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Intercellular adhesion molecules and mortality for sepsis in infants younger than 1 year of life

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ABSTRACT

Background. Adhesion molecules in sepsis syndrome are correlated with the severity of illness and may be considered as predictors of survival outcome in adults. However, only few studies have been performed in infants and none using international criteria for sepsis. Objective. To determine whether adhesion molecules during the first 7 days of the disease could predict sepsis outcome and its severity. Material and methods. We performed a prospective study in 88 infants with sepsis and 30 controls. Soluble intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and E-selectin levels were determined at days 1, 3 and 7 of follow-up in those patients with sepsis and only one determination in the control group. The main outcome measure was mortality during 10 days of monitoring. Results. Positive hemoculture was reported in 64(72.7%). ICAM-1, VCAM-1, and E-selectin levels were higher in the group of sepsis than in the control group. However, no association was found between ICAM-1, VCAM-1 or E-selectin levels with sepsis severity. Mortality linked to sepsis was observed in 9 patients (10.2%). In the logistic regression analysis, those variables positively associated with mortality were the increase in ICAM-1 levels > 250 ng/mL between day 1 to 3, number of amines and the baseline severity of sepsis. However, we did not identify in those patients who died a specific pattern in adhesion molecules levels during follow up. Conclusions. ICAM-1 levels, number of amines and severity of sepsis levels predict mortality during 10 days of monitoring in infants younger than 1 year of age with sepsis.

Key words. Sepsis. E-selectin. ICAM-1. VCAM-1. Mortality.

Moléculas de adhesión intercelular y mortalidad por sepsis en niños menores de un año de edad

RESUMEN

Antecedentes. Las moléculas de adhesión en el síndrome de sepsis se correlacionan con la severidad de la enfermedad y se consideran como predictores de la sobrevida en adultos. Sin embargo, sólo escasos estudios se han realizado en niños y ninguno utilizando los criterios internacionales para definir sepsis. Objetivo. Determinar si durante los primeros siete días de la enfermedad las moléculas de adhesión predicen la severidad y desenlace de la misma. Material y métodos. Se realizó un estudio prospectivo en 88 niños con sepsis y 30 controles. La molécula de adhesión intercelular soluble (ICAM)-1, la molécula de adhesión vascular (VCAM)-1 y los niveles de E-selectina se determinaron en los días 1, 3 y 7 del seguimiento en los pacientes con sepsis y sólo una determinación se efectuó para el grupo control. La variable primaria de desenlace fue la mortalidad en los diez días de seguimiento. Resultados. Se reportó hemocultivo positivo en 64 casos (72.7%). Los niveles de ICAM-1, VCAM-1 y E-selectina fueron mayores en el grupo de sepsis que en el grupo control. Sin embargo, no se encontró asociación entre los niveles de ICAM-1, VCAM-1 o E-selectina y la severidad de la sepsis. La mortalidad asociada a sepsis se observó en nueve pacientes (10.2%). En el análisis de regresión logística, las variables positivamente asociadas a mortalidad fueron el incremento en los niveles de ICAM-1 > 250 ng/mL entre el día 1 a 3, número de aminas y la severidad basal de la sepsis. Sin embargo, no se identificó un patrón específico en los niveles de las moléculas de adhesión en el grupo que falleció. Conclusiones. Los niveles de ICAM-1, el número de aminas y la severidad de la sepsis predicen la mortalidad a diez días de seguimiento en niños menores de un año de edad con sepsis.

