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ORIGINAL

CD64 surface expression on neutrophils is transiently upregulated in patients with septic shock

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Abstract *Objective*: To clarify the changes in total leukocyte counts, CD64 neutrophil receptor expression and serum granulocyte colony-stimulating factor (G-CSF) concentrations in critically ill patients without infection and sepsis and in patients with septic shock.
Design: Prospective study.
Setting: Intensive care unit (ICU) and research laboratory of a university hospital.
Patients: Eleven critically ill patients without infections and 22 patients with proven infections in septic shock for the first time and of at least 3 days' duration.
Measurements and results: Over a 6month period, a longitudinal analysis of expression of the monomeric Fc receptor type I (CD64, FcγRI) on neutrophils was performed by flow cytometric analysis on a daily basis in all postoperative/post-traumatic patients admitted to the ICU until discharge from the ICU or death. Out of 273 patients, 11 patients without sepsis had organ failure and 22 patients with proven infections had septic shock for the first time and of at least 3 days' duration. Ten out of the 22 patients survived, 12 died. CD64 expression was greater in patients with septic shock than in patients without sepsis. Moreover, CD64 expression was only initially and transiently elevated in most survivors (9/10) and non-survivors (8/12) of septic shock. In survivors, G-CSF serum concentrations were markedly decreased in the 2nd week.
Conclusions: Decreased neutrophil CD64 expression in an acutely ill population with septic shock may reflect the development of a non-responsive state as well as the early downregulation of neutrophil activation prior to the resolution of an ongoing infection.

Keywords Granulocyte colony-stimulating factor · Sepsis · Shock, septic · CD64 · Neutrophils

Introduction

Non-responsiveness or an early downregulation of neutrophil activation before an ongoing infection has resolved may be detrimental in septic shock and may contribute to the persistence of septic shock, multiple organ dysfunction and death. In the clinical setting, total leukocyte counts, without our knowledge of their functional status, often serve to monitor the systemic reaction to an infection [1]. Granulocyte colony-stimulating factor (G-CSF), a hematopoietic growth factor, plays a central role in the proliferation, maturation and functional activation of immature and mature neutrophils [2]. An inappropriate response to endogenous or exogenous G-CSF has been shown to be associated with an adverse outcome of infections and sepsis [2]. Besides neutrophil counts, expression of the functional antigen CD64 (monomeric Fc receptor type I; FcγRI) on the surface of neutrophils may indicate G-CSF responsiveness [2].

Table 1 Demographic data (*M* male, *F* female, *APACHE* Acute Physiology And Chronic Health Evaluation score)

	Non-survivors ($n = 12$)		Survivors ($n = 10$)		Critically ill ($n = 11$)	
Gender (M/F)	7/5		6/4		7/4	
	Median	Min–max	Median	Min–max	Median	Min–max
Age (years)	69.5	56–83	66.5	22–81	63	23–78
APACHE II	21	13–33	19	10–33	15	15–21
Length of stay (days)	14	5–47	20	15–30	9	5–34

Upregulation of CD64 in vivo has been shown to be associated with enhanced neutrophil function [3, 4, 5]. Decreased surface expression of CD64 has been reported in patients with septic shock [6]. At the onset of sepsis or septic shock, expression rates of activation markers CD63 and CD64 on monocytes and neutrophils, and CD66b on the neutrophils of patients were greater than on those of healthy controls [7]. Moreover, poor prognosis was associated with a lower expression of the priming, adhesion and activation markers CD11b and HLE on neutrophils and ICAM-1 on monocytes [7]. Longitudinal data, although limited to only two patients, were suggestive of the fact that the compensatory anti-inflammatory response syndrome is associated with impaired activation of monocytes (decreased HLA-DR expression) and neutrophils (decreased CD66b expression), calling for more extensive studies to confirm this.

Longitudinal analyses of CD64 expression on neutrophils and G-CSF serum concentrations are missing in patients with septic shock. The hypothesis of the present study was that a low expression or an early decrease in CD64 expression during intensive care unit (ICU) stay could indicate non-responsiveness or early downregulation of neutrophil activation, especially in non-survivors of septic shock. The aim of the present study was to clarify the longitudinal expression patterns of CD64 on neutrophils of postoperative/post-traumatic, critically ill patients without infections and sepsis, and survivors and non-survivors of septic shock.

