EBioMedicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Lymphopenic Community Acquired Pneumonia (L-CAP), an Immunological Phenotype Associated with Higher Risk of Mortality

Jesus F. Bermejo-Martin [a], Catia Cilloniz [b,1], Raul Mendez [c,d,1], Raquel Almansa [a,1], Albert Gabarrus [b], Adrian Ceccato [b], Antoni Torres [b,*,1], Rosario Menendez [c,1], for the NEUMONAC group

[a] Group for Biomedical Research in Sepsis (Bio Sepsis), Hospital Clínico Universitario de Valladolid/IECSCYL, Av. Ramón y Cajal, 3, 47003 Valladolid, Spain
[b] Department of Pneumology, Institut Clinic del Tórax, Hospital Clinic of Barcelona - Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona (UB) - SGR 911-Ciber de Enfermedades Respiratorias (Ciberes), Carrer de Villarroel, 170, 08036 Barcelona, Spain
[c] Pulmonology Service, University and Polytechnic Hospital La Fe, Ciber de Enfermedades Respiratorias (Ciberes), Avinguda de Fernando Abril Martorell, 106, 46026 València, Spain
[d] PhD program in Medicine and Traslational Research, University of Barcelona, Gran Via de les Corts Catalanes, 585, 08007 Barcelona, Spain

ARTICLE INFO

Article history:
Received 31 July 2017
Received in revised form 17 September 2017
Accepted 18 September 2017
Available online xxxx

Keywords:
Community
Acquired
Pneumonia
Lymphocyte
Mortality

ABSTRACT

The role of neutrophil and lymphocyte counts in blood as prognosis predictors in Community Acquired Pneumonia (CAP) has not been adequately studied. This was a derivation-validation retrospective study in hospitalized patients with CAP and no prior immunosuppression. We evaluated by multivariate analysis the association between neutrophil and lymphocyte counts and mortality risk at 30-days post hospital admission in these patients. The derivation cohort (n = 1550 patients) was recruited in a multi-site study. The validation cohort (n = 2846 patients) was recruited in a single-site study. In the derivation cohort, a sub-group of lymphopenic patients, those with <724 lymphocytes/mm^3, showed a 1.93-fold increment in the risk of mortality, independently of the CURB-65 score, critical illness, and receiving an appropriate antibiotic treatment. In the validation cohort, patients with <724 lymphocytes/mm^3 showed a 1.86-fold increment in the risk of mortality. The addition of 1 point to the CURB-65 score in those patients with <724 lymphocytes/mm^3 improved the performance of this score to identify non-survivors in both cohorts. In conclusion, lymphopenic CAP constitutes a particular immunological phenotype of the disease which is associated with an increased risk of mortality. Assessing lymphocyte counts could contribute to personalized clinical management in CAP.

1. Introduction

Community-acquired pneumonia (CAP) is a serious health problem causing high morbidity and mortality worldwide (Mandell et al., 2007). The rates of patients hospitalized due to CAP are increasing, with 22%–42% of adults needing admission to the hospital. In addition, CAP has an associated mortality of 5%–14% in patients requiring hospitalization. Approximately 5% of the patients hospitalized with CAP require admission to an intensive care unit (ICU). In these severe cases mortality rises to 35% (Lim et al., 2009).

