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# Interleukin-12 in children with sepsis and septic shock

*Interleucina-12 em crianças com sepse e choque séptico*

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## ABSTRACT

**Objective:** To examine the behavior of interleukin-12 and verify whether it can be used to differentiate septic conditions in children.

**Methods:** Septic children aged between 28 days and 14 years, prospectively enrolled from 01/2004 to 12/2005, were divided into sepsis (SG; n=47) and septic shock (SSG; n=43) groups. Interleukin-12 levels were measured at admission (T0) and 12 hours later (T12). Disease severity was assessed by the PRISM score.

**Results:** Interleukin-12 levels did not differentiate children with sepsis from those with septic shock at admission [SSG: 0.24 (0-226.4)=SG: 1.23 (0-511.6); p=0.135)] and T12 [SG: 6.11 (0-230.5)=SSG: 1.32 (0-61.0); p=0.1239)]. Comparing time points, no significant difference was observed in the SG [SG, T0: 1.23 (0-511.6)=T12: 6.11 (0-230.5); p=0.075]. In SSG however, interleukin-12 increased from T0 to T12 (SSG, T0: 0.24 (0-226.4)<T12: 1.32 (0-61.0); p=0.018]. The mean percentage agreement between the clinical diagnosis and laboratory findings was 59.7% and 58.5% for the SG and SSG, respectively, with no significant difference between groups and time points (p>0.05). There was no correlation between onterleukin-12 levels at admission and the PRISM score for either group.

**Conclusion:** Interleukin-12 levels cannot differentiate between septic conditions and are not related to disease severity at admission. In septic shock patients, interleukin-12 increases with time.


**Keywords:** Interleukin-12; Critical care; Shock; Septic shock; Child

## INTRODUCTION

The early identification of sepsis is still one of the greatest challenges in critical care medicine and is pivotal for its clinical management.[1,2] The clinical signs of sepsis or most typical laboratory findings occur later, when multiple organ system failure (MOSF) has already occurred and mortality has considerably increased. The effectiveness of new treatment strategies are therefore directly related to the speed with which the diagnosis is established.[3]

In response to several infectious and non-infectious stimuli, monocytes/macrophages release a number of mediators, including cytokines, which are involved in the proinflammatory response that underlies sepsis. The excessive release of these mediators results in the development of whole-body inflammation and plays an important role in the pathogenesis of sepsis and septic shock.[4] The initial systemic pro-inflammatory phase involves

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**Conflicts of interest:** None.

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tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), IL-6 (IL-6), and interferon-gamma, which act synergistically with interleukin-12 (IL-12) to induce septic shock in animal models.[5]

Interleukin-12, one of the most important inflammatory cytokines, is a heterodimeric cytokine produced mainly by antigen-presenting cells and plays a key role in determining the nature of the immune response to exogenous or endogenous antigens. IL-12 is composed of two disulfide-linked protein subunits, designated p35 and p40, which are encoded by two different genes.[6] This cytokine is an important intermediary between innate and adaptive immunity, as it is secreted upon the stimulation of monocytes/macrophages and dendritic cells and activates interferon production and proliferation and the cytolytic activity of natural killer cells and T lymphocytes.[7]

The role of IL-12 in sepsis and septic shock are a matter of controversy both in the experimental and clinical fields of investigation. The administration of live *E. coli* elicits the release of biologically active IL-12 subunit p70. Its neutralization provides a clear survival benefit after a high lipopolysaccharide (LPS) dose challenge to mice.[8-10] Other experimental findings, however, support the concept that impaired IL-12 production may severely limit the host's defense against some infections and therefore is associated with a higher mortality rate.[11,12]

Clinically, in a recent study of 1,113 patients who underwent elective surgery of the upper and lower digestive tract, monocytes were isolated from peripheral blood, stimulated with IFN-gamma and LPS, and the IL-12 production in these cells was quantified to correlate with survival rate. The authors found that preoperative monocyte secretion of IL-12 p70 and IL-12 p40 was significantly reduced in patients who developed lethal postoperative sepsis compared with sepsis survivors and patients with an uneventful postoperative recovery. Moreover, preoperative monocyte IL-12 production was an independent predictive factor for a lethal outcome in postoperative sepsis.[8] Similarly, in 66 patients who developed postoperative sepsis, Emmanuilidis et al.[13] observed significantly lower IL-12 levels in the control group but no difference in values between survivors and non-survivors.

