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MARKERS OF SEPSIS

Heinz Redl
Andreas Spittler
Wolfgang Strohmaier

Sepsis related organ failures are among the major causes of prolonged intensive care, placing a heavy burden upon health care. It is, therefore, imperative to monitor biochemical/immunological markers and to identify those predicting isolated or multiple organ failure. Traditionally the term 'sepsis' has been used to describe the process of infection accompanied by the host's systemic inflammatory response. Based upon that understanding, previous clinical studies have been designed to include only patients with positive blood cultures [1]. However, the frequent occurrence of an inflammatory response without detection of microorganisms in the circulation has led to a new understanding and definition of sepsis, mainly as the systemic host response to a microbiological, event which is often undetectable as such, or non-microbiological, process, with detectable levels of cytokines independent of the presence of documented infection [2]. Initially, a hyperinflammatory stage is present, which can be rapidly replaced by a hypoinflammatory phase with considerable overlap and then may lead to immunoparalysis if no recovery has taken place. In fact pro- and anti-inflammatory stimuli exist simultaneously in every patient [3]. Therefore, it is often difficult to identify the status of a patient. Despite this lack of knowledge and the limited monitoring possibilities, enormous resources have been invested into therapeutic studies.

Basically, there are two approaches for biochemical/immunological monitoring (Figure 1):

1) to study *body fluids,* mainly plasma, since the sepsis response can be
    highly compartmentalized, e.g., broncho-alveolar lavage (BAL) fluid
    may be more relevant [4] in certain situations;
2) to study *cells,* usually blood derived peripheral cells, but local cells, e.g.,
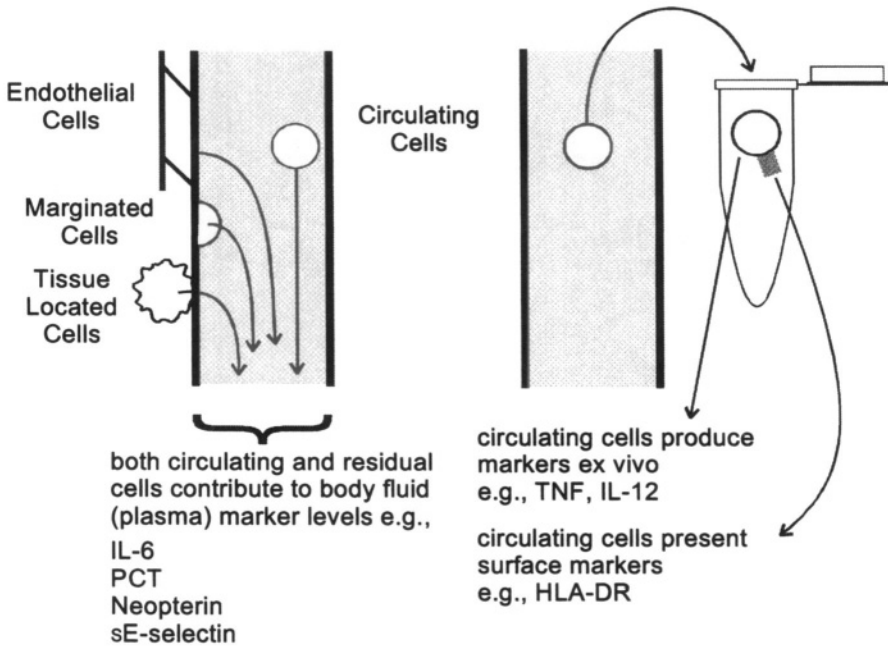   pulmonary macrophages could be more relevant on some occasions.

Endothelial Cells
Circulating Cells
Marginated Cells
Tissue Located Cells

both circulating and residual
cells contribute to body fluid
(plasma) marker levels e.g.,

IL-6
PCT
Neopterin
sE-selectin

circulating cells produce
markers ex vivo
e.g., TNF, IL-12

circulating cells present
surface markers
e.g., HLA-DR

Figure 1: *Possible approaches in sepsis monitoring*

Body fluids are usually easier to obtain. The measured markers are products
of both circulating and resident cells (e.g., polymorphonuclear leukocytes
[PMN], Kupffer, or endothelial cells). Apart from the rates of product
synthesis and release or shedding of surface molecules, clearance and
iatrogenic dilution are factors affecting concentrations. On the other hand *ex
vivo* measurements of cells are dependent on the mode of collection
(separation) and represent the non-resident fraction of cells only. However,
such measurements probably reflect the activation potential and the receptor
status of the peripheral immune system. For each of the two approaches some
examples will be discussed below.

