6.

Immature Myeloid Cell Detection (IMI, IG) as a Predictive Marker in Adult Human Sepsis

Axel Nierhaus

Introduction

During sepsis, the bone marrow is activated, which leads to leukocytosis as a result of increased white blood cell production, margination onto the blood and lymph vessels and elimination of leukocytes in the peripheral tissues and the spleen. The entry mechanisms of white blood cells into the circulation have not yet been entirely clarified. In the bone marrow, immature myeloid cells adhere to the stromal network until pro-inflammatory signals activate mature granulocytes and cause an increase in the circulating pool of leukocytes. The so-called shift-to-the-left, i.e. the presence of precursor forms of differentiating neutrophils, appears during full-blown reactions. In the latter situation, immature cells, positive for the CD34 cell surface marker, enter the circulation. It has been shown that in the neonate the number of peripheral immature granulocytes (IG) is an early indicator for the presence of sepsis and also negatively correlates with mortality. The present preliminary study gives the first evidence that the number of immature granulocytes is also indicative for survival in adult patients with sepsis and septic shock.

Sepsis and Septic Shock

Sepsis and septic shock are major problems for the international Intensive Care community because patient mortality remains high and advances in treatment are slow to emerge. As a prelude to the description of the role of predictive markers (new and old) for patients in Intensive Care Units, the pathophysiology of sepsis and septic shock must first be summarized.

Septic shock is defined as the systemic response to infection with circulatory failure. It occurs in 5–15 % of all patients in Intensive Care Units, both surgical and medical, and carries a mortality rate of 50–80 %. These statistics have not really altered in the past 20 years. The cytotoxic effects of the many mediators released during the early and late phases of sepsis result in endothelial dysfunction, the so-called ‘capillary leak’ syndrome, which, in turn, leads to micro-circulatory failure with remote organ damage. In the early stages this is basically a harmless ‘alarm’ reaction but as sepsis progresses major immune disturbance will ultimately ruin the system.

The role of the clinician is to secure and stabilise organ function albeit with a variety of crude, macro-style interventions and to diagnose and initiate appropriate antibiotic therapy. More effective goal-directed adjunctive therapy remains elusive.

Immune System and Immune Response

The immune system is an organ, not just a collection of cells. The immune response is divided into two major pathways, the innate immune response and the

adaptive immune response. The innate immune response consists mainly of mechanical, chemical and bacteriological barriers, the complement system, a variety of cytokines, (both pro-inflammatory and anti-inflammatory), macrophages and natural killer (NK) cells. The innate immune system is very conservative and is found in most eukaryotic organisms. Mammals have a very defined and refined adaptive immune system exhibiting both a cellular and a humoral response. The cellular system, which involves cytotoxic T cells (CD8) and helper T cells (CD4), eliminates pathogens that have invaded cells and regulates the body-'s entire immune response. The CD4 helper T cells can be further subdivided into Th 1 (pro-inflammatory) and Th 2 (anti-inflammatory) cells. The humoral system makes antibodies to eliminate pathogens and their products thus providing memory cells capable of mounting a very rapid and efficient immune response.

So what happens at a cellular level when a monocyte/macrophage encounters lipopolysaccharide (LPS) endotoxin? The rather complex series of events is illustrated in **figure 1**. One of the most prominent receptors involved is Toll-like receptor 4 (there are at least seven Toll-like receptors now defined) that interacts with the antigen that in turn activates the macrophage and leads to shedding of the pro-inflammatory mediators IL-1 and TNF alpha. In addition there is increased expression of a special HLA-DR receptor on the monocytes which is important for the presentation of antigen to T cells. Without a functioning HLA-DR receptor, there will be a poor and slow processing of exogenic antigen and the innate immune response will not work as it should.

