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# Acute Inflammatory Response of Patients with *Pseudomonas aeruginosa* Infections: A Prospective Study

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The severity of *Pseudomonas aeruginosa* (PA) infection may be determined by the interaction with the host immune system. We designed a prospective study to assess the relationship between the inflammatory response and the clinical presentation and outcome of PA infection. We also investigated whether there are differences in the inflammatory response depending on the resistance profile of PA. Interleukin-6 (IL-6), IL-10, procalcitonin (PCT), and C-reactive protein (CRP) were measured. Sixty-nine infection episodes were recorded; 40 caused by non-multidrug-resistant (non-MDR) strains [29 (73%) respiratory; 8 (20%) bacteremia], 12 by MDR non-extensively drug-resistant (MDR-non-XDR) [9 (75%) respiratory; 3 (25%) bacteremia], and 17 by XDR strains [9 (53%) respiratory; 7 (41%) bacteremia]. All inflammatory parameters were significantly higher in patients who developed acute organ dysfunction and bacteremia. PCT levels were higher in patients with early mortality [$p = 0.050$]. Inflammatory biomarkers were higher in patients with XDR than in those with non-MDR PA [IL-6 430 (67–951) vs. 77 (34–216), $p = 0.02$; IL-10 3.3 (1.5–16.3) vs. 1.3 (0–3.9), $p = 0.02$; and PCT 1.1 (0.6–5.2) vs. 0.3 (0.1–1.0), $p = 0.008$]. The intensity of inflammatory response was associated with the severity of PA infection, particularly if bacteremia occurred. Only PCT was documented useful to predict the outcome. XDR infections presented a higher inflammatory response; related in part to the larger number of bloodstream infections in this group.

**Keywords:** *Pseudomonas aeruginosa*, microbial drug resistance, nosocomial infections, infections

## Introduction

*Pseudomonas aeruginosa* (PA) is a common and serious cause of nosocomial infections. Mortality due to PA bacteremia may be as high as 50% within the first 24–72 hr.[1,2] Poor outcomes in PA infections have been associated with factors related to the antibiotic treatment, microorganism, and host. The presence of multidrug-resistant (MDR) strains reduces treatment options and raises the risk of receiving an inadequate initial antimicrobial treatment. Nonetheless, previous studies by our group found that early mortality was higher in non-MDR than MDR PA infections, suggesting that non-MDR PA infections have a more virulent behavior.[1–3] It is known that the severity of acute illness presentation is associated with a higher mortality,[4,5] and probably differences in the presentation of acute illness is determined not only by the production of PA virulence factors, but also by the interaction between the bacteria and the host innate immune system.

The early phase of sepsis is dominated by a hyperinflammatory state mediated by a systemic production of cytokines, as interleukin 6 (IL-6), which are required for adequate host defense against infection. Occurring almost simultaneously, the production of anti-inflammatory cytokines (IL-10),

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reduces local and systemic toxicity, and serves to balance the inflammatory state. However, an excessive inflammatory response may progress to sepsis and, ultimately, multiple organ dysfunction syndrome; on the other hand, an excessive anti-inflammatory response may hamper the resolution of the infection.[6,7] Several researchers have shown that the levels of both pro- and anti-inflammatory cytokines may be correlated with illness severity and that unbalanced inflammatory response is a strong predictor of mortality.[8–10] In addition, other biomarkers of inflammation have been widely used as clinical markers of bacterial infections. C-reactive protein (CRP) is an acute-phase reactant synthesized by the liver during the inflammation with both proinflammatory and anti-inflammatory effects.[11] More recently, Procalcitonin (PCT), the prohormone of calcitonin, has demonstrated to be an useful marker for the diagnosis of sepsis. Among its advantages, PCT shows a favorable kinetic profile in sepsis, as appears in plasma earlier and longer than CRP or cytokines. Moreover, PCT levels correlate with the severity of the infection.[11,12] Despite its usefulness, the biological role of PCT in sepsis is still unknown.[11]

To date, several studies in severe pneumonia[13–15] and in sepsis[16,17] have evaluated the inflammatory response; however, data regarding the inflammatory response in acute PA infections are limited[18,19] and in fact the issue has not been examined in humans. Nor has the possible correlation of multidrug resistance with the ability of the pathogens to elicit immune response been assessed.[18]

We designed a prospective study of PA infections in an ICU unit to assess the profile of the inflammatory response in PA infection. We measured several molecular inflammatory markers at two serial time points to: (i) determine the sequential changes in the expression of proinflammatory and anti-inflammatory cytokines and other markers of inflammation (PCT and CRP) in blood, during the first 48 hr after the onset of PA infection, (ii) analyze the relationship between these markers and the severity of the presentation of the acute illness and outcome, and (iii) establish whether there are differences in the molecular inflammatory response depending on the antimicrobial resistant profile of the PA strain.

