9 (0.9–1)	0.322
IL-4	0.3 (0.3–0.3)	0.3 (0.1–1.4)	0.385
IL-5	1.4 (1.2–6.3)	1.4 (0.8–4.7)	0.222
IL-6	21.1 (2.4–57.3)	41 (12.8–145.2)	0.013
IL-7	5.8 (1–14.7)	7.7 (1–16.9)	0.271
IL-8	10.1 (2.7–18.3)	16 (2.7–31.2)	<0.001
IL-10	2.2 (2–2.8)	2.6 (2–12.1)	0.099
IL-12	2.5 (2.2–2.8)	2.5 (2.2–14.3)	0.394
IL-13	0.4 (0.4–0.4)	0.4 (0.4–1.9)	0.627
IL-17	1.8 (1.6-3)	2.3 (1.8–17.1)	0.175
G-CSF	50.9 (26.3–100.9)	78.3 (31.3–185.7)	0.026
GM-CSF	0.8 (0.7–0.8)	0.8 (0.7–0.8)	0.904
IFN-γ	1.7 (1.2–32.2)	1.7 (1.2–33.3)	0.693
MCP-1	1.6 (1.5–31.9)	1.6 (1.5–50)	0.257
MIP-1β	44.9 (30–66.4)	50 (28.7–79.3)	0.402
Immunoglobulins			
IgG1	687.8 (516.6–911.3)	813.1 (512.2–1128.2)	0.411
IgG2	447.3 (250.2–486.7)	290.3 (217.4–523.8)	0.366
IgG3	52.2 (36.6–93.2)	87.2 (57.2–112.3)	0.096
IgG4	31.6 (8.6–81.5)	28.5 (8.4–83.3)	0.963
IgM	195.4 (131–305.5)	217.8 (162.9–274.6)	0.606
IgA	181.4 (78.5–238.4)	142.4 (98.8–172.6)	0.725

Lymphocytes are expressed in cells/μL. Cytokine levels are expressed in pg/mL. Immunoglobulin levels are expressed in mg/dL. Data are median (interquartile range). Abbreviations: CURB-65, Confusion, Urea, Respiratory rate, Blood pressure, Age ≥ 65; ALC, absolute lymphocyte count; CD, cluster differentiation; NK, natural killer; TNF-α, tumor necrosis factor alpha; IL, interleukin; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; MCP-1, monocyte chemoattractant protein-1; MIP-1β, macrophage inflammatory protein 1-beta; Ig, immunoglobulin.

found on day 4 for: $CD4^+$(rho 0.94), $CD8^+$ (rho, −0.82), $CD19^+$ (rho, −0.64), and NK cells (rho, −0.50) ($P < 0.001$).

There was a negative correlation between the severity scores (SOFA and CURB65) and the total lymphocyte count or subset counts on day 1, as follows: 1. SOFA score. ALC, rho −0.353 ($P < 0.001$); $CD4^+$ cells, rho −0.337 ($P < 0.001$); $CD8^+$ cells, rho −0.276 ($P < 0.001$); $CD19^+$ cells, rho −0.160 ($P = 0.019$); and NK cells, rho −0.179 ($P = 0.008$). 2. CURB65 score. ALC, rho −0.344 ($P < 0.001$); $CD4^+$ cells, rho −0.360 ($P < 0.001$); $CD8^+$ cells, rho −0.310 ($P < 0.001$); $CD19^+$ cells, rho −0.238 ($P < 0.001$); and NK cells, rho −0.093 ($P = 0.171$). The data of lymphocyte subsets, cytokines and immunoglobulins according to the initial severity measured by CURB-65 score are shown in Table 2.