BODY FLUID (PLASMA) MARKERS

The spectrum of markers to be measured in plasma comprises: a) sepsis inducers such as lipopolysaccharide (LPS) and related molecules; b) products of responding cells; and c) products of humoral activation cascades.

Inducers

Endotoxin (and associated LPS binding protein [LBP], LPS-Ab)

Bacterial endotoxin (LPS) is a primary inducer in the pathophysiology of gram-negative bacterial sepsis. Therefore, LPS is considered an important monitoring parameter. However, there are several obstacles to LPS measurements. A single measurement of endotoxin may be misleading. The often transient appearance of bacteria/LPS in the circulation makes it difficult to document the occurrence in patients and this might be one possible explanation for conflicting clinical results [5], although in a recent study Opal et al. [6] were able to demonstrate in > 250 sepsis patients that median LPS levels at study entry were more highly elevated in non-survivors. A Kaplan-Meier survival plot revealed a 28 day mortality of 35 % in LPS positive, and 22 % in LPS negative, patients. From a practical point of view LPS assays using current techniques cannot be recommended for routine use.

In the same study LBP, the principle protein responsible for transporting endotoxin to effector cells, was measured. While there was no correlation between LPS and LBP, LBP levels were less highly elevated in non-survivors [6]. Plasma levels of LBP correlate with outcome in sepsis and other patients [7].

Beside LPS and LBP, the levels of anti-LPS core antibodies in plasma have been considered for monitoring with the rationale of identifying those sepsis patients who might benefit from endotoxin-neutralizing therapy. Decreased levels of anti-LPS core antibodies were associated with increased mortality [8, 9].

No routinely applicable analytical tools are available for Gram-positive cell components.

Products of Responding Cells

One important aspect in the understanding of the pathophysiological network in sepsis is the ability to detect and measure cytokines. This is a difficult task due to local production and action, low levels and short half-lives [4]. The

short half-life in plasma results partly from binding to receptors, including circulating soluble cytokine receptors. Not only can these soluble receptors neutralize cytokines, they are diagnostic tools in themselves [9].

An approach to avoiding difficulties associated with cytokine monitoring is the use of so-called surrogate markers for sepsis monitoring, of which the macrophage activation marker neopterin [10] and procalcitonin [11] are the most well-known.

Plasma Cytokines

From the large pool of cytokines, IL-6 has been most widely used, due to favorable characteristics such as fast kinetic response, independence of soluble receptor-binding in most assays, and relative independence of kidney function. Relevant concentrations in the low nanogram range are suitable for reliable commercial immunoassays. Elevated levels of interleukin (IL)-6 were found to be associated with fatal outcome, while tumor necrosis factor (TNF) levels did not prove to be a prognostic indicator [12], thus indicating that IL-6 is a reliable marker of sepsis [12-14]. Furthermore, plasma IL-8 [15], IL-10 [16], IL-18 [17], and natural antagonists such as IL-1 receptor antagonist (IL-1ra) and soluble TNF receptors [9] have been repeatedly used for monitoring purposes in sepsis patients.

Plasma IL-6 levels have also given useful information regarding therapy. In some anti-TNF trials, the overall study population showed no increase in survival after sepsis upon treatment. Retrospective stratification of patients by IL-6 concentrations suggested beneficial effects of the drug for patients with baseline circulating IL-6 concentrations of > 1000 pg/ml [18]. Interestingly, the administration of an anti-TNF antibody resulted in a decrease in IL-6 and not in **TNF-α** [14]. As a result of these findings, two prospective studies (RAMSES in Europe, MONARCS in North America) were set up to evaluate the effect of anti-TNF antibody therapy in patients presenting with a hyperinflammatory state based on IL-6 plasma levels at the time of study entry. In the MONARCS study, of the 2,634 patients enrolled, 998 had IL-6 levels > 1000 pg/ml and 1,636 did not. Patients with IL-6 > 1000 pg/ml had a significantly higher mortality (47.7 vs. 28.6 %) (Panacek et al. unpublished data). In those with elevated IL-6 levels, TNF antibody reduced risk-adjusted 28-day mortality (6.9 %) compared to placebo (41.5 % vs. 48.4 %, p = 0.041). Mortality at 28-days was also reduced (3.6 %) in the overall population (32.3 % vs. 35.9 %, p = 0.049). In other words, anti-TNF therapy was twice as effective in patients with IL-6 > 1000 pg/ml indicating the potential of markers of sepsis. Anti-TNF therapy also significantly reduced serum IL-6 levels (p > 0.05).

