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REVIEW ARTICLE

Biomarkers of endothelial dysfunction: can they help us deciphering systemic inflammation and sepsis?

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Abstract

The endothelial integrity, as mechanical barrier against microorganisms and as natural "anticoagulant", is crucial for physiologic organ function. Systemic activation of the endothelium upon inflammation, sepsis, and septic shock is always ending in blood–tissue barrier disruption. With increasing dysfunction, uncontrolled clotting activation, capillary microthrombi formation, tissue edema, local hypoxia, and ischemia are initiated. This in turn enhances a vicious circle leading to multiple organ failure and death.

Therefore, biomarkers reflecting this special compartment may help in the early detection of systemic inflammation and its complications. This review provides an overview of the most important endothelial biomarkers and their possible use in sepsis.

Keywords: Systemic inflammation, sepsis, endothelial dysfunction, biomarkers, intensive care

Introduction

Sepsis represents the third leading cause of death in the industrialized countries (Anderson 2002; Andreu Ballester et al. 2008; Angus 2001a, 2001b; Dombrovskiy et al. 2007; Engel et al. 2007; McDonald et al. 2005). The early diagnosis and therapy of sepsis still remain a major challenge, where time plays a crucial role (Kumar et al. 2006; Lever and Mackenzie 2007; Zambon et al. 2008). It is well known that an increase in the number of failing organs during the first 48 h following admission to an intensive care unit (ICU) is a good indicator of mortality in septic patients (Ferreira et al. 2001). Therefore, the early diagnosis of sepsis and prevention of organ dysfunction progression to multiorgan failure (MOF) is the primary goal in the treatment of septic patients (Slade et al. 2003). In the daily clinical routine, many markers reflecting i.e. immune status, liver, kidney, or heart failure are commonly used to efficaciously detect the course of sepsis. However, one of the largest and most important organs, the endothelial and vascular system, is not routinely tracked in the daily clinical routine. No recommendations about measuring the extent of endothelial damage are embedded in the latest sepsis guidelines.

As one of the largest "organs", the vascular and capillary system occupies a central role in the homeostasis of organ functions. It provides a mechanical barrier, which keeps liquids within the vasculature, ensuring transport of nutrients to the different organs. It builds up a natural barrier that prevents microorganisms to invade tissues and it exerts a natural anticoagulant action that prevents from uncontrolled activation of the coagulation system.

Almost every stimulus leading to a systemic inflammatory response i.e. severe infection, trauma, excessive tissue breakdown, solid tumors, leukemia, pregnancy-associated complications, vascular anomalies, liver failure, and toxicological or immunological responses and activation of the coagulation system can be associated with endothelial damage. Systemic inflammation and sepsis are mostly accompanied by an overwhelming activation of the coagulation system, preceded by the endothelial dysfunction and the loss of its antithrombotic properties (Knoebl 2010; Levi 2010; Levi et al. 2002; Levi and van der Poll 2010). Clinically, this

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pathology first manifests i.e. as a general hemostasis reaction that may vary greatly from a simple drop in platelet counts or subclinical prolongation in clotting times to the full picture of a disseminated intravascular coagulation (DIC) and, finally ends up in a global consumption of clotting factors (Levi 2007; Levi and Ten Cate 1999; Taylor et al. 2001). During sepsis, the release of endotoxin or proinflammatory cytokines initiates an activation cascade in endothelial cells (ECs) leading to impaired homeostasis (Ince 2002; Tyagi et al. 2009). Due to rapid changes in endothelial function (early contraction followed by a rapid loss of function), microthrombi occur by activation of the clotting system leading to changes in blood rheology and causing organ failure due to misperfusion (Lee and Slutsky 2010; Lehr et al. 2000). Thus, in septic patients, levels of factors that directly reflect endothelial dysfunction might be changed (Fareed et al. 1998; Iba et al. 2005).

In sepsis, endothelial activation and dysfunction are critical determinants of the host response and represent an explanation for the complex sepsis pathophysiology (Fareed et al. 1998; Iba et al. 2005; Shapiro et al. 2010). Given this central place of the endothelium, biomarkers reflecting ECs' state might be useful in the tracking of sepsis (Pierrakos and Vincent 2010). However, direct markers reflecting endothelial damage are not commonly used in daily clinical routine.