Inflammatory response and lymphopenia

L-CAP patients had significantly higher concentrations of IL-8, IL-10, G-CSF, and MCP-1 in comparison to non L-CAP on day 1. In patients with <1000 lymphocytes/μL the same cytokines were elevated along with IL-2, IL-17, and MIP-1β (Table 3). There was a negative correlation between ALC and the cytokines: IL-8 (rho −0.285; $P < 0.001$), IL-10 (rho −0.245; $P = 0.001$), G-CSF (rho −0.246; $P = 0.001$), and MCP-1 (rho −0.242; $P = 0.001$). At day 4, a decrease in the levels of cytokines in plasma was observed (i.e., IL-6 and G-CSF), but levels of IL-8 and MIP-1β remained higher in patients with <1000 lymphocytes/μL.

Immunoglobulins and lymphocytes

Immunoglobulin levels were measured in 44 patients (20.3%), of whom two of them died. Ig levels were compared depending on the lymphocyte counts (Table 4). There was a significant lower concentration of IgG2 in those patients with L-CAP, whereas in those with <1000 lymphocytes/μL, the difference did not reach statistical significance. Levels of IgG2 were not correlated with age (rho −0.066, $P = 0.671$).

A direct correlation was found between IgG2 levels and the ALC (rho, 0.415; $P = 0.005$), $CD4^+$ count (rho, 0.385; $P = 0.010$), and $CD19^+$ count (rho, 0.341; $P = 0.023$). By contrast, no correlation was found between the remaining Ig subclasses and the lymphocyte subtypes.

Lymphocyte subsets and clinical outcomes

Length of stay was weakly and negatively correlated with the ALC (rho, −0.155; $P = 0.023$) and $CD4^+$ count (rho, −0.191; $P = 0.005$). Treatment failure developed in 27 patients (12.4%) and ALC <724 cells/μL was present at diagnosis in 16 of these (59.3%). Patients with treatment failure had lower levels of ALCs, $CD4^+$, and $CD8^+$ counts (Supplementary File 2).

The lymphocyte subsets counts were analyzed by mortality at 30 days (Fig. 3). Non-survivors had lower ALCs and lower, $CD4^+$, $CD8^+$, and NK cell counts compared with survivors. This difference was most prominent for $CD4^+$ cells ($P < 0.001$), while no differences were found in the number of $CD19^+$ cells. The areas under the receiver operating characteristics (AUROC) curve to predict 30-day mortality were calculated for CURB65 score, and to predict survival at 30 days with ALC and lymphocyte subsets: CURB65 score 0.83 (0.71–0.94 95% Confidence Interval [CI]), $P < 0.001$; ALC 0.79 (0.65–0.92), $P = 0.001$; $CD4^+$ 0.80 (0.67–0.93), $P < 0.001$; $CD8^+$ 0.72 (0.56–0.89), $P = 0.009$; $CD19^+$ 0.56 (0.37–0.76), $P = 0.455$; NK cells 0.74 (0.59–0.88), $P = 0.006$.

The independent odds ratios (OR) for predicting 30-day mortality are shown in Table 5. $CD4^+$ cells were the strongest lymphocyte subset for predicting 30-day mortality, with an odds ratio of 5.07 and a 95% confidence interval of 1.12–23.01.

Discussion

The main findings of the study are as follows. First, among the patients hospitalized with CAP, 39.2% of the patients had L-CAP at diagnosis with reduced $CD4^+$, $CD8^+$, $CD19^+$, and NK cell counts. Second, L-CAP patients presented higher frequency of severe episodes, had higher systemic levels of inflammatory cytokines, and lower IgG2 levels. Finally, a low $CD4^+$ cells count was an independent risk factor for mortality, independently of age, sex, CURB-65, and all the remaining lymphocyte subsets.