One of the most recently identified substances acting as a cytokine is high mobility group 1 (HMG-1) [19], which does not appear in the plasma of endotoxic animals until 8 hours after endotoxin administration. However, even late therapeutic blockade is associated with improved survival. Although currently there are only limited data, HMG-1 could become a valuable marker in the future, since sepsis patients who succumb to infection have increased serum HMG-1 levels [19].

Neopterin

Neopterin (NEO), a member of the ubiquitous family of unconjugated pteridines, is derived from guanosine triphosphate (GTP) [20] and released from monocyte/macrophages upon stimulation with interferon (IFN)-γ and LPS [21]. Related molecules such as biopterins have gained widespread interest since tetrahydrobiopterin is the essential co-factor for aromatic amino acid monoxygenases and thus for neurotransmitter synthesis, as well as for nitric oxide synthases (NOS) [22].

After previous use in graft versus host disease, a strong correlation between NEO levels and the occurrence of septic events in intensive care patients was first described in 1987 [10] with discrimination between survivors and non-survivors among these 21 patients as early as day one. An investigation on 56 patients [23] was in agreement; in that study NEO testing (96% sensitivity and 73% specificity for NEO < 40 nmol/1) yielded an overall accuracy of 83%. NEO also predicted the Goris multiple organ failure (MOF) score (if > 5) when measured one day before the evaluation. In this study NEO always differentiated between septic and non-septic survivors and non-survivors as well. Increased NEO concentrations were also reported [24] in parallel with demonstrating that freshly isolated monocytes stimulated *ex vivo* with LPS are exhausted to produce NEO in surgical patients. The authors concluded that resident, tissue-bound macrophages are responsible for the high serum levels. In another study [25] a clear discrimination between survivors and non-survivors starting as early as day two post trauma in a 14 day observation period could be observed. One hundred patients with severe polytrauma (mean injury severity score [ISS] = 37) were studied. Both NEO and soluble IL-2 receptor were identified by Delogu et al. as significant predictors of shock states in Gram-negative sepsis [26]. Slightly divergent data were presented in a prospective study of 56 patients, evaluating various inflammatory mediators as predictors of MOF after blunt trauma (ISS > 33) with significant changes in the later course of sepsis [27]. The authors used the NEO/creatinine ratio in their calculations to compensate

for kidney dysfunction, since NEO is cleared via the kidneys in a creatinine-like fashion [28].

For well over ten years now, NEO has been assessed routinely in the ICU at the Lorenz Böhler Trauma Hospital and has gained acceptance as part of monitoring. Moreover, it has turned out that NEO lends itself to a number of additional applications. First, selective puncture of corresponding veins and arteries allows a comparison of their respective NEO concentrations. The resulting arterio-venous difference may be an indication for the existence, or absence, of septic foci [29], the latter information often being of more importance. Second, in 1996, Strohmaier et al. [30] showed that NEO blood levels can provide a reliable basis for the decision on whether or not to use antibiotics in our ICU. After a two-year evaluation period with 536 patients enrolled and the definition of a few exceptions (e.g., open head fracture), we ultimately adopted the present procedure: In cases of suspected infection, antibiotics are given only if serum NEO levels exceed 40 nmol/1. This approach is supported by bedside infection screening using Gram-stained smears [31]. The cut-off value of 40 nmol/1 serves as a discriminator between colonization, which remains untreated, and systemic infection. The main results of this strategy have been a marked reduction in infectious episodes and isolated microorganisms, particularly *Pseudomonas* spp. and staphylococci, as well as in the cost of therapy.

Procalcitonin

Procalcitonin (PCT) is a 116-amino acid propeptide, which undergoes proteolysis into the hormone calcitonin. PCT has been suggested as an excellent early and discriminating marker of bacteria-associated sepsis in patients (with low levels in virus-induced infection) [32]. An increasing number of clinical studies have been performed, since a commercial assay has become available (for review see Meisner [33]. Although the source of calcitonin has been generally considered to be the thyroid cell (and other neuroendocrine cells), this cell is probably not the source of PCT, as an infection-associated rise in PCT has also been shown in thyroidectomized sepsis patients [11]. The source of PCT in sepsis is currently unclear. Some of the inducers of PCT, such as endotoxin and *E. coli* are known. Given to chimpanzees and volunteers [34], and to baboons [35], these agents resulted in increased serum PCT concentrations.