## Materials and Methods

### *Setting and design*

This prospective cohort study was performed at one of the three general ICUs at the Hospital Universitari de Bellvitge, a 700-bed tertiary-care institution for adult patients in Barcelona. The ICU is a 12-bed, private room ward providing care to medical and surgical patients, including those who have undergone solid organ transplantation. The study included all patients admitted to the ICU for more than 48 hr during an 18-month period (1 January, 2012 to 1 July, 2013). An active surveillance study was performed as previously reported.[20] Patients were prospectively monitored from ICU admission to ICU discharge, through daily clinical evaluation to detect whether they presented any sign of infection. In addition, a daily review of microbiological cultures was performed. When any patient presented clinical parameters of infection, clinical and blood sample were collected for the microbiological diagnostic and to analyze inflammatory biomarkers, respectively. Despite the prospective design of the study, the differentiation between infection and colonization is often difficult in critically ill patients. For this reason, we excluded nonbacteriemic urinary tract infections from the study, in addition to skin and soft tissue infections. Only evident acute infections with clinical significance were included: respiratory tract infections, bacteremia, or other infections in which the clinical sample has been obtained by surgery or sterile puncture (*e.g.*, intra-abdominal). All patients with PA infection were recruited in the study prospectively. Patients with any of the following criteria were excluded: length of ICU stay lower than 48 hr, polymicrobial infection; patients with other concomitant infection; and patients with do-not-resuscitate orders. The local Ethics Committee of our hospital approved the study, and patients or family provided informed consent.

### *Definitions*

The following data were recorded: age and sex; comorbidities and severity of underlying diseases calculated using the Charlson comorbidity index[21] and severity of illness estimated by the Simplified Acute Physiology Score (SAPS II) in patients at ICU admission and at the onset of infection.[22] Additionally, to analyze data according to the immune status, we created the variable ''immunocompromised,'' which included solid or hematologic malignancy, neutropenia, or patients under immunosuppressant treatment. The onset of PA infection was defined as the day in which the patient presented any sign of infection and clinical samples were collected for the microbiological diagnostic. In patients with multiple episode of infection, a second episode was defined when a new infection occurred at least 72 hr after the clinical resolution of the preceding episode. The source of infection was established according to the Centers for Disease Control and Prevention Criteria[23]; in addition, ventilator-associated tracheobronchitis and ventilator-associated pneumonia were classified according to the clinical pulmonary infection score,[24] and the severity of acute bacteremic illness at presentation and 48 hr according to the Pitt bacteremia score.[25] Acute inflammatory response was classified as sepsis, severe sepsis, septic shock, and multiorgan dysfunction syndrome (MODS) according to consensus definitions.[26] We also used the sepsis-related organ failure assessment (SOFA) score and defined acute organ dysfunction as the dysfunction of three or more organs in any of six organ systems.[27] For outcomes, overall mortality was defined as death from any cause occurring in the 30 days after the onset of PA infection; mortality was considered as early in patients who died within the first 5 days.

The phenotype stratification of PA isolates was made in accordance with recent standard definitions.[28] MDR PA was defined as a strain nonsusceptible to ≥1 agent in ≥3 antipseudomonal antimicrobial categories. Extensively drug-resistant (XDR) PA was defined as nonsusceptible to ≥1 agent in all but ≤2 antipseudomonal antimicrobial categories; thus, XDR PA isolates were included as MDR PA. To study the specific epidemiology of XDR PA, MDR PA isolates were distributed as follows: XDR PA as defined above, and MDR-non-XDR PA defined as PA strains nonsusceptible to ≥1 agent in ≥3 antipseudomonal antimicrobial categories, but susceptible to at least >2 antipseudomonal antimicrobial classes. All other PA isolates, including those nonsusceptible to ≥1 agent in <3 antimicrobial categories, were considered non-MDR PA.