Around 40% of our patients had L-CAP (<724 lymphocytes/μL on their CBC) when CAP was diagnosed. These patients had higher severity at presentation with higher SOFA and CURB-65 punctuations; that pattern of severity was similar in those patients with <1000 lymphocytes/μL. L-CAP patients exhibited an increase in the neutrophil to lymphocyte ratio, a recognized predictor of poor prognosis in pneumonia especially in the elderly.[18] In severe ARDS, lymphocyte depletion was correlated with severity of lung injury, suggesting that a limitation in the mobilization of immune cells may result in lung damage.[13] Our findings showed that L-CAP could be a marker for recognizing severe episodes beyond prognostic CAP scales. The cause of lymphopenia is not well known although several reasons have been proposed such as an apoptosis increase, a limitation of the host immune system to mobilize these cells or to a compartmentalization in the infection site.[13]

Table 3
Cytokine levels on days 1 and 4 according to absolute lymphocyte count on day 1.

Cytokines day 1						
N	≥1000 lymphocytes/μL 89	<1000 lymphocytes/μL 128	*P* Value	≥724 lymphocytes/μL 132	<724 lymphocytes/μL 85	*P* Value
TNF-α	3.7 (3.3–3.9)	3.7 (1.9–18.9)	0.954	3.7 (3.3–7)	3.7 (1.9–25.6)	0.682
IL-1b	0.5 (0.5–0.5)	0.5 (0.4–1.4)	0.831	0.5 (0.5–0.5)	0.5 (0.4–1.4)	0.573
IL-2	0.9 (0.8–1)	1 (0.9–1)	0.026	0.9 (0.9–1)	1 (0.9–1)	0.290
IL-4	0.3 (0.3–1.1)	0.3 (0.1–1.1)	0.842	0.3 (0.3–1.1)	0.3 (0.1–1.2)	0.835
IL-5	1.4 (1.2–4.7)	1.3 (0.8–6.1)	0.069	1.4 (0.8–6.1)	1.3 (0.8–1.5)	0.183
IL-6	21.1 (7.5–58.7)	30.6 (6.6–131.6)	0.302	25.2 (10.3–80.6)	27.2 (2.3–137.4)	0.931
IL-7	6.4 (1–13.1)	7.7 (1–19.3)	0.846	6.5 (1–14.4)	7.7 (1–18.2)	0.932
IL-8	2.7 (2.6–16.7)	15.8 (9.6–30.3)	<0.001	11.2 (2.7–19.3)	17.8 (10.1–31)	0.005
IL-10	2.0 (2–2.6)	2.9 (2–13.2)	<0.001	2.2 (2–2.9)	2.9 (2–12.5)	0.031
IL-12	2.2 (2.2–2.8)	2.8 (2.2–13.2)	0.119	2.5 (2.2–10.3)	2.8 (2.2–16.7)	0.333
IL-13	0.4 (0.4–0.4)	0.4 (0.4–1.6)	0.973	0.4 (0.4–0.4)	0.4 (0.4–1.6)	0.814
IL-17	1.8 (1.6–3)	3 (1.8–15.8)	0.042	2.3 (1.6–10.2)	3 (1.8–20)	0.239
G-CSF	39.3 (20.5–93.4)	82.4 (44.3–181.6)	<0.001	47.2 (23.3–114.3)	84.5 (50.8–193.7)	0.001
GM-CSF	0.8 (0.8–0.8)	0.8 (0.7–0.8)	0.247	0.8 (0.7–0.8)	0.8 (0.7–0.8)	0.844
IFN-γ	1.7 (1.7–31)	1.7 (1.2–40.9)	0.570	1.7 (1.2–33.3)	1.7 (1.2–80.3)	0.850
MCP-1	1.5 (1.5–1.6)	1.6 (1.5–59.3)	0.001	1.6 (1.5–26.9)	1.6 (1.5–59.4)	0.012
MIP-1β	44.2 (26–59.6)	50.5 (31–82.5)	0.032	45.3 (30–66.4)	49.9 (29.2–88.1)	0.344
Cytokines day 4						
N	≥1000 lymphocytes/μL 54	<1000 lymphocytes/μL 83	*P* Value	≥724 lymphocytes/μL 86	<724 lymphocytes/μL 51	*P* Value
TNF-α	3.7 (3.3–7)	3.3 (1.9–3.9)	0.093	3.7 (1.9–3.9)	3.5 (1.9–3.9)	0.841
IL-1b	0.5 (0.4–0.5)	0.5 (0.4–0.5)	0.185	0.5 (0.4–0.5)	0.5 (0.4–0.5)	0.567
IL-2	0.9 (0.9–1)	1 (0.9–1)	0.078	0.9 (0.9–1)	1 (0.9–1)	0.083
IL-4	0.3 (0.3–1)	0.3 (0.1–0.3)	0.278	0.3 (0.1–0.3)	0.3 (0.1–0.3)	0.922
IL-5	1.4 (0.8–4.7)	1.3 (0.8–1.5)	0.143	1.4 (0.8–2.2)	1.3 (0.8–4.7)	0.560
IL-6	2.4 (2.3–11)	2.3 (2.3–16.6)	0.981	2.4 (2.3–15)	2.3 (2.3–14.8)	0.594
IL-7	6.1 (1–11.9)	5.7 (1–11.9)	0.623	6.5 (1–13.1)	5 (0.6–11.8)	0.418
IL-8	2.7 (2.6–10.1)	7.2 (2.6–18.3)	0.019	2.7 (2.6–14.4)	7.2 (2.6–15.7)	0.830
IL-10	2.2 (2–2.6)	2.6 (2–2.9)	0.100	2.2 (2–2.9)	2.6 (2–2.9)	0.436
IL-12	2.2 (2.2–2.5)	2.5 (2.2–2.8)	0.068	2.2 (2.2–2.8)	2.5 (2.2–2.8)	0.487
IL-13	0.4 (0.4–0.4)	0.4 (0.4–0.4)	0.471	0.4 (0.4–0.4)	0.4 (0.4–0.4)	0.651
IL-17	1.8 (1.6–3)	2.3 (1.6–3)	0.140	2.3 (1.6–3)	2.3 (1.6–3)	0.896
G-CSF	33.6 (13.2–62.4)	34.3 (14.4–59.1)	0.967	34.3 (15.8–62.4)	34 (7.9–56.3)	0.525
GM-CSF	0.8 (0.7–0.8)	0.8 (0.7–0.8)	0.489	0.8 (0.7–0.8)	0.8 (0.7–0.8)	0.891
IFN-γ	1.7 (1.2–31)	1.7 (1.2–1.7)	0.111	1.7 (1.2–1.7)	1.7 (1.2–1.7)	0.838
MCP-1	1.6 (1.5–1.6)	1.6 (1.5–1.6)	0.400	1.6 (1.5–1.6)	1.6 (1.5–1.6)	1.000
MIP-1β	37.3 (21.4–54.3)	49 (33.7–69.5)	0.009	44.1 (22.2–60.7)	46.5 (34.6–67.3)	0.147

