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# Cytokine Activation Patterns and Biomarkers Are Influenced by Microorganisms in Community-Acquired Pneumonia

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***Background:*** **The inflammatory response in community-acquired pneumonia (CAP) depends on the host and on the challenge of the causal microorganism. Here, we analyze the patterns of inflammatory cytokines, procalcitonin (PCT), and C-reactive protein (CRP) in order to determine their diagnostic value.**

***Methods:*** **This was a prospective study of 658 patients admitted with CAP. PCT and CRP were analyzed by immunoluminometric and immunoturbidimetric assays. Cytokines (tumor necrosis factor-α [TNF-α], IL-1β, IL-6, IL-8, and IL-10) were measured using enzyme immunoassay.**

***Results:*** **The lowest medians of CRP, PCT, TNF-α, and IL-6 were found in CAP of unknown cause, and the highest were found in patients with positive blood cultures. Different cytokine profiles and biomarkers were found depending on cause: atypical bacteria (lower PCT and IL-6), viruses (lower PCT and higher IL-10), *Enterobacteriaceae* (higher IL-8), *Streptococcus pneumoniae* (high PCT), and *Legionella pneumophila* (higher CRP and TNF-α). PCT ≥ 0.36 mg/dL to predict positive blood cultures showed sensitivity of 85%, specificity of 42%, and negative predictive value (NPV) of 98%, whereas a cutoff of ≤ 0.5 mg/dL to predict viruses or atypicals vs bacteria showed sensitivity of 89%/81%, specificity of 68%/68%, positive predictive value of 12%/22%, and NPV of 99%/97%. In a multivariate Euclidean distance model, the lowest inflammatory expression was found in unknown cause and the highest was found in *L pneumophila*, *S pneumoniae*, and *Enterobacteriaceae*. Atypical bacteria exhibit an inflammatory pattern closer to that of viruses.**

***Conclusions:*** **Different inflammatory patterns elicited by different microorganisms may provide a useful tool for diagnosis. Recognizing these patterns provides additional information that may facilitate a broader understanding of host inflammatory response to microorganisms.**

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**Abbreviations:** CAP = community-acquired pneumonia; CRP = C-reactive protein; GNB = gram-negative bacilli; GPC = gram-positive cocci; NPV = negative predictive value; PCT = procalcitonin; TNF-α = tumor necrosis factor-α

The respiratory tract is constantly exposed to environmental agents and potentially pathogenic microorganisms. The ciliated epithelium, alveolar macrophages, and neutrophils are able to destroy and remove pathogenic agents and prevent the progression of tissue invasion.[1,2] When the innate response is overcome, local reactions, with activation of cytokines and inflammatory markers, promote a specific immune response against the microorganism.[2] This reaction is not limited to the lungs; there is also a systemic response that has repercussions on the course of the infection and its outcome.[1,3,4]

Community-acquired pneumonia (CAP) is the leading cause of mortality due to infection in developed countries.[5] The host inflammatory response is crucial to fighting the microorganism, and that interplay determines the outcome. Nevertheless, the mechanisms that trigger activation of the cytokine cascade and its different patterns (responsible for the outcome) are not sufficiently understood. An exuberant systemic activation of cytokines has been associated with a poorer outcome, although in some patients it is an adequate response, suggesting that this feature is far from understood.[6] Kellum et al[6] pointed out the

heterogeneous cytokine pattern activation with different combinations of high, medium, and low IL-6 and IL-10 levels, although they did not evaluate the influence of causal microorganisms.

Our hypothesis is that causal microorganisms play a key role in the host response and may trigger different inflammatory responses, depending on their intrinsic properties, the presence of a capsule, lipopolysaccharides in the cell wall, virulence factors, and infection spread.[1] Understanding the response of the host to the different pathogens is essential to increasing our knowledge of the course of infection in order to improve the diagnostic process and, possibly, for developing targeted therapeutic strategies.

Our objective was to investigate the cytokine systemic activation patterns (tumor necrosis factor-α [TNF-α], IL-1β, IL-6, IL-8, and IL-10) together with the biomarkers procalcitonin (PCT) and C-reactive protein (CRP) provoked by causal microorganisms in hospitalized patients with CAP. A secondary objective was to evaluate their usefulness in a causal-diagnosis approach. An abstract with some results has been published.[7]

## Materials and Methods

We performed a prospective study of hospitalized patients with CAP in two centers from October 2004 to September 2005. The inclusion criteria were a new radiologic infiltrate and at least two compatible clinical symptoms. The exclusion criteria were admission within the previous 15 days, immunosuppressive treatments, and being HIV positive. This study was approved by the ethics committee (Comité Ético de Investigación Clínica del Hospital Universitario y Politécnico La Fe, approval number 2004/69) and patients signed informed consents. Data recorded were age, sex, toxic habits, comorbidities, and prior antibiotic treatment for the same episode prior to admission.

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### *Cytokines, PCT, and CRP*

Blood samples were taken the morning after admission, and the serum was frozen at −80°C. Determination of IL-1β, IL-6, IL-8, and IL-10 and TNF-α was made using an enzyme immunoassay (Biosource). Limits of detection were 3 pg/mL for TNF-α, 2 pg/mL for IL-6, 0.7 pg/mL for IL-8, and 1 pg/mL for IL-10. PCT was measured using an immunoluminometric technique (Liason Brahms PCT) with a detection limit of 0.3 ng/mL and CRP using an immunoturbidimetric test (Bayer Diagnostics) with a detection limit of 1.5 mg/dL.

