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Immunoglobulins concentration and B cell counts as severity markers in adult community-acquired pneumonia

Cross sectional study

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Abstract

Community-acquired pneumonia (CAP) is a worldwide cause of morbidity and mortality. Immunoglobulins (Igs) and B cells quantification studies in CAP are few and show discrepancies. Serum IgA acts as a powerful natural anti-inflammatory factor, but its role in the CAP has not yet been defined. The highly sensitive xMAP Luminex technique allows better immunoglobulins quantification. The aim of this study was to analyze the relation between clinical severity and circulating Igs and B cells in adults with CAP.

Igs (M, A, G1, G2, G3, and G4) and B cells were quantified in peripheral blood of 190 Chilean patients ≥18 years old hospitalized for CAP and in 21 adults without respiratory disease, using xMAP Luminex and flow cytometry, respectively. Clinical history was recorded and PSI and CURB-65 scores were calculated for evaluation of clinical severity.

The total IgM, IgG2 and total IgG levels were lower in CAP than in asymptomatic adults ($P < .05$). No significant differences of Igs levels were found between patients classified as severe and mild by PSI and CURB-65 scores. Fatal cases had higher levels of IgA ($P < .05$). No differences in $CD19^+$ B cells frequency was found between CAP and asymptomatic adults ($P = .40$). In PSI severe cases, $CD19^+$ B cells were significantly lower than in mild cases ($P = .008$). No differences were found in CURB-65 severe and mild groups ($P = .11$). In fatal cases (11/82) group, $CD19^+$ B cells frequency was lower than in 71 survivors ($P = .2$). No differences in memory B lymphocytes were detected between asymptomatic and CAP adults, severe and mild patients, survivors and fatal cases ($P > .05$).

Serum IgA levels were significantly higher in fatal CAP cases, raising it as a potential biomarker for severe disease considering its relatively universal availability. In PSI severe patients, B cells showed lower levels and could have a role on its physiopathology. Finding new markers rooted in physiopathology could improve the possibility of scoring severe CAP cases. Luminex technology showed promising quantification serum Igs.

Abbreviations: CAP = community-acquired pneumonia, Igs = immunoglobulins, ICU = intensive care unit (ICU), PSI = Pneumonia Severity Index, CURB65 = confusion-ureic nitrogen-respiratory rate-blood pressure, PB = peripheral blood, EDTA = ethylenediaminetetraacetic acid, IQR = interquartile range, SD = standard deviation, COPD = chronic obstructive pulmonary disease, LR = likelihood ratio, HLA = human leukocyte antigen.

Keywords: pneumonia, community-acquired pneumonia, B cells, immunoglobulins, severity markers

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This study was approved by the ethics committees at the Clinic University of Chile Hospital (Act N12), the University of Chile Faculty of Medicine (Act N009), and the South Metropolitan Health Service (Act N 142/2017). Written informed consent was obtained from all subjects who participated in this study.

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All of the datasets in the current study are available from the corresponding author on reasonable request.

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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1. Introduction

Community-acquired pneumonia (CAP) is responsible for most of the burden of pneumonia,[1] being a worldwide leading cause of death. [1] CAP is the seventh cause of death in Chile, mostly among the elderly.[2]

Among CAP patients, 10% to 20% will require admission to intensive care unit (ICU), [1] and 5% to 10% of them will die.[2] Some well-known severity risk factors as age and comorbidities are included in most clinical severity scores like Pneumonia Severity Index (PSI) and CURB-65,[1,3] but some severe cases do not display them at early stage and patients are not adequately classified. Thus, finding biomarkers linked with early disease phase could improve assessing morbidity and mortality risk at admission.

The humoral immune response seems to be critical in the prevention and the recovery of the infection, CAP being more frequent in people with primary immunoglobulin deficiency. Antibodies in respiratory infections have effect mostly at the mucosal level, but since it is difficult to study at pulmonary site, immunoglobulins (Igs) protective function is usually studied using blood antibody titers.[4] The few available studies measuring Igs in adult CAP have shown lower levels in patients as compared with controls and also in hospitalized vs ambulatory cases.[5] However, they disagree on the class/subclass of Igs compromised.[5–7] The use of antibodies level as a severity biomarker in these patients is also controversial, and while some studies found no relationship,[6,8,9] others have associated low IgG or IgA levels with severity and/or lethality.[5,10,11]

IgA is the predominant immunoglobulin isotype in the respiratory secretions, being relevant on infection control and also for stimulating the inflammatory mucosal response.[12,13] Physiological role of serum monomeric IgA has not been clarified yet,[14] and it has been described as a powerful natural anti-inflammatory action controlling an excessive and harmful tissue response triggered by lung infection.[15] Consequently, manipulation of IgA functions and its receptor arise as interesting and new therapeutic options. However, the IgA role in the CAP cases has not been well defined,[6,11] showing lower levels in patients than in healthy controls,[6] and higher in patient that required inpatient treatment and in fatal cases.[11]

The Igs quantification usually is performed through nephelometry measuring the intensity of scattering light from antigen-antibody complexes (aggregation).[16] The new technology Luminex xMAP reads fluorescence intensity of aggregated enzyme-labeled antibody with flow cytometry, increasing 1000 times its ability to detect smaller proteins amount than nephelometry.[16] In addition, this technique allows the simultaneous detection of classes and subclasses of antibodies in a very small volume of sample, which represents a great clinical advantage.

