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REVIEW PAPER

In search of biomarkers for diagnosing and managing neonatal sepsis: the role of angiopoietins

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Abstract

Angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) are antagonistic ligands that bind to the extracellular domain of the Tie-2 receptor, which is almost exclusively expressed by endothelial cells. Angiopoietins can directly stimulate both endothelial cells and neutrophils for an overall proinflammatory and proangiogenic response. An increasing number of experimental and clinical studies gave evidence that in the course of sepsis the serum levels of Ang-1 and Ang-2 as well as their ratio significantly differ from those in healthy subjects, in non-septic hospitalized patients, and in patients with non-infectious systemic inflammatory response syndrome (SIRS) or critical illness. Further evidences have demonstrated that the magnitude of Ang-2 dysregulation correlates with the severity of sepsis and the mortality rate. Since the onset of neonatal sepsis is often subtle and the diagnosis occurs later, Ang-1 and Ang-2 appear to be very promising biomarkers for improving the diagnosis and the management of septic newborns.

Keywords

Angiopoietin-1, angiopoietin-2, endothelial cell, inflammation, neonatal sepsis, Tie receptors

History

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Introduction

Microvascular changes play a strategic and pivotal role in inflammation. Sepsis is a clinical syndrome characterized by excessive vascular permeability, microvascular thrombosis and inflammation that results from diffuse endothelial cell dysfunction [1]. Inflammation activates endothelium by increased expression of luminal adhesion molecules, leukocyte recruitment and altered vasomotor tone, resulting in vascular barrier breakdown. Endothelial barrier disruption plays a key role in the pathogenesis of sepsis and septic shock, contributing to the complex pathway of sepsis and making it an attractive target for studies aimed to identify new therapeutic targets [2]. The degree of endothelial activation and subsequent dysfunction contributes to the severity of illness and progression of disease. Such molecules, like vascular endothelial growth factors (VEGFs) and angiopoietins are critical in the recruitment of inflammatory cells as well as in angiogenesis. In particular, the endothelial-specific angiopoietin–Tie ligand–receptor system has recently emerged as a non-redundant regulator of endothelial activation. Thus, biomarkers reflecting endothelial cells state might be useful for tracking sepsis [3]. However, a potentially ideal biomarker should be specific in reflecting the pathophysiological mechanism of the disease affecting endothelial activation or dysfunction; in addition, changes in the biomarker concentration should be closely related with disease activity over time and reproducible across patients and populations. Angiopoietin-1 and -2 (Ang-1 and Ang-2) are two of the most widely studied biomarkers of endothelial activation/dysfunction in infectious diseases.

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Angiopoietins and Tie receptors

Angiopoietins are angiogenic factors belonging to a family of glycoproteins (growth factors) acting primarily on the vasculature to control blood vessel development and stability [4]. Angiopoietins contain an amino-terminal angiopoietin-specific domain, a coiled-coil domain, a linker peptide and a carboxyl-terminal fibrinogen homology domain. Four distinct angiopoietins have been described: Ang-1, Ang-2, Ang-3 and Ang-4. Ang-1 is produced constitutively, primarily in the pericytes and smooth muscle cells that surround the endothelial cell monolayer [5]. Ang-2 is produced by endothelial cells themselves and stored in Weibel-Palade bodies for rapid release upon exposure to various noxious or inflammatory stimuli. Ang-3 and Ang-4 have different tissue distributions: Ang-3 is expressed in multiple mouse tissues, whereas Ang-4 is specifically present at high levels only in human lungs. Ang-1 and Ang-2 are antagonistic ligands of the Tie-2 receptor, which belongs to a family of vascular tyrosine kinase receptors expressed primarily in endothelial cells. The