Palabras clave. *Sepsis. E-selectina. ICAM-1. VCAM-1. Mortalidad.*

INTRODUCTION

Sepsis remains an important health problem in children and adults.[1,2] Endothelial damage accounts for much of the pathology of sepsis, resulting in capillary leak, hypotension, microvascular thrombosis, and finally, multiple organ dysfunction (MOD) and lethal outcome.[3] Members of the selectin family of glycoproteins such as endothelial (E)-selectin mediate the initial step of leucocyte adherence and rolling on activated endothelium, and members of adhesion molecules, such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, have been reported to mediate firm adhesion between leucocytes and endothelium and subsequent diapedesis.[4-6] Furthermore, some studies have reported that levels of endothelium derived soluble adhesion molecules in severe sepsis syndrome are correlated with the severity of illness and may be considered as predictors of survival outcome.[7-12] However, only few studies have been performed in children and none of them in a representative population of younger than 1 year of age, when sepsis is highly prevalent. For example, recently Figueras, *et al.*,[11] reported that high baseline increases in ICAM-1 and VCAM-1 levels in newborn infants with sepsis and positive hemoculture may be secondary to the inflammatory process leaded by the next microorganisms: *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Streptococcus agalactiae*, *Enterobacter cloacae*, *Klebsiella oxytoca*. However, they did not consider the International Pediatric Sepsis Consensus Conference,[13] for sepsis definitions. Therefore, we performed a prospective study of patients younger than 1 year of age with sepsis to define the relationship between ICAM-1, VCAM-1 and E-selectin levels and clinical severity of the disease as well as to identify whether these adhesion molecules levels during the first 7 days of the disease could predict mortality.

MATERIAL AND METHODS

Eighty eight consecutive infants with sepsis, severe sepsis, multiple organ dysfunction (MOD) or septic shock admitted to the Pediatric Intensive Care Units in the Hospital of Gynecology and Obstetrics in the General Hospital in Leon, Mexico, were recruited. Control group consisted of 30 infants with diagnosis other than sepsis, including transient tachypnea of the newborn and those programmed for simple surgical interventions such as hydrocele, cleft lip and palate, or equinovarus foot. None of these patients had evidence of systemic inflammatory response syndrome and in all cases blood sample for adhesion molecules determination was obtained before the surgery.

All infants had gestational age older than 35 weeks, and at least 2 kg at birth weight. Exclusion criteria were patients with innate immunodeficiency, severe congenital anomalies, metabolic-diseases or complex cardiac diseases.

The study protocol was approved by the local Ethical Committee in both hospitals. The purpose of the study was fully explained to each parent, and written informed consent was obtained before enrollment.

Sepsis, severe sepsis, septic shock, and MOD were defined according to the International Pediatric Sepsis Consensus.[13]

Mortality was considered the outcome measure. Day of suspecting sepsis was defined as day 1 or baseline determinations.

By chart review and asking to the parents we obtained infant's age, antenatal and postnatal steroids use, pregnancy complications, cause of sepsis and other comorbidities. Body weight and height were determined with the patient naked using a scale with 10 g and 0.5 cm of precision respectively.

Infection was demonstrated microbiologically when the growth of at least one microorganism was observed in a biological fluid that should have been sterile (blood, pleural or peritoneal liquid, urine, cerebrospinal fluid). *Stafhylococcus epidermidis* bacteremia was regarded as true bacteremia when the same strain was obtained from at least two positive blood cultures.

At the two participating hospitals routine culture to bacteria, such as blood cultures, urine culture, cultivation of tracheal aspirate, cerebrospinal fluid (CSF), from catheter tip cultures, secretion and stool culture were conducted. The type of culture was made at the discretion of the requested physician according to the clinical suspicion of sepsis focus. Studies such as pharyngeal throat culture, antigen rotavirus detection, and other special studies at CSF for virus and parasites identification were only processed in specific cases. In all cases of negative hemoculture, antibiotherapy was maintained for at least 7 days in case of the attending physician considered that the syndrome was probably due to sepsis, especially when patients had received previous antibiotics in private or public medical assistance.

Measurements

A 2 mL venous blood sample was obtained from central catheter or by peripheral venipuncture on

days 1, 3, and 7 of sepsis. In the control group, a blood sample was obtained only at one time when general blood studies were request. Samples were centrifugated immediately for 10 min and serum was stored at -70 °C until it was processed (no longer than 6 months).

