Biomédica 2013;33:643-52
doi: http://dx.doi.org/10.7705/biomedica.v33i4.805

ARTÍCULO ORIGINAL

Characterization of nCD64 expression in neutrophils and levels of s-TREM-1 and HMGB-1 in patients with suspected infection admitted in an emergency department

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Introduction: The nCD64 receptor, the soluble triggering receptor expressed in myeloid cells (s-TREM-1), and the high mobility group-box 1 protein (HMGB-1) have been proposed as significant mediators in sepsis.
Objective: To evaluate the prognostic value of these markers in patients with suspected infection recently admitted in an emergency department (ED).
Materials and methods: All patients who presented to the ED with suspected infection were eligible for enrollment in this study. Baseline clinical data, Sequential Organ Failure Assessment score (SOFA) score, APACHE II score, HMGB-1 levels, s-TREM-1 levels, and nCD64 levels were analyzed. The HMGB-1 and sTREM-1 serum concentrations were determined using commercially available ELISA kits, and CD64 on the surface of neutrophils was measured by flow cytometry.
Results:. A total of 579 patients with suspected infection as their admission diagnosis were enrolled in this study. The median patient age was 50 years (IQR = 35-68). Morbidity during the 28-day follow-up period was 11.1% (n=64). The most frequent diagnosis at the time of admission was community-acquired pneumonia (CAP) in 23% (n=133) patients, followed by soft tissue infection in 16.6% (n=96), and urinary tract infection in 15% (n=87). After multivariable analysis, no significant association was identified between any biomarker and 28-day mortality.
Conclusion: In the context of a tertiary care hospital emergency department in a Latin-American city, the nCD64 receptor, s-TREM-1, and HMGB-1 biomarkers do not demonstrate prognostic utility in the management of patients with infection. The search continues for more reliable prognostic markers in the early stages of infection.

Key words: Prognosis, biomarkers infection, neutrophils, patients, emergencies

doi: http://dx.doi.org/10.7705/biomedica.v33i4.805

Caracterización de la expresión de nCD64 en neutrófilos y de los niveles de s-TREM-1 y HMGB-1 en pacientes con sospecha de infección admitidos en el departamento de emergencias

Introducción. El receptor CD64, receptor soluble 'desencadenador' expresado en células mieloides (sTREM-1) y la proteína del grupo Box-1 de alta movilidad (HMGB-1), se han propuesto como mediadores en la sepsis.
Objetivo. Evaluar el valor pronóstico de estos marcadores en pacientes con sospecha de infección, recientemente admitidos en un departamento de emergencias.
Materiales y métodos. Se incluyeron en el estudio pacientes que consultaron al hospital con sospecha de infección. Se analizó la base de datos clínica, el puntaje SOFA, el puntaje APACHE II, los niveles de HMGB-1, los niveles de sTREM-1 y los niveles de nCD64. Se determinaron las concentraciones

Contribución de los autores:
Juan D. Matute, Laura Y. Gámez, Pablo J. Patiño and Fabián A. Jaimes: research question and study design.
Fabián A. Jaimes, Martha L. Valencia and Gisela De La Rosa: recruitment of participants.
Sergio Velásquez, Juan D. Matute, Laura Y. Gámez, Luis E. Enriquez, Iván D. Gómez, Fabiola Toro and Pablo J. Patiño: data collection and processing.
Sergio Velásquez, Laura Y. Gámez and Fabián A. Jaimes: data analysis.
Sergio Velásquez and Fabián A. Jaimes: draf of manuscript.
All the authors reviewed and approved the final version of the manuscript and are responsible for the research as a whole.

en suero de HMGB-1 y sTREM-1, usando kits de ELISA disponibles comercialmente, y la de CD64 se midió por citometría de flujo.

Resultados. Se analizaron 579 pacientes con sospecha de infección al ingreso. La edad media fue de 50 años (rango intercuartílico=35-68), y 11,1 % (n=64) murieron durante el seguimiento de 28 días. El diagnóstico más frecuente en el momento del ingreso fue neumonía adquirida en la comunidad, en 23 % (n=133) de los pacientes, seguida de infección de tejidos blandos, en 16,6 % (n=96), e infección urinaria, en 15 % (n=87). Después de un análisis multivariado, no hubo asociación significativa entre ningún biomarcador y la mortalidad a los 28 días.