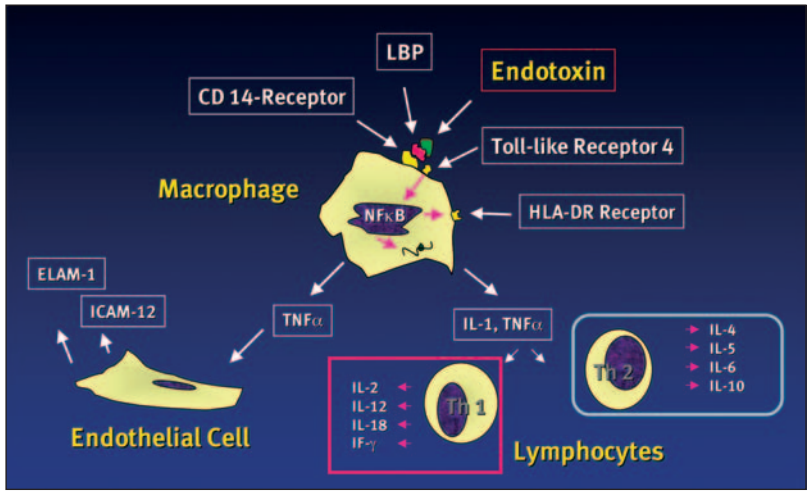


Figure 1
Endotoxin and monocyte/macrophage pathways.
ICAM = intercellular adhesion molecule
ELAM = endothelial leucocyte adhesion molecule
TNF = tumor necrosis factor
HLA = human leucocyte antigen
IL = interleukin

The innate and adaptive immune responses are essential for man to defend against infection. The effect of compromised or genetically inactive responses is illustrated in **figure 2**. The only situation that is really favourable is when both innate and adaptive immune responses are active so that the control of micro-organism growth over time is effective.

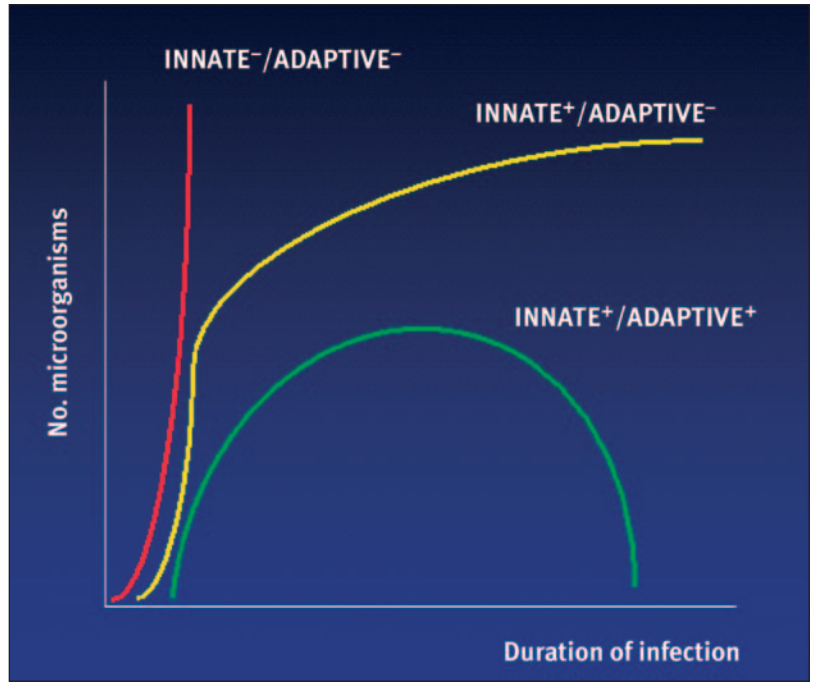


Figure 2
Innate and adaptive immune responses.