Assays

Soluble E-selectin, ICAM-1, and VCAM-1 concentrations were evaluated by ELISA according to the instructions of the manufacturer (BioSource Europe S.A.). The intra-assay CV was 5.4, 5.6, 4.8%, and the interassay CV was 6.0, 7.8, 6.7%, for E-selectin, ICAM-1, and VCAM-1 respectively. The sensitivity limit was 0.5 ng/mL for all this determinations.

Statistical analysis

Results are expressed as mean ± SD or 95% CI. Differences between the control and sepsis group were assessed by χ^2 for proportions. The Mann-Whitney U test or Student's t-test was used for variables displaying non-normal or normal distribution respectively. We evaluate associations between stage of sepsis and adhesion molecule levels on days 1, 3 and 7 by Spearman correlation analysis. Cumulative influence of adhesion molecule levels, age, sepsis focus, type of antibiotic therapy, and septic state on mortality was investigated by backward logistic regression analysis considering enter a variable if $p < 0.05$ and remove it if $p > 0.1$. Statistical analysis was performed using the STATISTICA software version 6.0 (Statsoft Inc. Tulsa OK).

RESULTS

We evaluated 118 infants younger than 1 year of age (88 with a diagnosis of sepsis and 30 controls). Infants in the control group never developed SIRS, not even after surgery. There was no difference in anthropometric characteristics, age or gender among infants with sepsis and controls, however, the levels of ICAM-1, VCAM-1 and E-selectin were significantly higher at baseline in the group with sepsis than in the control group (Table 1). The majority of patients in this group were included at ear-

Table 1. General characteristics and baseline markers of inflammation in infants with and without sepsis.

Variable	Infants with sepsis (n = 88)	Infants without sepsis (n = 30)	p
Gender (M/F)	46/42	15/15	0.85
Age (months)	3.5 (2.8-4.2)	2.9 (1.5-4.2)	0.37
Weight (kg)	4.1 ± 2.0	4.5 ± 2.2	0.43
Height (cm)	53.7 ± 7.8	54.9 ± 7.2	0.43
VCAM-1 (ng/mL)	1726 (1611-1841)	251.8 (187.2-316.3)	< 0.0001
ICAM-1(ng/mL)	374.4 (293.6-455.2)	233.1 (182.2-284.0)	0.04
E-Selectin (ng/mL)	229.8 (187.7-271.9)	135.1 (105.0-165.3)	0.01

Values are showed as mean ± SD or mean (95% CI) according to the distribution of variables.

Table 2. Endothelial adhesion molecule values in different days of monitoring in infants with and without mortality associated to sepsis.

Days of follow- up	Variable	Alive (n = 79)	Dead (n = 9)	p value
1	VCAM-1	1709 (1586-1832)	1869 (1503-2235)	0.41
	ICAM-1	371.7 (284.0-459.5)	397.8 (173.1-622.5)	0.84
	E-selectin	218.2 (175.2-261.2)	331.7 (148.0-515.3)	0.10
3	VCAM-1	2244 (1999-2488)	2357 (1287-3427)	0.77
	ICAM-1	572.3 (482.8-661.8)	873.0 (220.7-1525)	0.06
	E-selectin	194.8 (144.9-244.8)	437.9 (200.8-2534)	0.03
7	VCAM-1	2660 (2312-3007)	3462 (1893-5032)	0.24
	ICAM-1	568.8 (483.3-654.3)	458.8 (244.0-673.6)	0.50
	E-selectin	153.4 (125.6-181.1)	189.2 (69.7-595.1)	0.54

Results are expressed as mean (95% CI).

Table 3. Characteristics of patients with sepsis who died during follow up.