### *Collection of blood samples and laboratory processing*

For all PA-infected patients, two serial blood samples were obtained at infection onset and 48 hr later. We measured circulating levels of IL-6 as marker of the proinflammatory cytokine response and IL-10 as a marker of the anti-inflammatory cytokine response; PCT and CRP biomarkers were also analyzed. The blood obtained was placed in tubes containing EDTA, immediately centrifuged, and stored at −80°C. Cytokines were measured by flow cytometry using the cytometric bead arrays technology. Samples were analyzed using a BD FACSCalibur flow cytometer (BD Biosciences), according to manufacturer's instructions. Samples were analyzed in duplicate. The detection limit was 5 pg/ml for IL-6 and 1 pg/ml for IL-10. PCT levels were measured by electrochemiluminescence immunoassay using the Modular Analytics e170 immunoassay analyzer (Roche Diagnostics), with a detection limit of 0.02 ng/ml. CRP levels were measured using an immunoturbidimetric assay in a Cobas c711 analyzer (Roche Diagnostics), with a detection limit of 1 mg/L.

### *Microbiological studies*

The isolates were identified and tested for antimicrobial susceptibility by a MicroScan automated microdilution system using CN1S and CO1S panels (Dade International, West Sacramento, CA). The following antimicrobial agents were tested: aztreonam, ceftazidime, cefepime, piperacillin, piperacillin-tazobactam, imipenem, meropenem, gentamicin, tobramycin, amikacin, levofloxacin, ciprofloxacin, colistin, and fosfomycin. Clinical categories were determined according to the CLSI breakpoints.

### *Statistical analysis*

Quantitative variables were expressed as the mean ± SD, medians and interquartile range (IQR). The variables were compared by the two-tailed *t*-test or Mann–Whitney *U*-test. The chi-square test or Fisher's exact test were used to compare categorical variables. Variables with a $p < 0.05$ were considered statistically significant. Cytokine measurements in the different PA phenotypes were compared with nonparametric test (Mann–Whitney *U*-test or Kruskal–Wallis test). A stratified analysis based on the type of infection (*i.e.*, respiratory tract infection and bacteremia) was performed. In addition, as an immunosuppression status may modulate the immune response during the infection, we also carried out a stratified analysis according to the immune status of the patient. Data were analyzed using SPSS version 19.0.

## Results

### *Baseline characteristics of the included population*

The epidemiological characteristics of the patients with PA infection are summarized in Table 1. Sixty-nine episodes of infection were recorded in the 61 patients included. Six patients presented a second episode of infection: five with the same strain isolated in the first episode (two non-MDR and three XDR PA) and one patient with initial MDR non-XDR infection presented a second episode by non-MDR strain. Only one patient had a third episode of infection, caused by the same XDR PA strain. Forty-eight episodes were respiratory tract infections (three of them with bacteremia) and 18 bloodstream infections. Among the remaining six episodes, three episodes were intra-abdominal infections

Table 1. Baseline Characteristics of Patients with *Pseudomonas aeruginosa* Infection According to Phenotype

