Is TNF-α a prognostic factor in patients with sepsis?

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Objective: To determine tumor necrosis factor-α (TNF-α) levels in a prospective study in 58 hospitalized patients in a department of internal medicine (63 episodes, 29 in immunocompromised patients) during a 7-month period.

Methods: Patients fulfilling the following criteria were included: clinical evidence of acute infection, temperature >38.2°C, tachycardia >90 beats/min, tachypnea >20 breaths/min. Samples were taken from day 1 up to day 13 after an infection was diagnosed, and TNF-α was determined by enzyme immunoassay.

Results: In 29 episodes (46.0%) the infection was microbiologically documented. The median of the TNF-α levels in the Gram-negative episodes was significantly higher than that in the Gram-positive episodes (p=0.002). Thirteen of 63 episodes (20.6%) had a fatal outcome. With respect to all measured values, the non-survivors had a significantly higher median of TNF-α levels than the survivors (p=0.0001). There was, however, great interpatient and intrapatient variability in TNF-α levels; thus, no unequivocal correlation between TNF-α and outcome could be documented.

Conclusions: Our data indicate that the influence of the infecting organism on TNF-α kinetics is less pronounced than that of the underlying disease.

Key words: TNF-α, sepsis, immunocompetent, immunocompromised, microbiologically documented and non-documented infections, outcome

INTRODUCTION

Lipopolysaccharide (endotoxin) and lipid A are considered to cause shock and organ failure in Gram-negative sepsis [1–4]. Endotoxin injection into animals and human volunteers results in symptoms similar to those in sepsis and septic shock [5–12]. Furthermore, injection of endotoxin into animals and human volunteers induces release of tumor necrosis factor-α (TNF-α) in serum within minutes [7–11,13]. Infusion of TNF-α into animals and human volunteers produces fever, hypotension, tissue necrosis, tachypnea, tachycardia, edema, increased pulmonary vascular permeability and glomerular damage, similar to the effects seen in sepsis [6,7,14–17]. TNF-α, or elevations of TNF-α, in serum or cerebrospinal fluid have been detected in patients with serious bacterial infections, sepsis or septic shock [11,18–38]. However, injection of endotoxin into healthy volunteers sometimes resulted in similar or higher TNF-α levels without life-threatening symptoms [11]. Kiener et al. showed that both the toxic forms of lipid A and its non-toxic derivative, monophosphoryl lipid A, induced equal amounts of TNF-α in mice, but only the mice given toxic lipid A or endotoxin died [39].

TNF-α elevation has also been reported in non-infectious disorders (e.g. rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, allograft rejection) and in infections caused by parasites, Gram-positive bacteria and viruses [33,35,36]. The protective effects of TNF-α in some infectious diseases are also being discussed [32–34]. In some studies the degree of TNF-α elevation was closely related to the severity of sepsis [11,20,22,24,26,27,29,30,38], while in other studies the serum levels of TNF-α varied widely from patient to patient without correlation with severity or outcome [21,23,25,28,31,37].

Most authors measured TNF-α only at the beginning of the infection or during the first 48 h after

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the infection was diagnosed. Only a few studies followed TNF-α levels during the whole course of the infection, with diverging results and conclusions [22,24,27,31]. We therefore planned to determine TNF-α levels during the entire course of microbiologically documented infections (MDI) and to compare these results with those in microbiologically non-documented infections (MNDI).

PATIENTS AND METHODS

Fifty-eight hospitalized patients, 21 women and 37 men of mean age 46 years (±15.8 years) in the internal medicine ward, with 63 infection episodes during a 7-month period, were studied prospectively. Patients fulfilling the following criteria were included:

1. Clinical evidence of acute infection.
2. Temperature >38.2°C.
3. Tachycardia (>90 beats/min).
4. Tachypnea (>20 breaths/min, while the patient was spontaneously breathing) or patient on assisted ventilation.