Reith et al. [36] reported significant falls in plasma PCT concentrations in patients with peritonitis after successful focal ablation. When surgical removal of septic foci failed and patients died, mean PCT levels remained high. Brunkhorst et al. [37] clearly discriminated between an infectious and a

non-infectious etiology of acute respiratory distress syndrome (ARDS) using PCT levels. In a series of 17 consecutive patients with very similar Murray scores, PCT distinguished between the septic and the non-septic origin of ARDS. TNF and NEO yielded equivalent results, while IL-6 and C-reactive protein (CRP) proved inadequate. Scoring the patients by means of the APACHE II also clearly discriminated septic from non-septic etiology. Another group highly vulnerable to infection are burns victims. Nylen et al. [38] investigated 41 patients and demonstrated a preferential release of PCT from the lung; these authors concluded that serum PCT levels might have prognostic power regarding the severity of inhalational injury. Circulating PCT was measured in 40 burns patients with total body surface area (TBSA) > 30% by Carsin et al. [39] up to one week after admission and compared to levels of IL-6, TNF-α and endotoxin. PCT levels proved to be of prognostic value for mortality and to correlate with IL-6 and the severity of skin burn injury, but were not associated with inhalation injury. The finding that mortality was decreased when septic animals were treated with an antiserum reactive to PCT, suggests that PCT is more than a marker of bacterial sepsis and can actually be regarded as an active player in inflammatory processes [40].

Endothelial Markers

Since it is difficult to obtain the few circulating endothelial cells [41], plasma measurement is the method of choice. Amongst many other events during sepsis is an activation of endothelial cells with an up-regulation of cell surface adhesion molecules such as P-selectin, E-selectin and intercellular adhesion molecule (ICAM)-l. This up-regulation increases leukocyte adherence. A small proportion of these adherence molecules is shed into plasma and thus is accessible for plasma analysis. In a polytrauma study, from the 4th day onwards sE-selectin and sICAM-1 were different in three outcome groups, namely lethal, reversible, and no organ failure (Jochum et al., unpublished data). Endothelial cells are also considered to be a possible source of increased IL-6 production observed in situations such as stress or septic shock, in which catecholamines are elevated due to endogenous production or exogenous application [42].

It is not only interesting to monitor endothelial activation but also endothelial damage. One factor is thrombomodulin. Besides being present on the endothelial surface, a soluble form of thrombomodulin (in reality, several fragments) has been found in plasma and urine of normal subjects. *In vitro* [43] and *in vivo* [44] experiments in rabbits suggest that soluble thrombomodulin is not shed from the endothelial surface but is the result of

cellular damage. Several investigators have reported that plasma thrombomodulin levels are elevated in disease states commonly associated with perturbation of the vascular endothelium, such as ARDS [45] and sepsis [46]. Experimental results provide evidence for an *E. coli* dose-related and TNF-dependent thrombomodulin release into the plasma of septic baboons and suggest a possible role of anti-TNF for the protection of the endothelium [47].

   Apart from thrombomodulin, plasma levels of soluble endothelial cell protein C receptor are elevated in patients with sepsis. However, there is lack of correlation with thrombomodulin, which suggests the involvement of different pathological processes [48].

Humoral Factors

Humoral cascades become activated in the plasma of sepsis patients partly as a direct reaction with sepsis inducers (e.g., during opsonization), but mainly upon reaction with activated cells (e.g., monocytes and tissue factor). These reactions lead to the induction of coagulation, fibrinolysis, and the complement cascade (for review see Hack [49]). Since clinical intervention trials are underway or completed (antithrombin [AT]III, tissue factor pathway inhibitor [TFPI], activated protein C [APC]), the obvious question is whether plasma levels of elements of the humoral cascades can be of use in monitoring sepsis. Two examples worth pointing out in this context are the good correlation of low ATIII [50] and low protein C [46, 51] with higher risk in sepsis patients. ATIII levels < 70 % at the onset of fever predicted a lethal outcome with 85 % sensitivity and 85 % specificity [50].

Kinetics of Plasma Markers

One must be aware that different plasma markers have different appearance kinetics. This is easily demonstrated in a non-human primate sepsis model (Figure 2). While TNF or IL-10 follow short time kinetics, NEO exhibits a slow but stable response. Other parameters such as IL-6 or PCT occur between these extremes. On the one hand, although a fast response is desirable for the early detection of sepsis complications, a fast response is usually associated with unstable plasma levels. Thus, the time of sampling is a possible source of inaccurate results. One of the slow reacting markers is CRP. Since CRP is elevated in all inflammatory conditions, however, its value as a sepsis marker is limited.