Cytokine levels are expressed in pg/mL. Data are expressed as median (interquartile range).
Abbreviations: TNF-α, tumor necrosis factor alpha; IL, interleukin; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; MCP-1, monocyte chemoattractant protein-1; MIP-1β, macrophage inflammatory protein 1-beta.

Table 4
Immunoglobulin levels according to absolute lymphocyte count.

N	≥1000 lymphocytes/μL 18	<1000 lymphocytes/μL 26	*P* Value	≥724 lymphocytes/μL 25	<724 lymphocytes/μL 19	*P* Value
IgG1	806.1 (547.8–936.7)	661.0 (477.8–1087.1)	0.551	830.2 (575.4–1008)	622.3 (413.6–1049.2)	0.205
IgG2	423.1 (304.2–485.7)	268.1 (209.4–523.8)	0.072	457.9 (304.2–592)	228.4 (162.2–460.5)	0.005
IgG3	82.0 (54.5–131.9)	57.9 (37.9–92.5)	0.050	78.9 (48.7–131.9)	58.6 (37.9–90)	0.090
IgG4	44.6 (16.8–102.5)	23.1 (4.1–68.2)	0.115	31.9 (15.7–83.2)	23.2 (3.6–68.2)	0.241
IgM	237.2 (133.7–274.6)	212.4 (144.3–318.6)	0.905	243.2 (178.4–305.5)	180.7 (125–251.1)	0.105
IgA	167.3 (87.2–218.8)	151.2 (95.8–228.0)	0.830	151.4 (96.9–222.7)	150.9 (87.6–172.6)	0.578