Blood samples were taken (10 mL in a heparin-coated endotoxin-free tube) from a central line or a peripheral vein every day except Saturdays and Sundays between 7:00 AM and 10:00 AM. Within 30 min the samples were centrifuged, and the serum was stored in aliquots in endotoxin-free tubes at −80°C, for measurement of TNF-α concentration at a later date. TNF-α concentrations were determined by enzyme immunoassay (TNF-α-EASIA, Medgenix Diagnostics, No. 40 175 00, Medgenix Group Deutschland GmbH) according to the manufacturer's instructions. The detection limit was 5 pg/mL TNF-α. Duplicate determinations were carried out on each sample.

The first day of fever was defined as day 1 for each episode. All data collected consecutively were numbered accordingly. The underlying disease, clinical course and outcome, medication, body temperature, heart rate, blood pressure, blood count, other laboratory data and microbiological findings were documented. Data were evaluated from day 1 to 3 days after defervescence, to a maximum of 13 days. For analysis and comparison, patients were divided according to the following features: outcome; body defense status; underlying disease; microbiological findings.

Statistical analysis

In this study median TNF-α levels were used because the number of data collected in separate episodes differed. For each group of patients (episodes), defined by the above criteria, three median values were calculated. M_{total} was the median of all the TNF-α levels determined in all the episodes in the group. $M_{maximum}$ was the median of the highest TNF-α values in the individual episodes included in the group. $M_{episode}$ was the median of the series composed of the median values calculated for the individual episodes included in the group. M_{total} is thus based on all values of all episodes of one group, and in this calculation episodes with many TNF-α values have more importance than episodes with less values. The advantage of $M_{maximum}$ and $M_{episode}$ is that every episode becomes equally important; however, groups might be composed of different numbers of episodes. The data were analyzed exploratively and descriptively using the *F*-test and the Mann-Whitney rank sum test, because there was no standard distribution of the data. The false probability 'α' was 5%. A probability of $p<0.05$ showed a significant difference between two groups.

RESULTS

Thirteen (22.4%) of 58 patients died and 13 of 63 episodes (20.6%) had a fatal outcome. Nine patients died during the study period; four patients died within 10 days after the last blood sample for TNF-α measurement had been taken. M_{total} of the non-survivors was significantly higher than M_{total} of the survivors (Table 1). The same was true for $M_{episode}$ ($p=0.032$). There was no significant difference with respect to $M_{maximum}$ ($p=0.056$).

In 29 episodes (46.0%) the infection was microbiologically confirmed. Blood cultures yielded Gram-positive bacteria in 13 episodes and Gram-negative bacteria in nine. In five episodes *Plasmodium falciparum* was found in the blood smear, and in two episodes *Aspergillus fumigatus* at post-mortem examinations. Figure 1 shows M_{total} calculated for each individual day ($M_{total(day)}$) from day 1 to day 13, for MDI and for MNDI. $M_{total(day)}$ was greater than 53.5 pg/mL only on days 1 and 5, and only on these two days was the difference between MDI and MNDI greater than 16.5 pg/mL. Neither MDI nor MNDI $M_{total(day)}$ was consistently the higher on the various days. There was no significant difference between MDI and MNDI with respect to M_{total} (for the whole investigation; Table 1), $M_{maximum}$ or $M_{episode}$ ($p>0.05$, in each case). However, M_{total} of the episodes with Gram-negative bacteria was significantly higher than M_{total} when Gram-positive bacteria were found (Table 1). There was also a just significant difference with respect to $M_{episode}$ ($p=0.049$) but not $M_{maximum}$ ($p=0.217$).

In 29 episodes (46.0%) the patients were immunocompromised: 14 patients, acute myeloid leukemia (AML) (16 episodes); five patients, acquired immuno-

Table 1 Episodes, number of samples and median of TNF-α levels in pg/mL (M_{total}) with minimum and maximum and p-value with respect to survivors and non-survivors, microbiological findings and underlying disease

	Episodes	Number of samples	Minimum	Maximum	M_{total}	p-value
Survivors	50	188	0.0	587.3	30.3	0.0001
Non-survivors	13	53	16.0	554.9	55.3	
MDI	29	110	0.2	242.2	37.3	0.724
MNDI	34	131	0.0	587.3	42.2	
Gram-positive bacteria	13	53	0.2	242.2	23.7	0.002
Gram-negative bacteria	9	30	0.5	222.9	48.4	
Primary diagnosis sepsis	7	26	0.0	225.0	42.8	0.018
Falciparum malaria	5	20	22.9	153.9	78.6	
AIDS	5	21	41.2	479.0	88.8	0.0001
AML	16	58	0.2	554.9	16.8	

AIDS = acquired immunodeficiency syndrome; AML = acute myeloid leukemia.
MDI = microbiologically documented infection; MNDI = microbiologically non-documented infection.

