Original Article

Prognostic serum tumor necrosis factor-α in paediatric patients with sepsis

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Abstract

Objective: To study the association of tumor necrosis factor-α (TNF-α) in paediatric patients with different etiological agents and levels of sepsis.

Methodology: Seventy-nine patients with sepsis were studied. Blood cultures, along with other relevant specimens, were processed for bacterial and fungal etiology. TNF-α was detected by enzyme immunoassay.

Results: In total, 42 (53.2%) of the patients had a microbiologically documented cause for sepsis. Of the gram-negative bacilli, *Escherichia coli* was the most common isolate followed by *Klebsiella pneumoniae*. *Enterobacter* spp. *Serratia marscecens* as well as *Citrobacter koseri*. *Streptococcus pneumoniae* and *Staphylococcus aureus* predominated among the gram-positive cocci. Patients with a positive culture had significantly higher TNF-α levels than patients with a negative culture (70 pg/ml vs. 33 pg/ml $p = 0.01$). Patients with a pure gram-negative infection had significantly higher TNF-α levels than those with pure gram-positive infection (83 pg/ml vs.52 pg/ml). The geometric mean TNF-α concentrations in patients with severe sepsis and those with late septic shock were 47 pg/ml (range 5-2720) and 59 pg/ml (range 5-3310) respectively.

Conclusion: TNF-α was significantly raised in culture-positive cases in general and in gram-negative infections in particular. It can be used as a surrogate marker of sepsis and aggressive treatment initiated in patients with elevated levels of TNF alpha.

Key words: sepsis, pediatric, tumor necrosis factor-α

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Introduction

Sepsis syndrome and septic shock remain significant causes of morbidity and mortality in critically ill patients. In spite of great strides in therapeutic and technological fronts in the care of these patients, the average mortality rate for sepsis syndrome remains approximately 40%, and the incidence of this disorder continues to increase [1,2]. Bacteremia and endotoxemia elicit the production of a cascade of endogenous mediators that cause a metabolic and immunologic host systemic inflammatory response. Both gram-negative and gram-positive bacteria, as well as fungi and exogenous microbial components, can initiate this cascade [3,5]. In this study, we focused on the association of serum tumor necrosis factor-α (TNF-α) and sepsis in paediatric patients. We compared TNF-α levels in patients with microbiologically confirmed infection with clinically diagnosed cases of sepsis. We also correlated TNF-α levels with different types of organisms isolated in patients with sepsis. We compared post-mortem TNF-α levels with initial TNF-α levels in the expired patients.

Materials and Methods

The study was conducted in the Departments of Microbiology and Paediatrics, Maulana Azad Medical College, and associated Lok Nayak Hospital, New Delhi. Seventy-nine children were evaluated in the study. The samples were collected from the neonatal intensive care unit, the high-risk nursery unit, and the paediatric intensive care unit from the Department of Paediatrics, Lok Nayak Hospital, New Delhi. The mean age of the patients was three years (range 18 days – 13 years). Forty-three children were below one year of age; 21 children were between one and five years; and 15 children were older than five years of age. Seven patients expired during the course of the study. TNF-α levels in their serum were examined at the time of death.

The criteria for inclusion in the study were as follows: (1) objective signs of acute infection; (2) $\geq$ 3 recognized signs of systemic inflammatory response; and (3) evidence of $\geq$ 2 organ dysfunctions [3].

Cultures of blood and specimens from other relevant sites were obtained to identify a microbial

cause for the infection. All samples were cultured and isolates were identified by standard microbiologic procedures [4].

Collection of blood samples

Blood samples were collected prior to initiation of antibiotic therapy. Using aseptic technique, 0.5ml to 2ml of blood was collected from children by venipuncture and inoculated into blood culture bottles containing 5 ml brain heart infusion broth. The bottles were incubated at 37°C for seven days and examined daily for bacterial growth. Subcultures were performed on the first, second, third, fifth and seventh days of incubation on chocolate agar, 5% sheep blood agar, and MacConkey agar [4].

TNF assay

TNF-α levels at the time of enrollment were determined by standard enzyme linked immunosorbent assay (Titerzyme TNF-α EIA kit PerSeptive Biosystems). TNF-α levels of expired patients were also determined.

Statistical Procedures

Comparisons of cytokine concentrations were made using analysis of variance techniques employing log transformed data. The distribution of residuals of each model was tested for normality by Shapiro Wilkes test. When the normality assumption was violated, a non-parametric test (Wilcoxon) was applied to confirm the results.