Case No.	Gender	Hemoculture	Adhesion molecules	Day of dead
13	Female	*Staphylococcus aureus* coagulase-negative.	Only E-selectin levels were higher than survivors on days 1 and 3.	4
30	Female	*Enterobacter sp* + *Staphylococcus aureus* coagulase-negative.	VCAM-1 levels were higher than survivors on days 1 and 3 but similar on day 7. ICAM-1 and E-selectin levels were higher than survivors only on day 1.	8
31	Female	Negative	All adhesion molecules were higher than survivors only at baseline.	6
48	Male	*Pseudomonas sp*	Only VCAM-1 on days 1 and 3 were higher than survivors.	10
52	Male	Negative	All adhesion molecules were higher than survivors on days 1 and 3.	4
66	Male	Negative	VCAM-1 and ICAM-1 were higher than survivors on day 3, whereas E-selectin levels were higher on days 1 and 3.	5
69	Female	*Klebsiella pneunoniae* + *Pseudomona aeruginosa.*	All adhesion molecules were higher than survivors on days 1 and 3.	6
76	Male	*Klebsiella pneunoniae* + *Pseudomona aeruginosa.*	All adhesion molecules were similar to those who survived.	10
80	Female	*Candida albicans* + *Escherichia coli.*	Baseline levels of all adhesion molecules were similar to those who survived, but they were higher than survivors on days 3 and 7.	8

Table 4. Logistic regression analysis for variables associated with mortality within 10 days of surveillance on infants with sepsis.

Variable	Coefficient	p
ΔICAM-1 > 250 ng/mL from day 1 to 3	0.22	0.01
Baseline sepsis severity	0.46	< 0.0001
Each number of amines > 1	0.69	< 0.0001

Intercept 0.6059, Std. Error 0.14. $R^2 = 0.38$; $p < 0.0001$ for the model.

ly stages of the process of sepsis: 65(73.8%) in sepsis, 11(12.5%) in severe sepsis, 5(5.7%) in multiple organ dysfunction and 7(8.0%) in septic shock.

The primary sites of sepsis focus were mainly respiratory tract and neonatal sepsis. Positive cultures were reported in 64 patients (72.7%). However 10 of the 24 patients with negative hemoculture had received at least two antibiotics in private or public assistance in the last 10 days. Isolated multiresistant pathogens corresponding to *Acinetobacter*, *Pseudomonas* and *Klebsiella* occurred in 24 patients (27.3%). These pathogens trend to be more frequently isolated in those infants who maintained or progressed to the diagnosis of multiple organ dysfunction or septic shock during follow up (5/26 = 19.2%) compared with those who maintained or changed to sepsis or severe sepsis (5/62 = 8.0%; p = 0.07) but were not related to mortality or adhesion molecules levels.

No association was found between ICAM-1(rho = -0.10: p = 0.32), VCAM-1(rho = 0.11; p = 0.35) and E-selectin (rho = -0.20; p = 0.28) levels evaluated at day 1 or even during follow up with sepsis severity.

Mortality occurred in 9 (10.3%) of all infants with sepsis. In these patients, E-selectin levels on day 3 were higher than those who survived (Table 2). Five of them were female and in 3 cases no pathogens were isolated. Adhesion molecules did not show a specific pattern (Table 3).

In the logistic regression analysis, those variables positively associated with mortality were the increase in ICAM-1 levels > 250 ng/mL between day 1 to 3, number of amines needed during the period of evaluation and the baseline severity of sepsis (Table 4).

DISCUSSION

We performed a study in infants with sepsis within the first 10 days of surveillance at diagnosis. In more than half cases included, the sepsis origin was respiratory tract, which is consistent with tho-

se reported by Watson, *et al.*,[14] in the pediatric population of the United States.

Overall mortality rate in these children was 10.2%, similar to that reported by Whalen, *et al.*,[9] in children of ~2 year of age.