Immunological Markers in Sepsis: Study Design

The study group consisted of 29 consecutive adult patients (8 female; 21 male) admitted to two operative Intensive Care Units in the University Clinic Hamburg-Eppendorf. All patients fulfilled the ACCP/SCCM [**1**] criteria for severe sepsis and septic shock, respectively, with the onset of sepsis being defined as Day 1. All patients were assessed by recognised scoring systems (APACHE II [**2**], MODS [**1**], SOFA [**3**]). Diagnostic and monitoring parameters included Interleukin 6 (Il-6), lipopolysaccharide binding protein (LBP), Procalcitonin (PCT), lipopolysaccharide (LPS), C-reactive protein (CRP), tumour necrosis factor alpha (TNFa), HLA-DR, TNFa (ex-vivo stimulation) Immature Granulocyte (IG) and Immature Myeloid Information (IMI) cell counts and the ICU-Mortality [**4**]. The observation period was 14 days.

Results

The median age of the population was 58 years (range 21–73 years); the median APACHE II score was 18 (range 9–31), 21 in non-survivors and 16 in survivors, the difference being statistically significant; ICU-mortality as it is described in many other populations of this severity is rather high at 38 % (8 of 29 patients). All patients were infected as judged by the Centers for Disease Control criteria.

The flow cytometric analysis of HLA-DR receptor density on monocytes (**figure 3**) confirmed the observations of other groups in that this parameter clearly differentiates between survivors and non-survivors already on Day 1 and for the duration of the study was always worse in the non-survivors than in the survivors.

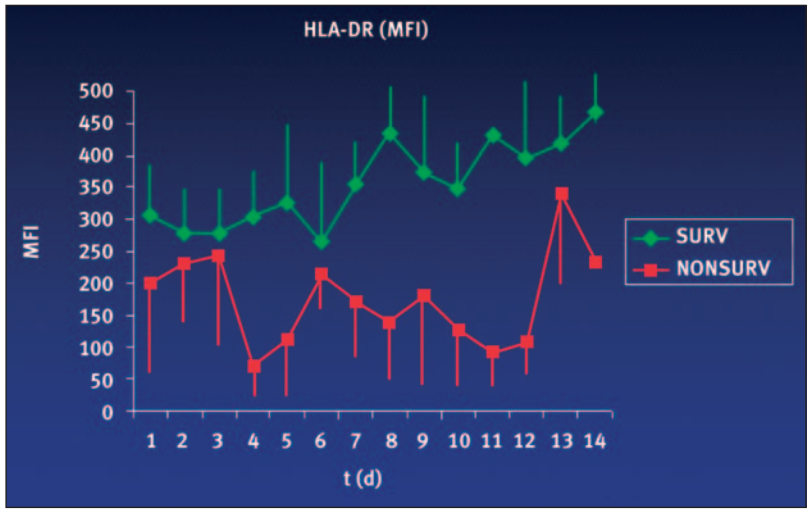


Figure 3
Immunological markers in sepsis:
HLA-DR on monocytes.
MFI = mean fluorescence intensity;
t(d) = time in days;
SURV = survivor;
NONSURV = non-survivor.

Interleukin-6 (IL-6) is usually considered to be a useful gauge of severity at the onset of sepsis and so it proved to be in the present study (**figure 4**). The non-survivors exhibit much higher activation of the immune system and a much higher pro-inflammatory status than the survivors. This is predicted on day 1 but for the remainder of the study the difference between the two groups was small.

