Initial levels of organ failure, microbial findings and mortality in intensive care-treated primary, secondary and tertiary sepsis

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ABSTRACT

Objective: Analysis of whether patients with primary, secondary and tertiary sepsis, defined by the presence or absence of recent systemic inflammation-inducing events before the onset of sepsis, differ in clinical presentation, microbiological test results, treatment received and outcome.

Design, setting and participants: A retrospective observational study in a single, general intensive care unit, of all patients treated for severe sepsis or septic shock from 2006 to 2011. Patients with haematological malignancies, with immunosuppressive diseases or being treated with immunosuppressive drugs were excluded.

Interventions: None.

Main outcome measures: Sequential Organ Failure Assessment score, incidence of organ failure, microbiological results of blood cultures and mortality.

Results: We included 213 patients, who were classified as having primary ($n = 121$), secondary ($n = 65$) or tertiary sepsis ($n = 27$). The groups differed significantly in SOFA score, the incidence of kidney failure and coagulation failure at onset of sepsis in the ICU, as well as in blood culture findings. No differences in 7-day or 28-day mortality were seen, but the time of death occurred earlier among non-survivors in the primary sepsis group.

Conclusions: Inflammatory insults before the onset of sepsis affect the clinical picture, blood microbial findings, and in non-survivors, the time of death. These results could, if validated in a prospective study, form a basis for a novel and simple strategy for stratifying patients in clinical studies for immunomodulation therapies in sepsis.

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Sepsis is one of the leading causes of death in the intensive care unit, with mortality rates approximating 20%.[1,2] Symptoms of sepsis are caused by systemic inflammatory reactions in response to the invading microorganisms.[3] This systemic inflammatory response syndrome (SIRS) may also be triggered by non-infectious stimuli.[4] After acute sepsis-induced and SIRS-induced inflammation, an anti-inflammatory response may ensue, which results in immunosuppression that, in its more advanced stages, has been called immunoparalysis.[5,6]

One major area of interest in sepsis research has been immunomodulation therapies, such as corticosteroids, antiendotoxin antibodies, cytokine antagonists and activated protein C. Earlier studies showed reduced mortality[7-9] that was not reproduced in subsequent larger randomised trials.[10-13]

Interventional research in patients with severe sepsis and septic shock is inherently difficult for many reasons. Among these, patient heterogeneity and problems with enrolment, due to the acuity of the conditions involved, represent special problems.[14] Efforts to classify septic patients in order to reduce the heterogeneity and predict outcome, including the predisposition, infection, response and organ failure (PIRO) classification,[15] have not resulted in functional tools to guide immunomodulation therapy.

Evidence has mounted that proinflammatory and anti-inflammatory systems are activated concurrently with a net result of an initial proinflammatory response followed by a predominantly hypoinflammatory one.[5,16] The existence of inflammatory-induced immunosuppression leads to a largely unexplored hypothesis that the activity of the innate immune system before the onset of sepsis may influence the clinical presentation and course of a "second hit". Most experimental sepsis models use animals unexposed to disease or inflammatory stimuli before the septic challenge, but there are a limited number of experimental models in which sepsis is induced as a second hit and in which signs and symptoms have been shown to be attenuated.[17,18]

Clinically, it seems easy to identify at least three septic patient phenotypes: patients who were healthy before the onset of sepsis (primary sepsis), patients in whom sepsis occurs as a second hit (secondary sepsis) and patients with sepsis after multiple hits or prolonged critical illness (tertiary sepsis). Only a few publications describe the inflammatory response and outcome in these patient phenotypes.[19-21] In these studies, no comparison between the different phenotypes and their clinical characteristics and courses was made.

Table 1. Criteria* for systemic inflammatory response syndrome, sepsis, severe sepsis and septic shock used by the intensive care doctors

Systemic inflammatory response syndrome (SIRS)

At least two of the following criteria:

- heart rate > 90 beats/min
- respiratory rate > 20 breaths/min, or $PaCO_2$ < 4.3 kPa
- total leukocyte count < 4×10^9/L, or > 12×10^9/L, or > 10% immature forms
- body temperature > 38°C or < 36°C.

Sepsis

SIRS + probable infection or verified infection.

Severe sepsis

Sepsis + one of the following criteria:

- hypotension (systolic blood pressure < 90 mmHg or mean arterial pressure < 70 mmHg)
- hypoperfusion
- one sepsis-related organ dysfunction.

Septic shock

Severe sepsis + persistent hypotension and signs of hypoperfusion or organ dysfunction, despite adequate hydration.