Conclusión. Los resultados sugieren que en el contexto de un departamento de emergencias de tercer nivel de una ciudad latinoamericana típica, los tres marcadores evaluados no ofrecieron ninguna ventaja en el pronóstico de infección. La búsqueda de marcadores pronósticos más confiables en estadios tempranos de la infección aún continúa abierta.

Palabras clave: pronóstico, infección, neutrófilos, pacientes, urgencias médicas

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Infectious disease and sepsis are serious conditions with significant morbidity and mortality. The prevalence of sepsis among inpatients varies between 2% and 11% (1,2), but little is known about how to identify patients who have increased risk for mortality from infection. A need exists to identify biomarkers to accurately identify subpopulations of individuals with infection who are at risk for increased mortality, for the purposes of prognostication and development of targeted therapies towards specific biomarkers or related biologic processes. Moreover, previous reports have shown that early therapy improves survival and clinical outcomes (3,4).

Several markers have been suggested to facilitate and inform prognosis in patients with infection. The cluster of differentiation-64 (CD64), soluble triggering receptor expressed on myeloid cell-1 (s-TREM-1), and high-mobility group box-1 (*HMGB-1)* are considered to be promising new biomarkers in the context of infection, and they have been studied primarily in the intensive care unit setting (5). CD64 is a high affinity immunoglobulin Fc receptor I (FcRI) (6), which is expressed in monocytes and macrophages and is virtually undetectable on mature neutrophils from healthy individuals (1). CD64 levels increase within hours, both *in vitro* and *in vivo,* in the presence of inflammatory mediators such as interferon-gamma, granulocyte colony stimulating factor (G-CSF), immunoglobulin G (IgG), and complement opsonized particles (1,2,5). CD64 levels also have been shown to increase rapidly in infected patients (7). TREM-1 is a cell surface molecule present on neutrophils and mature monocytes and is actively expressed in response to infection by bacteria or fungi (8). TREM-1 can be released as a soluble molecule (s-TREM-1) in response to microbial products before the occurrence of the classic clinical findings of sepsis (8). Some reports have shown high levels of sTREM-1 in biologic fluids of patients with infections (8,9). Furthermore, Gibot et al found that sTREM-1 levels were constitutively elevated in all non-surviving patients, whereas sTREM-1 levels rapidly decreased in survivors during the first week after study admission (8). HMGB-1 is a cytokine-like mediator of inflammation, and it is released later in the inflammatory process as compared to the classical 'alarm-phase' cytokines, such as TNF and interleukin (IL)-1β. Clinical reports reveal that HMGB-1 levels were increased significantly in critically ill patients with sepsis (10), severe sepsis, and septic shock, with levels remaining high in non-survivors (5).

This clinical study aimed evaluate the prognostic value of nCD64, s-TREM-1, and HMGB-1 in patients with suspected infection admitted in an Emergency Department (ED), using 28-day mortality as a metric for prognosis. We selected 28-day mortality as our primary outcome because this period of time frequently covers the most significant changes in clinical response in sepsis and also is an accepted clinical end-point in the critical care population. Previous research with the same study population evaluating the same biomarkers as diagnostic tests has been previously published (11).

Materials and methods

We prospectively recruited patients with suspected infection who presented to the ED of a tertiary care level hospital in Medellin, Colombia.

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Recibido: 14/11/12; aceptado:05/07/13

Study design

This was a prospective cohort study. The patients in this study were part of the population of a larger study "Toward an operative diagnosis in sepsis: a latent class approach" (12). The local scientific ethical committee approved sample collection on the basis of verbal informed consent.