We have, therefore, reviewed the most common direct endothelial markers, with special view on their physiologic role and changes during systemic inflammation in humans.

von Willebrand factor and ADAMTS13

The acute phase glycoprotein von Willebrand factor (vWF) is a hemostatic cofactor that is physiologically produced in ECs, megakaryocytes, and subendothelial connective tissue. Usually, vWF is able to form large multimers, which circulate in the blood. The multimers undergo proteolysis by a specific plasma metalloprotease known as ADAMTS13 (a disintegrin and metalloprotease with thrombospondin motif). Under physiologic conditions, circulating ultra-large vWF (ULVWF) is barely detectable. Severe hereditary or acquired ADAMTS13 deficiency causes organ dysfunction and systemic inflammatory response syndrome (SIRS). In proinflammatory conditions, ADAMTS13 activity decreases, leading to increased levels of circulating vWF, which in turn causes platelet thrombus formation. Severe sepsis or septic shock results in the accumulation of substantial amounts of plasma ULVWF, which positively correlates with the severity of inflammation and the degree of organ failure (Claus et al. 2010).

The role of vWF as a biologic marker for the diagnosis of sepsis, or the discrimination between individuals at risk for developing acute respiratory distress syndrome (ARDS) or as prognosis marker is very inconsistent. In fact, an increase in plasma levels of vWF can be caused by various septic and non-septic insults (Reinhart et al. 2002). The hypothesis that vWF may be increased in patients suffering from ARDS vs. only septic patients could not be fulfilled. The same observation was done, when comparing sepsis vs. non-septic patients with hyperinflammation (Rubin et al. 1990). Thus, it seems that vWF is a marker for diagnosing hyperinflammation, without being able to discriminate between specific organ damage (i.e. ARDS) or inflammation accompanied by infection as it occurs in sepsis. Nevertheless, vWF levels could help to differentiate between survivors and non-survivors in sepsis and high levels of vWF were correlated to fewer days organ-failure-free days (Moss et al. 1996; Scherpereel et al. 2006). Furthermore, plasma vWF-antigen levels were associated with the development of acute lung injury and 28-day survival. However, these data demonstrated moderate sensitivity (87%) and specificity (77%) (Rubin et al. 1990; Sadler 1998). McGill et al. (1998) also demonstrated that vWF might be useful to serve as prognostic marker, predicting survival with a positive predictive value by 80%. Sensitivity was 80% and specificity was 87% (McGill et al. 1998). However, Moss et al. (1996) could not verify these findings.

Taken together, vWF might only serve as marker to detect systemic endothelial activation upon systemic inflammation. Also, high levels of vWF reflect endothelial damage and correlate with poor survival and fewer organ-failure days. However, it seems that discrimination between infection and specific organ damage is not possible with this marker as data are too inconsistent at current stage.

Concerning the vWF cleaving protease ADAMTS13, data seem to reflect the same tendency as for vWF. Unfortunately, the current literature does not provide any larger studies regarding its role in inflammation. In a small clinical study (50 patients), ADAMTS13 was decreased in septic patients with concomitant DIC, however, with a low predictive value. The incidence of acute renal failure in patients with decreased ADAMTS13 levels was 41.2%. Decrease of the protein was due to a combination of increased cleavage together with a decreased synthesis of new ADAMTS 13 in the liver (Furlan and Lammle 2001; Mimuro et al. 2008; Ono et al. 2006).

Ang-1 and -2

The angiopoietins (Ang)-1 and -2 belong to the family of growth factors and have been studied mainly in proliferative diseases such as cancer (neoangiogenesis) (Davis et al. 1996; Fiedler and Augustin 2006; Maisonpierre et al. 1997; Suri et al. 1996). Ang-1 and -2 are antagonistic factors that trigger endothelial cell activation, involving the most important intracellular pathways (nuclear factor-κB for inflammation, Rho-kinase for interendothelial cell contacts and PI3K/AKT pathway for cell survival). Ang-1 and -2 bind to the endothelial Tie-2 receptor. Activation of the Tie-2

pathway involves processes such as vessel integrity, vascular permeability, and the regulation of inflammation (Fiedler et al. 2003; Mammoto et al. 2007; Papapetropoulos et al. 2000; Witzenbichler et al. 2005; Wong et al. 1997; Yuan et al. 2000). The importance of the Ang/Tie system in systemic inflammatory disorders has been demonstrated in many studies. In critically ill patients, the release of Ang-2 directly reflects vascular barrier breakdown. Orfanos et al. (2007) could demonstrate that Ang-2 serves as marker to discriminate between sepsis and severe sepsis ($p < 0.05$). The authors investigated serum Ang-2 levels and could also show that Ang-2 acts similar to tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, two very important sepsis markers ($p < 0.001$). The clinical study was performed on a very small group (61 patients, 6 non-SIRS, 8 SIRS, 16 sepsis, 18 severe sepsis, and 13 septic shock patients).