Criteria for hypoperfusion or organ dysfunction include:

- hypoperfusion: P-lactate > 3 mmol/L or > 1 mmol/L above upper reference value
- kidney dysfunction: diuresis < 0.5 mL/kg/h for 2 h despite adequate hydration
- respiratory dysfunction: PaO_2/FiO_2 < 250 mmHg or < 200 mmHg if lung is the site of infection
- Central nervous system dysfunction: acute change of mental status, eg, confusion
- liver dysfunction: increase in bilirubin level to > 45 μmol/L above normal value
- haematological dysfunction: decrease in platelet level to < 100×10^9/L, or international normalised ratio > 1.5, or activated partial thromboplastin time > 60 s.

* As listed in the guidelines of the Swedish Intensive Care Registry.

Table 2. Criteria for primary, secondary and tertiary sepsis*

Primary sepsis

No history of:

- trauma or surgery within the past 30 days, or
- antibiotic-treated infection within the past 30 days, or
- active systemic inflammatory disease.

Secondary sepsis

One of:

- recent (< 7 days) trauma or surgery
- recent (< 7 days) antibiotic-treated infection
- active systemic inflammatory disease.

Tertiary sepsis

- > 1 criterion for secondary sepsis within the past 10 days (minimum of 24 hours between insults).

* Patients not fulfilling criteria for any group were to be categorised as unclassifiable; patients with a haematological malignancy, immunosuppressive disease, or ongoing or recent treatment with immunosuppressive drugs were excluded.

The aim of our pilot study was to retrospectively study whether there were differences in clinical presentation, microbiological test results, treatment received and outcome in patients with severe sepsis or septic shock. We categorised severe sepsis and septic shock into primary, secondary or tertiary sepsis, based on the presence or absence of recent systemic inflammation-inducing insults. We defined "recent" as during the 30 days before the onset of sepsis.

Materials and methods

Hospital and settings

We performed our study in a six-bed ICU admitting about 600 patients per year. The unit is located in a 400-bed county hospital in central Sweden. Patients in need of neurosurgical, thoracic or paediatric intensive care are referred to a tertiary university hospital. Our study was approved by the regional ethics review board in Stockholm, Sweden (permit 2011/2022-31/1).

Patients

All patients over the age of 18 years admitted to the ICU from 1 January 2006 to 31 December 2011, and diagnosed with severe sepsis or septic shock during their ICU stay, were evaluated for inclusion in the study. Exclusion criteria were haematological malignancy affecting leukocytes, immunosuppressive disease or ongoing treatment with immunosuppressive or cytostatic drugs within the past 30 days. We applied the international consensus criteria listed in Table 1 for the diagnosis of severe sepsis and septic shock.[22]

Definitions of primary, secondary and tertiary sepsis

The definitions of the groups into which the patients were categorised as primary (PRIM), secondary (SEC) and tertiary (TERT) sepsis are shown in Table 2. Before the review of patient records, category criteria were elaborated by the study group until consensus over the criteria was reached. Patients with an onset of severe sepsis 7 to 30 days after a systemic inflammatory response-induced event were categorised as unclassifiable. A poststudy intention was to separately analyse the group affiliation of these patients before designing a prospective study.

Data collection

The data we extracted from the charts included patient characteristics, medical diagnoses, admission and discharge dates, antimicrobial treatments and outcomes. We also

Table 3. Patient characteristics and comorbidities, APACHE II and SOFA scores, LOS, duration of antibiotic therapy and outcome for the patients with primary (PRIM), secondary (SEC) and tertiary (TERT) sepsis

Characteristics and comorbidities	All patients	PRIM	SEC	TERT	*P**
Women, *n* (%)	83 (39%)	46 (38%)	21 (32%)	16 (59%)	–
Median age, years (IQR)	68 (55–75)	68 (56–75)	70 (57–81)	68 (45–74)	0.52
Median bodyweight, kg (IQR)	77 (67–90)	77 (67–88)	77 (68 –90)	76 (67–94)	0.87
Median APACHE II score (IQR)	18 (14–23)	18 (14–24)	16 (14–21)	17 (12–24)	0.24
Diabetes, *n* (%)	45 (21%)	28 (23%)	12 (19%)	5 (19%)	0.71
Cardiovascular disease, *n* (%)	110 (52%)	65 (54%)	34 (52%)	11 (41%)	0.47
Pulmonary disease, *n* (%)	36 (17%)	26 (21%)	8 (12%)	2 (8%)	0.11
Median Day 1 SOFA score (IQR)	7 (4–9)	7 (4–10)	6 (4–9)	5 (3–8)	0.04
Median Day 2 SOFA score (IQR)	4 (0–8)	4 (1–8)	4 (0–8)	2 (0–6)	0.28
Median Day 3 SOFA score (IQR)	2 (0–6)	2 (0–6)	3 (0–7)	0 (0–4)	0.19
Median ICU LOS, days (IQR)	2 (1–7)	2 (1–5)	3 (1–10)	2 (1–9)	0.15
Median hospital LOS, days (IQR)	17 (6–42)	13 (4–34)	17 (8–42)	51 (19–89)	< 0.001
Median duration of antimicrobial therapy, days (IQR)	15 (8–29)	14 (4–23)	16 (11–31)	25 (17–59)	< 0.001
Non-survivors, Day 7, *n* (%)	46 (22%)	29 (24%)	12 (18%)	5 (19%)	0.63
Non-survivors, Day 28, *n* (%)	62 (29%)	33 (28%)	21 (32%)	8 (30%)	0.77
Deaths, Days 8–28, *n* (%)	16 (8%)	4 (3%)	9 (14%)	3 (11%)	0.03
Median survival time of non-survivors, days (IQR)	3 (1–9)	2 (0–4)	6 (1–12)	6 (1–14)	0.04
Total (%)	*213*	*121 (57%)*	*65 (31%)*	*27 (13%)*	–