Only one study was found in pediatrics; it indicated a positive correlation between plasma IL-12 levels and disease severity measured by the Pediatric Risk of Mortality (PRISM) score in critically ill patients diagnosed with meningococcemia.[14]

These conflicting results, together with the lack of studies in pediatrics assessing the role of IL-12, indicate a need to better define its usefulness for diagnosing sepsis and septic shock in children. The study objectives were to study the behavior of IL-12 in children with clinical diagnoses of sepsis or septic shock and to verify whether this mediator is also an indicator of disease severity and is able to differentiate between sepsis and septic shock in post neonatal children.

## METHODS

### Patients, definitions and monitoring

This was a prospective observational study performed between January 2004 and December 2005, enrolling children aged between 28 days and 14 years, admitted to the pediatric intensive care unit (PICU) at the University Hospital, Sao Paulo State University-UNESP, Botucatu Medical School, and diagnosed with sepsis or septic shock. Children were excluded if they had chronic systemic inflammatory diseases; degenerative neurological diseases; primary or acquired immunodeficiency diseases; were on corticoid therapy, nonsteroidal anti-inflammatories or antibiotics for more than 24 hours; had suffered trauma or burns, or were in postoperative care. The study was approved by the Ethics and Research Committee at Botucatu Medical School-UNESP. Written consent was obtained from parents or guardians before recruitment. Procedures were in accordance with the ethical standards of the committee responsible for human experimentation and with the Helsinki Declaration of 1975, revised in 1996.

Sepsis and septic shock were defined according to criteria established by the 2001 Consensus Conference.[15] As the latest consensus conference on sepsis definitions in children was published when the study was already underway, the diagnosis protocol adequacy was reviewed when needed.

Patients were assigned to one of two study groups at admission: the sepsis group (SG) or the septic shock group (SSG). The diagnosis of sepsis or septic shock was reached for each patient by a consensus of all researchers at weekly rounds. Because patients with sepsis can progress to septic shock, the initial diagnosis was considered the definitive criterion for assignment to the appropriate study group.

Patients were monitored and treated according to the task force set up by the Society of Critical Care Medicine.[16] MOSF was defined as the presence of at

least two dysfunctional organs diagnosed according to pre-established criteria.[17] The PRISM score was calculated for all patients at admission.[18]

### Sample collection tests

The first blood sample was collected at admission and labeled T0. Another sample was collected 12 hours later and labeled T12. The biochemical analysis results were not made available to the treating doctors. The blood samples for IL-12 assays were 2 mL at T0 and 2 mL at T12. After collection, samples were centrifuged and the plasma stored at -70°C until IL-12 measurement.

### IL-12 measurement

Concentrations of IL-12 were measured by ELISA (enzyme-linked immunosorbent assay) using the Duo Set system (R&D System, Minneapolis, MN). Each sample was tested in triplicate and the mean value obtained. The limit of detection was 4 pg/mL and intra- and inter-assay variations were 5% and 7%, respectively. This methodology allowed the identification of the IL-12 p70 subunit, the biologically active fraction of this mediator.

### Statistical analysis

An analysis of the gender distribution, infection foci, and PRISM score were performed at admission. Plasma IL-12 levels were compared by time and group.

Gender, infection foci, and time between groups were analyzed using the Goodman test for contrasts between multinomial populations. The evaluation of groups by age and PRISM scores was performed using the Mann-Whitney U test. A repeated measures analysis was used to compare IL-12 levels. The correlation between PRISM and IL-12 level was examined by Spearman's test. The agreement between the clinical diagnosis and IL-12 levels was analyzed by the percentage of patients with plasmatic levels above and below the detection limit, with results compared by Fisher's Exact test. All conclusions were drawn based on a 5% significance level.

## RESULTS

During the study period, 689 patients were admitted to the PICU, and 59 met the diagnostic criteria for sepsis and 65 for septic shock. Twelve patients were excluded from the SG (six were given antibiotics for more than 24 hours, four were postoperative patients, and two had degenerative neurological diseases), and 22 were excluded from the SSG (12 were given antibiotics for more than 24 hours, three had active chronic inflammatory diseases, two had degenerative neurological diseases, two had been given corticoids, and three died before samples could be collected). The final sample, therefore, comprised 90 patients, 47 in the SG and 43 in the SSG.