Patients and methods

The study was approved by the ethics committee of the Ulm University Medical School and conducted according to the principles of the "Declaration of Helsinki". For severe sepsis, organ dysfunctions were defined in accordance with limits and definitions of the sepsis score [1] and the SOFA score [8] (at least two points for a single organ system) without employing the Glasgow Coma Score.

Eleven healthy volunteers, seven female and four male, served as controls. A longitudinal analysis of all patients admitted to the ICU over a 6month period was conducted, examining the kinetics of total peripheral blood leukocyte counts, neutrophil surface expression of CD64, G-CSF serum concentrations and clinical indices of sepsis, severity of disease and organ failure on a daily basis until discharge from the ICU or death.

EDTA-anticoagulated blood (50 µl) was stained with monoclonal antibodies recognizing CD64 (clone 22, directly FITC-labeled conjugate, Immunotech, Krefeld, Germany), incubated for 15 min, suspended with 50 µl PBS and erythrocytes were removed by Q-Prep-lysis (Beckman Coulter, Krefeld, Germany). Granulocyte surface expression of CD64 was tested by flow cytometry using a Coulter Epics XL-MCL (Beckman Coulter Electronis, Krefeld, Germany). The results are expressed as the difference between the mean fluorescence intensity (MnI) with the CD64 antibody and the isotype control IgG_1. Serum concentrations of G-CSF were determined by sandwich-type ELISA (R & D Systems, Minneapolis, USA) with detectable concentrations in the range of 50–1,000 pg/ml.

Since the aim of the study was to generate hypotheses, an explorative data analysis was conducted using two-sided tests. Therefore, it was not adjusted for multiple testing. The Wilcoxon signed-rank test was applied to compare data within groups of patients, the Mann-Whitney U-test to compare data between groups on days 1, 7 and 14. Probability values less than 5 % were considered as remarkable changes or differences.

Results

Two hundred seventy-three patients admitted to the ICU were included. Hundred ninety-two patients had to be monitored for up to 55 days to yield 75 patients who stayed for longer than 4 days on the ICU. Out of these, 11 patients (6 without shock and 5 with shock) without infections and sepsis had organ failure lasting at least 3 days. Twenty-two patients were in septic shock for the first time and for at least 3 days. Twelve of these 22 patients died, ten survived. Demographic data of the patients are given in Table 1.

In healthy volunteers, CD64 expression and G-CSF serum concentrations were in the range of –0.6 to 0.2 (median –0.2 MnI) and less than 50 up to 254 (median 49 pg/ml), respectively.

Experimental data for the three groups of patients during the 14 day period are presented graphically as box plots in Fig. 1 and for individual patients in Fig. 2.

Initially, SOFA scores did not differ between the survivors and non-survivors of septic shock, but were higher than in patients without infections (day 1: $p = 0.015$ and 0.003, respectively). SOFA scores increased over