APACHE = Acute Physiology and Chronic Health Evaluation. SOFA = sequential organ failure assessment. LOS = length of stay. IQR = interquartile range. * Using Kruskall–Wallis and χ^2 tests, where appropriate, to analyse differences between PRIM, SEC and TERT groups.

gathered data on vital signs, laboratory parameters, results from blood cultures, ventilator treatment and vasoactive therapy during the first 24 hours in the ICU. The Acute Physiology and Chronic Health Evaluation (APACHE) II score[23] and the Sequential Organ Failure Assessment (SOFA) score[24] were calculated.

Statistics

Kruskall–Wallis, χ^2 or Fisher exact tests were used to analyse differences between groups. Differences in 7-day and 28-day survival were calculated using survival analysis and log-rank tests. We used Statistica (StatSoft) for our statistical analyses and $P < 0.05$ was considered significant. We present numerical variables as medians and interquartile ranges (IQRs).

Results

Of 3553 adult patients admitted to the ICU during the period 2006–2011, 273 were diagnosed with severe sepsis or septic shock during their stay in the ICU. We noted no differences between the groups in age or proportions of patients with diabetes, cardiovascular disease or pulmonary disease (Table 3).

Of the patients eligible for inclusion, 60 were excluded: 26 because of ongoing treatment for a haematological malignancy, seven because of ongoing cytostatic treatment for solid tumours, seven because of immunosuppressive treatment after organ transplantation, four because of immunosuppressive treatment for active systemic inflammatory disease, 15 because of corticosteroid therapy of prednisolone > 10 mg/day, and one because of acquired immunodeficiency syndrome.

Of the included 213 patients, all could be classified into the three groups PRIM ($n = 121$), SEC ($n = 65$) or TERT ($n = 27$). No patients were regarded as unclassifiable. Table 4 shows an overview of the inflammation-inducing insults in the SEC and TERT groups. An overview of suspected primary sites of infection is shown in Table 5.

Disease severity scores and length of stay

No significant differences between the groups were found for the APACHE II scores (Table 3). The groups differed in SOFA scores at sepsis onset. No differences between the groups were noted for length of stay (LOS) in the ICU. The LOS in the hospital after the onset of sepsis showed a significant difference between the groups, with the longest hospital LOS in the TERT group.

Table 4. Causes of inflammation in patients with secondary and tertiary sepsis

Cause	*n*
Secondary sepsis	
Surgery:	
Elective abdominal	14
Elective urology	5
Elective orthopaedic	4
Elective peripheral vascular	2
Elective soft tissue	1
Acute abdominal	6
Antibiotic treatment for infection in:	
Urinary tract	11
Respiratory tract	9
Skin and soft tissue	4
Gastrointestinal tract	3
Postpartum period	1
Trauma	2
Pancreatitis	3
Total	*65*
Tertiary sepsis	
Recent antibiotic treatment for infection and:	
Abdominal surgery	10
Thoracic surgery	2
Orthopaedic surgery	1
Urological surgery	3
Trauma	2
Abdominal surgery and reoperation	5
Trauma and surgery	4
Total	*27*

Table 5. Suspected primary sites of infection in patients with primary (PRIM), secondary (SEC) and tertiary (TERT) sepsis, *n* (%)

Suspected primary site	PRIM	SEC	TERT	*P**
Unknown	54 (45%)	23 (35%)	4 (15%)	0.005
Lung	30 (25%)	5 (8%)	1 (4%)	0.02
Urogenital tract	14 (12%)	11 (17%)	4 (15%)	0.34
Abdomen	15 (12%)	19 (29%)	10 (37%)	0.002
Skin or wound	4 (3%)	6 (9%)	7 (26%)	0.001
Upper respiratory tract	2 (2%)	0	0	0.46
Gastrointestinal tract	2 (2%)	1 (2%)	1 (4%)	0.54
Total	121	*65*	*27*	–

* Using χ^2 and Fisher exact tests, where appropriate, to analyse differences between PRIM, SEC and TERT groups.