B lymphocytes activity is paradigmatic in the prevention and recuperation of infections through the antibody production as well as in the IgA transport inside the airway,[17] cytokines secretion and in new regulatory functions described recently by certain B cell subpopulations.[4,18] Nevertheless, there are too few studies on the role of B lymphocytes in pneumonia, and most of them are performed in animal models. In the only one published about adult CAP, no differences were found in the number of $CD19^+$ cells between deceased and recovered patients.[19]

CD19 is one of the most reliable surface biomarker for B cells expressed from pre-B cells until the terminal differentiation to plasma cells. CD19 functions as the dominant signaling component of a multimolecular complex on the surface of mature B cells, and plays a critical role in mountaining an optimal immune response.[20] Initially, antibody production in the first week of infection begins with high IgM titres, and changes through the switch-class recombination to IgG, allowing for an increase in specificity and affinity to block viral or bacterial antigens.[21] Approximately 15% to 20% of peripheral blood (PB) B cells in adults are IgG^+ B cells, expressing mainly IgG1, IgG2 or IgG3, whereas IgG4 is low express.[22] Their proportions among memory B cells vary considerably among individuals, likely because of different infection histories, as switching to specific IgG subclasses is influenced by the type of antigen and various immunoregulatory factors like cytokines. The existence of IgG subclasses contributes to the versatility of humoral immunity because of specific effector functions.[23]

The aim of this study was to explore the humoral immune response, analyzing the relationship between B cells count, the Igs sera concentration using a very high sensitivity technique and the severity of the CAP in Chilean adults, investigation which has not been done so far.

2. Methods

2.1. Patients and study design

A cross sectional study was conducted in 190 patients over 18 years of age presenting with CAP. They were recruited in 3 hospitals (Clínico Universidad de Chile, de Infecciosos Dr. Lucio Córdova and Complejo Hospitalario San José) in Santiago, Chile from May 2017 to January 2019. CAP was defined by the presence of acute respiratory symptoms for less than 1 week and chest radiography showing new pulmonary infiltrates. Exclusion criteria included immunocompromising conditions (i.e., human immunodeficiency virus infection, active treatment for cancer, organ transplant, immunosuppressive and, or steroids therapy), hemodialysis and hospitalizations within 30 days preceding enrollment. Information on age, gender, co-morbidities, life style (smoking, alcohol consumption >80 g per day), clinical presentation, chest radiographic patterns and laboratory parameters were recorded for all patients in standardized files. The illness outcome such as mortality before 30 days after discharge, admission to ICU, renal failure, shock, mechanical ventilation, supplemental oxygen therapy and others were recorded. Patient severity was assessed during the first 48 hours after enrollment by PSI[3] and CURB$-$65.[1] Class IV and V of PSI and $\geq$3 CURB-65 score were considered as severe; class I and II of PSI and $\leq$1 CURB-65 score as mild, and class III of PSI and 2 CURB-65 score as moderate.

Basal values of B cells were determined in 21 adults without respiratory infection at least 45 days before enrollment and with no agent detected in microbiological screening. In 19 of them, basal levels of Igs were also determined. The study was approved by the University of Chile and Health Institutional Ethics Committee and all subjects at enrolment gave written informed consent.

2.2. Microbiological study

Urinary antigens for *Streptococcus pneumoniae* and *Legionella* were detected using immunochromatographic tests (Binax NOW, Portland, Oregon, USA). Adenovirus, human Bocavirus,

human respiratory syncytial virus, human metapneumovirus, human Coronavirus, human Parainfluenza virus, Rhinovirus/Enterovirus, Influenza A and B viruses, *Chlamydophila pneumonia, Mycoplasma pneumoniae*, and *Legionella pneumophila* were detected by Respiratory Multi well system r-gene Real Time RT-PCR kit Argene in nasopharyngeal aspirate/swabs, according to the manufacturers instructions in a MIC instrument (BMS). Total genetic material was extracted from each 300 μl respiratory sample using Favorprep Viral Nucleic Acid Extraction kit (Favorgen), according to manufacturers instructions and quantified in an EPOCH spectrophotometer.

2.3. Quantification of immunoglobulins

Serum levels of IgM, IgA, IgG1, IgG2, IgG3 and IgG4 were measured by Bio-Plex Pro Human Isotyping Magnetic Bead Panel (Biorad) with MAGPIX (Luminex) equipment.

2.4. Quantification of B lymphocytes by flow cytometry

Peripheral blood leukocytes were obtained from EDTA blood tubes by centrifugation (Boeco Centrífuge). Cells were stained with BD Horizon Brilliant Stain Buffer (563794 BD Pharmigen) and BD Pharmigen Stain Buffer (FBS) (554656 BD Pharmigen). After incubation with specific antibodies, cells were incubated with 1X BD FACS Lysing Solution (641776 BD Pharmigen). Surface staining of 1×10^6 peripheral blood mononuclear cells obtained from blood with EDTA by centrifugation (Boeco Centrífuge) were performed using the followings antibodies of BD Pharmigen: BV510 mouse anti-human CD45 (563204); APC mouse anti-human CD3 (555342); B-lymphocytes with PE-Cy7 mouse anti-human CD19 (557835) and BV421 mouse anti-human CD27 (562513). Isotype-matched controls were used in all experiments: BV510 mouse IgG1 (562946); APC mouse IgG2a (555576); PE-Cy7 mouse IgG1 (557872), and BV421 mouse IgG1 (562438). Non- viable cells were stained by BD Horizon Fixable Viability Stain 780 (565388). Flow cytometric acquisition and analysis of samples were performed on a FACSCANTO II (BD Biosciences©). The data analyses were evaluated using the FlowJo10.4 software.

2.5. Statistical analysis

Continuous variables were described as median and interquartile range (IQR) or mean and standard deviation (SD) (B cells) and categorical variables as frequency and percentage. Differences between groups were performed using the Fishers exact test for categorical data; Student *t* for B cells, and Mann–Whitney or Kruskal–Wallis tests for Igs. Pearson correlation coefficient was used to assess the relationship between B cells and age. Statistical significance was set at P value $<.05$. Data were analyzed using SigmaStat 13.0 and GraphPrism 8.4.2 softwares.

3. Results

3.1. Characteristics of studied population

Clinical characteristics from 190 patients hospitalized with CAP are shown in Table 1. Median of days of illness at enrollment was 5.0 (IQR: 3–7 days), being less 1 week in 87 (61.3%) of 142 adults with data. According to PSI, 97 (54.5%) cases were classified as severe, 46 mild (25.8%) and 35 moderate (19.7%) out of 178 patients and according to CURB-65, 65 (36.3%) cases were severe, 67 mild (37.4%) and 47 moderate (26.3%) out of 179 patients. In relation to the clinical outcome of 182 cases, radiologic progression was detected in 14 (7.7%), 38 (20.9%) needed mechanical ventilation, 20 (11.0%) had shock, 49 (27.1%) had sepsis and death occurred in 32/179 (17.6%) cases.