Other small clinical studies demonstrated that circulating Ang-2 levels correlated with the APACHE and SOFA scoring systems as well as with the 28-day mortality reflecting disease severity and prognosis (Fiedler et al. 2006; Kranidioti et al. 2009; McCarter et al. 2007; Orfanos et al. 2007; Thurston et al. 2000; Thurston et al. 1999; Witzenbichler et al. 2005).

Another study with 293 children demonstrated that low Ang-1 and higher Ang-2 concentrations are associated with an unfavorable outcome in children with severe bacterial infection. The authors concluded that alterations of these secreted factors are directly linked to the endothelial damage/dysregulation occurring in severe bacterial infection and that angiopoietins could be used for the early identification of patients at risk of a poor outcome (Mankhambo et al. 2010).

Recently, Ricciuto et al. (2011) confirmed, in their study with 70 patients, the predictive power of Ang-1 and -2 as prognostic marker in the course of sepsis. Ang-1 plasma levels at admission and during the course of disease, both Ang-1 and -2 highly correlated with 28-day mortality in severe sepsis. Moreover, Ang-2 levels also correlated with disease severity as reflected by markers of organ damage and clinical sepsis scores. With Ang-1 exists a marker that might be of use to predict probability of sepsis mortality already at admission reflecting the critical role of early endothelial dysfunction. Here, low levels correspond with high mortality.

Taken together, Ang-2 and -1 seem to be of interest as prognostic sepsis biomarker. When detected over time, Ang-2 levels show a similar pattern such as TNF-α and IL-6. Ang-2 might present a reliable marker reflecting the direct status of the endothelium that correlates with disease severity (SIRS and sepsis vs. severe sepsis) and outcome (i.e. 28-day survival). Whereas, Ang-1 levels might have a high predictive outcome value, when determined at admission to the ICU. The early detection of severe disease courses and the progression to another pathologic state are extremely important for early decision to therapy escalation; therefore, Ang-1 and -2 levels might be a valuable tool to help taking this decision.

Endocan

Endocan, or originally known as endothelial cell-specific molecule-1, is a proteoglycan that is constitutively expressed in human ECs as well as in the human lung and kidney. It is localized in the vascular network, but also in the corresponding epithelia and in adipocytes (Bechard et al. 2000; Janke et al. 2006; Wellner et al. 2003). The expression of endocan in ECs is up-regulated in response to TNF-α or IL1-β. Its secreted form circulates in the human bloodstream and can easily be detected (Bechard et al. 2001a, 2001b; Lassalle et al. 1996). It is suggested that in patients with septic shock serum levels of endocan may increase dramatically, representing the occurring endothelial damage and correlating to the severity of disease and the outcome (Scherpereel et al. 2006).

Scherpereel and colleagues investigated circulating endocan levels in serum samples from septic patients ($n = 63$). The data were compared to serum levels of healthy volunteers ($n = 20$) and to serum endocan levels of patients with SIRS ($n = 7$). The authors only measured endocan levels once, at admission to the ICU. They found that endocan levels significantly varied between patients in septic shock, severe sepsis, and sepsis yielding specificity from about 80% and 100% for SIRS vs. sepsis and a sensitivity of about 82%. Also, the single point detection of endocan at admission was useful to discriminate between 10-day survivors and non-survivors, latter displaying significantly higher levels. Endocan levels concerning 10- and 28-day survival had a high negative predictive power with 92%. Cutoff levels were set at 1.2 for all sepsis vs. SIRS and 3.0 for septic shock vs. all sepsis. Unfortunately, this study lacks kinetic data.