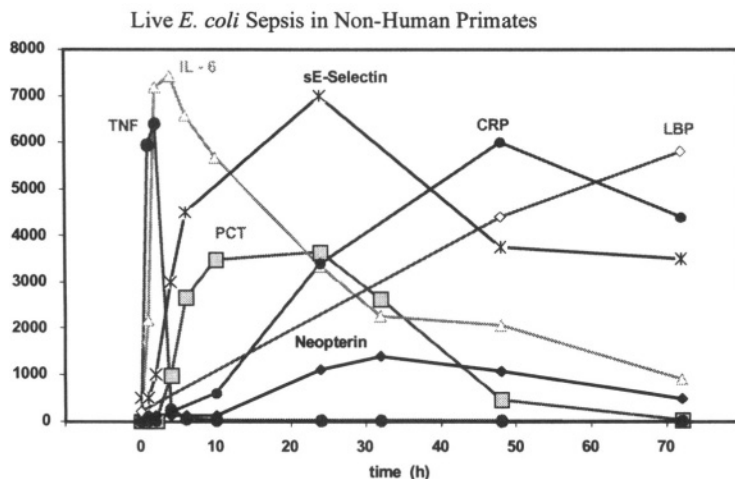Live *E. coli* Sepsis in Non-Human Primates

Figure 2: Kinetics of plasma markers. Data are displayed with the following dimension and correction factors: TNF, IL-6, PCT (pg/ml); NEO x 10 (nmol/l); LBP, CRP x 20 (mg/l); sE-Selectin x 500 (pg/ml).

CELL ASSOCIATED MARKERS

To define inflammatory stages, several cell surface molecules on various cell types have been investigated and functional assays performed to determine the *ex vivo* reactivity of immunocompetent cells after stimulation. Highly standardized methods (e.g., flow cytometry, ELISA), which are easily carried out, yield results in a short time. Immunological interventions and therapeutic strategies should now be applied depending on what immunological phase the patient exhibits.

HLA-DR expression as a prognostic and predictive parameter

The best characterized cell surface antigen in septic disease is the human leukocyte antigen (HLA) with its cluster DR. HLA-DR is part of the major histocompatibility class II antigen complex mainly expressed by antigen-presenting cells such as monocytes and macrophages but also by activated T and B cells. In this context, monocytes have been intensively studied since they play a central role in both specific and non-specific immunity against bacterial, viral, and fungal infection. As early as 1986, Polk and co-workers

correlated the percentage of HLA-DR expressing monocytes with the appearance of infection [52]. The authors investigated patients who had sustained major trauma and showed a positive correlation between low monocyte HLA-DR expression and the development or presence of major infection at days 7-8 and days 10-12. Some years later, Hershman and co-workers investigated 60 trauma patients divided into three groups [53]. Monocyte HLA-DR expression in those patients with uneventful recovery returned to normal range after one week, whereas in patients with recovery from severe sepsis, normal levels were reached after 3 weeks. In those patients who died, HLA-DR expression never returned to normal levels. Similar results have been observed by other investigators. Döcke et al. demonstrated that a diminished MHC class II antigen expression (< 30 % HLA-DR+) on monocytes over a period of over 5 days strongly correlated with fatal outcome after septic disease [54]. Immune monitoring in cardiac transplant recipients performed by our group showed that patients with a reduced HLA-DR density on monocytes after 5-7 days following transplantation were at a high risk of developing infectious complications [55]. Those patients with an increased HLA-DR expression in comparison to their individual HLA-DR density preoperatively had a high rejection risk.
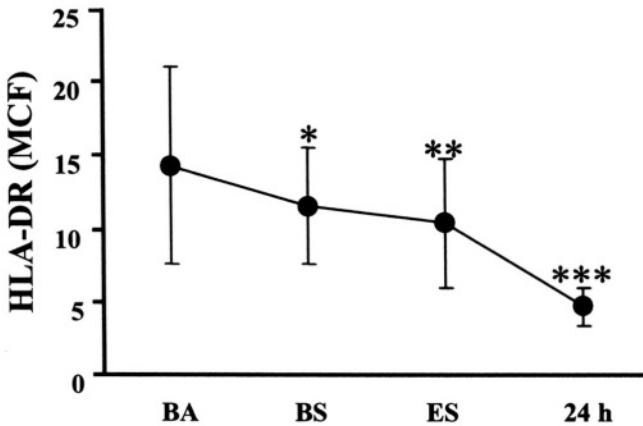

Figure 3. *Expression of HLA-DR on CD14⁺ monocytes before anesthesia (BA), before surgery (BS), at the end of surgery (ES), and 1 day after surgery (24 h). *p<0.05, **p<0.01, ***p<0.001 vs baseline.*

It must, however, also be pointed out that a decrease in density of HLA-DR on monocytes always occurs during surgery and that a significant decline

takes place immediately after induction of anesthesia, even before surgical intervention [56]. In this study the density of HLA-DR at day 1 after thoracic surgery was reduced to 30% of baseline levels (Figure 3). Interestingly, all patients had an uncomplicated post-operative course despite such a large decrease in HLA-DR density. Patients with severe pancreatitis also showed a decrease in HLA-DR on monocytes after surgery. However, repeated surgical interventions did not further influence HLA-DR expression [57].