### *Microbiologic Analysis*

The following studies were carried out: (1) blood cultures (n = 575), (2) urinary antigens for *Legionella pneumophila* (n = 626) and *Streptococcus pneumoniae* (n = 628), (3) sputum Gram stain (n = 319) (< 10 epithelial cells and > 25 leukocytes per field × 100) and culture, (4) nasopharyngeal swab (n = 162) to detect viral nucleic acids, (5) paired serologic studies (n = 629) for *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, *Coxiella burnetii*, and *L pneumophila*, and (6) invasive samples (n = 92) obtained by bronchoscopy and/or pleural fluid.

### *Microbiologic Diagnostic Criteria*

Bacterial cause was established using the following criteria: (1) isolation of microorganisms in respiratory samples above the cutoffs (BAL ≥ $10^4$ colony-forming units/mL; bronchoaspirate sample ≥ $10^5$ colony-forming units/mL) or in pleural fluid, (2) isolation of one predominant microorganism in sputum or *L pneumophila* in buffered charcoal yeast extract agar, (3) microorganisms in blood culture, (4) positive urinary antigens, (5) seroconversion or fourfold antibody increase with titers of IgG ≥ 1:512 for *C pneumoniae*, ≥ 1:160 for *M pneumoniae* and *C burnetii*, or IgM ≥ 1:32 for *C pneumoniae* and ≥ 1:80 for *M pneumoniae* and *C burnetii*,[8] and (6) positive detection of viral nucleic acids: ProDetect BCS RV CHIP (bcs Biotech SpA) for influenza virus A and B (gen NS), respiratory syncytial virus (gen NS2), parainfluenza virus I, II, and III (gen HN), SARS coronavirus (fragment BNI-1), and adenovirus (gen H).

### *Statistical Analysis*

The statistical analysis was carried out using SPSS software (version 15.0; SPSS Inc). PCT, CRP, and cytokines were presented as medians and interquartile ranges, and parametric data as mean ± SD. The hypotheses were tested using the Mann-Whitney *U* test. Significance was established at $P < .05$.

The microorganisms were analyzed individually and according to the following groups: no cause, bacteria subdivided into gram-positive cocci (GPC) and gram-negative bacilli (GNB), viruses, and atypical pathogens (*C pneumoniae*, *M pneumoniae*, and *C burnetii*).

A multivariate Euclidean distance model was performed. The graphics were generated by means of a hierarchic cluster analysis and multidimensional scaling of the distance matrix based on the significant differences observed in the pair comparison of microorganisms using the Mann-Whitney *U* test.

## Results

Six hundred eighty-five patients were included, and in 295 (43%) a causal diagnosis was reached: 118 *S pneumoniae* (17.2%), 24 *L pneumophila* (3.5%), 18 *Pseudomonas aeruginosa* (2.6%), 14 *Haemophilus influenzae* (2%), 13 *Staphylococcus aureus* (1.9%),

**Table 1—*Demographic Data, Comorbidities, Fine Risk Classes, and Mortality Depending on Causal Microorganism***

| Characteristics | NE (n = 390) | GPC (n = 134) | GNB (n = 69) | ATP (n = 24) | VIR (n = 12) |
|---|---|---|---|---|---|
| Sex | | | | | |
| M | 252 (64.6) | 78 (58.2) | 54 (78.3) | 14 (58.3) | 9 (75) |
| F | 138 (35.4) | 56 (41.8) | 15 (21.7) | 10 (41.7) | 3 (25) |
| Mean age, y | 67.2 ± 17.2 | 66.6 ± 17.8 | 68.8 ± 13.9 | 53.9 ± 22.3 | 62.1 ± 16.9 |
| Diabetes | 77 (19.8) | 29 (21.8) | 9 (13) | 1 (4.2) | 2 (16.7) |
| Heart failure | 77 (19.8) | 21 (15.7) | 16 (23.5) | 3 (13) | 1 (8.3) |
| Chronic renal failure | 22 (5.6) | 5 (3.7) | 5 (7.2) | 1 (4.2) | 0 (0) |
| Digestive disease | 58 (14.9) | 32 (23.9) | 17 (24.6) | 5 (20.8) | 1 (8.3) |
| Cirrhosis | 11 (2.8) | 5 (3.7) | 3 (4.3) | 1 (4.2) | 1 (8.3) |
| COPD | 80 (20.5) | 20 (14.9) | 22 (31.9) | 3 (12.5) | 0 (0) |
| Neurologic disease | 87 (22.4) | 28 (21.1) | 17 (24.6) | 1 (4.2) | 1 (8.3) |
| Smoking | 75 (19.3) | 36 (27.1) | 26 (37.7) | 9 (37.5) | 5 (41.7) |
| Alcohol consumption | 27 (7) | 17 (12.8) | 9 (13) | 2 (8.3) | 1 (8.3) |
| Fine I-III | 194 (49.7) | 70 (52.2) | 27 (39.1) | 17 (70.8) | 11 (91.7) |
| Fine IV-V | 196 (50.3) | 64 (47.8) | 42 (60.9) | 7 (29.2) | 1 (8.3) |
| No sepsis | 123 (31,5) | 38 (28,4) | 16 (23,2) | 11 (45,8) | 5 (41,7) |
| Sepsis | 132 (33.8) | 30 (22.4) | 20 (29.0) | 8 (33.3) | 4 (33.3) |
| Severe sepsis | 129 (33.1) | 59 (44.0) | 27 (39.1) | 5 (20.8) | 3 (25.0) |
| Septic shock | 6 (1,5) | 7 (5,2) | 6 (8,7) | 0 (0) | 0 (0) |
| Hipoxemia | 151 (38.7) | 66 (49.3) | 35 (50.7) | 1 (4.2) | 5 (41.7) |
| Mechanical ventilation | 7 (1.8) | 8 (6.0) | 6 (8.7) | 0 (0) | 0 (0) |
| Death | 19 (4.9) | 6 (4.5) | 10 (14.5) | 0 (0) | 0 (0) |

Data are presented as No. (%) or mean ± SD. ATP = atypical pathogen (*Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and *Coxiella burnetii*); F = female; GNB = gram-negative bacilli; GPC = gram-positive cocci; M = male; NE = no cause; VIR = viruses.