Immunoglobulin levels are expressed in mg/dL. Data are median (interquartile range).
Abbreviations: Ig, immunoglobulin; L-CAP, lymphopenic community-acquired pneumonia.

The reduction of ALC was characterized by a decrease in the $CD4^+$, $CD8^+$, $CD19^+$, and NK cell counts. In patients with ventilator-associated pneumonia, Pelekanou et al. have reported significant initial decreases in $CD4^+$ cell counts compared to those with other nosocomial infections.[19] This highlights how the origin of infection affects the immune system, and specifically, the marked drop in $CD4^+$ cell counts in respiratory infections. In the current study, at diagnosis > 40% of patients with CAP and > 75% of those who did not survive had a $CD4^+$ counts <350 cells/μL, while 93% of patients with ALCs <724 had $CD4^+$ counts <350 cells/μL. These $CD4^+$ counts are consistent with the immunological criterion for diagnosing acquired immune deficiency syndrome (AIDS) in HIV infected patients.[20] Although we do not know whether exhibiting a similar drop in $CD4^+$ cells could confer a similar degree of immunosuppression in CAP, this finding has potential clinical implications that should be considered. $CD4^+$ cells are probably the main actors in adaptive immunity,[21] being responsible for the proper functioning and transformation of $CD8^+$ cells, together with B cells, in memory cells and its decrease would lead to inappropriate adaptive immunity.[22,23]

In a murine model, Serbanescu et al. demonstrated the attrition of memory-phenotype $CD8^+$ cells in early sepsis, with improved