| *Characteristic* | *Non-MDR PA* n = *37 (%)* | *MDR non-XDR PA* n = *11 (%)* | p[a] | *XDR PA* n = *13 (%)* | p[b] |
|---|---|---|---|---|---|
| Age (mean ± SD) | 67.2 ± 12.3 | 58.9 ± 11.8 | 0.06 | 67.3 ± 17.2 | 0.98 |
| Male sex | 25 (67.6) | 9 (81.8) | 0.27 | 9 (69.2) | 0.92 |
| Days ICU-infection acquisition [median (IQT)] | 14.5 (3.75–31) | 14.5 (8.75–35.75) | 0.63 | 8.5 (3.5–15.0) | 0.21 |
| SAPS score mean (SD) | 42.0 ± 17.2 | 41.4 ± 13.0 | 0.91 | 44.3 ± 9.2 | 0.66 |
| Charlson index [median (IQT)]) | 1 (0.75–4) | 3 (2–4) | 0.18 | 2 (1.25–4) | 0.39 |
| Underlying condition | | | | | |
| Diabetes | 7 (19) | 1 (10) | 0.65 | 1 (8) | 0.66 |
| Chronic lung disease | 3 (8) | 3 (30) | 0.12 | 3 (23) | 0.17 |
| Heart Disease | 13 (35) | 2 (20) | 0.45 | 5 (38) | 0.83 |
| Solid malignancy | 6 (16) | 3 (30) | 0.40 | 3 (23) | 0.67 |
| Hematologic malignancy | 1 (3) | 1 (10) | 0.40 | 0 | — |
| Chronic renal failure | 3 (8) | 2 (20) | 0.31 | 1 (8) | 0.95 |
| Chronic neurologic disease | 3 (8) | 3 (30) | 0.12 | 1 (8) | 0.72 |
| Cirrhosis | 2 (5) | 3 (30) | 0.07 | 3 (23) | 0.06 |
| Neutropenia | 0 | 0 | — | 0 | — |
| Immunosuppression | 10 (27) | 1 (10) | 0.40 | 2 (15) | 0.47 |
| Outcome | | | | | |
| Early exitus | 6 (16) | 1 (10) | 0.56 | 1 (8) | 0.40 |
| Overall exitus | 7 (19) | 1 (10) | 0.65 | 2 (15) | 0.56 |
| Days ICU-discharge/exitus [median (IQT)] | 53 (26.5–87.25) | 59.5 (39.25–86.25) | 0.50 | 68.5 (21.0–87.75) | 0.72 |

[a]Comparison of non-MDR and MDR non-XDR.
[b]Comparison non-MDR and XDR.
SD, standard deviation; PA, *P. aeruginosa*; MDR, multidrug-resistant; XDR, extensively drug-resistant; ICU, intensive care unit; IQT, interquartile range; MODS, multiorgan dysfunction syndrome.

that required emergency surgery, two episodes were central nervous system infections and one episode was a mediastinitis. A stratified analysis according to the inmmune status of patients, which could account for a different immune response, did not show significant differences as compared with the overall analysis (data not shown). Higher early mortality was observed in patients with non-MDR PA infections versus MDR PA infections, though the difference was not statistically significant (16% for non-MDR vs. 10% for MDR non-XDR and vs. 8% for XDR PA).

### *Molecular inflammatory response and severity of acute illness*

Cytokine and biomarkers concentrations at presentation. Blood was obtained for assay of cytokine concentrations and markers of inflammation at presentation from 69 infections of the 61 subjects. IL-6, CRP, and PCT levels were detected in all infection episodes analyzed. However, 27.5% (19/69) of IL-10 samples had undetected concentrations. The medians of IL-6 and IL-10 were higher in XDR

Table 2. Episodes of *P. aeruginosa* Infection: Clinical and Molecular Inflammatory Response