### Comparison of groups: general characteristics

Table 1 shows a comparison of the groups by age, gender, and PRISM scores. The groups did not differ significantly in terms of age or gender. They were, however, significantly different in terms of disease severity, assessed by the PRISM score, which was greater in the SSG [SSG: 29 (19.6-33.5)>SG: 17.8 (16-21.7); p<0.05]. The frequency of progression to MOSF was significantly greater in the SSG [SSG: 42/43 (97.7%) vs. SG: 11/47 (23.4%); relative risk = 4.17; p<0.05]. Three (6.4%) patients in the SG and 11 (25.6%) patients in the SSG died.

**Table 1 -** Comparison of sepsis and septic shock groups for age, gender, and PRISM scores

| Variable | Sepsis (N=47) | Septic shock (N=43) | p value |
|---|---|---|---|
| Age (months) | 24 (2 - 167) | 12 (1 - 204) | p > 0.05 |
| Gender | | | |
| Male | 26 (57.3) | 26 (60.5) | p > 0.05 |
| Female | 21 (42.7) | 17 (39.5) | |
| PRISM | 17.8 (16.0-21.7) | 29 (19.6-33.5) | p < 0.05 |

N - number of patients; PRISM - pediatric risk of mortality. The results are expressed in median (minimum - maximum) or number (%). Mann-Whitney U test; Goodman test.

Microorganisms were isolated from blood or cerebrospinal fluid cultures in 54 patients. Blood cultures were positive in 40 patients, 29 (72.5%) from the SSG, and cerebrospinal fluid cultures were positive in 14 children, 11 (78.6%) from the SSG. In 38 (70.4%) patients, gram-negative germs were identified (*P. aeruginosa; H. influenzae; A. baumanii; and Klebsiella* species), 11 (20.4%) of these were gram-positive (*S. aureus*; *S. pneumoniae; S. epidermidis*), and in another four (7.4%), the infection was polymicrobial. A fungal infection (*C. albicans*) was diagnosed in one patient (1.8%).

The primary focus of infection was the lungs in the SG [lungs: 33 (70.2%) vs. central nervous system: five (10.6%) vs. intestines: four (8.5%) vs. others: five (10.6%); p<0.05] and in the comparison between the two groups [SG: 33 (70.2%) vs. SSG: 17 (39.5%); p<0.05]. The infection focus distribution was not significantly different in the SSG [lungs: 17 (39.5%) vs. central nervous system: 11 (25.6%) vs. intestine: eight (18.6%) vs. others: seven (16.3%); p>0.05].

### IL-12 at T0 and at T12 and the PRISM score

Comparing IL-12 at T0 [SG: 1.23(0-511.6) = SSG: 0.24 (0-226.4); p=0.135] and T12 [SG: 6.11 (0-230.5) = SSG: 1.32 (0-61.0); p=0.1239] revealed no significant difference between the groups (Figure 1). Comparing time points (T0 vs. T12) revealed no significant difference in the SG [SG- T0: 1.23 (0-511.6) = T12: 6.11 (0-230.5); p=0.075] but did reveal a significant increase in IL-12 levels from T0 to T12 in the SSG (SSG - T0: 0.24 [(0-226.4]<T12: 1.32 [0-61.0]; p=0.018) (Figure 1).

A higher proportion of patients had IL-12 levels above the laboratory established limit at both T0 and T12 [T0: above (SG=63.04%)=(SSG=53.6%) and T12: above (SG=56.5%)=(SSG=63.4%)] but with no significant difference between groups or time points (p>0.05). The mean percentage agreement between the clinical diagnosis and laboratory findings were 59.7% and 58.5% for the SG and the SSG, respectively, without any significant difference between groups or time points (p>0.05).