Various mediators have been shown to regulate the expression of HLA-DR. Among the main regulators are cytokines: e. g., IL-10 and transforming growth factor (TGF)-β are known to dramatically reduce HLA-DR expression on monocytes *in vitro,* whereas IFN-γ is a potent counter-regulator. The beneficial effect of IFN-γ was demonstrated in patients with severe sepsis [54]. Patients with a monocyte HLA-DR expression below 30% on two consecutive days were treated with IFN-γ until more than 50% of monocytes were HLA-DR$^+$ for three consecutive days. Recovery of monocyte HLA-DR expression led to clearance of sepsis in 8 out of 9 patients in this study.

An additional mediator which influences HLA-DR expression on human monocytes in cell culture experiments as well as *in vivo* is the amino acid glutamine (GLN). GLN is the most abundant amino acid in the blood and in the free amino acid pool of the body. During starvation and catabolic stress following trauma, surgical procedures or sepsis, GLN loss correlates with the severity of the disease and supplementation of GLN has beneficial effects in those patients. Low GLN concentrations have profound effects on cells of the immune system. These effects include the inhibition of mitogen-induced T-cell proliferation, a decrease in the capacity of macrophages to phagocytose opsonized particles, and the reduction of the differentiation of B-cells into plasma cells. Previously we have shown that GLN deprivation in the culture medium down-regulates HLA-DR antigen expression on monocytes and reduces their capacity to present tetanus-toxoid to CD4$^+$ T lymphocytes [58]. Recently we demonstrated that the treatment of patients with a GLN-containing solution in the postoperative phase reduced immunosuppression by partial prevention of the surgery induced decrease in HLA-DR expression on monocytes [59].

The regulatory capacities of mediators on the expression of HLA-DR on monocytes and macrophages raises the question whether the reduced number of MHC class II molecules determines the capacity to present antigen. In fact, in a previous study it was shown that occupancy of as few as 0.1% of all MHC class II molecules has been found to be sufficient for stimulation of quiescent T-cells [60]. Therefore, these data suggest that the capacity of circulating monocytes to present antigen and to stimulate proliferation of T-cells is not suppressed during uneventful recovery after major surgery,

despite a significant loss of cell surface HLA-DR proteins. Thus, the correlation of HLA-DR reduction and clinical outcome are based on other mechanisms. However, since there is a well documented correlation between infectious complications, clinical outcome and the expression of HLA-DR on circulating monocytes, this antigen seems to be a reliable marker in the course of sepsis [52-54, 56, 57, 61].

Despite the well characterized expression of HLA-DR on monocytes, its expression on other cell types is only partly documented. Wakefield et al. described that in contrast to monocytes the level of T-cell HLA-DR expression rose significantly on the first day after surgery in non-septic patients to a level higher than in those who developed infection [62]. The investigators concluded that these findings have important implications for biological response modification in patients at risk of developing sepsis after surgery. However, in patients with septic shock Lin et al. observed no significant alterations in lymphocyte activation and they concluded that the study of lymphocyte cell surface marker expression during sepsis may be too insensitive to measure transient changes in lymphocyte activation [63]. Furthermore, HLA-DR expression on circulating B-cells from severely injured patients was investigated by Ditschkowski et al. [64]. In this study HLA-DR was significantly reduced from days 6-14 after admission in patients with subsequent severe sepsis compared to those who did not develop sepsis.

A recently published study by Ditschkowski et al. showed that the soluble form of HLA-DR in septic patients immediately after trauma was significantly lower then in non-septic patients. This was paralleled by a reduced expression of HLA-DR on circulating T-cells [65].