Table 1

General characteristics of 190 adults with community-acquired pneumonia.

Characteristics	Adults
Age (years) median (IQR)	73.0 (59.0–84.0)
Female patients n (%)	93 (49.5)
Death n/N (%)	32/179 (17.9)
Hospital stay (days) median/N (IQR)	8/175 (6.0-–14.0)
ICU admission n/N (%)	34/182 (18.7)
Smokers n/N (%)	46/182 (25.3)
Alcohol use >80g/day n/N (%)	13/182 (7.1)
Comorbidity N	182
Any n (%)	144 (81.9)
Type 2 Diabetes mellitus n (%)	59 (32.4)
COPD[2] n (%)	43 (23.6)
Asthma n (%)	14 (7.7)
Cardiac failure n (%)	33 (18.1)
Liver damage n (%)	6 (3.3)
Renal disease n (%)	17 (9.3)
Antibiotics prior to admission n/N (%)	33/167 (19.8)
Mental confusión n/**N** (%)	64/182 (35.2)
Hypotension n/**N** (%)	37/182 (20.3)
Shock n/**N** (%)	20/182 (11.0)
Chest radiograph N	177
- Interstitial patterns n (%)	31 (17.5)
- Alveolar patterns n (%)	123 (69.5)
- Both n (%)	23 (13.0)
- Multilobar involvement n (%)	91 (51.4)
- Pleural effusion n (%)	52 (29.4)

IQR: 25 to 75th percentile range; ICU = intensive care unit; COPD = chronic obstructive pulmonary disease.

Respiratory pathogens were detected in 126 (66.3%) of 190 cases, corresponding to only bacteria in 32 (25.4%), only viruses in 68 (54.0%), and 26 cases (20.6%) positive for both (mixed infection). Among 94 patients with viral detection, viruses most frequently detected were: influenza (32 cases; 34.0%) and rhinovirus (31 cases, 33.0%). *S.pneumoniae* was the most frequently detected bacteria, in 47 cases (81.0%).

Age median of 21 adults without respiratory infection was 54.0 years (IQR: 43–63); 7 (36.8%) were men; 3 (15.8%) had comorbidities (1 type 2 diabetes mellitus, 1 asthma, and 1 hypothyroidism); 1 was smoker (4.8%) and no one had alcohol consumption >80 g/day.

3.2. Levels of immunoglobulins

Igs were quantified in 151 CAP and 19 asymptomatic adults (Fig. 1A) (see Table 1, Supplemental Digital Content (http://links.lww.com/MD/E913), which shows serum concentrations of Igs). IgM, IgG2 and total IgG levels were significantly lower in CAP patients than in adults without respiratory illness ($P \leq .04$). No significant differences of Igs levels were detected between severe and mild patients according to clinical scores PSI and CURB-65 (Fig. 1B) (see Table 1, Supplemental Digital Content, which shows serum concentrations of Igs), neither applying different clinical outcomes such as ICU admission, shock, hypotension,

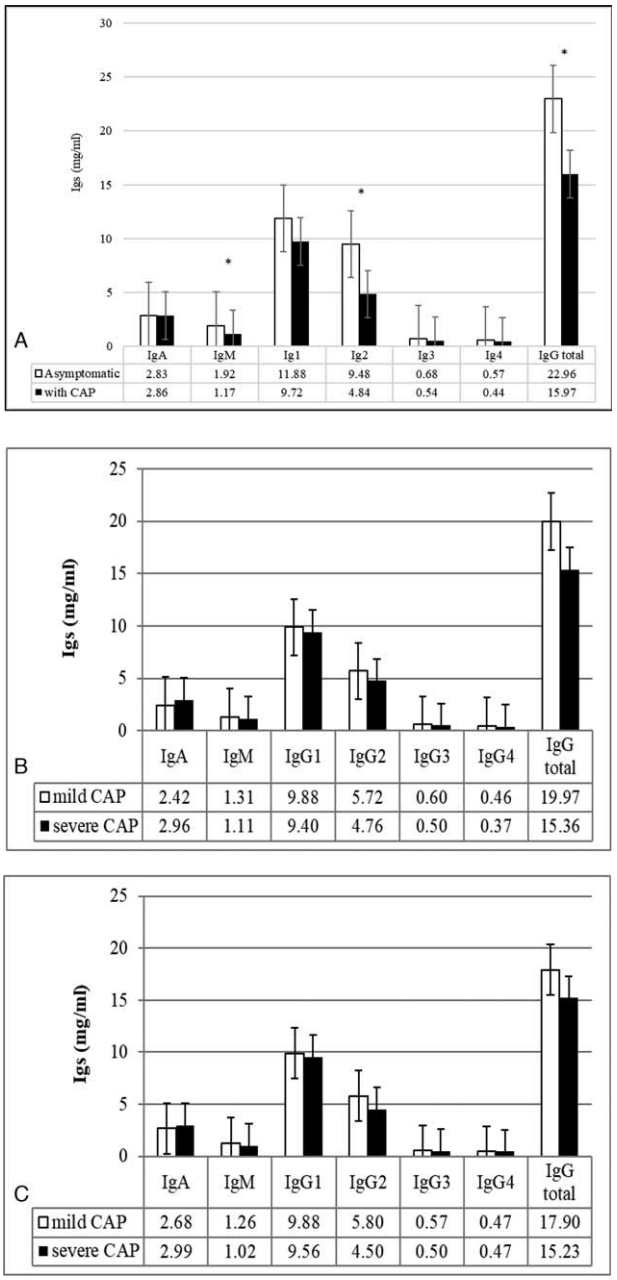

Figure 1. Serum immunoglobulins in 151 adults with community-acquired pneumonia and 19 adults without respiratory illness (A) and in adults with severe and mild CAP according to PSI (B) or CURB-65 (C). Data as median mg/ml; $^{*}P \leq .04$, Mann–Whitney test. CAP = community acquired pneumonia, PSI = Pneumonia Severity Index, CURB65 = confusion-ureic nitrogen-respiratory rate-blood pressure.

radiologic progression and renal failure, except mechanical ventilation (Table 2).