Organ dysfunction

The groups did not differ in body temperature or leukocyte count (Table 6). This was also true for the duration of invasive mechanical ventilation (data not shown) and the frequency of treatment with invasive mechanical ventilation. Nor were there any differences between the groups in respiratory frequency or Pao_2/Fio_2. Treatment with vasoactive drugs and mean arterial pressure did not differ between the groups. The groups showed a significant difference in haemoglobin concentration, with the highest values in the PRIM group.

The incidence of kidney failure at the onset of sepsis varied significantly between the groups, a difference that was mainly caused by the differences in plasma creatinine, with the highest values observed in the PRIM group. Urine output did not differ between the groups.

Ten patients fulfilled the SOFA criteria for liver failure at the onset of sepsis with no differences between the groups.

The incidence of coagulation failure at the onset of sepsis differed between the groups, as did platelet count, with the lowest values in the PRIM group.

Microbiological cultures and antimicrobial therapy

Blood cultures were obtained from 193 patients, and 56% of the cultures were positive (Table 7). The frequency of bacteraemia was higher in the PRIM group than in the SEC and TERT groups. The rates of gram-negative and gram-positive bacteraemia were lower in the TERT group than in the PRIM and SEC groups, but the opposite was evident for the occurrence of candidaemia. The most common microbes in the blood from the patients in the PRIM and SEC groups were Streptococcus spp, *Staphylococcus aureus* and *Escherichia coli,* together representing 77% (PRIM group) and 69% (SEC group), respectively, of all positive blood cultures in the groups. The most common microbes in the TERT group were Candida spp, representing 64% of all positive blood cultures. Coagulase-negative Staphylococci and Enterococci, ie, low-virulent bacteria, were represented in 4%, 14% and 27% of all positive blood cultures in the PRIM, SEC and TERT groups, respectively. In all five patients in the PRIM and SEC groups with growth of Enterococci, the bacteria grew together with more virulent gram-negative bacteria.

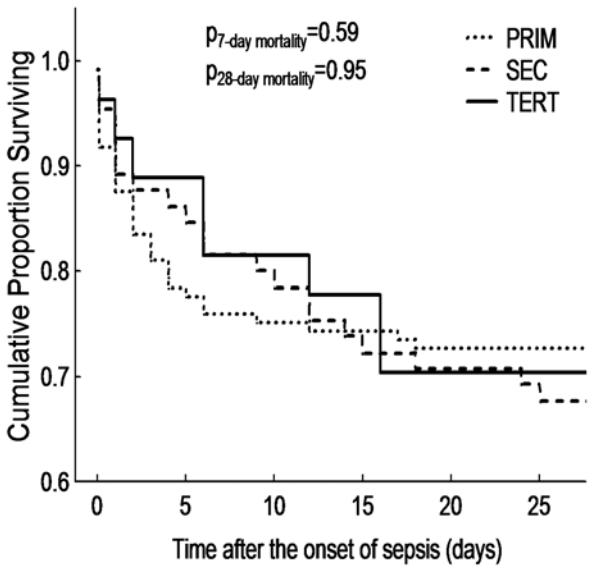
The duration of antimicrobial therapy differed significantly between the groups, with the longest duration in the TERT group (Table 3).

Mortality

Twenty-eight-day mortality after admission to the ICU was 29.1%; no differences were noted between the groups in 7-day or 28-day mortality (Figure 1). In the PRIM group, mortality during the first 7 days after the onset of sepsis

occurred in 88% of the non-surviving patients, but this was seen in only 57% of the SEC group and 63% of the TERT group. The median survival time among the patients who died was significantly shorter in the PRIM group compared with the other groups (Table 3).

Figure 1. Kaplan–Meier curves for cumulative proportion of survival over the first 28 days after admission to intensive care unit for patients classified as having primary (PRIM), secondary (SEC) and tertiary (TERT) sepsis*



* *P* values are results of log-rank tests.

Discussion

Key findings

Patients with primary sepsis had higher SOFA scores, a higher incidence of kidney failure and a lower platelet count than the other groups, with the largest differences in the comparison with the patients with tertiary sepsis. The microbiological results differed greatly, with common virulent bacteria in most of the blood cultures in primary and secondary sepsis and opportunistic microbes in tertiary sepsis. In patients with tertiary sepsis, the duration of antimicrobial treatment, as well as the hospital LOS, was longer, compared with patients with primary and secondary sepsis. There were no differences between the groups in the log-rank survival analyses at Day 7 and Day 28, but the time of death differed significantly. Almost 90% of the patients who died with primary sepsis died during the first week in the ICU, but 60% of the patients who died with secondary or tertiary sepsis died during the first week.