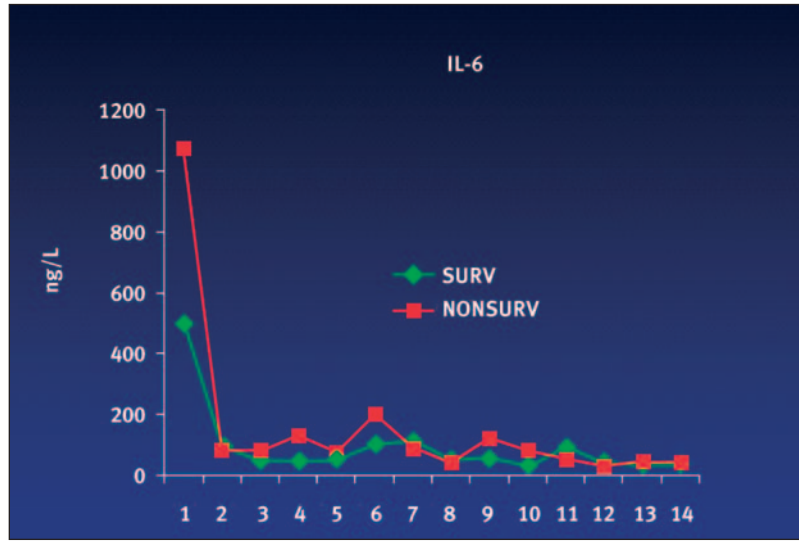


Figure 4
Immunological markers in sepsis: IL-6 data.

To our astonishment, the IG count was statistically significantly lower in the surviving group than in the non-surviving group (**figure 5**). It is not possible at this stage to speculate on why this is so. These data for Day 1 are, however, very important clinically because there are not many bedside signs which predict that the septic or inflamed patient is going to do well at that stage.

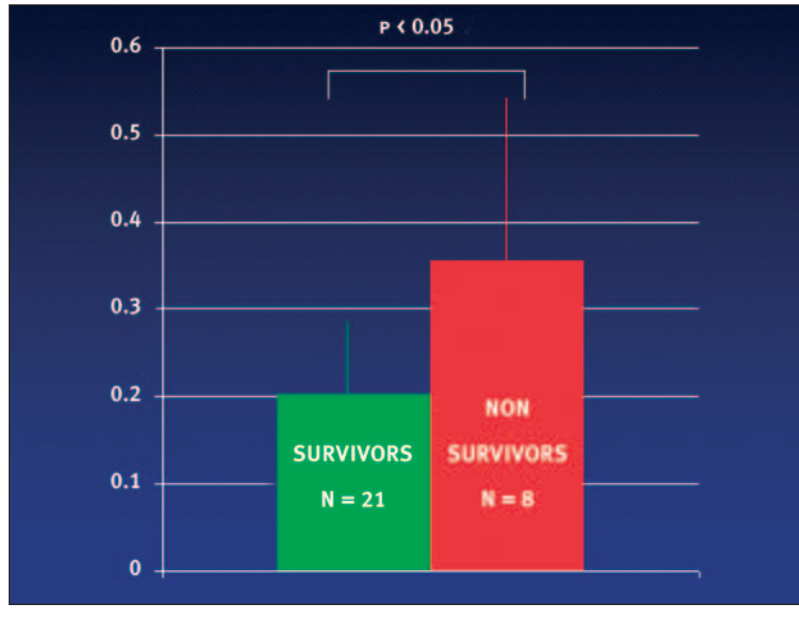


Figure 5
Immunological markers in sepsis: IG count data.

The IMI count was also helpful in distinguishing significantly between the non-surviving group and the surviving group in the first few days after the onset of sepsis (**figure 6**). A quite marked increase in the IMI was noted in the later stages in the non-survivors. Again, there was a statistically significant difference between the two groups, quite highly differentiating with good power.