Other studies described endocan as not specific for systemic inflammatory diseases. High levels have also been detected in patients with cancer (Aitkenhead et al. 2002; Depontieu et al. 2008; Grigoriu et al. 2006; Huang et al. 2009; Scherpereel et al. 2003; Zuo et al. 2008). Nonetheless, high levels may be of relevance for the promotion of systemic inflammation, as endocan mediates the recruitment of circulating lymphocytes to inflammatory sites as well as leukocyte adhesion and activation (Bechard et al. 2001b).

Large preclinical investigations underline the previous findings from the clinical studies. Taken together, endocan has a high prognostic value to discriminate between 10- and 28-day survivors. It serves also to distinguish between SIRS and sepsis with a specificity of 100%. However, no data exist for patients undergoing large cancer surgery, as cancer also upregulates endocan circulating levels (Sarrazin et al. 2006). Moreover, endocan kinetic data in the course of sepsis are missing. Endocan may be a very robust marker to solely detect endothelial

(as organ) damage at early time points, which has been verified in large preclinical trials.

Selectins and other adhesion molecules

Selectins (E-, P-, L-) represent key adhesion molecules during the inflammatory process. They are the key mediators of leukocyte trafficking and hemostasis implicated in many human diseases including systemic inflammatory disorders and sepsis (Kansas 1996; Laubli and Borsig 2010; Ley 2003; McEver 1997; Witz 2008). The selective recruitment of subpopulations of circulating leukocytes to the sites of injury or inflammation is a complex process orchestrating the interaction of primary (selectin) and activation-dependent (integrin) adhesion molecules. Selectins are expressed on the surface of activated ECs, platelets, and leukocytes. After activation, selectin receptors are shed from the surface and are measurable in the circulation. Severe injury and subsequent onset of systemic inflammation is resulting upon release of IL-1 β, IL-6, IL-8 (CXCL-8), and TNF-α contributing in turn to the release of adhesion molecules. Therefore, soluble selectins are directly linked to the secretion of proinflammatory mediators and reflect endothelial response upon inflammation (Carlos and Harlan 1994; Newman et al. 1993; Siemiatkowski et al. 2001).

Endothelial leukocyte adhesion molecule (E-selectin or ELAM-1) is exclusively expressed on activated ECs. E-selectin can rapidly be induced (within 2–6 h) in response to IL-1 or TNF-α released by damaged cells or upon disturbances in blood rheology (Kansas 1996; Morigi et al. 1995).

During the initiation of inflammation, neutrophils interact with ECs by binding to the endothelial intercellular adhesion molecule-1 (ICAM-1). ICAM-1 instead is constitutively expressed on ECs but is highly inducible upon stimulation (Kayal et al. 1998). Together with E-selectin, ICAM-1 plays an important role in the initiation or "rolling" phase of inflammation. E-selectin exists in a bound and a soluble form (Cummings et al. 1997; Rao et al. 2007).

P-selectin has a similar function, but is constitutively expressed in lung ECs, and therefore correlates with lung endothelial injury (Sakamaki et al. 1995). On demand, it can rapidly be released from the Weibel–Palade bodies of ECs or α-granules from thrombocytes *via* exocytosis (Kansas 1996; McEver 1997) after appropriate activation by histamine, thrombin, complement or reactive oxygen species. Increased soluble (s) P-selectin levels coincide with a raised number of leukocytes "rolling" along the endothelium thereby reflecting their activation (Rivera-Chavez et al. 1998). In addition, functionally active plasma-soluble P-selectin may modulate leukocyte adhesion to the P-selectin expressed on ECs (Siemiatkowski et al. 2001).

L-selectin is constitutively expressed on all myeloid cells, on naïve T-cells and some activated memory T-cells. Upon their activation, L-selectin is quickly proteolytically cleaved (Kansas 1996; Laubli and Borsig 2010; Ley 2003). Soluble L-selectin concentrations are reduced by widespread binding to activated endothelium making it a representative marker reflecting the activation status of the endothelium. Elevated levels of sL-selectin are detected in patients with AIDS, leukemia, and SIRS. Low concentrations can be found in ARDS, brain injury, and severe organ failure (Cocks and Chan 1997; Donnelly et al. 1994; Kerner et al. 1999; Maekawa et al. 1998; McKeating et al. 1998; Siemiatkowski et al. 2001).