Almost in a third of these patients we detected multiresistant pathogens. Bacterial resistance is currently one of the most important problems of infectious pathology and patients infected with Gram-negative resistant strains have shown increased mortality and longer hospitals stays.[15]

Compared with controls, in our study, all adhesion molecule levels in patients with sepsis were significantly higher as previously reported.[16-18] However, only the increase in ICAM-1 levels > 250 ng/mL between days 1 to 3 was related to mortality. This increase in ICAM-1 levels was probably due to the higher inflammatory response in patients who died. Consistent with our results Figueras, *et al.*,[11] found a cutoff level of ICAM-1 > 274 μg/L to predict positive hemoculture in neonatal sepsis. Also Whalen, *et al.*,[9] reported that, in children with sepsis-induced multiple organ dysfunction, plasma ICAM-1 levels independently predicted number of organ failures, development of more than three organ failures, and mortality.

We did not identify in those patients who died a specific pattern in adhesion molecules levels during follow up. The biological functions of soluble adhesion molecules in patients with sepsis are poorly understood. Some studies suggest that soluble forms of adhesion molecules can increase inflammation activating neutrophil integrin ligands and promoting neutrophil adhesion.[19,20] Furthermore, the formation of oxygen radicals by neutrophils originate the so-called "respiratory burning",[21,22] which can have deleterious consequences and explain many of the changes that have been observed in sepsis and septic shock. On the other hand, it is possible that these adhesion molecules could have anti-inflammatory effects *in vivo*. Soluble ICAM-1 may prevent leukocyte adhesion to endothelium by competitively binding to leukocyte counterreceptors.[23] Soluble E-selectin administered intravenously prevents intratracheal lipopolysaccharide-induced emigration of neutrophils into alveoli in rats.[24] Another objection might be that endothelial adhesion molecules are locally released, and thus measurement of concentrations in the circulating blood might not accurately reflect the concentrations of local active molecules. Thus we detected that the response to endothelial injury is complex and dynamic in the sepsis evolution.

Number of amines needed during follow up and baseline severity of sepsis were also predictive factors of mortality and together with ICAM increase levels > 250 ng/mL between days 1 to 3 predict 38% of this outcome.

Some limitations of this study include that we did not evaluate antimicrobial effectiveness and calculation of sample size was expecting a 20% of mortality. Subsequent studies with a higher sample size are needed to proof these adhesion molecules cut-off points according to specific therapies. However, the strength of the study is due to the repeated measurements of adhesion molecules and accurate assessment of patients according to the International Pediatric Sepsis Consensus which establishes definitions and criteria for facilities conducting clinical studies in children with sepsis.

CONCLUSIONS

We have shown that ICAM-1 levels, number of amines and severity of sepsis levels predict mortality during 10 days of monitoring in children younger than 1 year of age with sepsis. However, we did not identify in those patients who died a specific pattern in adhesion molecules levels during follow up.

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The authors declare that they have no conflict of interest.

REFERENCES

1. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001; 29: 1303-10.
2. Berger A, Salzer HR, Weninger M, Sageder B, Aspock C. Septicaemia in an Austrian neonatal intensive care unit: a 7-year analysis. *Acta Paediatr* 1998; 87: 1066-9.
3. Peters K, Unger RE, Brunner J, Kirkpatrick CJ. Molecular basis of endothelial dysfunction in sepsis. *Cardiovasc Res* 2003; 60: 49-57.
4. Bone RC. The pathogenesis of sepsis. *Ann Intern Med* 1991; 115: 457-69.
5. Parrillo JE. Pathogenetic mechanisms of septic shock. *N Engl J Med* 1993; 328: 1471-7.
6. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: The multistep paradigm. *Cell* 1994; 76: 301-14.
7. Cowley HC, Heney D, Gearing AJ, Hemingway I, Webster NP. Increased circulating adhesion molecule concentrations in patients with the systemic inflammatory response syndrome: a prospective cohort study. *Crit Care Med* 1994; 22: 651-57.