Study setting and population

The study setting was an ED at the Hospital Universitario San Vicente de Paúl (Medellin, Colombia). This is a 550-bed fourth level University Hospital with an admission rate of approximately 1,800 patients per month through the ED, and it is a reference institution for a region of approximately 4 million habitants. Patient enrollment began on July 2007 and concluded on September 2008. All patients were greater than or equal to 18 years of age, and were evaluated in the ED within 24 hours before recruitment to the study. The only inclusion criterion for entry to this study was suspected infection by the treating doctor. Exclusion criteria included the following: 1) screening for the study more than 24 hours after admission to the hospital; 2) refusal of the patients, their families, or the attending physician to be part of the study; 3) antimicrobial treatment received in other medical institution immediately before the beginning of the study; 4) medical decision to treat the patient ambulatory or in a different institution within 24 hours after admission; 5) homelessness or inability of the patient to follow-up at 28 days; and 6) previous participation in the same study.

Data collection and processing

Three physicians and two trained nurses recruited patients by checking admission lists and clinical records on a daily basis from Monday to Saturday. The general protocol for each patient after recruitment was as follows: 1) collection of baseline clinical data; 2) calculation of entrance Sepsis Organ Failure Assessment (SOFA) score (13) and Acute Physiology and Chronic Health Evaluation (APACHE II) score (14); and 3) blood sampling (two EDTA and two anticoagulant free tubes with separation gel). All of these steps occurred within 24 hours of the first ED evaluation. A standard case report form was used to record daily progression of patients by reviewing medical and nursing records until the time of discharge or death. In patients who left the hospital alive, the vital status was verified at day 28 through a telephone call.

Methods of measurement

Laboratory techniques

The serum samples (anticoagulant free tube with separation gel) were separated by centrifugation at 2,000 rpm over 20 minutes within 2 hours after bleeding to avoid false elevation of levels of HMGB-1 secondary to passive release from the intracellular compartment (15). Samples then were stored at -70ºC for later analysis of s-TREM-1 and HMGB-1. Blood specimens remain acceptable for up to 8 hours when held at room temperature (18-22°C) or for 48 hours when refrigerated (2-8°C) (16). The measurement of nCD64 from EDTA tubes was made less than 15 hours after blood extraction. All the procedures were performed by laboratory technicians who were blinded to clinical data. The handling of samples and the techniques for biomarkers measurement described below were similar to those used in previous reports (5,6).

Serum HMGB-1 levels were determined using commercially available ELISA following manufacturer instructions (Human HMGB-1 Kit cat No. 326052202, Shino –Test Corporation 2-29-14, Oonodai, Sagamihara-shi, Kanagawa 229-0011, Japan). The lower limit of detection of the test is 1 ng/ml. The cross reaction to HMGB-2 is less than 2% and the coefficient of variation was 10%. s-TREM-1 serum concentration was determined by ELISA (Kit Quantikine, Human TREM-1 Immunoassay, cat no. DTRM10, R&D Systems, Inc. Minneapolis, MN, USA). The lower limit detection of the test is 3.88 pg/ml. ELISA results were quantified using a microplate reader set POWER WAVE X (Biotek Instruments Inc). The inter- and intra-assay coefficient of variation for the s-TREM and HMGB-1 tests were <10%. nCD64 levels were measured with the Leuko 64 kit (Reference: LK64-250 – Trillium Diagnosis, LLC, Brewer, Maine, USA 04412) using a COULTER EPIC XL flow cytometer using Leuko64™ software (included in the kit). The results were expressed in Molecules of Equivalent Soluble Fluorochrome (MESF) units (17), and then compared with the expression of positive control (monocytes) and negative control (lymphocytes).

Data analysis

According to the available literature and the established biological basis of infection, we considered as potential confounders age, sex, lactate, APACHE II and SOFA scores, and comorbidity including diabetes mellitus, chronic renal disease, chronic obstructive pulmonary disease, use of steroids or chemotherapy in the previous three