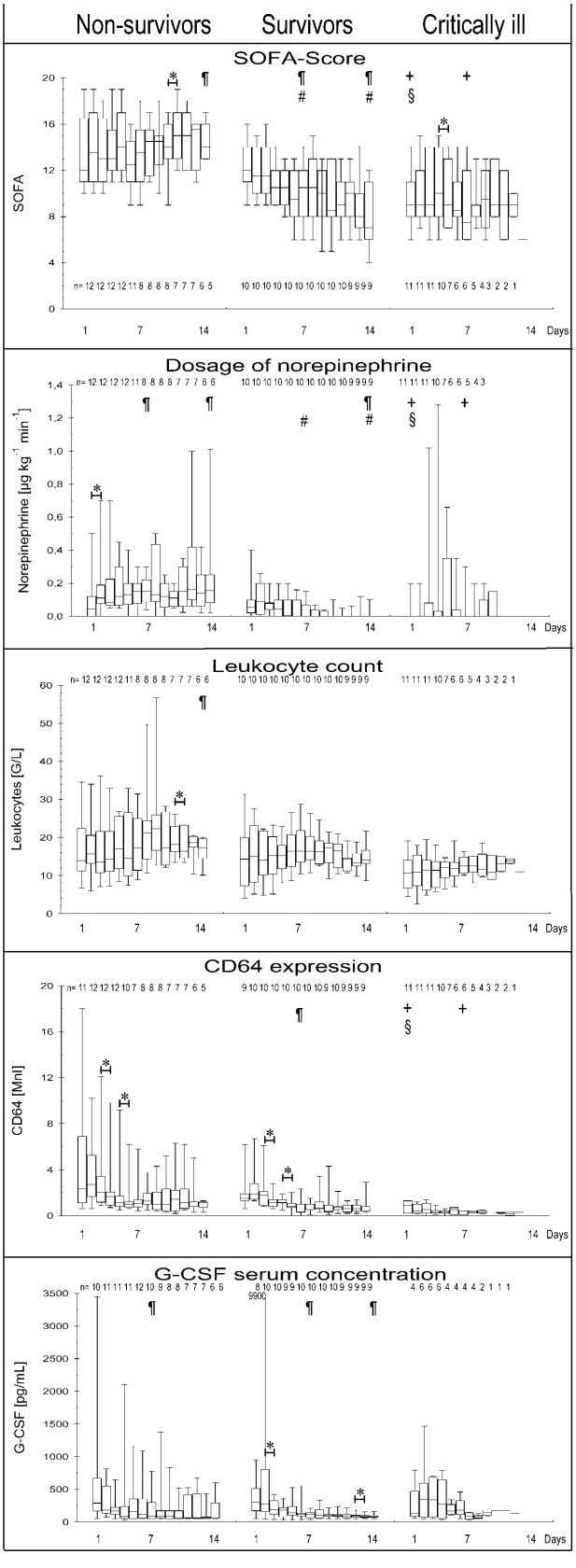


Fig. 2 SOFA scores, dosages of norepinephrine ($\mu g \cdot kg^{-1} \cdot min^{-1}$), leukocyte counts (G/l), CD64 expression on neutrophils (MnI) and G-CSF serum concentrations (pg/ml) in survivors and non-survivors of septic shock and in critically ill patients without infection and sepsis during the first 14 days presented as individual graphs ▶

time in non-survivors and decreased in survivors, being lower in survivors than in non-survivors within the 1st week (days 7 and 14: $p = 0.041$ and 0.003, respectively). Initially, dosages of norepinephrine, given intravenously to counteract hypotension, did not differ between survivors and non-survivors, but were higher than in patients without infections ($p = 0.010$ and 0.031, respectively). In non-survivors, the dosages of norepinephrine administered increased markedly over time and decreased in survivors, being lower in survivors than in non-survivors within the 1st week (day 7 and 14: $p = 0.005$ and 0.003, respectively).