Comparison with other studies

The more severe clinical picture in patients with primary sepsis in combination with increased mortality during the first week is consistent with the suggestion that if death occurs in the first few days of the illness, it is probably

Table 6. Frequencies of organ dysfunction and associated laboratory parameters at admission to the intensive care unit for patients with primary (PRIM), secondary (SEC) and tertiary (TERT) sepsis

Dysfunction type and parameters	PRIM	SEC	TERT	*P**
Patients with ⩾ 3 SIRS criteria, *n* (%)	73 (60%)	28 (43%)	14 (52%)	0.08
Median temperature, °C (IQR)	38.3 (37.2–39.2)	38.4 (37.1–39.3)	38.7 (37.1–39.7)	0.68
Median leukocyte count, 10^9/L (IQR)	15.8 (10.0–21.7)	12.9 (6.9–19.9)	16.4 (9.0–21.4)	0.41
Mechanically ventilated, *n* (%)	58 (48%)	31 (48%)	12 (44%)	0.91
Median respiratory rate, breaths/min (IQR)	32 (22–40)	30 (18–40)	26 (20–30)	0.22
Median Pa_{O_2}/Fi_{O_2}, mmHg (IQR)	187 (116–294)	233 (128–340)	255 (126–317)	0.21
Vasopressor therapy, *n* (%)	66 (55%)	34 (52%)	11 (41%)	0.43
Median arterial pressure, mmHg (IQR)	63 (55–79)	66 (54–75)	73 (58–77)	0.81
Median haemoglobin level, g/L (IQR)	117 (105–132)	112 (98–123)	95 (90–114)	< 0.001
Kidney failure (SOFA), *n* (%)	35 (29%)	13 (20%)	2 (7%)	0.04
Median urine output, mL/day (IQR)	1220 (190–2185)	605 (95–1245)	1400 (435–2165)	0.58
Median creatinine level, µmol/L (IQR)	199 (95–336)	145 (94–275)	95 (56–181)	0.002
Liver failure (SOFA), *n* (%)	4 (3%)	4 (6%)	2 (7%)	0.45
Median bilirubin level, µkat/L (IQR)	14 (9–23)	17 (9–31)	11 (6–21)	0.45
Coagulation failure (SOFA), *n* (%)	21 (17%)	4 (6%)	1 (4%)	0.05
Median platelet count, 10^9/L (IQR)	168 (89–258)	222 (138–291)	274 (127–499)	0.01

SIRS = systemic inflammatory response syndrome. IQR = interquartile range. SOFA = sequential organ failure assessment. * Using Kruskall–Wallis or χ^2 tests, where appropriate, to analyse differences between PRIM, SEC and TERT groups.

Table 7. Results of blood cultures at admission to the intensive care unit for patients with primary (PRIM), secondary (SEC) and tertiary (TERT) sepsis*

Blood culture result (*n*)	PRIM	SEC	TERT	*P*†
Blood not taken or sample not registered (20)	14	5	1	–
Patients from whom blood cultures were obtained (193)	107	60	26	–
Positive blood cultures (108/193; 56%)	68 (64%)	29 (48%)	11 (42%)	0.05
Gram-negative species (49/193; 25%)	30 (28%)	18 (30%)	1 (4%)	0.03
Escherichia coli	17	10	–	–
Klebsiella spp	5	3	–	–
Haemophilus influenzae	2	–	–	–
Proteus spp	2	–	1	–
Pseudomonas aeruginosa	1	–	–	–
Serratia marcecens	1	–	–	–
Neisseria meningitidis	1	–	–	–
Bacteroides fragilis	1	3	–	–
Enterobacter spp	–	2	–	–
Gram-positive species (60/193; 31%)	41 (38%)	14 (23%)	5 (19%)	0.05
Streptococcus pneumoniae	10	1	–	–
Streptococci group A	5	4	1	–
Alpha streptococci	2	2	–	–
Streptococci group B	1	–	–	–
Staphylococcus aureus	18	3	1	–
Coagulase-negative staphylococci	–	2	2	–
Clostridium perfringens	2	–	–	–
Enterococcus spp	3	2	1	–
Fungi (7/193; 4%)	0	0	7 (27%)	<0.001
Candida albicans	–	–	5	–
Candida glabrata	–	–	2	–
Total (213)‡	*121*	*65*	*27*	–

* Values are given as *n* (% of the cultures obtained) within each group. † Values are results of χ^2 tests analysing differences between the PRIM, SEC and TERT groups. ‡ The sum of all microbes found in the blood cultures is more than the sum of the positive cultures because of growth of multiple agents in eight patients; in the PRIM group, three patients had growth of enterococci together with another microbe (one case each of *E. coli*, Proteus spp and Klebsiella spp); in the SEC group, three patients had growth of multiple agents (Enterobacter spp together with enterococci in two patients, and *E. coli* together with Klebsiella spp in one patient); in the TERT group, two patients had growth of multiple agents (*Candida glabrata* together with *Enterococcus faecalis* in one patient, and (*Candida albicans* together with coagulase-negative staphylococci in one patient).