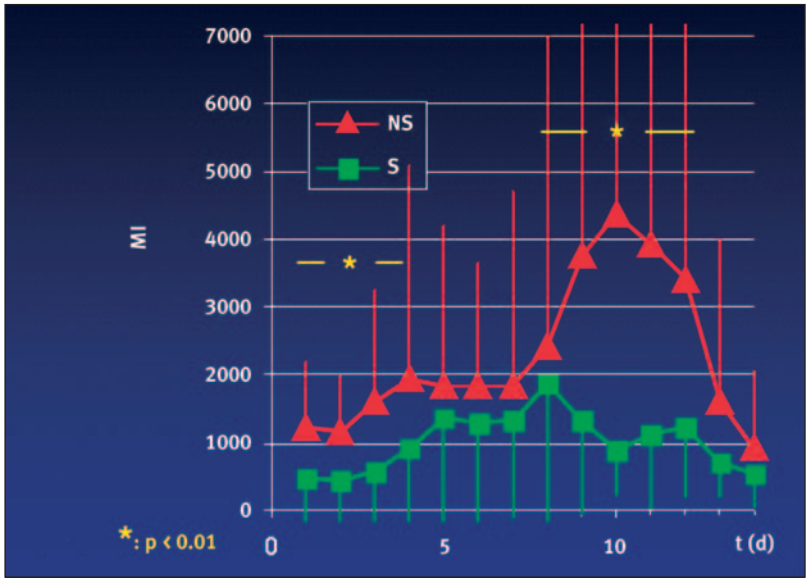
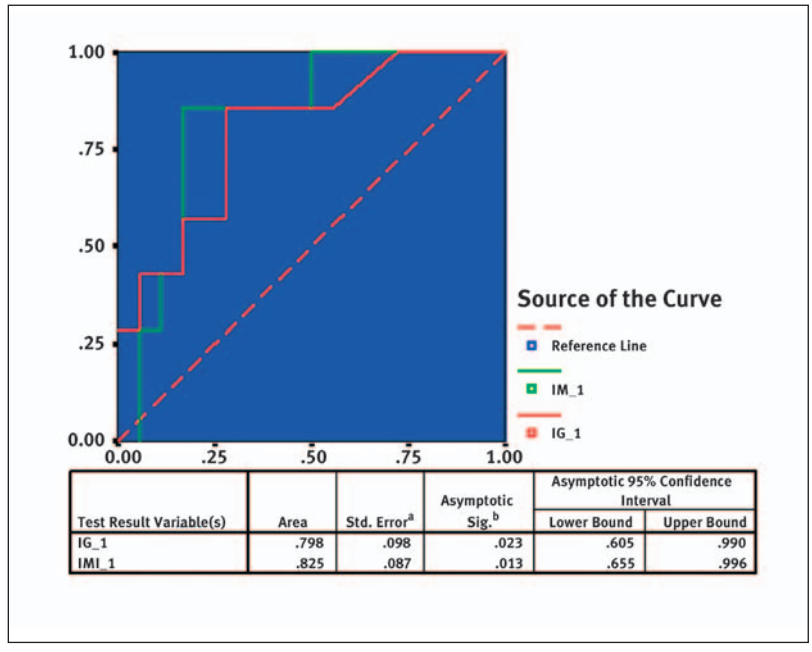


Figure 6
Immunological markers in sepsis: IMI channel count data.(/µL)

Receiver operating characteristics (ROC) curves (**figure 7**) for IG and the IMI counts on day1 indicate that both parameters are predictors of death with areas under the curve of 0.79 and 0.82 respectively. These correlate well with predictive scores derived from APACHE II and SOFA. As before the pathophysiological basis is as yet unclear.



Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
IG_1	.798	.098	.023	.605	.990
IMI_1	.825	.087	.013	.655	.996

Figure 7
Immunological markers in sepsis:
ROC of IMI and IG (day 1)
x-axis = 1 – specificity
y-axis = sensitivity

Case study

This is an example of a 58-year-old female patient with fulminant necrotising fasciitis of the left leg which extended into the pelvis. She was extremely septic; there was no sustained improvement over time and she died of multiple organ failure. As always in such patients there was a considerable signal in the IMI channel (**figure 8**). Looking at this case over time (**figure 9**), clinical severity scores (MODS and SOFA) were very high at the outset. There was some improvement subsequently as a result of surgery and antibiotic therapy followed by a preterminal increase in morbidity indices. The parallel behaviour of the IG count is noteworthy as it reflected the performance of the morbidity indices very closely. It is tempting to speculate that these new parameters, i.e., the IG and IMI counts, may be providing information on the progression and severity of sepsis.