Host individual variability is now recognized as a key factor influencing clinical expression and prognosis of CAP (Leoni and Rello, 2017). Learning from the successes of using precision medicine in the treatment of cancer, identifying individual phenotypes associated with poor outcome in CAP could help to better understand the pathogenesis of this disease and to improve its treatment (Rello and Perez, 2016; Prina et al., 2016; Aliberti et al., 2014). In this regard, Davenport et al., using a transcriptomic analysis, found a gene expression signature (SRS1) which identifies individuals with an immunosuppressed phenotype and higher 14-day mortality within a cohort of patients with sepsis secondary to CAP (Davenport et al., 2016). In addition, new biomarkers such as expression of HLA-DR on monocytes (Zhuang et al., 2015), soluble CD14 (presepsin) (Klouche et al., 2016), interleukins (Menéndez et al., 2009; Andrijevic et al., 2014), procalcitonin (Liu et al., 2016a), and mid-regional pro-ADM (Liu et al., 2016b) could help risk assessment in the early stages of the disease. As an alternative to these new sophisticated (and expensive)"omics" and biomarker-based approaches, a simple leukogram has shown potential to be a tool for classifying patients with CAP or sepsis based on their outcomes. The neutrophil/lymphocyte ratio (NLR) has been proposed as a candidate predictor of mortality for hospitalized CAP patients (Cataudella et al., 2017). Further, we have demonstrated that patients with septic shock who fail to expand circulating neutrophil counts in their blood present an increased risk of mortality (Bermejo-Martín et al., 2014).

* Corresponding author at: Department of Pneumology, Institut Clinic de Respiratori, Hospital Clinic of Barcelona-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona (UB)-SGR 911-Ciber de Enfermedades Respiratorias (Ciberes, CB06/06/0028) Villarroel, Barcelona, Spain.
E-mail address: ATORRES@clinic.cat (A. Torres).
[1] Equal contribution.

https://doi.org/10.1016/j.ebiom.2017.09.023

The objective of the present study was to evaluate the potential role of neutrophil and lymphocyte counts in blood as biomarkers of mortality risk in patients with CAP and no antecedents of immunosuppression. For this purpose, we performed a derivation-validation retrospective study employing two large cohorts of hospitalized patients with this disease.

2. Materials and Methods

2.1. Study Design

A derivation-validation retrospective study was performed to evaluate the association between neutrophil and lymphocyte counts at hospital admission and the risk of mortality at 30 days following admission, in patients hospitalized with CAP. The derivation cohort comprised patients recruited in the context of a multi-site study while those of the validation cohort were recruited in the context of a single-site study. Only those patients showing complete data for lymphocyte and neutrophil counts in the first 24 h following admission to the hospitaland the variables "admission to the ward or ICU", and "mortality at 30 days post-admission from hospital" were included in the analysis.

2.2. Patient Selection, Inclusion and Exclusion Criteria

2.2.1. Multi-Site (Derivation) Cohort

The patients included in this cohort had been admitted to 14 Spanish hospitals (NEUMONAC group), other than the Hospital Clinic (Barcelona). Patients were recruited from January 2012 to June 2015. Inclusion criteria were the presence of (assumed) new pulmonary infiltrate shown by chest radiograph and respiratory signs and symptoms compatible with CAP (cough, expectoration, chest pain, dyspnea, fever, among others). Exclusion criteria were as follows: nursing-home patients and immunosuppression status (human immunodeficiency virus-positive, acute leukemias, myelodysplastic syndromes, myelodysplastic and myeloproliferative syndromes, chronic myeloproliferative syndromes, lymphoproliferative syndromes, monoclonal gammopathies, marrow failure syndromes, primary immunodeficiencies and severe chronic neutropenia, solid-organ transplantation, >14 days of treatment with >20 mg/day of prednisone or equivalent, and other immunosuppressive drugs).

2.2.2. Single-Site (Validation) Cohort

This comprised consecutive patients admitted to the Hospital Clinic, Barcelona, Spain, between January 2005 and December 2015 with a diagnosis of CAP. Pneumonia was defined as an (assumed) new pulmonary infiltrate found on the hospital admission chest radiograph and symptoms and signs of lower respiratory tract infection. Patients with prior immunosuppression were excluded (for example, patients with neutropenia after chemotherapy or bone marrow transplantation, patients with drug-induced immunosuppression as a result of solid-organ transplantation, corticosteroid (>10 mg/day) or cytotoxic therapy, and patients with HIV-infection).