| *Characteristic* | *Non-MDR PA* n = *40 (%)* | *MDR non-XDR PA* n = *12 (%)* | p[a] | *XDR PA* n = *17 (%)* | p[b] |
|---|---|---|---|---|---|
| Origin of infection | | | | | |
| High risk origin | 38 (95) | 10 (83) | 0.22 | 14 (82) | 0.12 |
| Unknown | 3 | 0 | | 1 | |
| Respiratory tract | 29 | 10 | | 9 | |
| Abdominal | 3 | 0 | | 3 | |
| Other(s) | 3 | 0 | | 1 | - |
| Low risk origin | 2 (5) | 2 (17) | 0.22 | 3 (18) | 0.12 |
| Vascular catheter | 1 | 2 | | 2 | |
| Urinary tract | 0 | 0 | | 1 | |
| Pancreaticobiliary | 1 | 0 | | 0 | |
| SAPS score [mean (SD)] at infection | 47.1 ± 14.8 | 44.6 ± 12.7 | 0.60 | 56.5 ± 17.6 | 0.04 |
| Bacteremia | 8 (20) | 3 (25) | 0.70 | 7 (41) | 0.09 |
| Initial presentation | | | | | |
| Pitt score [median (IQT)] | 3 (2–5.50) | 1 (0–1) | 0.15 | 6 (6–6) | 0.22 |
| IL-6 [median (IQT)] | 360 (44–945) | 93 (64–93) | 0.50 | 183 (53–789) | 0.78 |
| IL-10 [median (IQT)] | 11.7 (0.2–22.7) | 13.5 (3.8–13.4) | 1.00 | 11.8 (2.76–19.6) | 1.00 |
| Procalcitonin [median (IQT)] | 3.75 (1.45–11.1) | 0.77 (0.50–0.77) | 0.085 | 2.99 (1.16–18.63) | 0.96 |
| CRP [median (IQT)] | 241 (67–361) | 34 (21–337) | 0.194 | 191 (87–394) | 0.95 |
| Sepsis (any severity) | 7 (87.5) | 2 (66.7) | 0.491 | 6 (85.7) | 0.73 |
| 48 after bacteremia | | | | | |
| Pitt score [median (IQT)] | 0 (0–5.25) | 0 | — | 3.5 (1.5–4.0) | 0.91 |
| IL-6 [median (IQT)] | 52 (25–167) | 60 (22–170) | 0.63 | 36 (25–138) | 0.66 |
| IL-10 [median (IQT)] | 1.15 (0.2–11.9) | 7.7 (3.3–12.9) | 0.19 | 7.21(0.5–13.2) | 1.00 |
| Procalcitonin [median (IQT)] | 2.42 (1.2–9.3) | 0.83 (0.4–0.9) | 0.50 | 1.39 (0.52–5.81) | 0.96 |
| CRP [median (IQT)] | 174 (79–298) | 43 (17–198) | 0.78 | 84 (51–303) | 0.62 |
| Sepsis (any severity) | 4 (50) | 1 (33) | 0.58 | 5 (83.3) | 0.24 |
| Initial clinical presentation | | | | | |
| Sepsis | 6 (15) | 4 (33) | 0.21 | 0 | - |
| Severe sepsis | 8 (20) | 1 (8) | 0.66 | 2 (12) | 0.70 |
| Shock | 9 (22.5) | 2 (17) | 0.50 | 11 (65) | 0.002 |
| MODS | 3 (7.5) | 0 | — | 4 (23.5) | 0.18 |
| Initial molecular inflammatory response | | | | | |
| IL-6 [median (IQT)] | 77 (34–216) | 38 (24–86) | 0.15 | 430 (67–951) | 0.02 |
| IL-10 [median (IQT)] | 1.3 (0–3.9) | 2.7 (0–11.8) | 0.41 | 3.3 (1.5–16.3) | 0.02 |
| Procalcitonin [median (IQT)] | 0.3 (0.1–1.0) | 0.3 (0.1–2.4) | 0.70 | 1.1 (0.6–5.2) | 0.008 |
| CRP [median (IQT)] | 191 (79–305) | 103 (41–184) | 0.12 | 202 (56–332) | 0.83 |
| Clinical presentation at 48 hr | | | | | |
| Sepsis | 2 (5) | 0 | — | 4 (25) | 0.05 |
| Severe sepsis | 5 (13) | 1 (8) | 0.56 | 6 (37.5) | 0.03 |
| Shock | 6 (15) | 1 (8) | 0.47 | 2 (12.5) | 0.57 |
| MODS | 2 (5) | 1 (8) | 0.56 | 0 | — |
| Molecular inflammatory response at 48 hr | | | | | |
| IL-6 [median (IQT)] | 58 (30–104) | 19 (15–49) | 0.02 | 86 (31–324) | 0.33 |
| IL-10 [median (IQT)] | 1.7 (0–7.5) | 0.98 (0–6.6) | 0.60 | 2.9 (0.7–7.8) | 0.52 |
| Procalcitonin [median (IQT)] | 0.2 (0.1–17) | 0.3 (0.1–1.1) | 0.96 | 0.9 (0.5–4.1) | 0.03 |
| CRP [median (IQT)] | 96 (47–256) | 44 (28–105) | 0.14 | 124 (41–299) | 0.58 |

[a]Comparison non-MDR and MDR non-XDR.
[b]Comparison non-MDR and XDR.
IL, interleukin; CRP, C-reactive protein.