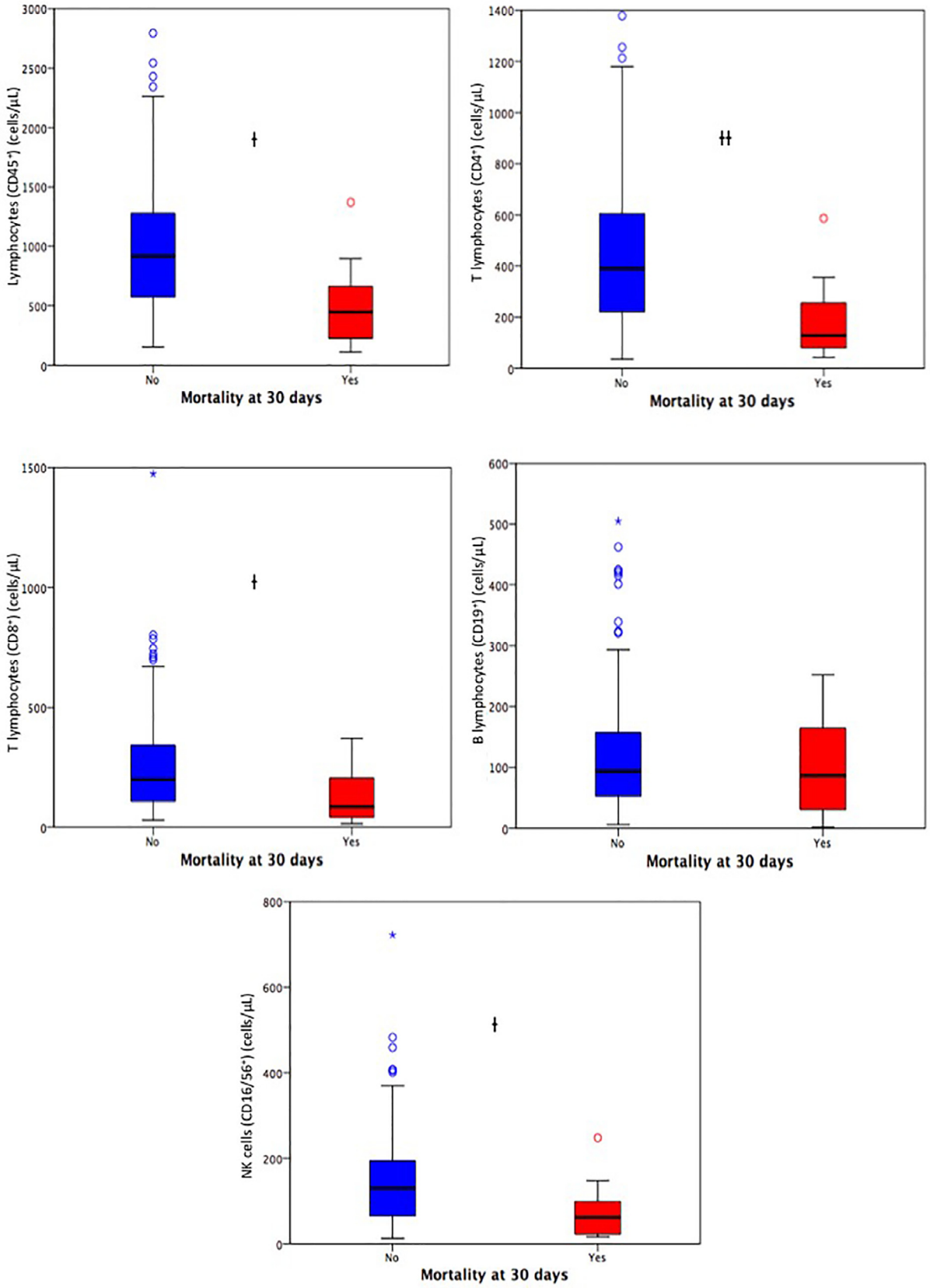

Fig. 3. Lymphocyte subsets by the 30-day mortality. I: $P < 0.05$; II: $P < 0.001$.

Table 5
Logistic regression analysis for the 30-day mortality.

	OR (95% CI)	*P* Value
Age > 70, years	1.56 (0.37–6.67)	0.546
Sex, male	0.90 (0.22–3.57)	0.875
CURB-65 ≥2	8.72 (0.98–78.00)	0.053
$CD4^+$ <129 cells/μL	5.07 (1.12–23.01)	0.035
$CD8^+$ <85 cells/μL	2.05 (0.45–9.43)	0.358
$CD19^+$ <87 cells/μL	0.27 (0.06–1.24)	0.093
NK <62 cells/μL	2.63 (0.59–11.72)	0.205

Abbreviations: OR, odds ratio; CI, confidence interval; CURB-65, Confusion, Urea, Respiratory rate, Blood pressure, Age ≥ 65; CD, cluster differentiation; NK, natural killer.

survival after reversal with immunotherapy.[24] By contrast, although circulating B cell counts were lower in septic patients, there were no differences in outcomes. Similar results were reported in a septic shock model regarding the absolute $CD19^+$ cell count.[25] However, differences were found when analyzing B cells subsets; with a low percentage of $CD23^+$ and high percentage of $CD80^+$ and $CD95^+$ in those that did not survive. Lastly, NK cells also play an important role when adaptive immunity is defective.[26] For example, low circulating NK cell counts have previously been associated with host defense compromise in a pneumonia mouse model and in patients with common variable immunodeficiency.[27,28]

Traditionally, the inflammatory response in sepsis and non-responding pneumonia has been considered a consequence of a cytokine storm that provokes a double-edged inflammatory scenario.[29] In this study, patients with L-CAP exhibited higher levels of pro-inflammatory cytokines and chemokines (IL-8, G-CSF, and MCP-1), plus increased levels of the anti-inflammatory cytokine IL-10. We found a significant negative relationship exists between lymphocyte counts and systemic cytokines, with a lower concentration of lymphocytes paralleling a higher degree of inflammation. This dysregulated host response implies a depression of adaptive immunity with a concurrent increase in the innate response probably as the main drivers of the boosted inflammatory state, leading to negative outcomes.