Similar to the selectins, vascular cell adhesion molecule-1 (VCAM-1) or CD106 participates in the adhesion of leukocytes to the endothelium at the site of inflammation. Besides its mechanical function, it also plays a role in the intercellular signal transduction. Upregulation of VCAM-1 in ECs is transcriptionally activated mainly by cytokines (TNF-α and IL-1). VCAM-1 protein is endothelial specific, and is a ligand for the $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins (Cook-Mills et al. 2010; Rao et al. 2007).

In a cohort of 25 consecutive newborns, the investigation of sICAM-1 revealed that levels increased during the course of sepsis, and being higher in patients with positive vs. negative microbial hemoculture (HC). Soluble VCAM-1 only increased slightly in HC-positive sepsis. The highest sICAM-1 levels could be positively correlated with scores of illness severity. Soluble VCAM-1 levels increased only slightly in HC-positive sepsis, while sL-selectin and sP-selectin did not change (Figueras-Aloy et al. 2007).

In another study including 119 critically ill patients, E-selectin serum levels were assessed and compared to healthy volunteers. The authors could show that sE-selectin levels were higher in serum of patients with microbiologically documented sepsis than in other critically ill medical ICU patients. The E-selectin levels at day 1 of sepsis diagnosis correlated highly with hemodynamic compromise and modestly with subsequent organ dysfunction and survival (Cummings et al. 1997).

In 63 septic patients with an infection in one or more major organs, serum sL-selectin could be identified as a predictor of survival in patients with sepsis. Patients with low sL-selectin (<470 ng/mL) were characterized by a high 1-year mortality (Seidelin et al. 2002).

In a pediatric study including 77 children with sepsis and 14 acutely ill children without sepsis, a pronounced and persistent increase in plasma VCAM-1 and ICAM-1 was observed in septic children and persistent MOF. The authors concluded that these changes correlated mainly with the proinflammatory status of the endothelium (Whalen et al. 2000).

Taken together, selectins and adhesion molecules may serve as markers for the detection of general endothelial activation and consequently sepsis. The studies investigating their use as biomarkers revealed that they can be used to monitor i.e. specific organ's endothelium damage (i.e. L-selectin for ARDS). Also, these molecules seem to be good markers of prognosis for the 1-year survival and for progression of disease in case general

endothelial damage has not yet occurred. However, the exact function and biological role of the soluble adhesion factors is so far unclear; some of the factors influencing their plasma levels are yet unknown. These markers, however, act otherwise to secreted factors described above. The soluble adhesion molecules are binding to the activated endothelium and are thus not detectable by tests. Therefore, low levels are often associated with the severity of disease and hence the severity of endothelial activation.

ET-1 and its precursor peptide proET-1

The endothelium-derived 21-residue venous and arterial peptide, endothelin (ET), has a potent and prolonged vasoconstrictor and vasopressor activity. ET has two functions: first, as circulating hormone and second, as paracrine factor mainly involved in the regulation of the vascular tone and systemic blood pressure (Wei et al. 1994; Yanagisawa et al. 1988). ET production is induced by substances such as epinephrine, IL-1, thrombin, and by conditions such as shear stress and damage to ECs. The ET family consists of three distinct isopeptides, ET-1, ET-2, and ET-3, which are derived from three distinct genes. ET-1 is the most potent vasoconstrictor out of the three. It is synthesized as a much longer polypeptide preproendothelin, being 203 amino acid residues long. Preproendothelin is cleaved by a signal peptidase to pro-ET-1, which in turn is cleaved by ET-converting enzyme (ECE) to the 21 amino acid long ET-1 (Brauner et al. 2000; Hemsen et al. 1996; Kido et al. 1998; Schuetz et al. 2011; Schweizer et al. 1997; Tschaikowsky et al. 2000). Using a sandwich immunoassay, Schuetz and colleagues evaluated proET-1 levels in patients with community-acquired pneumonia (CAP) and found that proET-1 levels at admission were independent predictors of short-term mortality and the need for ICU admission. They concluded that proET-1 levels improved the prognostic accuracy of the commonly used CURB-65 score to predict adverse outcome (Schuetz et al. 2007; Schuetz et al. 2009; Schuetz et al. 2008). Piechota and colleagues could demonstrate in another study including twenty patients with sepsis and severe sepsis that ET-1 levels highly correlate with levels of NT-proBNP, PCT, and CRP, as well as the SOFA score. Mean ET-1 concentrations were 8.39 ± 6.39 pg/mL. Correlation between ET-1 levels and levels of NT-proBNP, PCT, and CRP was 0.3879 ($p < 0.001$), 0.358 ($p < 0.001$), and 0.225 ($p = 0.011$), respectively. Correlation between the ET-1 levels and SOFA score was 0.470 ($p < 0.001$) (Piechota et al. 2007).