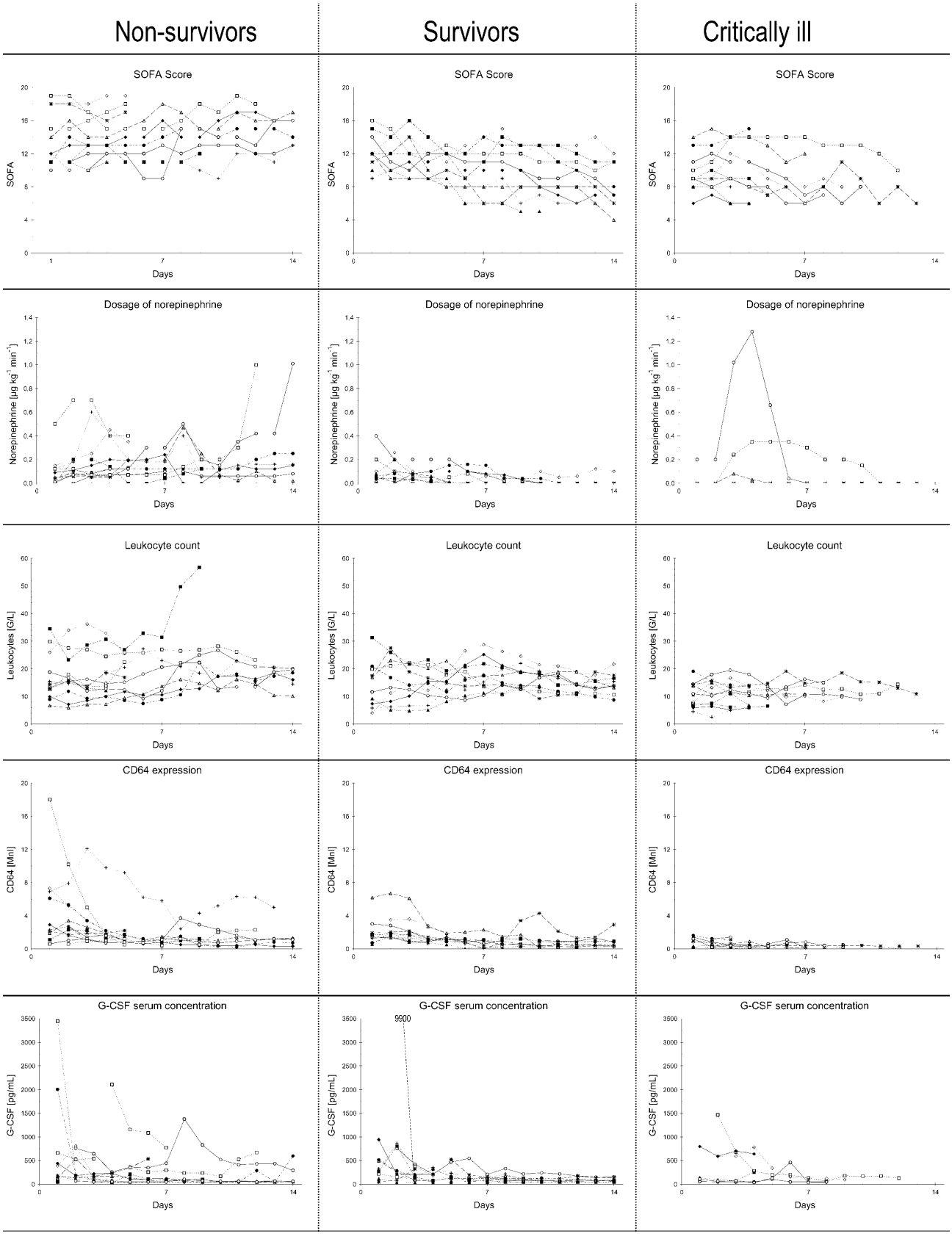
Leukocyte counts increased in non-survivors, but not in survivors and patients without infections, and did not differ among the three groups. CD64 expression on the neutrophils of survivors and non-survivors was profoundly elevated initially, decreased within the first 6 days and was markedly higher than that of controls and of patients without infections initially (day 1: $p = 0.039$ and 0.010, respectively). Three patterns of CD64 expression occurred in patients with septic shock (Fig. 2): expression transiently increased (8 non-survivors and 9 survivors), was persistently elevated (2 non-survivors) and occurred delayed (2 non-survivors).

Granulocyte colony-stimulating factor serum concentrations were elevated initially, decreased within the 1st week in survivors and non-survivors of septic shock, and remained decreased in survivors in the 2nd week. There were no marked differences among patients without infections, survivors and non-survivors.

Discussion

The main results of the present study are that CD64 expression on neutrophils was greater in patients with sep-

◀ **Fig. 1** SOFA scores, dosages of norepinephrine ($\mu g \cdot kg^{-1} \cdot min^{-1}$), leukocyte counts (G/l), CD64 expression on neutrophils (MnI) and G-CSF serum concentrations (pg/ml) in survivors and non-survivors of septic shock and in critically ill patients without infections and sepsis during the first 14 days presented as box plots demonstrating median, 25th–75th percentiles, 5th–95th percentiles. $^{*}p < 0.05$ between one day and the following day within groups; $^{¶}p < 0.05$ between day 1, and day 7 and/or 14, respectively, within groups; $^{\#}p < 0.05$ between survivors and non-survivors of septic shock at day 1, 7 or 14; $^{§}p < 0.05$ between survivors or $^{†}p < 0.05$ between non-survivors of septic shock and critically ill patients without infection and sepsis at day 1, 7 or 14 (n = number of patients at the various days)


Non-survivors
Survivors
Critically ill
SOFA Score
SOFA
Days
Dosage of norepinephrine
Norepinephrine [µg kg-1 min-1]
Leukocyte count
Leukocytes [G/L]
CD64 expression
CD64 [MnI]
G-CSF serum concentration
G-CSF [pg/mL]
9900

tic shock than in critically ill patients without infections initially, and was only initially and transiently elevated in most postoperative/post-traumatic patients with septic shock, regardless of whether they survived or died.