13 *M pneumoniae* (1.9%), 13 *Enterobacteriaceae* (1.9%), 12 viruses (1.8%) (nine influenza virus), eight *C burnetii* (1.2%), three *C pneumoniae* (0.4%), and 35 mixed infections (5%). Causal groups were 390 no cause (56.9%), 134 GPC (19.6%), 69 GNB (10.1%), 24 atypical pathogens (3.5%), and 12 viruses(1.8%) General characteristics, Fine risk classes, and mortality depending on cause are depicted in Table 1.

Bacteremia was found in 48 cases (7%): 36 *S pneumoniae*, seven *Enterobacteriaceae*, three *H influenzae*, four *S aureus*, three *P aeruginosa*, one *Streptococcus pyogenes* and one *Acinetobacter baumannii*. Six of the cases were mixed infections due to several bacteria, principally *S pneumoniae*.

### *CRP and PCT*

Patients with a causal diagnosis showed higher CRP and PCT in comparison with those without, and the highest levels were found in those with bacteremia (Table 2). The diagnostic value of PCT (≥ 0.36) to predict bacteremia was as follows: 85% sensitivity, 42% specificity (E), and 98% negative predictive value (NPV).

The medians of CRP, PCT, and cytokines depending on microorganisms are shown in Table 3, and those depending on sepsis status and on hypoxemia or mechanical ventilation are shown in Tables 4 and 5, respectively. The highest levels of CRP and PCT

**Table 2—*Results of CRP, PCT, and Cytokines According to Causal Microorganism and Bacteremia***

| | Cause | | | Bacteremia | | |
|---|---|---|---|---|---|---|
| Biomarkers | No (n = 390) | Yes (n = 295) | *P* Value | No (n = 527) | Yes (n = 48) | *P* Value |
| CRP, mg/dL | 13.7 (6.95-21.85) | 18.1 (9.7-27.3) | < .0001 | 16.1 (8.8-24.1) | 23.3 (14.9-35.1) | .002[a] |
| PCT, ng/mL | 0.37 (0.15-1.56) | 0.86 (0.27-4.12) | < .00001 | 0.51 (0.18-2.24) | 4.54 (0.49-11.16) | < .00001[b] |
| TNF-α, pg/mL | 25 (15-41) | 27 (15-48) | NS | 26 (15-45) | 30.5 (18-67) | .04[c] |
| IL-1, pg/mL | 15 (3-33) | 16 (4-32) | NS | 16 (4-33) | 16.5 (3-33) | NS |
| IL-6, pg/mL | 71 (25-175) | 105 (42-300) | < .0001 | 95 (34-235) | 192 (79.5-568.5) | .004[d] |
| IL-10, pg/mL | 5 (0-15) | 6 (0-19) | NS | 5 (0-17) | 12 (1-38.5) | NS |
| IL-8, pg/mL | 8 (2-17) | 9 (3-22) | NS | 10 (3-20) | 5 (2-30.5) | NS |

Data are presented as median (interquartile range). AUC = area under the curve; CRP = C-reactive protein; NS = not significant; PCT = procalcitonin; TNF-α = tumor necrosis factor-α.

[a]AUC, 0.7 (0.6-0.8); *P* < .001.

[b]AUC, 0.7 (0.6-0.8); *P* < .0001.

[c]AUC, 0.6 (0.5-0.7); *P* = .03.

[d]AUC, 0.7 (0.6-0.7); *P* < .001.

were found in CAP caused by *L pneumophila*, *Enterobacteriaceae*, and *S pneumoniae*. PCT was also higher in *S aureus*. CRP and PCT were higher in sepsis in those with GPC and GNB compared with those with unknown cause, and, after stratifying for hypoxemia or mechanical ventilation, the medians were higher in GNB. The differences among causal groups and between *S pneumoniae* and *L pneumophila* are shown in Table 6.

### *Cytokines*

Patients with a causal diagnosis had a higher IL-6 than those without, whereas those with bacteremia showed the highest IL-6 and TNF-α (Table 2). Causal microorganisms exhibited different cytokine patterns (Table 3): *L pneumophila* and *S aureus* had higher TNF-α, and the former also had higher IL-6; *Enterobacteriaceae* had higher IL-8, whereas influenza virus infections showed higher IL-10 (compared with bacteria, $P = .03$) and lower TNF-α. Concerning sepsis status (Table 4), IL-6 was higher in severe sepsis in those with GPC and GNB compared with those with unknown cause. In patients stratified by hypoxemia and mechanical ventilation, those with GPC and GNB had higher IL-6 (and IL-8 in GNB) compared with those with unknown cause (Table 5).

### *Prior Antibiotics and Inflammatory Markers*

Two hundred thirty-three patients (34%) had received antibiotics prior to admission and they had lower PCT ($P < .01$), IL-6 ($P < .03$), and IL-10 ($P < .01$), and higher IL-8 ($P < .05$) (Table 7). PCT was higher in those with known cause who did not take prior antibiotics compared with those who did ($P = .02$).