Table 3. Inflammatory Response According to Acute Organ Dysfunction

| | *At onset* | | | *48 hr* | | |
|---|---|---|---|---|---|---|
| | *No organ dysfunction (n = 11)* | *Organ dysfunction (n = 58)* | p (n = *18*) | *No organ dysfunction (n = 49)* | *Organ dysfunction* | p |
| IL-6 [median (IQT)] | 20 (10.8–52.8) | 104 (42.1–435.4) | <0.001 | 28.1 (12.4–49) | 66 (32.1–161.6) | <0.001 |
| IL-10 [median (IQT)] | 0.2 (0–1.7) | 2.5 (0.06–9) | 0.03 | 1.5 (0–4.2) | 2.4 (0–8.3) | 0.72 |
| Procalcitonin [median (IQT)] | 0.1 (0.06–0.8) | 0.5 (0.2–3.2) | 0.01 | 0.3 (0.1–0.7) | 0.5 (0.1–1.8) | 0.02 |
| CRP [median (IQT)] | 62 (22.4–129) | 200 (90.7–317) | 0.03 | 74 (20.4–144) | 97.4 (44.1–299.5) | 0.01 |

PA infections than in non-MDR PA infections ($p = 0.02$). In addition, the PCT level was also significantly higher in XDR PA infections ($p = 0.008$) (Table 2). We also observed a significant number of episodes subsequently developing septic shock ($p = 0.002$) in XDR PA infections than in non-MDR PA infections. A trend toward higher MODS in XDR PA versus non-MDR PA infections was found, although the differences were not statistically significant ($p = 0.18$). The analysis of the molecular and clinical inflammatory response in bacteremic group is shown in Table 2. No significant differences in the progression to sepsis were observed between PA phenotypes. However, non-MDR and XDR PA strains presented a higher number of bacteriemic sepsis patients than MDR non-XDR strains, as it is shown in Table 2. Regarding respiratory tract infections, the median levels of IL-6 were higher in non-MDR PA than in MDR non-XDR PA episodes, with a difference close to statistical significance [69 (36–126) vs. 36 (23–57); $p = 0.06$]. The level of PCT was significantly higher in XDR PA than in non-MDR PA [0.92 (0.24–1.80) vs. 0.24 (0.12–0.52); $p = 0.05$]. No other statistically significant differences were observed at the onset of the infection in the respiratory infection group.

Cytokine and biomarkers concentrations 48 hr after infection onset. We obtained blood for assay of concentrations of cytokine and inflammation markers 48 hr after presentation from 67 infections (Table 2). Concentrations of IL-10 were undetected in 26% (18/67) of samples; the other markers of inflammation were detected in all samples. The median level of IL-6 was higher in non-MDR infections than in MDR non-XDR infections ($p = 0.02$), and the level of PCT at 48 hr remained significantly higher in XDR PA infections than in non-MDR PA infections ($p = 0.03$). Data regarding the molecular inflammatory response at 48 hr of the infection in bacteriemic group are shown in Table 2. Regarding to respiratory infection group, we observed that IL-6 levels remained higher in non-MDR than in MDR non-XDR PA after 48 hr [65 (32–104) vs. 17 (15–30); $p = 0.001$]. No other significance differences were observed at 48 hr in this group when comparing the biomarkers levels according to PA phenotypes.

### *Molecular inflammatory response in acute organ dysfunction and in bacteremic infections*

Concentrations of cytokines and other inflammatory markers are presented in Table 3, according to the definition of acute organ dysfunction using the SOFA score. Medians of all parameters were significantly higher in patients who developed acute organ dysfunction at infection onset. These differences between the groups remained significant 48 hr after infection onset, except in the case of IL-10; IL-10 levels remained high at 48 hr but the differences were not significant. We also observed significant differences in the levels of IL-6, IL-10, and PCT at onset between bacteremic and nonbacteremic infections. However, after 48 hr, only PCT levels still presented significant differences (Table 4).

### *Molecular inflammatory response and outcome*

In general, plasma cytokine concentrations were higher in nonsurvivors than survivors; however, these differences did not reach statistical significance in either early mortality (those who died within 5 days) or overall mortality (Table 5). Only PCT levels presented significant differences [median, IQT: 0.4 (0.1–1.6) between survivors and 2 (0.4–5.7) in nonsurvivors; $p = 0.050$] at infection onset. None of the analyzed parameters—cytokines, PCT or CRP -, either at baseline or after 48 hr were related to higher overall mortality (Table 5).