The concentration of all Igs (except IgG3) was higher in the deceased than in the survivors, although only IgA was statistically significant (Table 2) and this trend was maintained after adjusting by age ($P = .07$). In relation to severity of CAP by PSI, level of IgA ≥1.76 mg/ml had sensitivity of 87.01%, specificity of 33.33%, 68.97% correctly classified LR+ of 1.30 and LR− of 0.38.

According to age, only serum levels of IgG4 were significantly lower in 101 adults with CAP ≥65 years than in 48 patients under

Table 2
Serum levels of immunoglobulins in 151 adults with community-acquired pneumonia according to outcome.

Serum levels of Igs (mg/ml)	Hypotension		Shock		Mechanical ventilation		ICU		Mortality at 30 days	
	Yes (n = 29)	No (n = 120)	Yes (n = 12)	No (n = 137)	Yes (n = 25)	No (n = 124)	Yes (n = 20)	No (n = 129)	Yes (n = 27)	No (n = 121)
IgA	3.01 (2.17–5.10)	2.84 (1.89–3.99)	2.70 (2.44–3.83)	2.86 (1.88–4.56)	2.54 (1.84–3.57)	2.98 (1.92–4.76)	2.86 (2.23–5.19)	2.86 (1.86–4.12)	3.56 (2.54–6.32)	2 77* (1.89-3.97)
IgM	1.52 (0.88–2.07)	1.10 (0.80–1.85)	1.37 (0.81–1.62)	1.15 (0.81–1.87)	0.92 (0.57–1.37)	1.26** (0.85–1.92)	1.25 (0.82–1.58)	1.15 (0.81–1.87)	1.58 (0.84–2.20)	1.10 (0.81-1.63)
IgG1	10.40 (6.68–15.0)	9.72 (6.78–13.53)	10.90 (7.24–11.76)	9.76 (6.64–13.92)	9.12 (6.24–11.24)	9.80 (7.00–13.96)	10.10 (6.89–11.76)	9.72 (6.68–14.56)	11.64 (7.04–16.80)	9.64 (6.42- 12.86)
IgG2	4.72 (3.36–8.78)	5.12 (3.46–7.32)	4.76 (3.99–8.35)	4.96 (3.41–7.46)	3.76 (2.64–5.52)	5.44** (3.90–7.60)	5.94 (3.55–-8.34)	4.84 (3.43–7.38)	6.68 (3.59–8.80)	4.76 (3.40-7.24)
IgG3	0.45 (0.29–0.94)	0.56 (0.37–0.86)	0.44 (0.29–0.57)	0.56 (0.36–0.94)	0.40 (0.33–0.59)	0.57** (0.57–0.97)	0.48 (0.29–0.84)	0.54 (0.36–0.91)	0.48 (0.54–0.6)	1.10 (0.35-0.84)
IgG4	0.46 (0.20–0.93)	0.3 (0.14–0.77)	0.58 (0.39–1.19)	0.40 (0.13–0.77)	0.47 (0.23–0.90)	0.43 (0.14–0.78)	0.57 (0.31–0.85)	0.38 (0.12–0.84)	0.58 (0.29–0.89)	0.38 (0.12-0.84)
IgG total	15.48 (11.96–24.27)	16.06 (11.40–23.36)	15.35 (13.30–-22.65)	16.14 (11.41–23.73)	13.31 (10.3–19.42)	16.91 (11.95–23.87)	16.48 (11.82–21.84)	15.48 (11.43–24.10)	21.01 (11.23–-28.95)	15.38 (11.50- 21.72)

* $P < .05$.
** $P < .01$, by Mann–Whitney test.
Data as median and IQR (25−75th percentile); all P values >.1, except.
ICU = intensive care unit.

Table 3

Serum levels of immunoglobulins in 149 adults with community-acquired pneumonia according to age and gender.

Serum levels of Igs (mg/ml)	<65 years (n = 48) Median (IQR)	≥65 years (n = 101) Median (IQR)	P*	Female (n = 73) Median (IQR)	Male (n = 77) Median (IQR)	P*
IgA	2.62 (1.90–4.23)	2.96 (2.06–4.34)	.5	2.77 (1.73–4.24)	3.02 (2.18–4.56)	.4
IgM	1.29 (0.89–1.90)	1.05 (0.80–1.79)	.1	1.27 (0.86–2.27)	1.08 (0.79–1.61)	.03
IgG1	10.26 (7.29–14.96)	9.40 (6.28–12.54)	.1	9.80 (6.94–16.16)	9.72 (6.28–12.42)	.1
IgG2	5.66 (4.06–8.01)	4.72 (3.24–7.40)	.1	5.08 (3.43–7.38)	4.84 (3.50–7.66)	.9
IgG3	0.56 (0.38−0.86)	0.51 (0.33–0.91)	.5	0.56 (0.39–0.86)	0.51 (0.35–0.96)	.6
IgG4	0.53 (0.31–0.98)	0.36 (0.11–0.75)	.02	0.35 (0.09–0.75)	0.49 (0.26–0.93)	.03
IgG total	19.00 (13.23–27.37)	15.23 (11.03–22.52)	.09	16.76 (12.08–25.20)	15.23 (11.43–22.57)	.4

* *P* values by Mann–Whitney test.

65 years and according to gender, only higher levels of IgM and lower levels of IgG4 in 73 women than 77 men were significant (Table 3). Levels of all Igs studied were similar between 35 smokers and 112 no smokers ($P \geq .12$; *t* test); while 9 alcoholic adults had higher levels of IgG2 (mean (SD):14.40 (22.64) vs 6.43 (7.51); $P = .01$) and IgG4 (mean (SD):1.130 (0.99) vs 0.61 (0.69); $P = .03$) than 138 non- drinking adults, and similar levels of the others Igs.