caused by an uncontrolled hyperimmune response.[25] The frequent microbiological finding of opportunistic agents in patients with tertiary sepsis underscores the presence of an anti-inflammatory state, which accords with Kalb and colleagues, who reported that the typical clinical presentation of sepsis in patients with prolonged critical illness was discrete and often caused by opportunistic agents.[26] A lower inflammatory response at repeated hits has been seen in experimental studies[17,27] and, clinically, the results of our study are supported by those of Charles and colleagues who found lower levels of procalcitonin in patients with bloodstream infections in the presence of a previous episode of sepsis.[20] Even if the time death suggests different mechanisms, 28-day all-cause mortality in the SEC and TERT groups was the same as that in the PRIM group. Unresolved infection has been shown to be a common finding in patients dying from sepsis in the ICU.[28] It may be thought that some of the mortality in primary sepsis might be caused by a hyperimmune response-induced organ dysfunction, but mortality in secondary and tertiary sepsis might in part be the result of an unresolved infection, subsequently caused by a low-grade or insufficient host defence against invading pathogens.

Limitations

The major weakness in our study is the design, being a retrospective, single-centre study with a limited number of patients. These limitations are exemplified by the large

percentage of patients with an unknown source of infection as well as the somewhat surprising lack of patients with an onset of severe sepsis in the interval of 7 to 30 days after an inflammation-inducing event. No comparison of inflammatory mediators was possible, further reducing the applicability of our study, which in this context should be considered as a base for future studies. Our results are also influenced by the elaboration of the criteria, but to minimise that, we elaborated the criteria before the data were collected. Also, the use of SIRS criteria in the selection of patients with sepsis has recently been shown to exclude a large number of patients with infection and organ failure.[29] Another limitation is the non-homogeneous feature within the groups. For example, subclinical inflammation may persist for more than 30 days after an infection, thus contributing to heterogeneity in the primary sepsis group.[30]

In our study, patients with haematological malignancies or immunosuppressive diseases and those receiving immunosuppressive treatment or cytostatic drugs were excluded, but these patients are often excluded in clinical trials evaluating the effect of immunomodulation in severe sepsis and septic shock.[10,11,13,31,32]

Implications

The different clinical phenotypes represented by primary, secondary and tertiary sepsis are realities of everyday life in the ICU. The most obvious differences in presentation between the groups were in the incidence of kidney failure, the platelet count and the haemoglobin level. Similarly (although not significant) the differences in respiratory rate, Pa_{O_2}/Fi_{O_2}, vasopressor requirement, blood pressure and bilirubin all share the same picture, with the most affected patients belonging to the primary sepsis group. One should recognise that these differences occur even though the patients in the SEC and TERT groups had suffered systemic inflammation before the current septic episode, and probably had laboratory values which were affected to a greater extent before the onset of sepsis than the patients in the PRIM group.

Our study shows the heterogeneity in patients fulfilling the criteria for severe sepsis or septic shock. In the clinical trials investigating the efficacy of immunomodulatory therapies in sepsis, patients were not stratified based on their immunological state.[10,12,26] The lack of analyses of immunological state in immunomodulation trials has been severely criticised.[33] An example that might show the importance of the sepsis phenotypes is a comparison between two clinical trials that investigated the effect of an interleukin-1 receptor antagonist in severe sepsis and in which the microbiological results were reported in more detail. The first Phase II trial showed a significant reduction in mortality,[9] but this was not observed in the subsequent Phase III trial.[13] In the Phase III trial, the most common microbes were Candida spp, coagulase-negative staphylococci and enterococci, isolated in 24%, 23% and 19% of blood cultures, respectively, which was more than twice as often as in the Phase II trial in which more virulent bacteria were isolated. When comparing the microbiological results with our present study, it might be speculated that a higher number of patients with tertiary sepsis were included in the Phase III trial. It might further be hypothesised that patients with a presumed critical illness-induced immunosuppression will have no effect, a low effect or even an adverse effect from an anti-inflammatory therapy, and consequently, that patients with a presumed tertiary sepsis might have contributed to the negative result of the Phase III trial.

Future research

To our knowledge, our study is the first to clinically identify different patient phenotypes at the onset of sepsis, based on an assumption of the inflammatory response. Our findings must be validated and further elaborated on in prospective multicentre studies, in which characterisation of the inflammatory response is pivotal. Such a study is underway, and if it reproduces the results of this study, a new and relatively simple approach might be available for categorising patients in clinical trials, and possibly also in future clinical practice, before the consideration of immunomodulation therapy in patients with severe sepsis or septic shock.

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Competing interests

None declared.

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References

1 Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; 348: 1546-54.

2 Kaukonen KM, Bailey M, Suzuki S, et al. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000–2012. *JAMA* 2014; 311: 1308-16.