## Discussion

The aim of this study was to analyze the profiles of cytokines and other biomarkers in blood of ICU patients with

Table 4. Inflammatory Response According to Bacteremic Infection

| | *At onset* | | | *48 hr* | | |
|---|---|---|---|---|---|---|
| | *No bacteremia (n = 51)* | *Bacteremia (n = 18)* | p (n = *51*) | *No bacteremia (n = 18)* | *Bacteremia* | p |
| IL-6 [median (IQT)] | 66 (28.7–199) | 202 (61.3–688) | 0.03 | 50.5 (24.8–111.5) | 46.2 (25.3–144.6) | 0.93 |
| IL-10 [median (IQT)] | 1.5 (0–3.3) | 12.5 (1.4–21.7) | 0.003 | 1.9 (0–5.5) | 4.0 (0.6–12.7) | 0.13 |
| Procalcitonin [median (IQT)] | 0.3 (0.1–0.9) | 3 (0.9–8.8) | <0.001 | 0.2 (0.1–0.9) | 2 (0.5–3.9) | <0.001 |
| CRP [median (IQT)] | 137 (75.3–280) | 183 (49.6–340) | 0.08 | 84 (38.4–219) | 92 (52–296.5) | 0.39 |

Table 5. Inflammatory Response and Outcome

| | *At onset* | | | *48 hr* | | |
|---|---|---|---|---|---|---|
| *Early mortality* | *Survivors (n = 51)* | *Nonsurvivors (n = 18)* | p (n = *51*) | *Survivors* (n = *18*) | *Nonsurvivors* | p |
| IL-6 [median (IQT)] | 81 (33.5–281) | 120 (44.8–507) | 0.44 | 45 (23.3–95.3) | 154 (45.3–483) | 0.07 |
| IL-10 [median (IQT)] | 1.7 (0–5.7) | 7.2 (0.6–16.1) | 0.21 | 1.9 (0–5.4) | 9.4 (0.2–38.3) | 0.11 |
| Procalcitonin [median (IQT)] | 0.4 (0.1–1.6) | 2 (0.4–5.7) | 0.050 | 0.3 (0.1–1.2) | 2.1 (0.3–3.1) | 0.06 |
| CRP [median (IQT)] | 137 (66.2–285) | 262 (63.2–338) | 0.41 | 89 (36.4–230) | 131 (46.5–339) | 0.45 |
| Overall mortality | | | | | | |
| IL-6 [median (IQT)] | 81 (34.5–281) | 120 (27.3–441) | 0.58 | 45 (21.1–95.3) | 104 (34.3–278) | 0.09 |
| IL-10 [median (IQT)] | 1.7 (0–5.7) | 2.1 (0–16) | 0.50 | 1.9 (0–5.4) | 6.3 (0.1–17.6) | 0.16 |
| Procalcitonin [median (IQT)] | 0.4 (0.1–1.6) | 0.8 (0.3–4.9) | 0.12 | 0.3 (0.1–1.2) | 1.2 (0.2–2.4) | 0.13 |
| CRP [median (IQT)] | 125 (66.2–285) | 259 (63.2–328) | 0.38 | 89 (36.4–239) | 88 (46.3–276) | 0.77 |

PA infection and to evaluate their associations with the clinical severity of infections, outcome, and the pattern of antimicrobial susceptibility of the different strains.

Systemic cytokine activation was the rule, with predominance of an initial hyperinflammatory phase; the scale of this phase may be conditioned, by many factors including pathogen virulence, bacterial load and host factors.[29] In line with previous reports[13,14] we found a clear, overall relationship between the intensity of the inflammatory response, shown by very high levels of cytokines and biomarkers, and the severity of the initial clinical presentation. The inflammatory response was mainly evident at admission, but some traces remained 48 hr later. This response was observed when patients' severity was classified according to the SOFA score, but particularly in the cases with bacteremia, who presented the highest levels. These findings corroborate previous reports that patients with respiratory tract infections may show lower levels of cytokines in plasma, due to a possible compartmentalized immunological response inside the lung.[30]

Among the cytokines and biomarkers used in this study, IL-6 and PCT were the most significant indicators of the inflammatory response. Compared with PCT, the sensitivity of CRP was significantly lower. On the other hand, previous reports have indicated that PCT may be a good marker of systemic inflammation and severe forms of sepsis,[12] but not for local infections,[31] such as pneumonia; therefore, these findings may suggest that a local bacterial infection does not induce significant amounts of PCT.