### *Multivariate Euclidean Distance Model*

Statistical differences among cytokines and biomarkers in microorganisms converted into distances in a Euclidean two-dimensional space are depicted in Figure 1. We found three microorganisms with higher distances: *L pneumophila*, *S pneumoniae*, and *Enterobacteriaceae*; a close group of bacteria (*S aureus*, *M pneumoniae*, *C pneumoniae*, *P aeruginosa*, *C burnetii*, and *H influenzae*, together with influenza virus); and a third group close to this last group, unknown-cause CAP. Hierarchic cluster analysis is also shown with microorganisms associated in similar cluster groups.

## Discussion

The most outstanding findings of this study were as follows: (1) The highest levels of cytokines, CRP, and PCT were found in patients who were bacteremic

**Table 3—*Results of CRP, PCT, and Cytokines According to Causal Microorganisms***

| Causal Microorganism | CRP, mg/dL | PCT, ng/mL | TNF-α, pg/mL | IL-1, pg/mL | IL-6, pg/mL | IL-10, pg/mL | IL-8, pg/mL |
|---|---|---|---|---|---|---|---|
| Unknown cause (n = 390) | 13.7 (6.95-21.9) | 0.37 (0.15-1.56) | 25 (15-41) | 15 (3-33) | 71 (25-175) | 5 (0-15) | 8 (2-17) |
| Gram positive | | | | | | | |
| *Streptococcus pneumoniae* (n = 118) | 19.85 (10.3-28.4) | 1.71 (0.48-7.37) | 27 (16-47) | 16 (4-30) | 144 (38-305) | 7 (0-21) | 6 (2-19) |
| *Staphylococcus aureus* (n = 13) | 16.4 (5.6-24.8) | 1.37 (0.3-7.86) | 40.5 (22-48) | 22 (0-47.5) | 125 (63-204.5) | 7.5 (0-32.5) | 10 (3.5-16.5) |
| Gram negative | | | | | | | |
| *Legionella pneumophila* (n = 24) | 24.9 (21.3-33.5) | 0.71 (0.5-3.15) | 49 (40-72) | 19 (5-35) | 202 (69-1548) | 3 (0-12) | 16 (11-35) |
| *Haemophilus influenzae* (n = 14) | 12.5 (2.7-17.4) | 0.36 (0.215-1.37) | 19.5 (11-33.5) | 17.5 (0-37) | 63.5 (6-155) | 7.5 (6-35.5) | 5 (1-9) |
| *Pseudomonas aeruginosa* (n = 18) | 10 (7.4-13.8) | 0.44 (0.14-0.62) | 23 (15-42) | 16 (4-29) | 105 (22-223) | 6 (3-12) | 12 (8-20) |
| *Enterobacteriaceae* (n = 13) | 20.1 (12.6-31.5) | 1.59 (0.56-8.99) | 14.5 (11.5-68) | 20 (8.5-41) | 168.5 (58-339.5) | 6 (0-25.5) | 54.5 (13.5-79.5) |
| ATP | | | | | | | |
| *Mycoplasma pneumoniae* (n = 13) | 13.9 (7.6-24.6) | 0.34 (0.1-0.62) | 27.5 (15-128) | 23 (4-54) | 77 (26-98) | 4 (0-14) | 17.5 (7-24) |
| *Chlamydophila pneumoniae* (n = 3) | 19 (0.2-24.3) | 0.23 (0.1-0.36) | 36 (23-39) | 31 (22-35) | 26 (8-59) | 17 (10-23) | 1 (0-12) |
| *Coxiella burnetii* (n = 8) | 9.45 (5.5-25.3) | 0.13 (0.09-0.19) | 22 (21-38) | 17 (6.5-30.5) | 41.5 (25-122.5) | 2.5 (0.5-13) | 17 (8.5-24) |
| VIR | | | | | | | |
| Influenza virus (n = 9) | 15.4 (12-21.6) | 0.36 (0.09-0.47) | 11.5 (8-22) | 10 (2-18) | 129 (39-405) | 24.5 (18-33) | 7 (3-16) |

Data are presented as median (interquartile range). See Table 1 and 2 legends for expansion of abbreviations.