months, history of cancer in the previous year, HIV/AIDS, solid organ transplantation, cirrhosis, and trauma (1,2,12). Comorbidity data were obtained from medical records of each patient, and a dichotomous variable was defined as the presence of at least one of the comorbidities mentioned above. For continuous variables, expressed as medians with interquartile ranges (IQR) or as means with standard deviations, we used the Mann-Whitney U or Student's t-test (depending on data distribution) to investigate the differences between survivors and non-survivors. We performed a logistic regression analysis including variables of interest in accordance to those previously defined. The potential linear relation between the continuous independent variables and the logarithm of odds of the outcome ("logit") was explored by graphical representations of locally weighted ("lowess") nonparametric regression models (18). Biomarkers were also explored through their transformation into dummy variables based on their classification in tertiles, and using the first tertile as reference. Multicollinearity was assessed by measuring the variance inflation factor (VIF) and verifying that its value for each variable was less than 10 (19). We evaluated the possible interaction between the biomarkers and sex, as well as the interaction between the biomarkers and the severity of sepsis as measured by SOFA, using a logistic model by means of the likelihood ratio test (LR test), comparing the full and the nested model, and considering as significant p values of less than 0.1 (20). Given the obtained sample size, we tried to follow the rule of at least 10 outcomes per independent variable in the logistic regression analysis whenever possible (21,22). Results are presented as Odds Ratio (OR) with their 95% confidence intervals. All tests were performed using STATA SE statistical software (Version 10, College Station, TX, USA).

Results

During the study period, 1340 eligible patients were admitted to the emergency service and 761 were excluded based on previously described exclusionary criteria (figure 1). We analyzed 579 patients with suspected infection as their admission diagnoses, 64 (11.1%) of whom died during the 28-day follow up period. The median age was 50 years (IQR = 35-68), and there were approximately equal numbers of males and females (298 females, 51.5%). The median time of symptoms before consultation was 72 hours (IQR = 36-192), and the median hospital length of stay was 9 days (IQR = 5-17). Two hundred and forty-six (37.6%) patients had no associated comorbidity. In those patients with comorbidity, the most frequent conditions

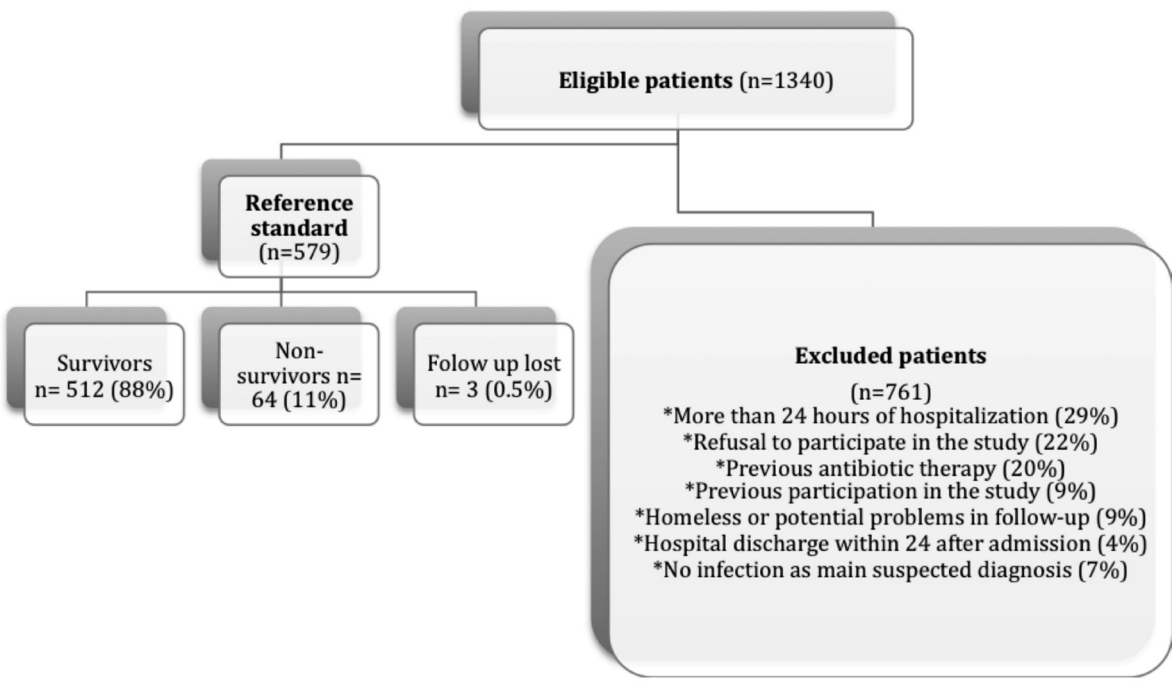

Figure 1. Flow chart of recruitment and patient status.

were diabetes mellitus in 125 (19.2%), chronic kidney disease in 72 (11.2%), chronic obstructive pulmonary disease in 70 (10.7%), exposure to steroids or chemotherapy during the previous 3 months in 57 (8.7%), and history of cancer during the previous year in 56 (8.5%) patients. The median APACHE II score on admission was 10 (IQR=6-15) and the median SOFA score was 2 (IQR=1-4). The main clinical and laboratory characteristics according to vital status are presented in table 1.