8. Kayal S, Jais JP, Aguini N, Chaudiére J, Labrousse J. Elevated circulating E-selectin, intercellular adhesion molecule 1, and von Willebrand factor in patients with severe infection. *Am J Respir Crit Care Med* 1998; 157: 776-84.
9. Whalen MJ, Doughty LA, Carlos TM, Wisniewski SR, Kochanek PM, Carcillo JA. Intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 are increased in the plasma of children with sepsis-induced multiple organ failure. *Crit Care Med* 2000; 28: 2600-7.
10. Cummings CJ, Sessler CN, Beall LD, Fisher BJ, Best AM, Fowler AA. Soluble E-Selectin levels in sepsis and critical illness. Correlation with infection and hemodynamic dysfunction. *Am J Respir Crit Care Med* 1997; 156: 431-7.
11. Figueras-Aloy J, Gómez-López L, Rodríguez-Miguélez JM, Salvia-Roiges MD, Jordán-García I, Ferrer-Codina I, et al. Serum Soluble ICAM-1, VCAM-1, L-Selectin, and P-Selectin levels as markers of infection and their relation to clinical severity in neonatal sepsis. *Am J Perinatol* 2007; 24: 333-8.
12. Boldt J, Muller M, Kuhn D, Linke LC, Hempelmann G. Circulating adhesion molecules in the critically ill: a comparison between trauma and sepsis patients. *Intensive Care Med* 1996; 22: 122-8.
13. Goldstein B, Giroir B, Randolph A. International pediatric sepsis consensus conference: Definitions for sepsis and organ dysfunction in pediatrics. *Pediatric Crit Care Med* 2005; 6: 2-8.
14. Watson RS, Carcillo JA, Linde-Zwirble WT, Lidicker J, Angus DC. The epidemiology of severe sepsis in children in the United States. *Am J Respir Crit Care Med* 2003; 67: 695-701.
15. Slama TG. Gram-negative antibiotic resistance: there is a price to pay. *Crit Care* 2008; 12(Suppl. 4): S4.
16. Gearing AJ, Newman W. Circulating adhesion molecules in disease. *Immunol Today* 1993; 14: 506-12.
17. Marui N, Offermann MK, Swerlick R. Vascular cell adhesion molecule-1(VCAM-1) gene transcription and expression are regulated through an antioxidant sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 1995; 92: 1866-74.
18. Krueger M, Heizmann A, Nauck M. Adhesion molecules in pediatric intensive care patients with organ dysfunction syndrome. *Intensive Care Med* 2007; 33: 359-63.
19. Lo SK, Lee S, Ramos RA, Lobb R, Rosa M, Chi-Rosso G, et al. Endothelial leukocyte adhesion molecule 1 stimulates the adhesive activity of leukocyte integrin CR3 (CD11b/CD18, Mac-1, alpha m beta 2) on human neutrophils. *J Exp Med* 1991; 173: 1493-500.
20. Barnett CC, Moore EE, Moore FA, Biffl WL, Partrick DA. Soluble intercellular adhesion molecule-1 provokes polymorphonuclear leukocyte elastase release by CD18. *Surgery* 1996; 120: 395-401.
21. Springer T. Adhesion receptors of the immune system. *Nature* 1990; 346: 425-33.
22. Carlos T, Harlam JM. Membrane protein involved in phagocyte adherence to endothelium. *Immunol Rev* 1990; 114: 5-28.
23. Rothlein R, Mainolfi EA, Czajkowski M, Marlin SD. A form of circulating ICAM-1 in human serum. *J Immunol* 1991; 147: 3788-93.
24. Ulich TR, Howard SC, Remick DG, Yi ES, Collins T, Guo K, et al. Intratracheal administration of endotoxin and cytokines: VIII. LPS induces E-selectin expression; anti-E-selectin and soluble E-selectin inhibit acute inflammation. *Inflammation* 1994; 18: 389-98.

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