**Table 4—*Biomarkers and Cytokines According to Causal Microorganisms and Sepsis Status***

| Biomarkers and Sepsis Status | NE | GPC | GNB | ATP | VIR | *P* Value |
|---|---|---|---|---|---|---|
| CRP | | | | | | |
| No sepsis | 13.5 (6-20) | 17.6 (5.1-30.2) | 13.8 (9.8-23.7) | 12.7 (7.6-19.1) | 4.9 (0.7-12.9) | NS |
| Sepsis | 12 (6.5-22.7) | 20.3 (12.2-28.2) | 18.5 (12.6-24.9) | 13.8 (7.5-24.3) | 12 (8.9-29.5) | < .05 |
| Severe sepsis/shock | 15.5 (8.4-23.4) | 20 (11.1-28.1) | 23.5 (10-32.8) | 11.3 (4-33.6) | 17.8 (14-21.6) | NS |
| PCT | | | | | | |
| No sepsis | 0.2 (0.1-0.6) | 0.7 (0.2-4.5) | 0.3 (0.1-1.1) | 0.2 (0.1-0.3) | 0.3 (0.2-0.4) | .004 |
| Sepsis | 0.3 (0.1-1.2) | 1.9 (0.7-4.1) | 0.6 (0.4-2.8) | 0.2 (0.1-0.5) | 0.1 (0.1-0.5) | < .0001 |
| Severe sepsis/shock | 0.8 (0.3-2.7) | 2.4 (0.8-9.1) | 0.7 (0.4-6) | 0.2 (0.2-0.6) | 1.2 (0.2-2.2) | .2 |
| TNF-α | | | | | | |
| No sepsis | 23 (13-41) | 35 (18-48) | 20 (11-58) | 23 (21-35) | 26.5 (18-35.5) | NS |
| Sepsis | 24 (14-34.5) | 29 (18-43) | 37 (13-52) | 26.5 (21-39) | 8 (8-13) | NS |
| Severe sepsis/shock | 29 (18-45) | 26 (16-48) | 37 (17-72) | 40 (36-128) | 18.5 (10-27) | NS |
| IL-1 | | | | | | |
| No sepsis | 10 (3-27) | 14 (3-34) | 12 (4-46) | 33 (5-50) | 14 (9-18) | NS |
| Sepsis | 17 (4-35) | 6.5 (2-19) | 22 (3-29) | 15.5 (4-26) | 2 (0-7) | NS |
| Severe sepsis/shock | 17 (2-37) | 18 (5-35) | 17.5 (6-36) | 22 (9-24) | 15.5 (13-18) | NS |
| IL- 6 | | | | | | |
| No sepsis | 66 (23-146) | 97 (29-225) | 71 (22-144) | 27.5 (17-77) | 54.5 (21-137.5) | NS |
| Sepsis | 65.5 (24.5-165.5) | 185 (92-380) | 79 (22-324) | 49.5 (39-79) | 39 (30-1549) | .005 |
| Severe sepsis/shock | 93 (30-273) | 150 (47-253) | 197 (69-1.288) | 166 (77-460) | 235.5 (66-405) | NS |
| IL-10 | | | | | | |
| No sepsis | 3 (0-9) | 2 (0-16) | 6 (0-9) | 7.5 (1-14) | 12 (1-28) | NS |
| Sepsis | 5 (0-12) | 2.5 (0-14) | 4 (0-15) | 1 (0-15) | 18 (0-27) | NS |
| Severe sepsis/shock | 8.5 (0-22) | 13 (1-31) | 6.5 (0-19) | 9 (3-14) | 29 (22-36) | NS |
| IL- 8 | | | | | | |
| No sepsis | 10 (4-18) | 10 (5-22) | 16 (12-35) | 16.5 (10-23) | 2 (0-4) | .006 |
| Sepsis | 7 (2-15) | 7 (3-18) | 10 (4-34) | 11 (5-19) | 5 (3-16) | NS |
| Severe sepsis/shock | 6 (2-19) | 5 (2-16) | 14 (5-61) | 13 (1-27) | 24 (9-39) | NS |

Data are presented as median (interquartile range). See Table 1 and 2 legends for expansion of abbreviations.

and the lowest in those with unknown cause; (2) the causal microorganisms elicited different inflammatory cytokine patterns and biomarkers as corroborated in the Euclidean distances model; (3) a cutoff of PCT ≤ 0.5 to differentiate viruses or atypicals vs bacteria showed sensitivity of 89%/81%, specificity of 68%/68%, positive predictive value of 12%/22%, and NPV of 99%/97%; (4) PCT ≥ 0.36 had an excellent NPV (98%) for predicting positive blood cultures; and (5) *L pneumophila* CAP showed higher IL-8 and TNF-α compared with *S pneumonia,* with high NPV (89% and 94%, respectively).

The study of the inflammatory profile in CAP may provide better knowledge of the host-microorganism interplay and may be useful for causal diagnosis. PCT, CRP, and, to a lesser extent, cytokines were studied for their diagnostic ability in CAP.[3,8-11] Interestingly, biomarkers and IL-6 were significantly lower when causal microorganisms were not found, even considering sepsis status, reflecting a lower microorganism load or virulence. On the other hand, in bacteremia, biomarkers and IL-6 were significantly higher, reflecting the greater dissemination of the infection,[12] which may be useful for selecting that specific CAP population.[8,13] Müller et al[13] found that PCT ≤ 0.25 ng/mL allowed a 37% reduction of blood cultures with high specificity. We chose a slightly higher cutoff (0.36 ng/mL) with very high NPV because we considered that a lower cutoff would include many atypicals and undiagnosed CAP. The role of cytokines in predicting bacteremia is less well known. We found that IL-6 ≥ 150 had an excellent NPV (96%).

Despite the interest in knowing the influence of microorganisms on triggering different inflammatory patterns, publications on the subject are few. Masiá et al[14] found lower PCT and CRP in atypicals compared with bacteria, although neither of them was useful for predicting that cause. Hedlund and Hansson[3] also reported lower PCT in atypical bacteria and/or viruses, with no differences in CRP. Our data confirm that PCT ≤ 0.5 provides high sensitivity and NPV for viral and/or atypical causes. Krüger et al[15] used a lower PCT cutoff (≤ 0.1) to differentiate *S pneumoniae* from atypical or viral causes and reported a high OR of 8.3. Nevertheless, the considerable overlap in levels among microorganisms should lead us to be cautious, to avoid prescribing insufficient antibiotics.