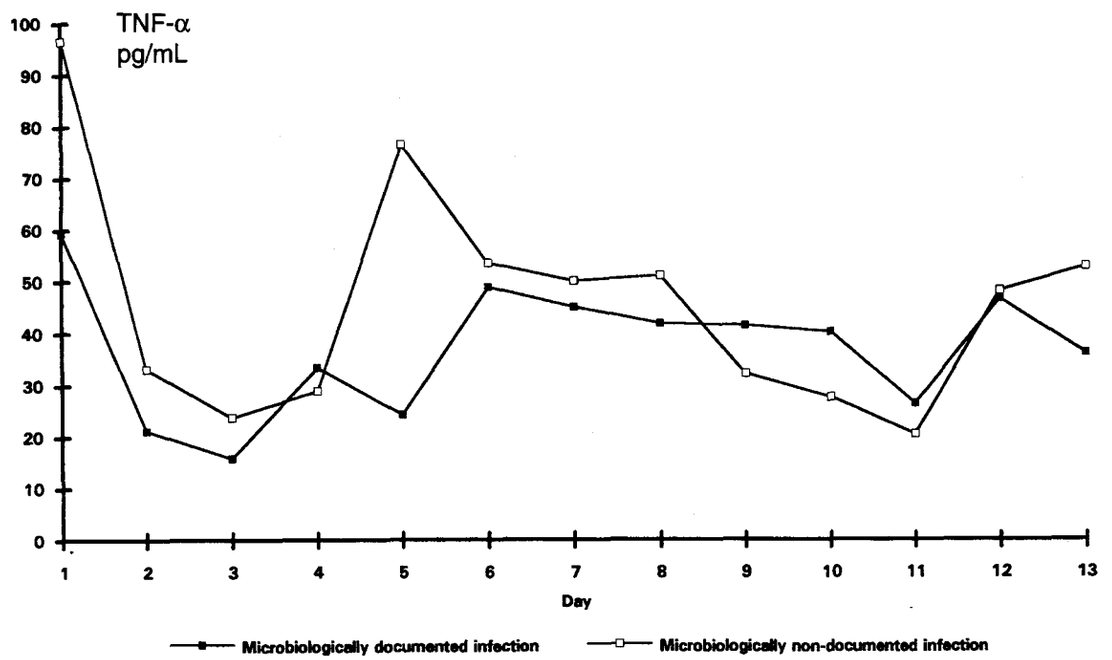

Figure 1 Median of TNF-α levels in pg/mL ($M_{total(day)}$) with respect to microbiologically documented and non-documented infections from day 1 to day 13.

deficiency syndrome (AIDS); two patients, Hodgkin's disease (three episodes); two patients, myelodysplastic syndrome; two patients, non-Hodgkin lymphoma; and one patient, chronic lymphoproliferative leukemia. In 34 episodes (54.0%) the patients were immunocompetent. The primary diagnoses were as follows: seven patients, sepsis; five patients, falciparum malaria; three patients, pneumonia (four episodes); one patient, tuberculosis (two episodes); three patients, pancreatitis; three patients, gastrointestinal disorders (two diverticulitis of the sigmoid, one ulcerative colitis), four patients, cardiac disorders (one endocarditis, two

aortocoronary vein bypass, one empyema of the pericardium); and six patients, other underlying diseases (bronchial asthma, chronic obstructive lung disease, hemolysis, angiosarcoma, terminal renal failure, hemolytic uremic syndrome). M_{total} of the immunocompetent patients was significantly higher than M_{total} of the immunocompromised patients. With regard to $M_{maximum}$ and $M_{episode}$ of TNF-α levels, there was no significant difference ($p>0.05$).