2.2.3. Other Definitions

In both studies, acute respiratory distress syndrome (ARDS) was identified in the first 24 h after hospital admission by applying the criteria described in the Berlin definition (ARDS Definition Task Force et al., 2012). The appropriateness of empiric antibiotic treatment was defined according to multidisciplinary guidelines for the management of CAP (Torres et al., 2013). Acute renal failure was defined using the criteria developed by the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group (Bellomo et al., 2004). Septic shock was defined according to the definition proposed by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (Bone et al., 1992).

2.3. Ethics

for the multi-site cohort, the Ethics Committee of the coordinating center approved the study (Code: 2011/0512). For the single-site cohort, the Ethics Committee of the Hospital Clinic of Barcelona approved the study (Code: 2009/5451). Given the observational and retrospective nature of the study, informed consent was waived. This study fulfils the standards indicated by the Declaration of Helsinki.

2.4. Leukocyte, Lymphocyte and Neutrophil Quantification

This measurement was performed on blood collected in ethylenediaminetetraacetic acid tubes by using the automatic analyzers available in each participating hospital at the central laboratories, under standard operative procedures approved for clinical use. Lymphopenia was considered as a total lymphocyte count $<1000/mm^3$, following the definition proposed in *Hematology*, 9th ed (Vasu S, Caligiuri MA, 2015).

2.5. Statistical Analysis (Supplementary file 1)

Differences in demographic and clinical characteristics between patient cohorts or sub-groups were assessed using the chi-squared test. The association between lymphocyte, neutrophil counts, C-reactive protein (CRP) levels, and the risk of mortality in the 30 days following hospital admission was evaluated by logistic regression analysis (Hosmer DW et al., 2013). Potential confounding variables were selected by assessing the association between those variables shown in Table 1 with mortality. Variables yielding a p value < 0.1 in the univariate regression analysis were further included in the multivariate analysis as adjusting variables. Final selection of the variables was performed by using the backward stepwise selection method (Likelihood Ratio) (pin < 0.05, pout < 0.10). Lymphocyte, neutrophil, and CRP concentrations were transformed to Naeperian log values in order to reach a normal distribution. Those variables showing a Spearman correlation coefficient > 0.3 with another inclusive variable were excluded from the multivariate analysis (Healey, 2014). The final number for each analysis (excluding the missing values) is shown in Tables 1 to 5. The ability of lymphocyte counts to differentiate survivors from non-survivors was assessed by using the area under the receiver operating characteristic curve analysis (AUC). The cut-off for lymphocyte counts regarding mortality prediction was obtained in the derivation cohort by calculating the optimal operating point (OOP) in the AUC, as previously described (Almansa et al., 2017). The OOP was considered the value for which the point on the curve had the minimum distance to the upper left corner (where sensitivity = 1 and specificity = 1). By Pythagoras' theorem this distance is:

$$\mathrm{OOP} = \sqrt{(1-\mathrm{sensitivity})^2 + (1-\mathrm{specificity})^2}$$

The ability of this cut-off value to predict 30-day mortality was further evaluated by using multivariate logistic regression analysis in the derivation cohort in a first step and in the validation cohort in a second step. Comparisons between the AUCs were assessed according to the method of DeLong et al. (DeLong et al., 1988). The level of significance was set at 0.05 (2-tailed). All analyses were performed with IBM SPSS Statistics 20.0 (Armonk, New York) and MedCalc 17.8.5 (Ostend, Belgium).

3. Results

3.1. Clinical Characteristics of the Patients (Table 1)

the derivation cohort comprised 1550 patients, and the validation cohort 2846. The proportion of patients older than 65 years was higher in the validation study. In turn, the proportion of those who had

Please cite this article as: Bermejo-Martin, J.F., et al., Lymphopenic Community Acquired Pneumonia (L-CAP), an Immunological Phenotype Associated with Higher Risk of Mortality, EBioMedicine (2017), https://doi.org/10.1016/j.ebiom.2017.09.023