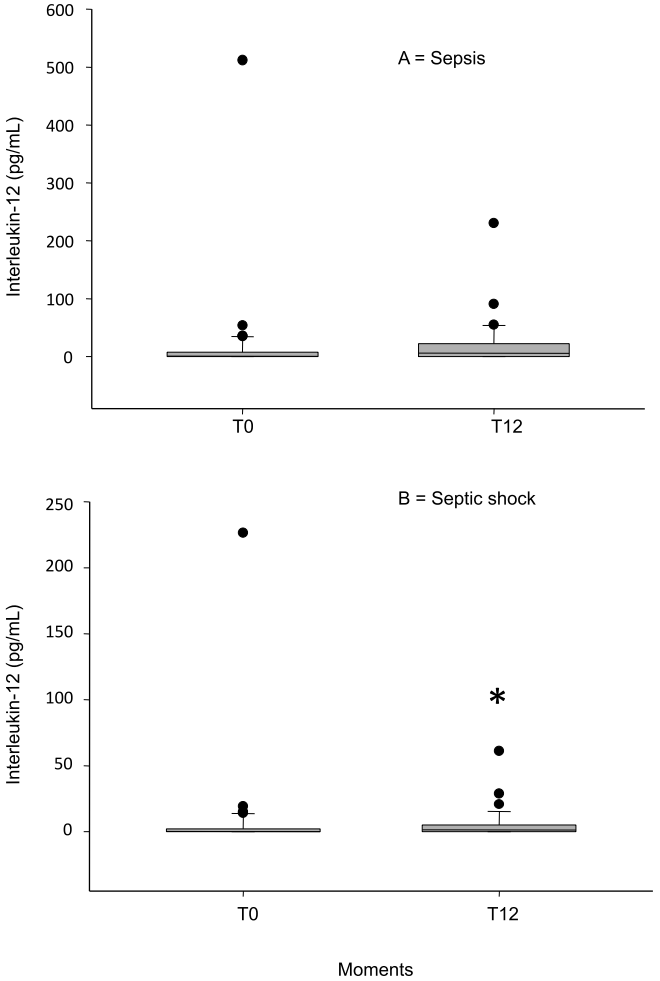


**Figure 1 -** Interleukin-12 plasmatic concentration for sepsis (panel A) and septic shock (panel B) groups at admission (T0) and 12 hours later (T12). Median, 10th, 25th, 75th, and 90th percentiles as vertical boxes with error bars and individual values. * p <0.05 T12 versus T0 for septic shock group. Wilcoxon test.

There was no positive correlation between IL-12 and the PRISM score at T0 in either the SG (r=0.19; p=0.028) or the SSG (r=0.20; p=0.215).

## DISCUSSION

We demonstrated that IL-12 p70 did not differentiate between children with sepsis and septic shock at admission. There was also no significant difference in the agreement between the clinical diagnosis of sepsis or septic shock and the IL-12 levels between groups. In contrast, while studying 117 septic neonates, Sherwin et al.[19] concluded that IL-12 p70 can be used to confirm sepsis. Previously, when we compared IL-12 with other cytokines, we found a higher percentage (above 90%) of patients for whom the clinical diagnosis of sepsis and septic shock was more strongly associated with laboratory findings for IL-6, procalcitonin, and C-reactive protein[20,21] than with IL-12.

Studies that have reported deleterious effects of IL-12 in both experimental models[22,23] and humans[14,24] observed a correlation between an increase in the p40 subunit and mortality. As with Sprong et al.,[25] in infants with meningococcal disease, we also did not observe a correlation between IL-12 p70 and disease severity as measured by the PRISM score. The same result was obtained by Livaditi[26] in 47 critically ill adult patients. In contrast, a correlation was reported between IL-12 and disease severity in children with burns.[24] Interestingly, we observed that IL-12 increased after 12 hours in our septic shock patients.

Many factors may have influenced our results. Disagreement between the findings from the IL-12 data in septic patients may be linked to the short half-life of IL-12,[27] its kinetics, the time of blood sample collection, intensity of the stimulus, differences in patient age and clinical condition,[28] and the IL-12 subunit studied.[29] It has been demonstrated that IL-12 p40 is produced immediately after stimulation, while p70[29] is produced from 12 h to 72 h after a stimulus, peaking at approximately 36 h,[30] which is later than the time at which we collected the second sample. However, immunological stimulation is more intense in septic shock patients, which made it possible for us to detect the increased IL-12 at T12, in contrast with the sepsis group in which stimulation is less intense. It

is also possible that at the beginning of the infectious stimulus, the earlier p40 subunit release downregulated p70 activity, a more pronounced effect in less severely ill patients.[31] This imbalance between bioactive isoforms, which is most likely designed to control excessive inflammation, may also cause septic patients to be more susceptible to nosocomial infection.[32] Another point worth mentioning is that depending on immune cell activation, ATP is released into the extracellular space. It has been demonstrated that ATP has multiple anti-inflammatory effects, playing a role in blunting an overactive immune response in infectious diseases. In situations such as sepsis and septic shock, ATP is released from death cells; this can downregulate IL-12 from the macrophages and enhance the release of the anti-inflammatory cytokine.[33]