The usefulness of CRP and PCT in distinguishing cause within the bacteria group is less clear. However, García Vázquez et al[8] found that CRP (> 25 mg/dL) may be useful in diagnosing *L pneumophila* with high

**Table 5—*Biomarkers and Cytokines According to Hypoxemia and MV***

| Biomarkers | NE | GPC | GNB | ATP | VIR | *P* Value |
|---|---|---|---|---|---|---|
| CRP | | | | | | |
| Hypoxemia | | | | | | |
| Yes | 16 (8.2-27.8) | 21.3 (10.8-28.9) | 21.3 (8.8-31.8) | 19 (19-19) | 14 (8.9-21.6) | NS |
| No | 11.7 (6.4-19.8) | 18.8 (9.2-27.3) | 17.3 (11.3-24.4) | 10.7 (7.5-24.5) | 10.5 (0.8-16.8) | .002 |
| MV | | | | | | |
| Yes | 15.8 (8.4-27) | 17.6 (4.9-39.9) | 26.5 (11-34.2) | … | … | NS |
| No | 13.7 (6.9-21.8) | 19.9 (10.2-28.3) | 17.8 (10.2-28) | 11.3 (7.5-24.3) | 12 (8.9-16.8) | .001 |
| PCT | | | | | | |
| Hypoxemia | | | | | | |
| Yes | 0.5 (0.2-2.7) | 1.8 (0.4-7.4) | 0.6 (0.4-3.6) | 0.4 (0.4-0.4) | 0.3 (0.2-2.2) | NS |
| No | 0.3 (0.1-0.9) | 1.6 (0.5-6.5) | 0.6 (0.3-2.8) | 0.2 (0.1-0.4) | 0.2 (0.1-0.5) | < .00001 |
| MV | | | | | | |
| Yes | 2.3 (0.8-7) | 1.2 (0.3-12.9) | 6.6 (0.9-38.1) | … | … | NS |
| No | 0.4 (0.1-1.4) | 1.7 (0.5-7.1) | 0.5 (0.3-2.3) | 0.2 (0.1-0.4) | 0.2 (0.2-0.5) | < .00001 |
| TNF-α | | | | | | |
| Hypoxemia | | | | | | |
| Yes | 27 (16-44) | 28.5 (15-45) | 37 (16.5-71) | 23 (23-23) | 27 (10-31) | NS |
| No | 25 (14-40) | 28 (19-48.5) | 26 (13-52) | 27.5 (21-39.5) | 13.5 (8-22) | NS |
| MV | | | | | | |
| Yes | 29 (16-33) | 44 (17-48) | 27 (6-166) | … | … | NS |
| No | 25 (15-41) | 28 (16-47) | 33 (15.5-56.5) | 25 (21-39) | 14 (10-27) | NS |
| IL-1 | | | | | | |
| Hypoxemia | | | | | | |
| Yes | 16 (3-36) | 16 (3-35) | 13.5 (4-35.5) | 31 (31-31) | 14 (13-18) | NS |
| No | 14 (3-29.5) | 14.5 (4-28) | 21.5 (6.5-38) | 23 (4.5-35) | 5.5 (2-14) | NS |
| MV | | | | | | |
| Yes | 7 (0-15) | 5 (0-16) | 36 (13-93) | … | … | NS |
| No | 15 (3-33) | 15 (4-30) | 18 (4-29) | 24 (5-35) | 13 (4-14) | NS |
| IL-6 | | | | | | |
| Hypoxemia | | | | | | |
| Yes | 95 (23-246) | 133.5 (42-284) | 197 (69.5-931.5) | 26 (26-26) | 66 (16-405) | NS |
| No | 65 (25.5-154.5) | 159.5 (59.5-302.5) | 72 (24.5-211.5) | 51 (24.5-92.5) | 61 (30-192) | .02 |
| MV | | | | | | |
| Yes | 34 (30-143) | 79 (71-644) | 380 (70-2.075) | … | … | NS |
| No | 72 (25-175) | 151 (47-284) | 105 (41-324) | 43 (26-87) | 66 (30-192) | .002 |
| IL-10 | | | | | | |
| Hypoxemia | | | | | | |
| Yes | 6 (0-18) | 10 (0-28) | 7 (2.5-17.5) | 10 (10-10) | 23 (22-36) | NS |
| No | 4 (0-12) | 5.5 (0-18.5) | 5 (0-9.5) | 4 (0.5-14.5) | 9.5 (1-27) | NS |
| MV | | | | | | |
| Yes | 25 (5-59) | 13 (1-35) | 20 (0-273) | … | … | NS |
| No | 5 (0-14) | 6 (0-21) | 6 (0-12) | 5 (1-14) | 22 (1-27) | NS |
| IL- 8 | | | | | | |
| Hypoxemia | | | | | | |
| Yes | 8 (2-18) | 6.5 (2-18) | 19.5 (11-70) | 12 (12-12) | 9 (0-39) | .005 |
| No | 9 (3-17) | 6.5 (4-19) | 11 (4.5-24) | 14 (5-23.5) | 4 (3-5) | NS |
| MV | | | | | | |
| Yes | 13.5 (2-49) | 10 (6-86) | 64 (0-70) | … | … | NS |
| No | 8 (2-17) | 6 (2-18) | 13 (6-34) | 13 (5-23) | 4 (3-9) | .007 |

Data are presented as median (interquartile range). MV = mechanical ventilation. See Table 1 and 2 legends for expansion of other abbreviations.

NPV (94%). We found that higher levels of TNF-α and IL-6 in *L pneumophila* had a high NPV compared with *S pneumoniae*. PCT and IL-8 showed different patterns among bacteria: higher IL-8 and lower PCT in gram-negative vs gram-positive germs. However, the clinical relevance of these findings has yet to be demonstrated, and it is possible that, for diagnostic purposes, several markers will be required.[16]

Prior use of antibiotics reduced the levels of PCT and cytokines, mainly IL-6 and IL-10, suggesting that the inflammatory phase was beginning to be downregulated.[11,17] We consider that PCT-guided antibiotic prescription in CAP requires extreme caution in patients with prior antibiotic treatment because it could underestimate bacterial cause,[9,10] and even in those without prior antibiotics it would appear that a low PCT has insufficient NPV to exclude pathogens.