Results

Microbiological findings

Overall, 42 patients (53.2%) had a microbiologically documented infection, while no infectious cause was identified in 37 patients (46.8%). Age-wise distribution of patients with and without microbiological confirmation of sepsis is given in Table 1. Among gram-negative bacteria, *Escherichia coli* was clearly the single most common isolate followed by *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens*, and *Citrobacter koseri*. Enterobacteriaceae thus constituted the most common cause of gram-negative sepsis (Table 2). *Pseudomonas aeruginosa* and *Acinetobacter baumanii* were isolated in two cases each.

Staphylococcus aureus was the most common gram-positive isolate followed by *Streptococcus pneumoniae*. *Staphylococcus epidermidis* was isolated in one patient who had intravenous catheterization for a prolonged duration. *Enterococcus faecalis* was isolated in two patients.

Among non-bacterial etiology, *Candida albicans* was isolated in two cases.

Overall, of the culture-positive patients, 29 cases (69%) had gram-negative bacterial infection, 11 (26.3%) had gram positive infection, and 2(4.7%) had fungal infection (Table 3).

Type of Infection

The foci of infection responsible for sepsis are given in Table 4. Pneumonia, meningitis, urinary tract infection, and intra-abdominal infections constituted the majority. Forty-six patients (58.2%) were categorized in the severe sepsis group and 33 patients (41.8%) in the septic shock group. There was no significant difference in the distribution of infection sites between the severe sepsis/early septic shock and the late septic shock groups. Seven children expired in our study. Five of them belonged to the septic shock group and the remaining two were in the severe sepsis group. Five of them had microbiologically documented infection: three had *Escherichia coli* infection, one had *Klebsiella pneumonia*e infection, and one had *streptococcus pneumoniae* infection.

Interactions with serum TNF-α concentrations

Data on baseline TNF-α levels were available for all 79 patients. The geometric mean serum TNF-α concentrations in patients with severe sepsis was 47 pg/ml (range, 5-2720), and 59 pg/ml (range, 5-3310)

Table 1. TNF-α levels in different age groups with culture positive and negative reports.

Age group	Total	Microbiologically documented infection (TNF-α range)	No documented microbial infection (TNF-α range)
Neonates 1-28	25	8 (23 -910 pg/ml)	15 (5-730 pg/ml)
Infants < 1 yr.	22	11 (33 -1870 pg/ml)	9 (10 -1270 pg/ml)
1 – 5 yrs.	21	16 (100-3310 pg/ml)	5 (43 -1675 pg/ml)
> 5 yrs.	15	7 (250-3310 pg/ml)	8 (175-1870 pg/ml)

in patients with late septic shock. Age-wise analysis of TNF-α in relation to confirmed or unconfirmed sepsis is given in Table 1. Neonates had the lowest geometric mean of TNF-α levels of the expired patients at the time of enrollment was 58 pg/ml (range: 50-2520; n = 7) and 64 pg/ml (range: 5-2720)

Table 2. Distribution of microbial isolates associated with severe sepsis/early septic shock and late septic shock.

Category	Bacterial isolates (from blood cultures only)	
	Severe sepsis/early septic shock	Late septic shock
Gram negative *Escherichia coli** K✞ ESC# *Pseudomonas aeruginosa* *Acinetobacter* spp.	7(3) 9(2) 0 1(1)	4(2) 5(2) 2(1) 1
Gram-positive *Staphylococcus aureus* *Streptococcus pneumoniae*✞ *Staphylococcus epidermidis* *Enterococcus* spp.	2 2 1 1	3(2) 11(1) 0 1
Fungal *Candida albicans*	2(1)	0

(KESC) *Klebsiella spp*., *Enterobacter spp*., *Serratia marscecens*, *Citrobacter spp*.
* 3 patients expired, ✞ 1 patient expired , ✟ 1 patient expired

Table 3. TNF-α levels in patients categorized by microbiologic infection.

Category	**Total (%)**	**Geometric mean TNF-α**
Pure gram-negative infection	29(69.1%)	83 pg/ml
Pure gram-positive infection	11(26.2%)	52 pg/ml
Pure fungal infection	2(4.7%)	47 pg/ml

levels whereas the older children had higher levels. In all age groups, TNF levels were higher in cases of confirmed sepsis.

Patients with a positive culture had significantly higher TNF-α levels than those from whom no pathogen was isolated. The geometric mean (co-efficient of variation [CV]) levels were 70 pg/ml (range 50-3310) versus 33 pg/ml (range 5-1870) p = 0.0001 (Table 1). Among patients with a positive bacterial culture, those with a pure gram-negative infection had significantly higher TNF-α levels (geometric mean 83 pg/ml: range 50-2520; n = 29) than patients with a pure gram-positive infection (52 pg/ml: range 50-3310; n = 11) or fungal infection 47 pg/ml: range 100-1675; n = 2) (Table 2). The at the time of expiry. This difference was not significant.