In the present study, initially elevated G-CSF serum concentrations remained decreased in survivors, but not in non-survivors, of septic shock. Thus, G-CSF may have been utilized more effectively in survivors. On the other hand, the fall in G-CSF serum concentrations in survivors may reflect a reduction in stimuli for G-CSF secretion. Polymorphonuclear leukocytes (PMNL) collected from donors treated with G-CSF showed decay in CD64 expression after 20 h in cell culture, but treatment with interferon-(IFN)-gamma + G-CSF preserved expression [9]. Thus, despite comparable G-CSF serum concentrations, concomitant IFN-gamma elevations in septic patients, in contrast to patients without infections, may explain the greater CD64 expression in our septic patients.

The clinical relevance of impaired neutrophil responsiveness is underlined by a longitudinal study that showed ongoing dysregulation of neutrophil function in patients whose systemic inflammatory response syndrome (SIRS) was complicated by multiple system organ failure and death [10]. In survivors of the present study, the early decrease in CD64 expression did not appear to be detrimental. The second peak expression of CD64 in one survivor might indicate an adequate response to a recurrent invasion of microorganisms, avoiding a relapse into septic shock. Since two non-survivors with consistently low expression during the 1st week exhibited a profoundly increased expression during the 2nd week, stimuli for increasing CD64 expression may not have been sufficient during the 1st week. Only 2/12 non-survivors demonstrated persistently elevated CD64 expression. Thus, despite ongoing bacterial infections, CD64 expression declined or remained persistently low in most non-survivors.

In healthy volunteers [4, 5] and postoperative/post-traumatic patients at high risk of sepsis [2], in vivo administration of G-CSF resulted in enhanced CD64 expression and neutrophil functions, such as ex vivo phagocytosis and oxygen radical production. It has to be clarified whether CD64 expression is an appropriate surrogate marker for neutrophil activation and functions in critically ill patients, and whether its restoration can be achieved by application of G-CSF, if CD64 expression is low or declines within the first days of septic shock despite ongoing infections and persistence or aggravation of septic shock, which may be indicated by increasing dosages of norepinephrine.

Early detection of unsuccessful responders to infection is warranted. The results of the present study indicate that a low expression or an early decrease in CD64 expression during ICU stay could indicate non-responsiveness or an early downregulation of neutrophil activation in patients with septic shock, which may be deleterious when it occurs before an ongoing infection has resolved.

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References

1. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee (1992) American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 20: 864–874
2. Weiss M, Moldawer LL, Schneider EM (1999) Granulocyte colony-stimulating factor to prevent the progression of systemic non-responsiveness in SIRS and sepsis. Blood 93: 425–439
3. Repp R, Valerius T, Sendler A, et al. (1991) Neutrophils express the high affinity receptor for IgG (Fc gamma RI, CD64) after in vivo application of recombinant human granulocyte colony-stimulating factor. Blood 78: 885–889
4. Turzanski J, Crouch SP, Fletcher J, et al. (1997) Ex vivo neutrophil function in response to three different doses of glycosylated rHuG-CSF (Lenograstim). Br J Haematol 96: 46–54
5. Hoglund M, Hakansson L, Venge P (1997) Effects of in vivo administration of G-CSF on neutrophil functions in healthy volunteers. Eur J Haematol 58: 195–202
6. Simms HH, D'Amico R (1994) Granulocyte colony-stimulating factor reverses septic shock-induced polymorphonuclear leukocyte dysfunction. Surgery 115: 85–93
7. Muller Kobold AC, Tulleken JE, Zijlstra JG, et al. (2000) Leukocyte activation in sepsis; correlations with disease state and mortality. Intensive Care Med 26: 883–892
8. Vincent JL, Moreno R, Takala J, et al. (1996) The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 22: 707–710
9. Cohen DM, Bhalla SC, Anaissie EJ, et al. (1997) Effects of in vitro and in vivo cytokine treatment, leucapheresis and irradiation on the function of human neutrophils: implications for white blood cell transfusion therapy. Clin Lab Haematol 19: 39–47
10. Simms HH, D'Amico R (1994) Polymorphonuclear leukocyte dysregulation during the systemic inflammatory response syndrome. Blood 83: 1398–1407