**Table 6—*Comparison of Main Groups and Microorganisms, and Diagnostic Cutoff Values***

| Biomarkers | Bacteria vs ATP | Bacteria vs VIR | Gram Negative vs Gram Positive | Gram Negative vs ATP | *L pneumophila* vs *S pneumoniae* |
|---|---|---|---|---|---|
| CRP | NS | NS | NS | NS | 24.9 vs 19.9[a] *P* = .01 |
| PCT | 1.12 vs 0.19[b] *P* < .0001 | 1.12 vs 0.2[c] *P* = .003 | 0.62 vs 1.67 *P* = .02 | 0.62 vs 0.19 *P* = .002 | NS |
| TNF-α | NS | 29 vs 14[d] *P* = .03 | NS | NS | 49 vs 27[e] *P* = .0002 |
| IL-6 | 144 vs 43[f] *P* = .01 | NS | NS | 116 vs 43 *P* = .04 | NS |
| IL-8 | NS | NS | 13 vs 6.5 *P* = .003 | NS | 16 vs 6[g] *P* = .003 |

E = specificity; NPV = negative predictive value; PPV = positive predictive value; S = sensitivity. See Table 1-3 legends for expansion of other abbreviations.

[a]CRP ≥ 22 mg/dL as cutoff to compare *L pneumophila* and *S pneumoniae*: S, 70%; E, 59%; PPV, 27%; and NPV, 90%. AUC, 0.7 (0.6-0.8); *P* = .01.

[b]PCT ≤ 0.5 mg/dL as cutoff to compare bacteria and atypical: S, 81%; E, 68%; PPV, 22%; and NPV, 97%. AUC, 0.8 (0.6-0.9); *P* < .0001.

[c]PCT ≤ 0.5 mg/dL as cutoff to compare bacteria and virus: S, 89%; E, 68%; PPV, 12%; and NPV, 99%. AUC, 0.8 (0.7-0.9); *P* < .01.

[d]TNF-α ≤ 27 pg/mL as cutoff to compare virus and bacterial: S, 78%; E, 51%; PPV, 7%; and NPV, 98%. AUC, 0.7 (0.6-0.9); *P* = .03.

[e]TNF-α ≥ 30 pg/mL as cutoff to compare *L pneumophila* and *S pneumoniae*: S, 88%; E, 57%; PPV, 39%; and NPV, 94%. AUC, 0.7 (0.6-0.8); *P* = .001.

[f]IL-6 ≤ 100 pg/mL as cutoff to compare bacteria and atypical: S, 81%; E, 56%; PPV, 17%; and NPV, 96%. AUC, 0.7 (0.6-0,8); *P* = .01.

[g]IL-8 ≥ 15 pg/mL as cutoff to compare *L pneumophila* and *S pneumoniae*: S, 57%; E, 69%; PPV, 27%; and NPV, 89%. AUC, 0.7 (0.6-0.8); *P* = .004.

On the other hand, IL-8 was raised, as reported in in vitro experiments,[18] probably because of enhanced cytokine secretion secondary to bacterial wall destruction. In fact, it was higher in those previously treated with β-lactams (12 vs 8.5, *P* = .06; data not shown).

Our findings highlight the differences in inflammatory cytokine activation, which were corroborated in the Euclidean distances model. The scenario with the least inflammation was found in unknown cause, whereas the greatest inflammation, though with specific expressive patterns, was found in *L pneumophila*, *S pneumoniae*, and *Enterobacteriaceae*. These distances reflected differences in the inflammatory profiles, probably due to variations in virulence and the recognition of different molecular patterns that activate different pathways and innate immunity, such as Toll-like receptor-9 in the case of *L pneumophila*, Toll-like receptor-4 in gram-negative bacteria, and Toll-like receptor-2 in gram-positive bacteria.[19-21] *Enterobacteriaceae* showed an increase in IL-8,[22] as reported in urinary infections.[23] Surprisingly, *P aeruginosa* presented an inflammatory response closer to that in CAP of unknown cause. This colonizing microorganism, which is associated with elderly patients or severe diseases, may take on importance depending on the characteristics of the patient; furthermore, it was associated with the use of oral or inhaled corticosteroids (nine patients). Influenza virus was associated with higher IL-10[24] and a lower TNF-α. This response profile plays a detrimental role in the host responses against the influenza A virus, as found in animal models.[25] In fact, although IL-10 activates the

**Table 7—*Effect of Antibiotic Treatment on Cytokines and Biologic Markers***

| | Prior Antibiotic Treatment | | | | | |
|---|---|---|---|---|---|---|
| | No | | | Yes | | |
| | Causal Diagnosis | | | Causal Diagnosis | | |
| Biomarkers | No (n = 256) | Yes (n = 196) | *P* Value | No (n = 134) | Yes (n = 99) | *P* Value |
| CRP, mg/dL | 13.5 (7-21.6) | 19.1 (10-28.3) | < .0001 | 15.3 (6.4-22,7) | 17 (8.7-26) | NS |
| PCT, ng/mL | 0.41 (0.16-1.86) | 1.11 (0.3-5.28) | < .0001 | 0.32 (0.14-0.9) | 0.54 (0.2-2.28) | .017 |
| TNF-α, pg/mL | 26 (16-40) | 29 (15-49) | NS | 23 (15-44) | 24 (14-44) | NS |
| IL-1, pg/mL | 16 (3-34) | 15 (4-30) | NS | 13.5 (4-31) | 19 (3-35) | NS |
| IL-6, pg/mL | 81 (25-197) | 122 (43-352) | < .001 | 60 (24-149) | 79 (29-235) | .036 |
| IL-10, pg/mL | 6 (0-19) | 8 (0-23) | NS | 3 (0-9) | 3 (0-13) | NS |
| IL-8, pg/mL | 7 (2-16) | 6.5 (2-21) | NS | 10 (5-19) | 12 (5-24) | NS |

Data are presented as median (interquartile range). See Table 2 legend for expansion of abbreviations.