Table 2 lists the documented microorganisms according to the immune status of the patients. In 17 episodes in both groups the infection was not documented microbiologically. Twelve of 29 infections in the immunocompromised, as against 17 of 34 in the immunocompetent, were microbiologically proven (Table 2). M_{total} values of TNF-α levels according to immune status and microbiological findings are given in Table 3. M_{total} of the immunocompromised patients with MNDI was significantly higher than M_{total} of those with MDI. This difference was not seen in the immunocompetent patients. However, M_{total} of the TNF-α levels in the immunocompetent patients with MDI was significantly higher than that in the immunocompromised patients with MDI, whereas in patients with MNDI no significant difference was seen. A significant difference was found between Gram-positive bacteria and Gram-negative bacteria in the case of immunocompromised patients, and between immunocompromised and immunocompetent patients with respect to Gram-positive bacteria. Because of the small number of patients in each group (Gram-negative or Gram-positive), the statistical strength was reduced and $M_{maximum}$ and $M_{episode}$ of the TNF-α levels were not calculated. In the other cases there was no significant difference with regard to $M_{maximum}$ and $M_{episode}$, except in MDI. For those cases, there was a higher $M_{episode}$ among the immunocompetent patients ($M_{episode}$=57.7 pg/mL TNF-α) than among the immunocompromised patients ($M_{episode}$=17.6 pg/mL TNF-α) (p=0.021).

There was a significant difference between patients with AIDS and those with AML, and a significant difference between patients with a primary diagnosis of sepsis and patients with falciparum malaria, with respect to M_{total} (Table 1).

Table 2 Microbiological findings according to the immune status of the patients

Microorganism	Immuno-competent patients	Immuno-compromised patients
Staphylococcus aureus[a]	3	3
Streptococcus mitis[a]	0	3
Streptococcus pneumoniae[a]	1	0
Streptococcus group B[a]	1	0
Streptococcus viridans[a]	0	1
Enterococcus faecalis[a]	0	1
Escherichia coli[a]	3	3
Stenotrophomonas maltophilia[a]	1	0
Pseudomonas aeruginosa[a]	1	0
Bacteroides fragilis[a]	1	0
Aspergillus fumigatus[b]	1	1
Plasmodium falciparum[c]	5	0
Subtotal	17	12
Microbiologically non-documented infections	17	17
Total	34	29

[a]Blood culture; [b]post-mortem examination; [c]blood smear.

Table 3 Episodes, number of samples and median of TNF-α levels in pg/mL (M_{total}) with minimum and maximum and p-value with respect to immune status and microbiological findings

	Episodes	Number of samples	Minimum	Maximum	M_{total}
Immunocompetent	34	130	0.0	587.3	45.5[a]
MNDI	17	65	0.0	587.3	42.2[b]
MDI	17	65	2.7	242.2	46.8[c]
Gram-positive bacteria	5	20	20.0	242.2	44.5[d]
Gram-negative bacteria	6	21	3.8	199.5	46.8[e]
Immunocompromised	29	111	0.2	554.9	30.3[f]
Gram-negative bacteria	3	9	0.5	222.9	94.4[g]
Gram-positive bacteria	8	33	0.2	130.2	15.7[h]
MDI	12	45	0.2	222.9	19.9[i]
MNDI	17	66	0.3	554.9	44.7[k]

Additional statistical comparisons:
a versus f: p=0.014; b versus c: p=0.232; b versus k: p=0.980; c versus i: p=0.0001; d versus e: p=0.540; d versus k: p=0.0001; e versus f: p=0.619; g versus h: p=0.034; i versus k: p=0.032.
MDI=microbiologically documented infection including *Aspergillus fumigatus* and *Plasmodium falciparum*. MNDI=microbiologically non-documented infection.