The most frequent diagnosis at the time of admission was community-acquired pneumonia (CAP) in 133 patients (23%), followed by soft tissue infection in 96 patients (16.6%), urinary tract infection in 87 patients (15%), intra-abdominal infection in 65 patients (11.2%), and clinical sepsis according to CDC definitions (21) in 56 patients (9.7%). Other identified infections included gastroenteritis, reproductive tract infection, deep surgical site infection, skin infection, catheter-associated urinary tract infection, endometritis, other lower respiratory tract infections, tracheobronchitis, other gastrointestinal infections, upper respiratory tract infection, otitis and mastoiditis, endocarditis, meningitis or ventriculitis, arthritis, osteomyelitis, bloodstream infection, and organ/space surgical site infection. A microbiological diagnosis was confirmed in 287 (43.9%) patients, 112 (39%) of them with positive blood cultures.

Prognostic value of biomarkers and severity scores

Figure 2 shows a representative flow cytometry analysis of nCD64 in the study population, and the values of each biomarker stratified by clinical diagnosis are shown in table 2. There were not significant differences between biomarker values in the study population according to previous use of steroids/chemotherapy or history of cancer (data not shown). The factors potentially associated with 28-day mortality, according to the logistic regression analysis, are presented in table 3. Although the univariable analysis showed a significant association between 28-day mortality and the highest tertiles of nCD64 and s-TREM-1 levels (OR = 2.06, 95% CI: 1.06; 4.00 and OR = 4.5, 95% CI: 2.12; 9.55, respectively), this relationship disappeared after multivariable analysis. These findings were confirmed in a model considering biomarkers as continuous variables, as well as in logistic models with backward and forward stepwise estimation procedures. There was no evidence of multicollinearity (VIF < 2) or interaction between biomarkers and sex (p > 0.2 for all the

Table 1. Baseline clinical data according to the 28-day survival status*

Characteristics	Survivors (n=512)	Non-survivors (n=64)
Age in years	49 (33-66)	64 (41-77)
SOFA[a] score	2 (1-4)	4 (3-6)
APACHE[b] II score	9 (5-14)	12 (10-18)
CD64 (MESF[c])	1.8 (1.2-3.1)	2.4 (1.4-3.6)
S-TREM (pg/ml)	140 (19.6-352.9)	373 (169-700)
HMGB1 (ng/ml)	8.1 (2.3-16.3)	9.3 (4.1-19.0)
Maximum temperature (°C)	37 (36-37)	37 (36-37)
Maximum heart rate (beats/min)	93 (82-108)	107 (91-119)
Maximum respiratory rate (breaths/min)	20 (18-27)	28 (20-32)
Minimum systolic blood pressure (mm Hg)	106 (95-121)	100 (87-121)
Minimun diastolic blood pressure (mm Hg)	62 (54-70)	60 (50-67)
Mimimun mean blood pressure (mm Hg)	77 (68-87)	72 (61-90)
Leucocytes (cells/mm^3)	11,600 (8,500-16,600)	11,450 (8,100-14,000)
Neutrophils (%)	80 (70-88)	81 (73-90)
Platelets (cells/mm^3)	291,000 (218,000-391,000)	223,000 (111,000-361,000)
Bilirrubine (mg/dl)	0.7 (0.5-1)	0.9 (0.6-2.2)
Creatinine (mg/dl)	1 (0.8-1.5)	1.3 (0.8-2.2)
PaO_2/FiO_2	317 (238-379)	235 (140-301)
Comorbidity[d] (%)	264 (51.6)	41 (64.1)
Positive blood cultures (%)[e]	61 (21)	11 (24)

* Survival status at 28-day was unknown for three participants.