Survivors increased T cell counts at day 4. Indeed, the $CD4^+$ counts had almost doubled from day 1 to day 4. The recovery in $CD8^+$ counts was similar except in $CD19^+$, and NK cells that was lower. Simultaneously, on day 4, systemic cytokine levels showed a significant decrease mainly for IL-6, and G-CSF. This finding reinforces the notion of a strong interplay between T cell counts and systemic cytokines in CAP. On the other hand, there is a persistent inflammation, as measured by IL-8 and MIP-1β confirmed in those with <1000 lymphocytes/μL although it did not reach significance in L-CAP. Persistent inflammation has previously been associated with worse prognosis.[17]

Patients with lymphopenia <724 cells/μL had significant lower levels of IgG2. Moreover, there was a positive correlation between IgG2 levels and ALC, $CD4^+$, and $CD19^+$ cell counts in a subgroup of patients. IgG2, is the main immunoglobulin responsible for bacterial polysaccharide capsular antigens,[30] and recently, it has been shown levels <301 mg/dL were an independent risk factor for mortality in intensive care.[10,31] Interestingly, the protective role of this Ig trends to be greatest in patients with moderate disease severity (SOFA <8),[32] as observed in our cohort (SOFA score 2). It should be emphasized that > 50% of patients with lymphopenia had IgG2 levels below the previously mentioned threshold, which has important prognostic implications.

A low $CD4^+$ cell count was the strongest independent risk factor for predicting 30-day mortality in this study, after adjusting for age, sex, and CURB-65 score. Moreover, the number of $CD4^+$ cells could be approximately estimated from the ALC from CBC ($CD4^+$ below 129 cells/μL corresponds with an ALC below decile 2). To date, therapy directed against inflammatory cytokines has not been effective, even though some preclinical studies aimed at restoring T cell function have shown encouraging results.[24,33,34]

This study has several limitations. First, it was a single center study. Second, Ig levels were not monitored over time, as has been done in other models of sepsis. Third, lymphocyte subsets were analyzed on day 4 in a small group only. Although the results are consistent on the initial severity, the group size precluded meaningful evaluation of clinical outcomes. Finally, other lymphocyte subsets and their functions were not included (e.g., T helper 17, T regulatory cells).[35,36]

In conclusion, this study provides new insights into the L-CAP immunophenotype, which is mainly caused by a fall in $CD4^+$ cell counts. L-CAP was associated with higher severity at presentation and with features denoting the existence of a dysregulated immune response, (a greater inflammatory expression along with a reduction in Ig levels) which was in turn associated with a greater initial severity and higher mortality. Our results support that immunophenotyping CAP could help in the future to implement personalized treatments in this disease.

Conflict of Interest Statement

All authors declare that they have no conflicts of interest.

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Author's contribution

RM conceived the study design. R. Méndez, I. A., L. F., and P. R. developed and recruited the cohorts that were used in this study. R. Méndez, I.A., and L. F. collected the data used in this study. AS, AO and JB performed the laboratory analysis of the biological samples. R. Méndez, and R. M. analyzed the data. R. Méndez, R. M., J. B., and A. T. contributed to interpretation of the results. R. Méndez wrote the manuscript with assistance from R. M., A. S., J. B., and A. T. All authors read and approved the final manuscript.

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The funding institutions had no role in the design, data collection, data analysis, data interpretation, writing, review, or approval of the manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.04.006.

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