Newborns physiologically present a reduced liver function. This is associated with low CRP levels, as CRP is produced in the liver. Hence, ET-1 might also be a good marker to detect sepsis especially in newborns with the same reliability than CRP or PCT in adults. Figueras-Aloy et al. (2004) demonstrated that plasma ET-1 levels in neonatal sepsis are related to the severity of clinical manifestations, especially oliguria, acidosis, and systemic hypotension. Taken together, proET-1 levels in CAP patients at admission may be independent predictors of short-term mortality and the need for ICU admission, correlating strongly with the CURB-65 score. With disease progression from sepsis to severe sepsis, ET-1 levels strongly correlated with markers of cardiac failure and markers of infectional status, reflecting disease severity. In newborns, where CRP production is physiologically low and thus not reliable, ET-1 might be a valuable marker to monitor disease courses.

VEGF and soluble VEGF-receptor-1 (Flt-1)

Vascular endothelial growth factor (VEGF) is a hypoxia-inducible cell mitogen. It stimulates endothelial cell migration along with vessel permeability (Dvorak et al. 1995), and promotes survival of the newly formed blood vessels (Ferrara and Davis-Smyth 1997). VEGF is crucial for endothelial cell survival (Gerber et al. 1999). Although VEGF is highly specific for ECs, it has become increasingly clear that it also elicits responses in non-ECs types. For example, it is chemotactic for monocytes and can inhibit the maturation of dendritic cells (Barleon et al. 1996; Clauss et al. 1990; Gabrilovich et al. 1996).

The family of VEGF-related molecules contains five members: VEGF, placenta growth factor, VEGF-B, VEGF-C, and VEGF-D, whereas VEGF-A plays the most important role during sepsis. VEGF-A is 50,000 times more potent than histamine and is a potent inducer of tissue edema (Dvorak et al. 1995). VEGFs bind to their receptors, which are mainly expressed on ECs. Currently three types are described: fms-like tyrosine kinase-1 and -4 (Flt-1, -4) and kinase-insert-domain-containing receptor (VEGFR-2 or KDR) (de Vries et al. 1992; Terman et al. 1992). A soluble form of Flt-1 is produced by alternative splicing and acts as a natural occurring inhibitor of the VEGF-signaling. Recent studies have demonstrated the direct role of this receptor in sepsis concerning disease progression, severity, and survival (Ebihara et al. 2008; Pickkers et al. 2005; van der Flier et al. 2005; Yano et al. 2006).

All studies investigating VEGF-A or its soluble receptor in sepsis were significantly associated with sepsis severity and organ dysfunction (high correlation with organ dysfunction scores). A recent study conducted in a small cohort of 18 patients with severe sepsis and in 40 healthy controls could show that plasma VEGF levels were increased during severe sepsis. Moreover, the authors associated plasma VEGF levels with disease severity and mortality and explained these results by the VEGF-triggered capillary leak (van der Flier et al. 2005). Karlsson et al. (2008) confirmed the relationship between high VEGF-levels and severe sepsis in a large clinical study including 470 patients. With progression of disease to septic shock, the levels of VEGF decreased, as a reflection of endothelial dysfunction. With this downregulation of VEGF, EC apoptosis rate will in turn increase, reinforcing the vascular disorder and finally leading to death. The

authors could also demonstrate this relationship, where low VEGF-levels correlate with survival. These data indicate that VEGF levels increase while sepsis progresses. At the same time, the physiologic counter-regulation *via* VEGF scavenging by soluble Flt-1 is induced. Therefore, Flt-1 represents an interesting biomarker candidate, recently demonstrated in 101 patients (Yang et al. 2011). High plasma sFlt-1 levels significantly correlated with pneumonia-related septic shock and negative 28-day survival with median values of 659 pg/mL.