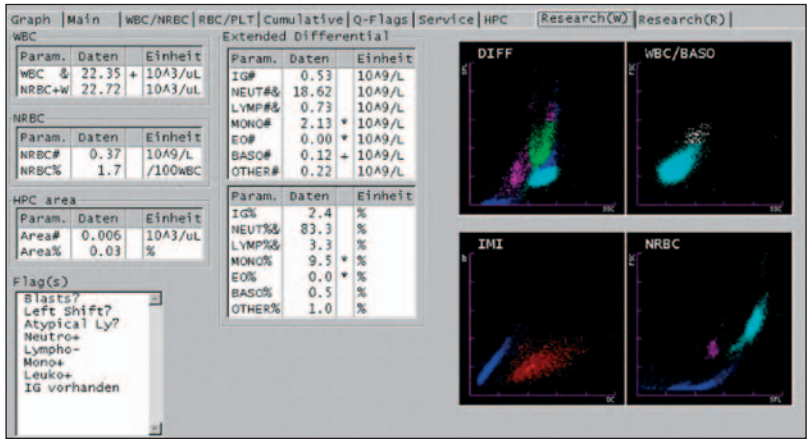


Figure 8
Case study: 58-year female with necrotising fasciitis and sepsis. XE-2100 *results.*

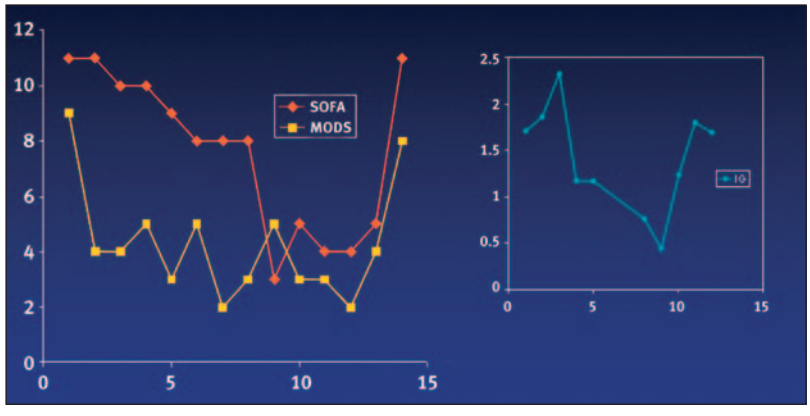


Figure 9
Case study: 58-year female with necrotising fasciitis and sepsis. Morbidity over time. SOFA, MODS see text.

Conclusions

Without any doubt, it is possible to perform much monitoring in patients with sepsis by measuring a plethora of parameters. However, the majority of these measurements are expensive; they are nonspecific; they are very sensitive; but at the end of the day all you have is a confirmation of diagnosis. The problem is – what can be done therapeutically in terms of modulating an overtly unbalanced immune system? During the immuno-paralysis one could imagine that immuno-stimulation by interferon or GM-CSF would be beneficial once the overwhelming pro-inflammatory phase has terminated. Likewise, it may be beneficial to reduce the amount of pro-inflammatory mediators by immuno-suppression or extracorporeal elimination. During chronic sepsis it may become important to provide immuno-nutrition. There are some promising data suggesting that glutamine, for example, is beneficial for patients with chronic peritonitis. Equally there are data

suggesting that treatment with intravenous immunoglobulins may be beneficial in the early phase of sepsis. Unfortunately there is insufficient evidence to make strong recommendations for all patients right now. I still think the emphasis should be on identifying better methods of monitoring in the first place. Perhaps these inexpensive haematologic parameters will tell us more in the future.

Acronyms

ACCP/SCCM = American College of Chest Physicians/Society of Critical Care Medicine
APACHE II = Acute Physiology and Chronic Health Evaluation
MODS = Multiple Organ Dysfunction Score
SOFA = Sequential Organ Failure Assessment

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