Lethal cases were older than the recovered (median: 85 vs 70 years; IQR: 80−89 vs 57.5−79.5; Mann–Whitney test; $P < .001$) and both groups had similar proportion of gender (40.74% vs 53.71% in men, $P = .2$).

Levels of all IgG subclasses were similar between cases with only viruses, only bacteria and mixed infections (Table 4). IgA levels were significantly higher in 23 patients with rhinovirus than in 22 cases with influenza virus ($P = .02$) and in 3 cases with respiratory syncytial virus than 22 patients with influenza virus ($P = .03$) (Table 5).

3.3. Quantification of B cells

$CD19^+$B cells were quantified in blood from 83 adult with CAP and 21 asymptomatic adults, detecting similar proportions in both groups (mean: 39.09% ± 3.14 vs 43.40% ± 2.45 mg/ml; *t* test; $P = .40$) (Fig. 2A). Compared to 16 adults with mild CAP classified by PSI, 45 patients with severe CAP had significantly lower B cells proportions (39.85% ± 3.01 vs 57.32% ± 6.52 mg/ml; *t* test; $P = .008$) (Fig. 2B). Although this difference was also detected among cases classified by CURB-65 (Fig. 2C), it did not reach statistical significance (40.36% ± 3.60 vs 49.73% ± 4.73; *t* test; $P = .11$). Proportions of B cells were lower in 11 deceased than in 71 survivors patients of 82 cases with this information, but it was not statistically significant (mean: 35.31% ± 6.11 vs 44.32% ± 2.67; *t* test; $P = .2$) (Fig. 3). Percentages of $CD19^+CD27^+$ B lymphocytes were similar between asymptomatic/CAP adults, severe/mild patients, and survivors/dead adults (Fig. 4).

Systemic levels of Igs and B cells were similar between 56 patients with and 21 without co-morbidities such as diabetes mellitus, hypertension and/or chronic obstructive pulmonary disease (B cells: 41.47% ± 2.87 vs 45.63% ± 5.29; *t* test; $P > .1$).

B cells was higher in 23 adults under 65 years than 59 adults ≥65 years, but this difference was not statistically significant (mean: 49.07% ± 5.15 vs 40.61% ± 2.71; *t* test; $P = .1$); Pearson coefficient r between cells and age was−0.27 (95%IC: −0.4633 to −0.0630; $P = .01$) (Fig. 5A). Proportions of B cells were similar between 39 men and 43 women with CAP (mean: 44.12% ± 3.86 vs 42.94% ± 3.22), just like above 65 years (43.59% ± 5.90 vs 59.36% ± 9.29; n = 15 and 8) and ≥65 years (44.45% ± 5.16 vs 38.06% ± 2.95; n = 24 and 34, respectively) (*t* test; $P > .1$) (Fig. 5B). However, 34 women above 65 years had lower B cells than 8 under 65 years (38.06% ± 2.95 vs 59.36% ± 9.29; *t* test; $P = .007$), what was not observed among men (44.45% ± 5.16 vs 43.59% ± 5.90; n = 24 and 15, respectively; t test; $P = .9$) (Fig. 5B).

$CD19^+$B cell proportion was higher in 18 patients with mixed infection than 47 viral CAP and 16 bacterial CAP (53.89% ± 5.32 vs 40.51% ± 3.22 vs 43.07% ± 5.13), but only was statistically significant the first one (t test; mixed vs viral $P = .03$; mixed vs bacterial $P = .19$ and viral vs bacterial $P = .6$) (Fig. 5C). However, there were no differences of $CD27^+$ (viral: 28.11% ± 2.6; mixed: 29.01% ± 4.5 and bacterial: 32.75% ± 5.6; $P > .1$); neither $CD27^-$ cells (viral: 67.29% ± 3.2; mixed: 69.48% ± 4.5; bacterial: 66.73% ± 5.6; $P > .7$) by agent CAP. According to severity and agent, proportions of total and memory B cells were similar between severe and mild cases in each type of infection both applying PSI and CURB65 scales (see Fig. 1, Supplemental Digital Content, which shows proportions

Table 4

Serum levels of immunoglobulins according to detected agent in 88 adults with community-acquired pneumonia.

Igs (mg/ml)	Viruses only (n = 46) median (IQR)	Bacterial only (n = 21) median (IQR)	Mixed (n = 21) median (IQR)	P*
IgA	2.86 (1.94– 3.85)	2.58 (2.06–4.44)	1.70 (2.36–3.92)	.7
IgM	0.91 (0.71–1.48)	1.08 (0.71–1.51)	1.03 (0.66–1.67)	.9
IgG1	8.94 (6.06–11.64)	8.64 (5.72–13.56)	7.64 (5.86–11.44)	.9
IgG2	4.52 (3.10–5.91)	4.40 (3.34–8.32)	3.94 (2.83–5.78)	.4
IgG3	0.50 (0.34–0.62)	0.47 (0.37–0.80)	0.47 (0.27–0.78)	.7
IgG4	0.33 (0.11–0.78)	0.43 (0.16–0.67)	0.38 (0.07–.58)	.6
IgG total	14.75 (10.47–20.33)	14.50 (9.99–24.99)	13.31 (9.88–17.36)	.8

* Kruskal–Wallis test.

IQR = interquartile range (25th−75th percentile).

Table 5

Serum levels of immunoglobulin A according to detected agent in 88 adults with community-acquired pneumonia.

Agent detected		n	IgA (mg/ml)	
			Median	IQR
Bacteria	*Streptococcus pneumoniae*	33	2.39	1.69–3.93
	Legionella pneumophila	6	2.67	2.27–3.74
	Escherichia coli	4	4.61	3.23–10.64
	Klebsiella pneumoniae	2	1.98	1.32–2.64
	Mycoplasma pneumoniae	1	1.97	N.A.
	Haemophilus influenzae	1	3.71	N.A.
	Staphylococcus aureus	1	8.8	N.A.
Virus	Influenza virus	22	2.27	1.24–3.07
	Rhinovirus	23	2.86	2.36–4.68
	Human Bocavirus	7	3.09	1.91–4.84
	Adenovirus	5	1.84	1.69–5.25
	Human Coronavirus	3	3.87	1.69–3.97
	Parainfluenza virus	3	2.21	1.69–2.72
	Respiratory syncytial virus	3	3.90	3.16–5.04
	Human metapneumovirus	1	3.84	N.A.