Additional Cell Associated Markers in Sepsis

Since monocytes play a decisive role in the development and course of sepsis and the expression of cell surface antigens is correlated with functional properties of these cells, numerous additional markers have been studied. One of these, the CD14 antigen, plays a central role in innate immune responses. CD14 is highly expressed on monocytes, binds bacterial LPS and LBP, and functions as an opsonin presenting LPS to CD14. Moreover, it is found as a soluble CD14 (sCD14) form in circulation, and the plasma level has been shown to be increased in some infectious diseases, including sepsis. The membrane-bound CD14 is down-regulated during sepsis and it has been suggested that decreased expression possibly indicates a poor prognosis [63, 66]. Recently, it has been shown that the signaling pathway of the complex LPS-LBP-CD14 is triggered by binding to Toll-like receptor (TLR) 4 and its

co-factor MD2. So far, only limited data are available concerning the regulation of TLR4 on monocytes and further studies will clarify its role as a suitable sepsis marker in an immune monitoring system.

In addition, a subset of monocytes with weak expression of CD14 and increased CD16 expression has been described in healthy subjects. This subset is expanded up to 80 % in sepsis patients and is characterized with features of tissue macrophages [67]. Interestingly, the appearance of these cells was paralleled by a high concentration of IL-6 in those patients. As previously mentioned, IL-6 has a high predictive value in the course of sepsis. However, in contrast to recently published work which demonstrated a down-regulation of HLA-DR, these CD16 + monocytes express high levels of MHC class II products. These different findings may be explained by different time points of measurement. Patients with high levels of HLA-DR and IL-6 are possibly in a state of transition from the hyper-inflammatory phase to immunosuppression. Our own observations have confirmed these results, since we have demonstrated that patients in the very early phase of sepsis had high IL-6 levels and showed an increase in the expression of HLA-DR and the production of **TNF-α** as well as in their capacity to phagocytose [68]. Therefore, sepsis patients with low IL-6 and **TNF-α** plasma levels, in combination with a progressively reduced monocytic phagocytic capacity and HLA-DR expression are possibly in a later phase of septic disease eventually leading to immunoparalysis.

To characterize the systemic inflammatory status, additional markers on monocytes as well as on various other cells have been described. The CD1ib/CD18 complex functions as an adhesive molecule in promoting cell interaction and mediates binding of iC3b-coated particles, leading to their ingestion and destruction. Takala et al. demonstrated that the expression of CD11b/CD18 increased with the severity of systemic inflammatory response syndrome (SIRS) and sepsis [69]. Since the increase in CD11b/CD18 expression does not require time-consuming *de novo* protein synthesis and occurs *in vivo* within minutes after stimulation, its expression may serve as an extremely early and sensitive cell-associated marker of phagocyte activation. On neutrophils the reduced expression of CD11b and CD16 after severe traumatic injury was correlated with the severity of injury and therefore possibly explains the increased incidence of septic complications seen in the more severely injured group after injury [70].

Ex vivo Inducible Sepsis Markers

Over the last few years techniques and measurements have been established to differentiate between the phases of the hyper-inflammatory phase of the

sepsis disease and immunoparalysis. One of these techniques is the stimulation of peripheral blood with LPS to determine the ability of leukocytes to produce and release pro-inflammatory cytokines into the plasma. Ertel et al. demonstrated that the LPS induced release of **TNF-α,** IL-**1β** and IL-6 into whole blood from sepsis patients was significantly depressed when compared with a control group [71]. This depression persisted up to 10 days after study enrollment. Interestingly, the half-life and consequently the expression of **TNF-α** and IL-6 mRNA were strongly reduced in the septic group. Despite the well-documented fact that the excessive secretion of pro-inflammatory cytokines in the early phase of sepsis has detrimental effects upon the patient, the reduced capacity of peripheral blood mononuclear cells (PBMCs) to synthesize and secrete pro-inflammatory cytokines may result in immunodeficiency, since these cytokines are involved in the up-regulation of essential cellular and humoral immune functions. This hypothesis was confirmed by Döcke et al [54]. As previously described, the administration of **IFN-γ** to sepsis patients reconstituted the expression of HLA-DR on monocytes. Simultaneously, their capacity to produce **TNF-α** after LPS stimulation recovered to normal levels when patients were cured from sepsis [54]. Further results in the immunosuppressive perioperative phase were obtained by Weighardt et al. who demonstrated that sepsis after major visceral surgery is associated with sustained and **IFN-γ-resistant** defects of monocyte cytokine production [66]. In this study an immediate defect of endotoxin-stimulated monocyte production of IL-12, **IL-1β,** and IL-10 was detected in both surviving and non-surviving patients. However during the final phase of postoperative sepsis, a significant recovery of IL-12 and IL-1, though interestingly not of IL-10, production, correlated with survival. The authors concluded that because both the pro- and anti-inflammatory cytokine secretion was affected immediately, immunosuppression is a primary rather than a compensatory response to a septic challenge.