Taken together, the VEGF and sFlt-1 data seem to be very specific in detecting progression of disease from non-sepsis to severe sepsis and to septic shock. The progression from systemic inflammation, the most common syndrome on ICUs after major surgery, to sepsis needs further evaluation. VEGF and its soluble receptor served also to reliably detect short-term and 28-day survival, where low VEGF and high Flt-1 levels correlated with poor outcome. This may be explained by the massive intrinsic counter-regulation to VEGF-overproduction during sepsis. Compared to well-established daily clinical tests, especially sFlt-1 strongly correlates with Il-6 levels (Shapiro et al. 2010). Whether VEGF levels in cancer patients suffering from sepsis following large tumor surgeries are reliable remains to be elucidated as VEGF is also a marker for cancer progression.

PDGF

Platelet-derived growth factor (PDGF) was first described in the 1970s as a serum factor that stimulates proliferation of smooth muscle cells. Its name "platelet-derived," is misleading due to the fact that the endothelium presents the major source of PDGF during sepsis (Battegay et al. 1994; Thommen et al. 1997; Yaguchi et al. 2004). The PDGF family compromises four different members, PDGF-A, -B, -C, and -D, forming biological active homodimers (Betsholtz 2003). Lack or loss of PDGF-B leads to vascular dysfunction and vascular leakage (Gaengel et al. 2009; Reigstad et al. 2005; Tallquist and Kazlauskas 2004; Trojanowska 2008).

In a study, involving 46 patients at day 3 of severe sepsis, PDGF-BB levels were measured correlating PDGF-BB levels with the outcome of septic patients and evaluated PDGF-BB as response marker for treatment with recombinant human-activated protein C (rhAPC). The authors proposed PDGF-BB as a useful laboratory marker to predict survival in patients suffering from severe sepsis (Brueckmann et al. 2007).

uPA and PAI-1

Plasminogen activator inhibitor (PAI-1) belongs to the family of serine protease inhibitors (SERPINs). PAI-1 is the most potent inhibitor of the serine proteases tissue plasminogen activator (tPA) and urokinase PA (uPA). The latter are potent activators of plasminogen and hence fibrinolysis. They are described to be involved in the inflammatory process and endothelial cell migration. Moreover, uPA seems to play a critical role in the VEGF-induced vascular permeability change (Yang et al. 2011). Under physiological conditions, PAI-1 is released into the circulation and the extracellular space by liver cells, smooth muscle cells, adipocytes, and platelets. This results in plasma levels of only 5–20 ng/mL of active PAI-1, which is sufficient to control fibrinolysis and extracellular proteolysis (Binder et al. 2002). However, during pathological states such as systemic inflammation, large amounts of PAI-1 may be secreted by ECs. Here, mainly lipopolysaccharide derived from gram-negative bacteria, TNF-α, and IL-1 are responsible for the PAI-1 upregulation (Binder et al. 2002; Zhang et al. 1997).

A clinical trial with 101 patients (81 patients with pneumonia-related septic shock and 20 with pneumonia without organ dysfunction) demonstrated the direct link between VEGF and uPA. Together with the increase of VEGF receptor, the activity of uPA increased significantly in non-survivors with septic shock. uPA activity was also considered as an independent marker of renal and hematologic dysfunction as well as metabolic acidosis. With increase of the number of failing organs, the uPA activity also increased (Yang et al. 2011).

In another large clinical study including 117 patients with sepsis-induced DIC and 1627 patients with non-septic DIC, the potential of PAI-1 as biomarker for sepsis was evaluated. Data showed that in septic DIC patients, plasma PAI-1 levels were significantly higher than in non-septic DIC cases, moreover high PAI-1 levels (>90 ng/mL vs. <30 ng/mL) in septic DIC patients correlated with multiple organ dysfunction scores. The 28-day mortality after DIC diagnosis was higher in patients having high circulating PAI-1 levels in the blood.

Taken together, uPA was described as an independent marker for organ dysfunction, increasing with the number of failing organs. Also, uPA was a marker for survival in septic shock patients, where high levels correlated with poor survival, independent of infection status. More, PAI-1 levels at the time of DIC diagnosis were an independent risk factor for mortality in sepsis-induced and a marker for organ failure progression after DIC onset (Madoiwa et al. 2006). The close link between endothelial dysfunction and coagulation activation is directly reflected by uPA and PAI-1. Their activation shows on the one hand the endothelial homeostasis and on the other hand, the activation of the clotting system.