The sum of cases is greater than 88 because there were patients with more than 1 agent detected. $P > .2$; Kruskal–Wallis test.

Paired analyses were performed with the Mann–Whitney test and all P were >05, except influenza virus vs rhinovirus ($P = .02$) and influenza virus vs respiratory syncytial virus ($P = .03$).

IQR = interquartile range (25th−75th percentile), N.A = not applicable.

of LB $CD19^{+}CD27^{+}$ in CAP adult according to agent and severity).

4. Discussion

The lower immunoglobulins levels detected in adults with CAP in comparison with adults without respiratory diseases agree with results of previous studies, mostly in lower IgG2 level [7] and IgG total,[6] while lower levels IgM have not been previously described. These lower Igs levels in patients, who were developing an infectious process, could be a factor favoring the establishment of a pneumonia, considering that the humoral immune response should have a relevant role in the prevention and control of pneumonia.[4,17] However, the role of this response in the outcome could not be defined due to discrepant results in the Igs levels and B cells count in relation to the CAP severity. Thus, while the Igs levels were similar in severe and mild cases defined by PSI or CURB65 or by severity parameters applied, B cells were lower in severe than mild patients. Our results about Igs levels agree with some reports,[8] but not with others,[11] and it hampers to present Igs levels as severity biomarkers.

This study differs from previous in 2 aspects. Firstly by not having enrolled immunosuppressed patients and secondly by the use of Luminex technique for quantification of serum immunoglobulins, which is much more sensitive than the nephelometry commonly used in clinics. The better sensitivity of Luminex technique explains the fact that just a few patients displayed IgG levels under the values considered normal by some publications, while the majority were above them. Since those values do not represent universal cut off points, in our cases we compared proportions instead of absolute values of IgG, so we do not mention reference values. Furthermore, since Luminex is being applied mostly for research, it will be necessary to define the normal range values in order to extend its use to clinical practice, taking also advantages of other qualities like the small volume of sample required and the ability to detect simultaneously Igs

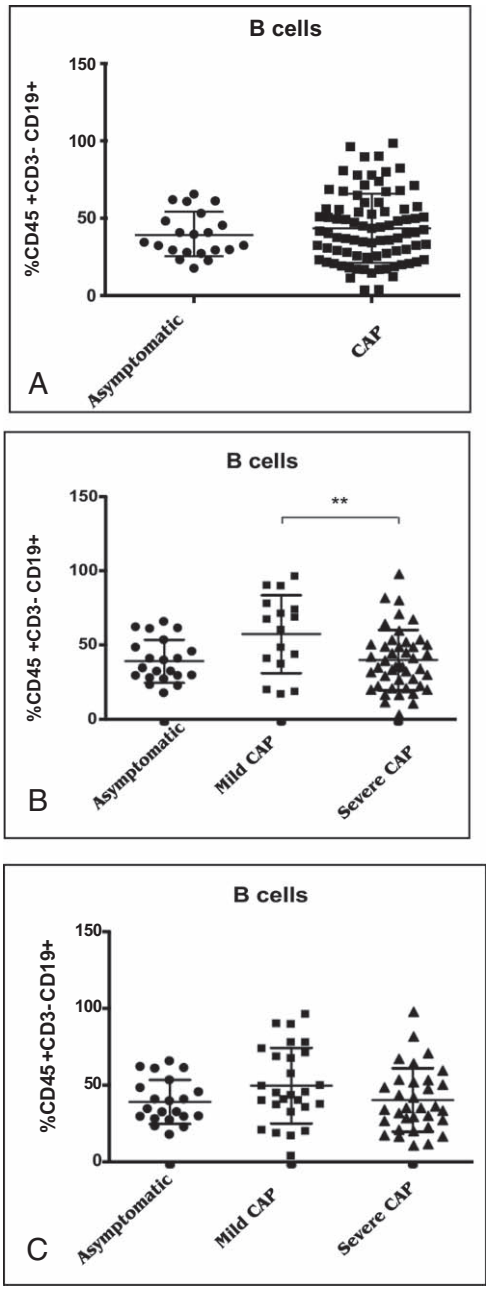

Figure 2. Proportion of B cells from asymptomatic and community-acquired pneumonia adults (A) and severe and mild adults with CAP (B, C) by flow cytometry. Asymptomatic and CAP adults $P > .05$ by t test. B. According to PSI n = 16 severe and 45 mild adults with CAP. C. According to CURB-65 n = 34 severe and 28 mild adults with CAP. CAP = community acquired pneumonia; t test; $^{**}P = .008$, PSI = Pneumonia Severity Index, CURB65 = confusion-ureic nitrogen-respiratory rate-blood pressure.

classes and subclasses. This technique is already being applied for human leukocyte antigen (HLA) typing and in other fields.[24]

Since increased level of IgA was the only significant difference found in fatal cases as compared with survivors, and it was also detected in severe as compared to mild cases ($P = .06$), this immunoglobulin could be candidate for a prognosis biomarker in the adult CAP. This increase has been previously reported in only one article [11] and raises the risk of lethal outcome in 1.1 times for each one IgA unit of rise, and the increment persists after age

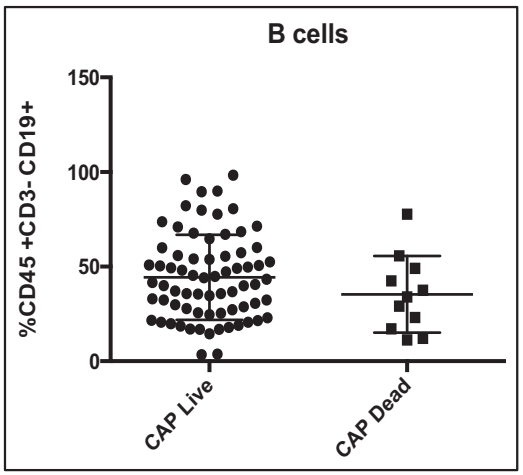

Figure 3. Proportion of CD19⁺ B cells in blood from 71 survivors and 11 deceased adults with community-acquired pneumonia by flow cytometry. $P > .05$; *t* test. CAP = community acquired pneumonia.

adjustment. Thus, it was explored the IgA level capable to correlate with PSI CAP severity founding that IgA ≥1.76 mg/ml would be an option.