Discussion

In the present study, 53.2% of the patients had a microbiologically documented infection and 35.7% of these patients had bacteremia. Other studies on sepsis have reported similar results [6-8]. The gold standard for diagnosing neonatal sepsis remains blood culture, even though in many cases blood cultures are negative in the face of strong clinical indicators of septicaemia, as well as in autopsy proven disseminated bacterial or fungal infection. Moreover, maternal antibiotics given in the majority of preterm deliveries may suppress the growth of bacteria in culture. Negative blood cultures in apparently septic neonates may also result from insufficient sample size [9]. Gram-negative infection occurred in a significantly larger number of cases than gram-positive infection, although most reports point to a very narrow difference between the two [6,7,10].

We investigated the microbial ecology comprehensively and our findings are consistent with other studies [11]. *Escherichia coli*, other enterobactericeae, *Staphylococcus aureus*, and *Streptococcus pneumoniae* accounted for the majority

of infection. *Candida albicans* was isolated in two patients.

Serum levels of TNF-α varied considerably even in patients belonging to the same clinical group (severe sepsis or septic shock group), which is evident from our data. The mean TNF-α levels were higher in the septic shock group in comparison to the severe sepsis group though not significantly so.

It was also found that those with bacterial infection had higher levels of TNF-α than those without documented infection in all age groups. TNF-α levels were significantly higher in patients with microbiologically documented infections than in patients with only clinically documented infections, a finding in conformity with the reports of other studies [11,12]. Performing TNF-α tests routinely in patients

Table 4. Focus of infection responsible for the development of sepsis syndrome.

Focus of Infection	No (%)	Severe sepsis/early septic shock (death)	Septic shock (death)
Respiratory tract	27(34.1)	14(1)	13(2)
Intra-abdominal/pelvis	17(21.6)	7(1)	10(1)
Urinary tract	12(15.2)	9	3
Skin/wound	8(10.1)	7	1
Pleural	2(2.5)	1	1
Cerebrospinal fluid	11(13.9)	6	5(2)
Foreign body/catheter	1(1.3)	1	0
Burn wound	1(1.3)	1	0
Total	79	46	33

These results are consistent with other reports [11**].** On analyzing the geometric means of different categories of sepsis, the range was from 47-59 pg/ml which suggests that levels at approximately 50 pg/ml can be the cutoff for sepsis.

Measurements of TNF-α at different stages of a disease's spectrum have not been conducted previously. Postmortem serum TNF-α levels, though raised, did not show significant elevations from the initial TNF-α levels in the seven expired patients. The observation that TNF-α levels were definitely raised in the postmortem samples, but not significantly, indicates that TNF-α cannot be utilized as a single prognostic marker in septic patients. A positive blood culture together with elevated TNF-α levels may be more efficient markers of prognosis.

When we analysed the TNF- α levels in relation to the ages of the children, we noted that the lowest levels of TNF-α were found in neonates, followed by infants. The highest levels were seen in children older than five years of age. This observation suggests immune competence is needed for TNF expression; however, normal levels of TNF are usually less than 3pg/ml. In this study, the majority of the cases had levels above 50 pg/ml across all groups. Levels above or equal to 50 pg/ml can be interpreted as the threshold for diagnosis of sepsis.

of sepsis can give rapid results in a day rather than the three to seven days required for bacterial culture and can act as surrogate markers of microbial infection. Thus, it appears that the distinction between "clinically documented" and "microbiologically documented" infection is far more significant than has been previously appreciated, as TNF-α represents a quantitative difference in the host response. A positive microbiological report with a raised TNF-α thus carries a much greater significance and the patient immediately should be classified in a higher risk category and aggressive treatment started. Five of the seven patients who expired had a positive culture report; three had *Escherichia coli* infection; one had *Klebsiella pneumoniae* infection; and one had *Streptococcus pneumonia* infection.

Furthermore, there was a significant difference in TNF levels between patients with gram-negative and gram-positive infections. This again stresses the differences in host responses between gram-negative and gram-positive infection, and this should be borne in mind while formulating supplementary or adjunctive therapeutic agents.

The present study yet again highlights the many diverse factors which work in tandem in sepsis syndrome**.** TNF-α is one such factor. Reports of TNF-α levels can be made available in a much shorter turnaround time than blood culture reports.

As seen in this study, assessment of TNF-α levels can give an indirect evidence of bacterial invasion and whether the septicaemia is due to gram-positive or gram-negative bacteria. This information will be invaluable in instituting timely appropriate treatment. The elevated TNF-α levels highlight the beneficial role of monoclonal anti-TNF-α antibody fragments in patients with microbiologically documented sepsis in general and in Gram-negative infection in particular [13].

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