Some of the differences in results when studying IL-12 may be related to the different populations analyzed. Upham et al.[28] studied IL-12 in healthy individuals at different ages: at birth, five years old, 12 years old, and adulthood. They observed that levels of the p70 subunit differed greatly in the four groups, being lower in younger patients. They also reported that the ability to synthesize IL-12 at comparable levels to adults seems only to be reached during adolescence. In our series, we studied a heterogeneous group in terms of age, encompassing one month to 15 years, with a greater proportion of younger individuals; this may have limited the detection of increased IL-12 and influenced our results.

### Limitations and implications of this study

First, our ethics committee did not allow us to include a control group; this made it difficult to calculate the sensitivity, specificity, and likelihood ratio. Second, the use of clinical diagnostic criteria may have introduced a bias in patient classification. Because the IL-12 results were hidden from treating physicians, they were not influenced by them, meaning that the lack of a gold standard for diagnosis does not compromise our results. Finally, the influence of different classes of antibiotics and corticosteroids on IL-12 levels should not be ignored and neither should the influence of organic dysfunction type or disease duration. A few of the meningitis patients only received corticosteroids in the first 48 h; we therefore believe this drug had little effect on our results. With respect to the organic dysfunction type or disease, the clinical character of our research implies that patients could possibly be at different phases of the disease at admission. We believe that excluding children who were on antibiotics for more than 24 h minimized this effect.

## CONCLUSION

IL-12 does not differentiate between septic conditions and is not related to disease severity at admission. In septic shock patients it increases with time.

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## RESUMO

**Objetivo:** Examinar o comportamento da interleucina-12 e verificar se pode ser utilizada para diferenciar condições sépticas em crianças.

**Métodos:** Foram inscritas, de forma prospectiva, entre janeiro de 2004 e dezembro de 2005, crianças com idades de 28 dias a 14 anos, subdivididas nos grupos sepse (SG; n=47) e choque séptico (SSG; n=43). A interleucina-12 foi avaliada quando da admissão (T0) e 12 horas mais tarde (T12). A gravidade da doença foi avaliada utilizando o escore PRISM.

**Resultados:** A interleucina-12 não diferenciou crianças com sepse das com choque séptico quando da admissão [SSG: 0,24 (0-22,64)=SG: 1,23 (0-511,6); p=0,135)] e na avaliação T12 [SG: 6,11 (0-230,5)=SSG: 1,32 (0-61,0); p=0,1239)]. Na comparação entre os momentos, não foi observada diferença estatística para SG [SG, T0: 1,23 (0-511,6)=T12: 6,11 (0-230,5); p=0,075]. Entretanto, em casos de SSG, a interleucina-12 aumentou entre as avaliações T0 e T12 [SSG, T0: 0,24 (0-226,4)<T12: 1,32 (0-61,0); p=0,018]. As porcentagens médias de concordância entre o diagnóstico clínico e os achados laboratoriais foram de 59,7% e 58,5% respectivamente para SG e SSG, sem diferença estatística entre os grupos e momentos de avaliação (p>0,05). Para ambos os grupos, não houve correlação entre interleucina-12 na admissão e o escore PRISM.

**Conclusão:** A interleucina-12 não diferencia as condições sépticas e não se relaciona com a gravidade da doença por ocasião da admissão. Em pacientes com choque séptico, a interleucina-12 aumenta com o tempo.

**Descritores:** Interleucina-12; Cuidados críticos; Choque; Choque séptico; Criança

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**Authors' contributions:** Dr. Martin had primary responsibility for protocol development, patient screening, enrollment, outcome assessment, preliminary data analysis, and writing the manuscript. Dr. Kurokawa participated in the development of the study protocol and analytical framework and contributed to the writing of the manuscript. Dr. Carpi participated in the development of the study protocol and analytical framework.

Dr. Bonatto and Dr. Moraes contributed in the same manner as Dr. Carpi. Dr. Fioretto supervised the study design and execution, performed final data analyses, and contributed to the writing of the manuscript.