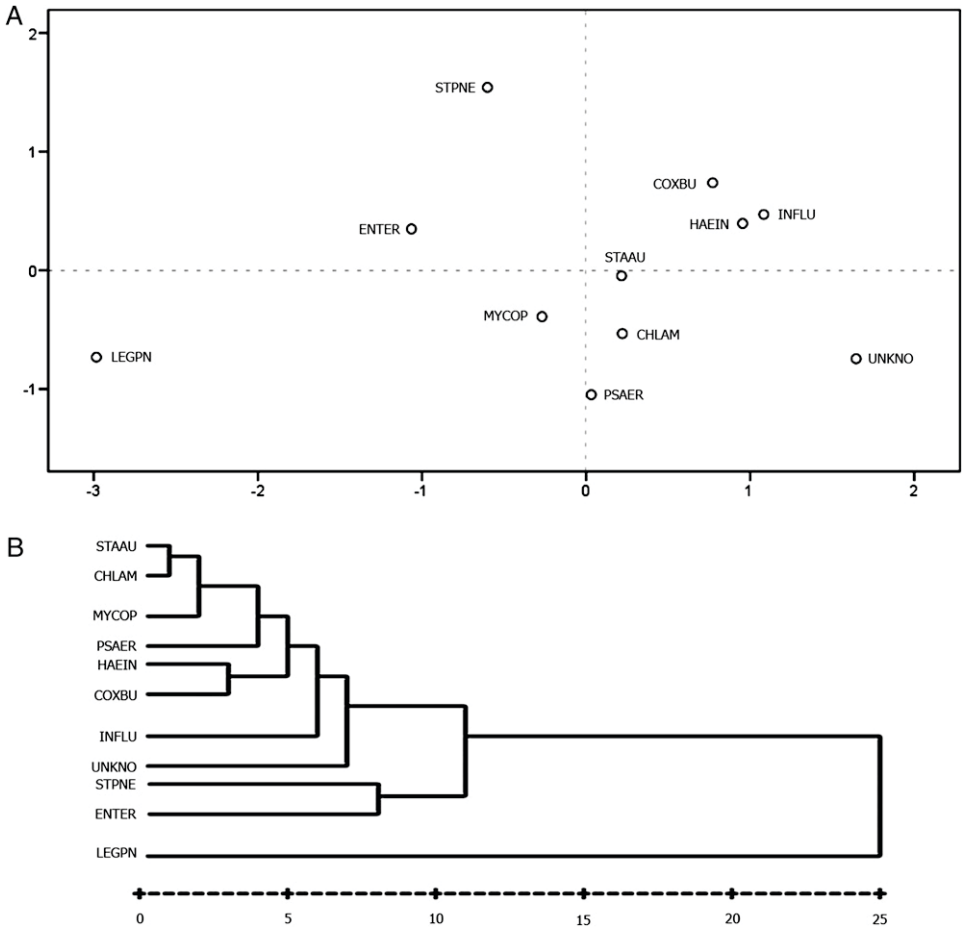


FIGURE 1. A, Multidimensional scaling in two dimensions that provides a visualization of similarities or dissimilarities among cytokine and markers and cause. B, Hierarchic cluster analysis for assigning microorganisms into cluster groups that are more similar to one or another in other clusters. CHLAM = chlamydophila; COXBU = *Coxiella burnetii*; ENTER = *Enterobacteriaceae*; HAEIN = *Haemophilus influenzae*; INFLU = influenza virus; LEGPN = *Legionella pneumophila*; MYCOP = *Mycoplasma pneumoniae*; PSAER = *Pseudomonas aeruginosa*; STAAU = *Staphylococcus aureus*; STPNE = *Streptococcus pneumoniae*; UNKNO = unknown cause.

natural killer lymphocytes and increases the antigen volume available to stimulate the immune system,[26] it has been shown that an increase of IL-6 and TNF-α protects against influenza virus pneumonia.[27]

Our findings suggest that interpretation of cytokine cascade activation should take into account not only host characteristics and sepsis status, but also the causal microorganism, bacteremia, and prior antibiotic treatment. Further studies of treatments designed to modulate the cytokine response should be designed to consider microorganism-specific inflammatory patterns. Prior failures of anticytokine treatments may be partially explained by this difference in cytokine patterns.

### *Limitations*

Not all microbiologic studies were performed on the whole population and unknown cause may correspond to underdiagnosed viruses or bacteria. Blood cultures were not obtained from patients at the same time intervals within the initial 24 h. Lower limits of PCT assay could be inadequate in milder CAP.

## CONCLUSIONS

In conclusion, the main causal agents of CAP presented different inflammatory patterns, which gave each group of microorganisms a specific profile, although their usefulness in diagnosis was limited. In bacteremia, inflammation was upregulated, whereas it was lowest in CAP of unknown cause. The most notable finding is that the knowledge of specific inflammatory patterns should enable us to better understand the host response in CAP. Further studies are needed to understand the mechanisms that lead each microorganism to present its own inflammatory response and to better define this response.

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