Fibrin degradation products

During systemic inflammation and sepsis, coagulation is partly activated or increased due to the loss of endothelium's antithrombotic properties and exposure of highly thrombogenic subendothelial structures (Gimbrone et al. 1982 Nawroth and Stern 1985; Semeraro and Colucci 1992). Here, massive turnover in coagulation factors and especially fibrinogen are observed. Along with this, the fibrinolysis system is activated, leading to a significant

amount of fibrin degradation products (Lord 2007; Mosesson 2005). Namely, X and Y fragments, D-dimers, D and E fragments, Bβ15–42 and smaller fragments (Olexa et al. 1981; Smith et al. 1990; Walker and Nesheim 1999).

Our understanding of fibrin degradation products until nowadays has been limited by their simple measurement as by-products of fibrinolysis without any biological activity. But, there is emerging evidence that fibrin degradation products also trigger inflammation. So far, only one molecular target has been reported to mediate this effect, namely vascular-endothelial-cadherin, an anchor protein that is part of the tight junctions and thus directly responsible for endothelial leak tightness (Petzelbauer et al. 2005). Even fibrinogen and fibrin monomers are able to bind to ICAM-1 on ECs and thereby promote the attachment of leukocytes and platelets which in turn may then result in vascular occlusion (Altieri et al. 1995) strong vasoconstriction by the stimulation of ET-1 (Anggrahini et al. 2009), or resulting in paracellular hyperpermeability and subsequent fluid and protein leak *via* RhoA-dependent signaling pathways (Guo et al. 2009).

Deitcher and Eisenberg (1993) observed high concentrations of fibrin degradation products in plasma in 100 patients with a gram-negative infection. The test (measurement of fibrin fragments by enzyme-linked immunosorbent assay) had a high negative predictive value of 100% in the case of gram-negative infection and 91% in the case of general bacteremia. The authors concluded that high levels of fibrin degradation products correlated with a high fibrinolytic activity in patients with gram-negative bacteremia. The fibrin fragment hemagglutinin test, which is—according to the authors—easy to perform (bed-side test that provides results within 3 min and yield a negative predictive value of 96%) could be useful to detect those patients who are less susceptible for gram-negative infections.

Discussion

Sepsis is known for centuries and still remains a current challenge as it is the third leading cause of death in industrialized countries. Novel therapies, and usually very expensive, have emerged in the last decades. However, treatment and its success also require a very specific and reliable diagnostic to be efficacious (De Waele 2010; Russel 2008).

Progressive organ dysfunction is a hallmark of this disease. One of the largest and most important organs is the endothelial system. Without a functional vascular system, blood rheology, coagulation, and organ perfusion is massively impaired. Endothelial dysfunction is a broad term, which is mainly used to refer to an imbalance of vasoconstriction, vasodilatation and vascular leakage, and edema formation. The endothelial injury, therefore, plays a key role during sepsis pathogenesis and is highly associated with mortality (Duffy et al. 2011). Tissue edema may rapidly impair organ function by elongating the oxygen diffusion distance, favoring the formation of microthrombi with consequent breakdown of adequate organ perfusion (Lee and Slutsky 2010). Therefore, biomarkers serving as indicators or as surrogate markers for endothelial cell activation and dysfunction are of particular importance (Filep 2006).

Today, the circulating levels of all these aforementioned factors, cytokines etc. are easily detectable and measurable. However, standardized chemistry tests to evaluate levels of creatinine, LDH, or GOT etc are missing for endothelial failure. Many tests described in this review seem to be robust and reliable tests. It is astonishing, due to the central role of the endothelium and its direct link to coagulation and homeostasis, that these markers have not been followed up until today. Some of the tests described in this review seem to be sensitive and specific enough to track the course of sepsis; however, they are not used broadly in the clinical routine. Economic considerations and physician's personal interest might surely play an important role in the propagation of such tests. Another aspect might also be the perception of the endothelium as central organ. We would like to encourage the message that the endothelium is far more than just the smallest brick in the vessel wall, but that it keeps upright important gradients in the body. Even, there is no organ damage when the vasculature is working correctly and efficiently.

Conclusion

Loss of endothelial function and subsequent organ damage are hallmarks of systemic inflammatory disorders and sepsis. The central role of the endothelium renders this compartment an attractive target for biomarker research. However, which markers to choose in clinical practice will depend on further validation of the existing markers, the clinical accessibility and feasibility, economic aspects, and the personal experience of the treating physician.

Declaration of interest

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