Data are presented as median (IQR) or absolute numbers and percentages. [a] SOFA: Sepsis-related Organ Failure Assessment score. [b] Acute Physiological and Chronic Health Evaluation. [c] MESF: Molecular equivalent soluble fluorochrome (17). [d] At least one comorbility: diabetes mellitus, chronic kidney disease, chronic obstructive pulmonary disease, use of steroids or chemotherapy in the previous 3 months or history of cancer during the previous year. [e] Percentage of positive blood cultures among total blood cultures requested.

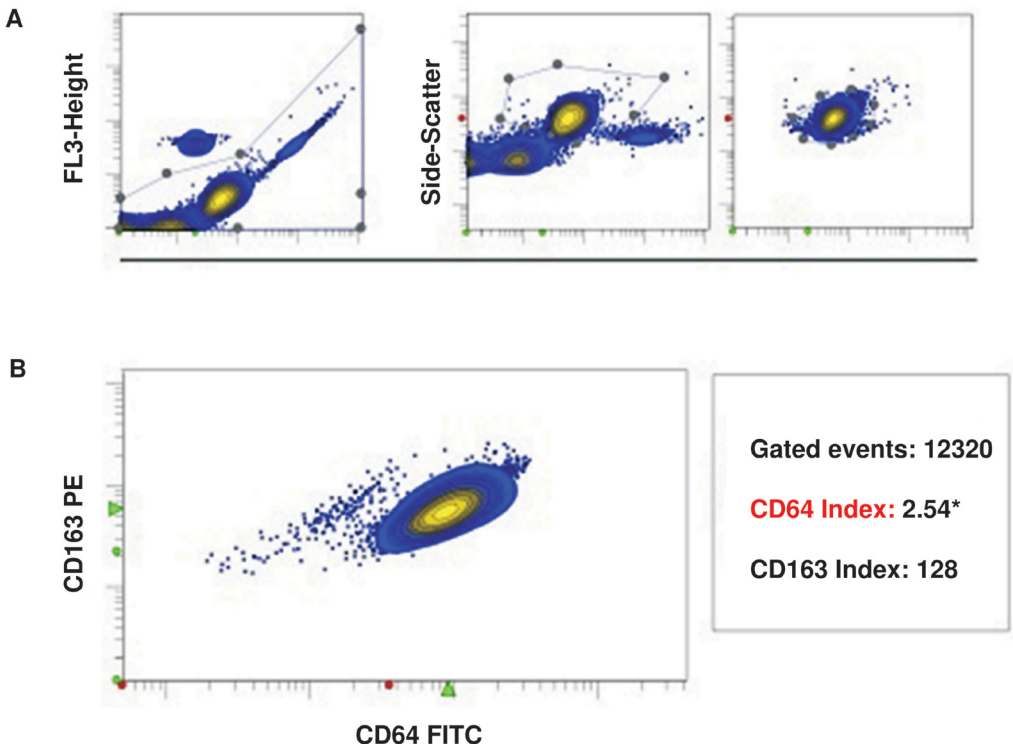

Figure 2. Flow cytometry analysis of nCD64 in the study population

Table 2. Main admission diagnosis and its respective biomarkers value

Main diagnosis at admission	nCD64 MESF	HMGB-1 pg/ml	s-TREM-1 ng/ml
Community-acquired pneumonia	2.1 (1.4-3.2)	8.2 (1.2-19)	185.4 (57.2-454.2)
Urinary tract infection	2.8 (1.6-4.2)	8.4 (4.1-16)	220.5 (77.1-409.2)
Soft tissue infection	1.7 (1.0-2.7)	9.6 (4.7-19)	212.3 (83.4-476.7)
Clinical sepsis	3.2 (2.3-4.5)	7.8 (3.3-16.3)	411.7 (153.6-694.7)
Intra-abdominal Infection	2.6 (1.8-3.5)	11.1 (5.3-23)	192 (81-465)
Superficial surgical site infection	1.7 (1.4-2.3)	9.6 (6.4-15.4)	174 (101.2-218)
Gastroenteritis	2.7 (1.7-4.1)	8.7 (2.3-12)	111.2 (0-282.7)
Reproductive tract infection	3.6 (3-5)	19.7 (9.8-25.8)	264.3 (55.3-354.3)
Deep surgical site infection	1.5 (0.9-2.3)	8.7 (16.2-4.7)	115 (0-190)
Skin infection	1.9 (0.9-2.3)	4.2 (1.9-9.8)	74.7 (15.0-116.4)
Catheter-associated urinary tract infection	3.4 (2.1-4.7)	9.7 (4.4-10)	540.8 (253-758.3)