    T lymphocytes play an important role in the septic disease since they are able to act as immunostimulators (Th1-cells) and as immunosuppressors (Th2-cells) as well. Both cell types are characterized by a specific cytokine pattern secreted under different immunological conditions. During the early postoperative course, reduced cytokine secretion is observed for IL-2, **IFN-γ** and **TNF-α,** which are associated with the Th1 phenotype of T helper lymphocytes [72].

    IL-4 production indicating the Th2 phenotype is also suppressed after major surgery, which demonstrates that major surgery is associated with a severe but transient reduction of T-cells to secrete a large panel of cytokines. The persistence of a diminished ability of T-cell cytokine secretion possibly leads to susceptibility of infectious complications.

A further study from this group with purified and stimulated peripheral T-cells demonstrated that selective T-cell proliferation and production of IL-2 and TNF were severely suppressed in patients with lethal intra-abdominal infection as compared with survivors and healthy controls [73]. TNF suppression in survivors was less severe than in non-survivors. Defective T-cell functions were also observed at the onset of sepsis and persisted throughout the entire observation period. Interestingly, the production of IL-4 and IL-10 was not affected by post-operative intra-abdominal infection. Since the immune defects were evident at the onset of sepsis, the authors concluded that immunosuppression may develop as a primary response to sepsis without preceding immune hyperactivity.

The importance of the cytokine IL-12 as a predictive parameter has been investigated by several groups. IL-12, mainly produced from phagocytes, is required for the production of IFN-γ by natural killer (NK)-cells and T lymphocytes and strongly supports the development of the Th1 phenotype of CD4+ cells. Furthermore IL-12 is required for the immediate defense mechanisms of the innate immune system as well as for the induction of subsequent adaptive immune responses. Hensler et al. demonstrated that monocyte IL-12 secretion was significantly impaired before surgery in patients developing post-operative sepsis and indicated that IL-12 may be crucial for establishing a protective immune response against post-operative infection [74]. Additionally, Ertel et al. were able to show diminished secretion of both IL-12 and IFN-γ in trauma or sepsis patients [75]. Because IL-12 and IFN-γ up-regulate essential immune functions, the marked inhibition of IL-12 and IFN-γ release may be pivotal for high susceptibility of critically ill patients to infection. Supporting results for a protective role of IL-12 were reported by Haraguchi et al. [76]. In a case report the authors demonstrated that IL-12 deficiency is associated with recurrent infections and that IFN-γ production from isolated and stimulated PBMCs was reduced.

'THE FUTURE IS AT THE BEDSIDE'

Thanks to progress in technology, sepsis markers are becoming more readily available with the use of automatic machines equipped with luminometric technique (cytokine analysis ≈ 20 minutes, e.g., DPC Bierman, Germany) or rapid bedside methods - IL-6 strips (Knoll, Germany) ± decision only [68], PCT cassettes (Brahms, Germany) semiquantitative, neopterin semiquantitative [77].

CONCLUSION

Considering the range of options available, one must be aware of the fact that the increase in parameters and compartments included in diagnosis, is directly proportional to the uncertainties arising [78]. Therefore, on the one hand, more prospective studies on sepsis markers are required to identify the most relevant and predictive markers (not to mention the financial aspects of costly analysis). On the other, the use of new approaches of data mining to identify specific patterns in specific patient groups is warranted. These efforts need the support of extensive pathophysiological research, since more extensive knowledge regarding patients' underlying diseases may facilitate the finding and choice of an appropriate marker (or set of markers). In conclusion we suggest improved monitoring of sepsis patients to enable a better selection of the appropriate cases and more precise timing for therapeutic interventions; much as vasopressors are not administered without pressure monitoring. We recommend the measurement of IL-6, PCT, and neopterin in plasma, particularly to determine the hyper-inflammatory phase. The most suitable marker for the immunoparalytic phase seems to be a combination of HLA-DR measurement on peripheral blood monocytes and the determination of the capacity of whole blood to secrete pro-inflammatory cytokines after stimulation. The fact that the use of sepsis markers for monitoring – though the actual parameter may ultimately be different – can be beneficial for the therapeutic regimen has recently been nicely demonstrated within the MONARCS, IL-6 monitored anti-TNF study. Furthermore, the active approach using the ACTH response test with monitoring of cortisol in plasma to identify specific sepsis patients [79] is a promising step for the future of sepsis trials.

The most recent research results raise hopes that sepsis markers in combination with increasing knowledge on the influence of polymorphisms and gender may enable us to identify patients at high risk of infectious complications. These patients may be those who will profit most from immune/biochemical monitoring, since individualized therapeutic interventions supporting the immune and humoral systems are within reach.

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