Male sex, old age and alcoholism had been significantly associated with serum IgA concentrations[14]; but given that lethal cases were older than the recovered and both groups had similar proportion of gender and alcoholics, only age could be influencing the increase of IgA in fatal cases in our study. On the other side, genetic conditioning of serum IgA levels in adults proposed by Dieguez et al[14] could explain the previous controversial publications with respect to IgA.[5,6,8]

To our knowledge this would be the first report of immunoglobulin levels and B cell counts on CAP distributed by gender. Therefore, it is interesting to comment the significant reduced IgM and the increased IgG4 responses in males in relation to females, which are not correlated to age or presence of co-morbidities, because both groups were similar ($P = .08$, Mann–Whitney test). Also these differences agree with previous observations of IgM reduction and male gender as risk factors for severe CAP,[6] although in this study, males and females had similar proportions of fatal and admitted to ICU cases. More studies on the impact of these gender differences are needed, because IgM and IgG4 have anti-inflammatory actions.[25,26] In relation to B cells, a significant difference was observed only in females under and over 65 years old, unaware of the consequence of this.

The advanced age is a well-known prognostic factor for severity on CAP because the immune senescence diminishes the adaptive response favoring infectious diseases.[27] In this study, we found lower levels of CD19⁺B cells, similar proportion of memory B cells CD27⁺, diminished IgG4 levels and increased IgA (Table 3, Fig. 5) in cases over 65 years old, which contrast with

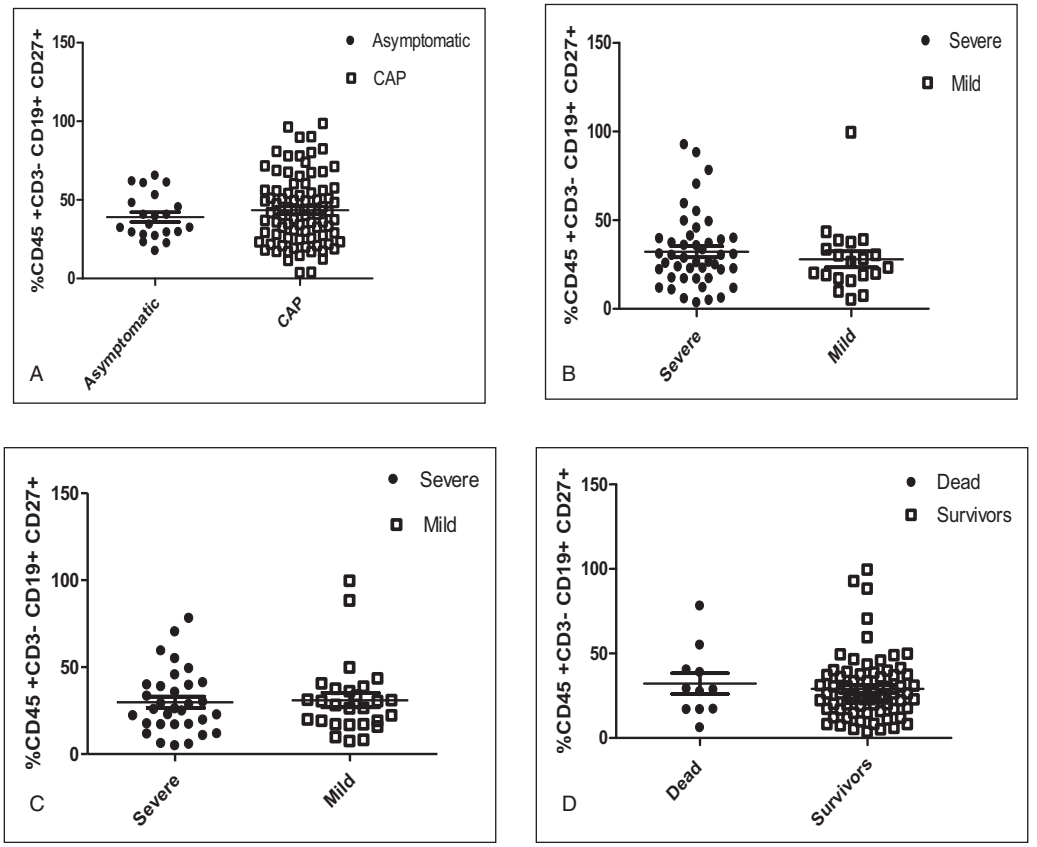

Figure 4. Proportion of CD19⁺CD27⁺ B lymphocytes in blood from asymptomatic and community-acquired pneumonia adults (A), severe and mild cases by PSI (B), severe and mild cases by CURB65 (C), and recovered and fatal cases (D). all $P > .05$; *t* test. CAP = community-acquired pneumonia adults, PSI = Pneumonia Severity Index, CURB65 = confusion-ureic nitrogen-respiratory rate-blood pressure.