Data are presented as median and interquartile range

comparisons) or between biomarkers and SOFA ($p > 0.3$ for all the comparisons).

Discussion

Cytokines are key mediators during early phases of infection and sepsis, and increased serum levels of cytokines are associated with the intensity of the inflammatory response. The value of prognostic markers in sepsis lies in their ability to identify subpopulations of patients with increased mortality risk who might benefit from specific therapies targeted at biochemical pathways identified by the respective biomarker. In this study, we evaluated the prognostic value of three biomarkers on mortality outcomes of patients with suspected infection. Following comprehensive analysis, we show that measurement of nCD64, s-TREM-1 and HMGB-1 serum levels does not have clinical utility in prognosis of 28-days mortality for infected patients in the ED of this hospital. Only the SOFA score was a useful prognostic marker for mortality in this study population.

Table 3. Logistic regression analysis for 28 day mortality

Variable	Univariate OR [95% CI]	Multivariate OR [95% CI]
Age (years)	1.02 [1.01, 1.04]	1.02 [1.01, 1.04]
Sex	0.86 [0.51, 1.44]	0.62 [0.33, 1.15]
SOFA[a] score	1.49 [1.32, 1.67]	1.34 [1.12, 1.62]
APACHE[b] score	1.11 [1.06, 1.15]	0.98 [0.92, 1.06]
Lactate	1.43 [1.23, 1.67]	1.19 [0.98, 1.45]
Comorbilities[c]	1.67 [0.98, 2.87]	1.00 [0.52, 1.94]
CD64 tertiles (MESF) [d]		
nCD64 T1 0. < 1.4	REF	REF
nCD64 T2 ≥ 1.4 & < 2.68	1.45 [0.70-2.99]	1.10 [0.48-2.48]
nCD64 T3 ≥ 2.68	2.06 [1.06-4.00]	1.18 [0.55-2.55]
sTREM-1 tertiles (pg/ml)		
sTREM-1 T1 < 80.33	REF	REF
sTREM-1 T2 ≥ 80.33 & < 282.48	1.55 [0.65-3.69]	1.11 [0.43-2.87]
sTREM-1 T3 ≥ 282.48	4.50 [2.12-9.55]	2.05 [0.83-5.07]
HMBG-1 Tertiles (ng/ml)		
HMGB-1 T1 < 4.29	REF	REF
HMGB-1 T2 ≥ 4.29 & < 12.07	1.35 [0.68-2.69]	0.98 [0.45-2.12]
HMGB-1 T3 ≥ 12.07	1.49 [0.77-2.86]	0.95 [0.44-2.06]

[a] SOFA: sepsis-related organ failure assessment score
[b] Acute physiological and chronic health evaluation
[c] At least one comorbility: diabetes mellitus, chronic kidney disease, chronic obstructive pulmonary disease, use of steroids or chemotherapy in the previous 3 months, and history of cancer during the previous year.
[d] MESF: molecular equivalent soluble fluorochrome (17)

Previous studies have suggested the utility of these three biomarkers as diagnostic metrics in the workup of patients with presumed infection. Use of these biomarkers to predict outcomes, however, have yielded inconsistent results in the literature. Livaditis, *et al.* (23), recently showed that increased CD64 neutrophil expression correlates with stages of severity and is associated with poor outcome in patients with sepsis. Similarly, Song, *et al.,* demonstrated that increased neutrophil CD64 expression correlates to 28-day mortality of patients with disseminated intravascular coagulation, including patients with sepsis (24). In evaluating the prognostic value of a wide range of markers reflecting different stages of priming, adhesion, and activation of monocytes and neutrophils, Muller et al showed that overall increase in the expression of markers of neutrophil and monocyte activation is related to favorable outcome in septic patients, with the exception of CD64 expression, which was increased in the non-survivors and reduced in survivors (25). Conversely, Danikas et al. found that an increased expression of CD64 antigen on polymorphonuclear cells (PMNs) and monocytes was favorable, but not fully correlated to patient survival (26). Consistent with that report, our findings did not confirm any independent relationship between CD64 levels and 28-day survival.