DISCUSSION

Four lines of evidence support the suggestion that TNF-α is a pivotal mediator of sepsis and septic shock. TNF-α appears in the blood circulation within minutes in experimental sepsis and endotoxemia in humans and laboratory animals [7–11,13]. TNF-α is detected in the serum or cerebrospinal fluid of patients with sepsis and with septic shock, and correlated with the severity of the sepsis or the outcome in some studies [11,18–38]. Infusion of TNF-α into animals and humans resulted in symptoms and pathologic findings similar to those seen in sepsis or septic shock [6,7,14–17]. Administration of antibodies against TNF-α in animal models of septic shock protected the animals against metabolic derangements or death [35,40]. In our study, 13 of 58 patients (22.4%) died and 13 of 63 episodes (20.6%) had a fatal outcome. A similar death rate has been reported by Bone et al. in the sepsis syndrome [41,42].

Some studies have shown a correlation between TNF-α serum levels and severity of infection, sepsis or septic shock. Waage et al. examined serum samples from 79 patients with meningococcal meningitis, septicemia, or both [20]. TNF-α was detected in samples from 10 of 11 patients who died but in only eight of 68 survivors. All patients (five) with TNF-α levels over 100 pg/mL died. In addition, however, some patients with lower levels died, while some patients with higher levels (but lower than 100 pg/mL) survived. Girardin et al. reported a direct correlation between serum TNF-α concentrations and mortality rate in childhood infectious purpura [30]. At a TNF-α serum concentration over 500 pg/mL, the mortality rate was high. On the day of sepsis onset, Offner et al. found significantly higher TNF levels in patients who died within 24 h than in survivors or in non-survivors who died after 24 h [38]. Cannon et al. demonstrated a positive correlation between TNF-α level and Apache II score in patients with septic shock [11]. However, in the same study, similar or higher TNF-α levels were found in healthy volunteers after endotoxin infusion, without the development of life-threatening symptoms. Calandra et al. showed that TNF-α levels were associated with the outcome of patients in septic shock [22]. However, the combination of the severity of the underlying disease, the age of the patient, the documentation of infection, the urine output and the arterial pH contributed more significantly to prediction of outcome than serum levels of TNF-α. Debets et al. found that detection of TNF-α and the patient's outcome were correlated [29]. TNF-α was detected in 11 of 43 patients with sepsis. Eight of these 11 patients died, while only 11 of 32 patients without a detectable TNF-α level died. There was, however, a great range in TNF-α levels (10–100 pg/mL). In addition, Hammerle et al. and Marks et al. reported a correlation of TNF-α level and severity of sepsis or septic shock, and detection of TNF-α discriminated between sepsis or septic shock and shock from other cause [24,27]. According to Dofferhoff et al., TNF-α levels can discriminate between sepsis and non-sepsis, but they found no correlation between TNF-α and Apache II score, whereas interleukin-6 (IL-6) was correlated with the Apache II score [23]. Fisher et al. reported that IL-6, but not TNF-α, predicted a fatal outcome in patients with sepsis or septic shock, but neither TNF-α nor IL-6 correlated with Apache II score [31]. However, TNF-α levels were higher in patients with septic shock than in those without septic shock. Casey et al. found a correlation between IL-6 and outcome in patients with sepsis syndrome but not between TNF-α concentrations and outcome [21]. The combination of interleukin-1β, IL-6, TNF-α and endotoxin level (lipopolysaccharide–cytokine scoring) correlated more significantly with the patient's outcome. According to a study of Pinsky et al., there was no correlation between maximum TNF-α level and outcome, but a discrimination between septic shock and non-septic shock was possible [25]. de Groote et al. did not find a correlation between TNF-α level and the severity of sepsis [37]. Indeed, TNF-α was detected in only 14 samples from six patients among 188 samples from 38 patients with sepsis. Similarly, Harris et al. did not find a correlation between TNF-α and severity of disease in children with sepsis or enterocolitis, or both [28].