High levels of proinflammatory cytokines have been correlated with the severity of systemic inflammation and impaired outcome,[32,33] but the authors of previous studies have emphasized the lack of specificity and note that peak levels may change rapidly without clinical correlation. Higher mortality has also been observed in patients with increasing or persistently high PCT concentrations, although a recent study showed that single PCT values were not predictive for patients' results.[12] We found higher levels of inflammatory cytokines and biomarkers in nonsurvivors in the early mortality group, but this correlation was quite weak in comparison with the findings of cytokine levels and severity of infection. In fact, PCT was the only biomarker of those studied that showed significant association with early mortality. No significant association was found with overall mortality. All these results show that a variety of factors may condition the outcome of these infections and they do not receive sufficient consideration when only data of blood inflammatory markers are evaluated.

Curiously, the analysis of inflammatory markers according to the antimicrobial susceptibility of the strains involved revealed significantly higher values in patients with XDR PA infections. In fact, a higher score of acute illness at infection onset and a more severe initial clinical presentation were observed in this group of XDR PA infection episodes, which may explain this correlation better than the antimicrobial pattern of resistance by itself. Thus, bacteremic infections were particularly frequent in the XDR PA group, and the levels of markers in these bacteremic patients were especially high.

In fact, we conducted a subanalysis including only bacteremic infections and no significant differences in the molecular inflammatory response were observed when comparing PA phenotypes. Moreover, levels of biomarkers were higher in bacteremic infections caused by non-MDR PA than in those caused by MDR non-XDR and XDR PA strains (Table 2), although no significance differences were observed. Respiratory tract infections were also less frequent in the XDR PA group (53%) than in the other two groups (non-MDR PA: 72,5% and MDR non-XDR group: 83%); as noted above, low levels of cytokines in plasma may be due to a compartmentalized inflammatory response in the lung.[30] In addition, there are several factors linked to XDR profile that could have an impact on the inflammatory response. In fact, XDR profiles are linked to defined high-risk clones,[34] and these clones are associated with certain biological markers that could well play a role in the inflammatory response.[35] Also, XDR high-risk clones show defined type III secretion system genotypes, which could certainly have an impact in the inflammatory response.[2] Thus, if the association of high marker levels and the XDR PA group is due to clinical factors and not only to microbiological factors, this may also account for the absence in our study of a stronger correlation between marker levels and outcome. We observed a tendency toward higher early mortality in the non-MDR PA group and in fact, in previous work we found early mortality to be lower in patients with XDR PA bacteremia than in patients with non-MDR PA bacteremia.[1]

Our study has several limitations. The first and major limitation is the small number of infections recruited, especially with respect to the analysis of the association between the results of biomarkers and the resistance profile of PA. Calculations to estimate power and sample size, based

on the epidemiological data of our hospital, were performed before the prospective recruitment. However, during the period of the study the epidemiology of PA infection changed in our center and we observed less episodes of infection than expected. Sufficient data for statistical analysis might have added statistical power and increased the reliability of the clinical interpretation of the results. Second, our work is a descriptive study of the inflammatory response of patients with PA infection and controls were not included. The use of ICU patients as controls would have improved our study. Third, we measured circulating levels of biomarkers at infection onset and 48 hr afterward, and this may have limited our ability to explore whether more time-sensitive patterns existed. Fourth, as the patients included in our study were critical patients, some of them were under corticosteroid or appropriate antibiotic treatment at the time of the infection onset; a situation that may have altered their cytokine levels.[6,29] In addition, other treatments and other patient conditions such as age or comorbidities may also have interfered with the results. Finally, extrapolating our findings in PA infections to other types of infection may be risky, because certain studies have shown PCT levels, to be significantly higher in the Gram-negative rod group than in the Gram-positive cocci.[36]

In conclusion, our study contributes toward a better understanding of the dynamics of biomarkers' inflammatory response in PA infections in ICU patients. A clear association between the intensity of molecular response and the severity of initial clinical presentation was observed. However, it was not clear whether a greater response of inflammatory biomarkers involves a worse prognosis. Inflammatory response was considerably higher in patients with bacteremia than in those with respiratory tract infections. Regarding the antimicrobial susceptibility of PA strains, no differences were documented in mortality between XDR and non-MDR PA. XDR PA infection presented higher values of inflammatory markers, but a greater number of bloodstream infections in this group may explain these results; thus should be interpreted carefully. Further investigation is needed to elucidate the clinical implications of these findings.

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## Disclosure Statement

No competing financial interests exist.

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