The soluble triggering receptor expressed on myeloid cells (s-TREM-1) is a recently identified molecule involved in the inflammatory response (27). Although the membrane-bound TREM is substantially up-regulated during infection (28), the soluble form appears to be specifically released in pneumonia and sepsis, where high levels of s-TREM are observed in serum samples from septic shock patients but not in controls. s-TREM-1 has been shown to be a promising diagnostic tool, both in ventilator-related pneumonia (29) and in the diagnosis and prognosis of sepsis in critical care (30). Reduction of serum values of s-TREM-1 is also useful to assess the antibiotic response in patients with community acquired penumonia (31). For patients with ventilator-associated pneumonia, Routsi C et al. reports that a sustained increased in s-TREM-1 levels from the time of diagnosis is associated with poor prognosis (30). Similarly, Tejera et al showed that initially increased s-TREM-1 levels were related to short-term mortality in patients with community-acquired pneumonia, and its prognosis value was independent of advanced age, other markers of inflammation, severity indexes, and nutritional status (32). There remain inconsistencies in the literature, however, with Gibot et al reporting an association between low initial serum s-TREM-1 levels and mortality in

septic patients, and Latour-Pérez et al showing no association between s-TREM-1 and mortality after adjustment by severity according to SAPS 3 score (29,33,34). Our results suggest that the role of s-TREM-1 as prognostic marker in infection still warrants further study.

HMGB-1 was originally identified as a nuclear DNA-binding protein critical for proper transcriptional regulation (35). More recently, HMGB-1 has been found to act as a "late" inflammatory cytokine that contributes to the pathological progression of sepsis and other inflammatory disorders (36). In patients with severe sepsis, the kinetic of circulating HMGB1 may differ from the classic mouse model findings depending on the primary source of infection (37). HMGB-1 has been proven to be a successful therapeutic target in experimental models of diverse infectious and inflammatory diseases (39). Higher levels of HMGB-1 have been shown patients with fatal outcome (36, 38), as well as in patients with infection as compared to healthy controls (38, 40). Other studies, however, have shown no statistically significant difference in serum levels of HMGB-1 between infected and the non-infected patients (40, 41), using the same commercial kit utilized in this study. In the absence of general consensus in the literature, the role of HMGB-1 remains debatable, as this molecule parallels other severity markers and may not provide further specific information regarding outcome (5). Our study confirms that serum HMGB-1 measurement does not have a role at this time in the prognosis of patients with infection.

An important limitation in this study involves kinetics of the biomarkers. Specifically, the production and release of these molecules varies according to the clinical course of the patients and the timing of measurements. Patients present to medical attention at time points during the course of illness, and biomarker peaks may be missed based on timing of presentation.

Our results suggest that, although the biomarkers nCD64, s-TREM-1, and HMGB-1 are increased during early phases of infection and sepsis, their prognostic value is limited. At this time, they are not ideal candidates as therapeutic targets, as they do not identify subpopulations with increased risk. In the context of an Emergency Department of a tertiary care hospital in a typical Latin-American city, these markers do not offer an advantage in infection prognosis. The search for more reliable prognostic markers in the early stages of infection remains open, and the relative strengths and weaknesses of biomarkers must be recognized in order to use them rationally.

Acknowledgements

To Bruce Davis for the reduced cost of nCD64 Flow cytometry kit; and Dr. Erica C. Kaye from St. Jude Children's Research Hospital, Memphis, TN, for the language review of the manuscript.

Conflicts of interest

We declare that we have no any potential conflict of interest.

Financing

Funded by Servicio Nacional de Aprendizaje (SENA), Colciencias Grant 1115-343-19153, Colombia, and "Estrategia de Sostenibilidad Universidad de Antioquia 2013-2014".

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