3 Lever A, Mackenzie I. Sepsis: definition, epidemiology, and diagnosis. *BMJ* 2007; 335: 879-83.

4 Castellheim A, Brekke O-L, Espevik T, et al. Innate immune responses to danger signals in systemic inflammatory response syndrome and sepsis. *Scand J Immunol* 2009; 69: 479-91.

5 Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. *Lancet Infect Dis* 2013; 13: 260-8.

6 Van der Poll T, Meijers JCM. Systemic inflammatory response syndrome and compensatory anti-inflammatory response syndrome in sepsis. *J Innate Immun* 2010; 2: 379-80.

7 Schumer W. Steroids in the treatment of clinical septic shock. *Ann Surg* 1976; 184: 333-41.

8 Ziegler EJ, Fisher CJ Jr, Sprung CL, et al. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. A randomized, double-blind, placebo-controlled trial. The HA-1A Sepsis Study Group. *N Engl J Med* 1991; 324: 429-36.

9 Fisher CJ Jr, Slotman GJ, Opal SM, et al. Initial evaluation of human recombinant interleukin-1 receptor antagonist in the treatment of sepsis syndrome: a randomized, open-label, placebo-controlled multicenter trial. *Crit Care Med* 1994; 22: 12-21.

10 Sprung CL, Annane D, Keh D, et al. Hydrocortisone therapy for patients with septic shock. *N Engl J Med* 2008; 358: 111-24.

11 Bernard GR, Vincent J-L, Laterre P-F, et al. Efficacy and safety of recombinant human activated protein c for severe sepsis. *N Engl J Med* 2001; 344: 699-709.

12 Ranieri VM, Thompson BT, Barie PS, et al. Drotrecogin alfa (activated) in adults with septic shock. *N Engl J Med* 2012; 366: 2055-64.

13 Fisher CJ Jr, Dhainaut JF, Opal SM, et al. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. *JAMA* 1994; 271: 1836-43.

14 Dellinger RP, Vincent J-L, Marshall J, Reinhart K. Important issues in the design and reporting of clinical trials in severe sepsis and acute lung injury. *J Crit Care* 2008; 23: 493-9.

15 de Groot B, Lameijer J, de Deckere ER, Vis A. The prognostic performance of the predisposition, infection, response and organ failure (PIRO) classification in high-risk and low-risk emergency department sepsis populations: comparison with clinical judgement and sepsis category. *Emerg Med J* 2014; 31: 292-300.

16 Bone RC. Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). *Ann Intern Med* 1996; 125: 680-7.

17 Castegren M, Lipcsey M, Söderberg E, et al. Differences in organ dysfunction in endotoxin-tolerant pigs under intensive care exposed to a second hit of endotoxin. *Shock* 2012; 37: 501-10.

18 Gierer P, Hoffmann JN, Mahr F, et al. Sublethal trauma model with systemic endotoxemia for the study of microcirculatory disorders after the second hit. *J Surg Res* 2008; 147: 68-74.

19 Gårdlund B, Sjölin J, Nilsson A, et al. Plasma levels of cytokines in primary septic shock in humans: correlation with disease severity. *J Infect Dis* 1995; 172: 296-301.

20 Charles PE, Ladoire S, Snauwaert A, et al. Impact of previous sepsis on the accuracy of procalcitonin for the early diagnosis of blood stream infection in critically ill patients. *BMC Infect Dis* 2008; 8: 163.

21 Kox WJ, Bone RC, Krausch D, et al. Interferon gamma-1b in the treatment of compensatory anti-inflammatory response syndrome. A new approach: proof of principle. *Arch Intern Med* 1997; 157: 389-93.

22 Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; 101: 1644-55.

23 Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; 13: 818-29.

24 Moreno R, Vincent JL, Matos R, et al. The use of maximum SOFA score to quantify organ dysfunction/failure in intensive care. Results of a prospective, multicentre study. Working Group on Sepsis related Problems of the ESICM. *Intensive Care Med* 1999; 25: 686-96.

25 Skrupky LP, Kerby PW, Hotchkiss RS. Advances in the management of sepsis and in the understanding of key immunologic defects of the disorder. *Anesthesiology* 2011; 115: 1349-62.

26 Kalb TH, Lorin S. Infection in the chronically critically ill: unique risk profile in a newly defined population. *Crit Care Clin* 2002; 18: 529-52.

27 Murphey ED, Fang G, Sherwood ER. Endotoxin pretreatment improves bacterial clearance and decreases mortality in mice challenged with *Staphylococcus aureus*. *Shock* 2008; 29: 512-8.

28 Torgersen C, Moser P, Luckner G, et al. Macroscopic postmortem findings in 235 surgical intensive care patients with sepsis. *Anesth Analg* 2009; 108: 1841-7.

29 Kaukonen KM, Bailey M, Pilcher D, et al. Systemic inflammatory response syndrome criteria in defining severe sepsis. *N Engl J Med* 2015; 372: 1629-38.