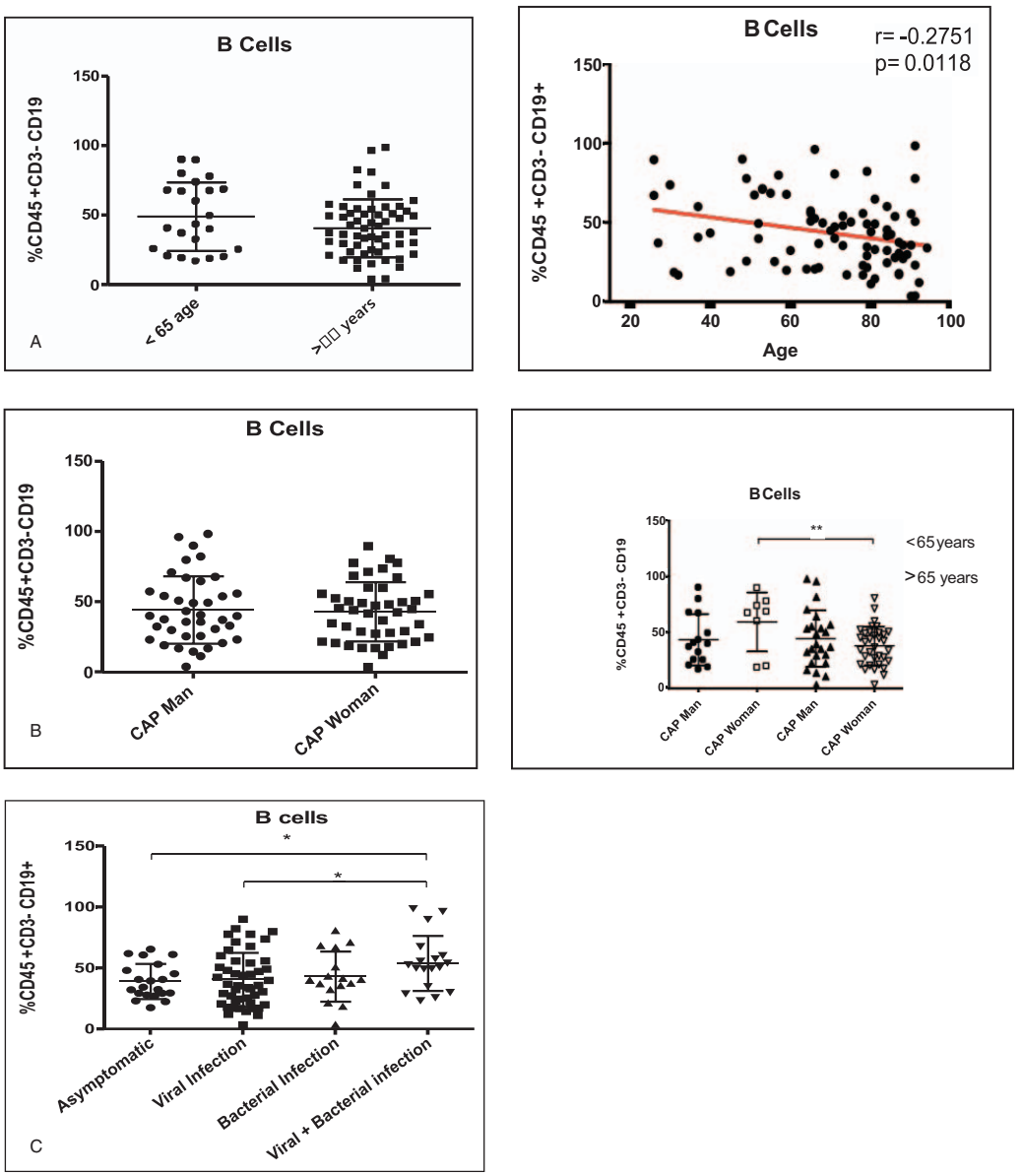

Figure 5. Proportion of B cells from adults with community-acquired pneumonia by flow cytometry according age (A), gender (B) and detected agent (C). A. Under and ≥65 years adults with CAP. $P = .1$, *t* test Relationship between age and B cells; Pearson coefficient *r*. B. 39 males vs 43 females adults with CAP $P > .05$, *t* test. Age and gender $^{**}P = .007$, *t* test. C. according to detected agent. $^{*}P = .03$, *t* test. CAP = community acquired pneumonia.

the increase of memory B cells, $CD27^{-}$ and immunoglobulins, and lower levels of IgM previously reported.[23,27,28] Probably the differences could be explained by the different techniques used.

The immunoglobulins levels were similar in cases with viruses, bacteria and mixed detections. The B cell proportion was higher in mixed cases than in viral or bacterial infections. The seric IgA was lower in 22 adults suffering influenza infection than in 45 with other viruses (median: 2.27 vs 3.01 mg/ml; IQR: 1.24–3.07 vs 2.00–4.34; Mann–Whitney test, $P = .01$), which is in agreement with previous reports.[29] Also IgG3 was significantly lower (0.41 vs 0.54 mg/ml; IQR: 0.27–0.58 vs 0.38–0.73; $P = .04$).

The use of systemic immunoglobulins quantification is a limitation in relations to local pulmonary determinations; however, it is assumed that the levels of serum samples reflex what is happening in the lower respiratory tract.[4] Also, without a following up it is not possible to know if the lower values found are permanent or transient, assuming that the last option is more probable, because cases with known immune deficiencies were not enrolled and normally recovered patients normalize their values.[6]

The low levels of the markers analyzed could be due to a higher use of them by the infectious process or by a limited production.[6] The analysis of B cells favors the first hypothesis because the proportions were similar among sick and asymptomatic cases, and although were lower in severe than mild cases, only the difference with PSI was significant. Furthermore, the similarities in the proportion of memory B cells among fatal and recovered cases do not argument in favor of an association

between these cells and the severity of the respiratory disease. Therefore these cells are not recommended as severity biomarkers in adults with CAP.[14,30] However, the subset diversity of B cells and their functions makes necessary to characterize the subpopulations in CAP to better understand their influence.

In this study, the counts of lymphocyte populations were evaluated in the acute phase of the disease for analyzing circulating lymphocytes post stimulation by the infectious agent of CAP and to evaluate its role as a biomarker of illness severity. It would be of interest to broaden the study to other components of the immune response – such as T lymphocytes – which is outside the scope of this paper.

In conclusion, since the level of serum IgA was significantly higher in fatal cases of CAP, it could be considered as a biomarker for severe prognosis. Decrease in B cells could contribute to the severity of the adult with CAP. xMAP Luminex technology is a good option to quantify serum immunoglobulins, which requires defining normal parameters for its wide clinical use.

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