In most of the studies [11,20,21,23,25,28–30], TNF-α was measured only at the beginning of the episodes or within the first 48 h after infection was diagnosed. In contrast, Calandra et al. measured TNF-α levels on day 0, day 1 and day 10 and described a decrease of TNF-α levels in survivors, in contrast to non-survivors [22]. Marks et al. found that TNF-α levels were highest in the initial samples and decreased during the subsequent 24 h [24]. In accordance with other studies, including some of those which described a correlation of TNF-α level and severity of sepsis [11,20–27,37], in our study TNF-α concentrations varied widely from patient to patient, from non-detectable up to 587.3 pg/mL. We found not only high initial TNF-α levels but also an increase in the course of sepsis. There were also episodes during which low initial levels gave place to high levels, and vice versa. Thus the TNF-α levels varied widely from patient to patient, but also widely within each individual patient episode. The interpatient heterogeneity of the TNF-α response might be explained by the findings of Stüber et al. [43]. They found in patients with severe sepsis higher circulating TNF-α concentrations as well as

higher multiple organ failure scores in patients homozygous for the allele TNFB2 compared with heterozygous (TNFB1/TNFB2) patients. In contrast to most other investigators [20,21,24,29,37], who detected TNF-α in only 16–54% of cases, we detected TNF-α in 87% of the samples. In only four patients was TNF-α not detected at any time during the whole episode. Calandra et al. detected TNF-α in 79% of the patients, Dofferhoff et al. in 94% and Girardin et al. in 91% [22,23,30]. In our study the non-survivors had a significantly higher M_{total} of TNF-α levels than the survivors ($p=0.0001$), but there was not an unequivocal correlation between TNF-α level and outcome or severity of the infection, as described in other studies [11,20,22,24,26,27,29,30,38]. There was also a positive correlation with regard to $M_{episode}$ ($p=0.032$), but not with regard to $M_{maximum}$ ($p=0.056$). Pinsky et al. reported a positive correlation of TNF-α levels with respect to all measured values at 0, 1, 2, 4, 12, 24 and 48 h but not to the peak levels [25]. In a longitudinal study of Munoz et al., initial or maximum levels of TNF-α among surviving and non-surviving septic patients were not statistically different, whereas IL-6 plasma levels (initial as well as maximum) correlated with outcome [44]. Baud et al. described no correlation between serum TNF-α levels in patients with septic shock on admission and outcome. However, persistent high TNF-α serum levels during the initial 3 days of hospitalization were always associated with fatal outcome, whereas there was a progressive decline of its concentration in all but one of the patients who survived [45]. Hammerle et al. found an increase of TNF-α levels in patients with sepsis 3 days before multiple organ failure occurred [27]. In some cases we also detected high TNF-α levels before patients died. However, the interpatient and intrapatient variability in our study reduced not only the statistical strength of most comparisons but also the prognostic value of TNF-α.

Several factors could potentially confound the comparison of the TNF-α levels: the lack of standardization of the assays used in the different studies, the short half-life of TNF-α, the time at which the samples were collected relative to the onset of sepsis, the time delay before the aliquots were processed, the temperature at which the samples were stored, the effects of circulating soluble TNF-α receptors, or the addition of, for example, heparin or EDTA [21,46–50].

In accordance with other studies [41,42,51] with similar inclusion criteria, in our study 46% of the episodes were microbiologically documented. With respect to M_{total}, there was no significant difference of TNF-α levels between microbiologically documented versus non-documented episodes ($p=0.724$), but there was between Gram-positive and Gram-negative bacterial causes ($p=0.002$). This difference was in agreement with other studies [21,31] which did not show similarly high values in the patients with microbiologically non-documented infection. Other authors [24–26,28] did not report different TNF-α levels in microbiologically proven and unproven sepsis.

As reported by other authors, we found high TNF-α levels in falciparum malaria [52–56], and also [33,57, 58] high TNF-α levels in patients with AIDS. A direct correlation between TNF-α and severity of disease in patients with HIV infection has been reported [57]. In our study, lower levels of TNF-α, with regard to M_{total}, were found in patients with AML; some patients, however, had very high levels. Only a few reports are available on the relevance of TNF-α in patients with leukemia, especially AML [59–63]. Foa et al. reported that TNF-α plays an important role in the pathogenesis of the pancytopenia commonly associated with hairy cell leukemia [62].

The results of the present study cannot be compared directly with the published data, because of vast differences in the inclusion criteria and the time period during which TNF-α levels were monitored. The influence of the infecting organism on TNF-α kinetics seems to be less pronounced than that of the underlying disease. The role of TNF-α and its prognostic value in severe sepsis or septic shock remains to be defined.

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