30 Yende S, D'Angelo G, Kellum JA, et al. Inflammatory markers at hospital discharge predict subsequent mortality after pneumonia and sepsis. *Am J Respir Crit Care Med* 2008; 177: 1242-7.

31 Opal SM, Fisher CJ, Dhainaut J-FA, et al. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, doubleblind, placebo-controlled, multicenter trial. *Crit Care Med* 1997; 25: 1115-24.

32 Opal SM, Laterre P, Francois B, et al. Effect of eritoran, an antagonist of md2-tlr4, on mortality in patients with severe sepsis: the access randomized trial. *JAMA* 2013; 309: 1154-62.

33 Schefold JC. Immunostimulation using granulocyte- and granulocyte-macrophage colony stimulating factor in patients with severe sepsis and septic shock. *Crit Care* 2011; 15: 136. ❑

Appendix 1. This appendix was part of the submitted manuscript and has been peer reviewed. It is posted as supplied by the authors.

Supplement to the manuscript; Rationale for the elaboration of criteria for primary, secondary and tertiary sepsis

Primary sepsis was defined as sepsis without any systemic inflammatory-inducing insult before onset. It is well documented that severe infections, surgery, major trauma and severe burns affect the inflammatory response for a period of a week or more [Castellheim A et al. *Scand. J. Immunol.* 69(6):479-491, 2009, Hotchkiss RS et al. *N. Engl. J. Med.* 348(2):138-150, 2003, Munford RS et al. *Am. J. Respir. Crit. Care Med.* 163(2):316-321, 2001]. Evidence has mounted that pro-inflammatory and anti-inflammatory systems are activated concurrently with a net result of an initial pro-inflammatory response that is followed by a predominantly hypo-inflammatory one [Skrupky LP et al. *Anesthesiology.* 115(6):1349-1362, 2011]. To ascertain with a reasonable margin that the sepsis-induced inflammatory response was not affected by a prior insult it was stated that infections, surgery, trauma or major burns <30 days before the septic insult should exclude patients from the primary sepsis category.

Secondary sepsis was defined as sepsis with a probable systemic inflammatory reaction prior to onset. Thus, the reverse criteria to primary sepsis were used, but with a shorter time span from the first inflammatory-inducing stimulus. The shorter time span was chosen to have a reasonable likelihood of a persisting effect on the inflammatory response at the time of the onset of sepsis. Patients with an onset of severe sepsis in the time interval of 7 to 30 days after a prior systemic inflammatory response-induced event were categorized as unclassifiable. A post-study intention was to analyze these patients separately before designing a prospective study but, unfortunately, no such patients were observed during the study period.

The group of patients with tertiary sepsis was more difficult to define. Following injury or infection-induced local or systemic activation, an anti-inflammatory response dominates systemically [Munford RS et al. *Am. J. Respir. Crit. Care Med.* 163(2):316-321, 2001.]. This systemic anti-inflammation is often reflected by diminished monocyte human leukocyte antigen-DR expression (mHLA-DR) [Monneret G et al. *Mol. Med.* 14(1-2):64-78, 2008]. Reduced mHLA-DR expression is independently associated with nosocomial infections [Landelle C et al. *Intensive Care Med.* 36(11):1859-1866, 2010]. One hit may result in a hypo-inflammatory response for >1 week if it is severe enough [Hotchkiss RS et al. *N. Engl. J. Med.* 348(2):138-150, 2003, Skrupky LP et al. *Anesthesiology.* 115(6):1349-1362, 2011, Grimaldi D et al. *Intensive Care Med.* 37(9):1438-1446, 2011] but severity is difficult to define and quantify. To increase the likelihood of an anti-inflammatory state two preceding insults were selected as criteria for tertiary sepsis. In these patients circulating monocytes are expected to have a reduced response to bacterial agonists, yet their ability to produce anti-inflammatory mediators is retained [Munford RS et al. *Am. J. Respir. Crit. Care Med.* 163(2):316-321, 2001] and the numbers of dendritic cells and T-cells are often reduced [Skrupky LP et al. *Anesthesiology.* 115(6):1349-1362, 2011, Grimaldi D et al. *Intensive Care Med.* 37(9):1438-1446, 2011].

In this study patients with hematological malignancies or immunosuppressive diseases and those receiving immunosuppressive treatment or cytostatic drugs were excluded. It cannot be ruled out that these underlying conditions more specifically and profoundly affect the inflammatory response. Leukocytes have a central role in the initiation of the inflammatory response [Castellheim A et al. *Scand. J. Immunol.* 69(6):479-491, 2009] and T-cell attenuation suppresses LPS-induced Toll-like receptor 4 signaling in innate immune cells [Kobayashi Y et al. *Proc. Natl. Acad. Sci. U. S. A.* 110(13):5121-5126, 2013]. Moreover, these patients are often excluded in clinical trials evaluating the effect of immunomodulation in severe sepsis and septic shock.