acronym Tie stands for tyrosine kinase with immunoglobulin and epidermal growth factor (EGF) homology domains. In fact, Tie-2 is a 140-kDa tyrosine kinase receptor with homology to immunoglobulin and EGF. Tie receptors have an amino-terminal ligand binding domain, a single transmembrane domain and an intracellular tyrosine kinase domain. Tie-2 is shed from the endothelial cell and can be detected in soluble form in normal human serum and plasma; soluble Tie-2 may be involved in ligand scavenging without signaling. Angiopoietins bind the second immunoglobulin motif of Tie-2 whereby they activate Tie-2 and, indirectly, Tie-1 in Tie-1/Tie-2 heterodimers [6]. Tie-2 receptors are believed to play an important role in stabilization or destabilization of endothelial integrity as well as angiogenesis by involving processes such as vessel integrity, vascular permeability and the regulation of inflammation [7]. Ang-1 and Ang-2 trigger endothelial cell activation, involving the most important intracellular pathways (nuclear factor-κB for inflammation, Rho-kinase for inter-endothelial cell contacts and PI3K/AKT pathway for cell survival). Under physiological conditions, the serum concentration of Ang-1 exceeds that of Ang-2, allowing Ang-1 to preferentially bind the Tie-2 receptor and thereby initiate pro-survival pathways and inhibit pro-inflammatory pathways. Inflammation induces Weibel-Palade body exocytosis and Ang-2 release, allowing Ang-2 to preferentially bind the Tie-2 receptor and promote pro-inflammatory and pro-thrombotic pathways, as well as microvascular leak. Ang-1 serves as an agonist for Tie-2, whereas Ang-2 was initially thought to serve primarily as a functional antagonist [8]. Ang-1 closely interacts with VEGF in initiating angiogenesis; they act in a coordinated way during embryogenesis for vascular development [9]. However, VEGF is more involved in the initial formation of vasculature, whereas Ang-1 is integral in vascular remodeling and maturation into functional blood vessels [10]. Both Ang-1 and VEGF stimulate expression of inflammatory cytokines before angiogenesis. In the mature vessel, Ang-1 acts as a paracrine signal to maintain a quiescent *status quo,* whereas Ang-2 induces or facilitates an autocrine endothelial cell response [11]. In general, Ang-1 can be viewed as a stabilizing messenger, causing continuous Tie-2 phosphorylation, and Ang-2 as a destabilizing messenger preparing for action. Angiopoietins can directly stimulate both endothelial cells and neutrophils for an overall proinflammatory and proangiogenic response. Ang-1's chemotactic effects on neutrophils are regulated by PI-3K activation [12]. Both Ang-3 and 4 are agonists of Tie-2 receptor signaling, with Ang-3 being a specific ligand for Tie-2 receptors of its own species [13].

Potential clinical applications

Several investigations have demonstrated the importance of the Ang/Tie-2 system in systemic inflammatory disorders. In critically ill patients, the release of Ang-2 directly reflects vascular barrier breakdown; in addition, Ang-2 serves as marker to discriminate between sepsis and severe sepsis ($p < 0.05$), being its variations closely similar to those of tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, as recently reported [14]. In patients with febrile neutropenia (FN), it was found that the relative concentration of Ang-1 and Ang-2 were different in subgroups of patients with FN evolving to non-complicated sepsis compared to patients developing septic shock, and that the evaluation of these two proteins within the first 48 h after FN is a promising tool to discriminate high risk patients with FN before the development of any signs and symptoms of septic shock [15]. Further 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 [16–18]. In children with severe bacterial infection, circulating low Ang-1 and higher Ang-2 concentrations are associated with an unfavorable outcome [19]; thus, angiopoietins could be considered useful markers for the early identification of patients at risk of a poor outcome, being directly linked to the endothelial damage/dysregulation occurring in severe bacterial infection. The predictive power of Ang-1 and -2 as prognostic marker in the course of sepsis has been recently confirmed [20]: in 70 septic patients, both Ang-1 and -2 highly correlated with 28-day mortality. In addition, Ang-2 levels also correlated with disease severity as reflected by markers of organ damage and clinical sepsis scores. These studies suggest that Ang-1 and -2 seem to be of interest as prognostic sepsis biomarker, reflecting the direct status of the endothelium which correlates with disease severity and outcome. At admission to the NICU, Ang-1 levels might predict outcome, whereas Ang-2 might be of interest for monitoring septic newborns, showing similar pattern such as TNF-α and IL-6 [21].

Conclusions

Based on the remarkable convergence of experimental and human observational data, the Angpt-Tie-2 system seems to be a strong candidate vascular pathway involved in inflammation and sepsis. Thus, it is not surprising that a number of candidate biomarkers of endothelial dysfunction have been recently proposed in the literature for managing various infectious disease [22]. These markers are molecules reflecting the extent of the pathophysiologic abnormalities induced by the disease process; they may be more sensitive indicators of worsening disease severity than are traditional laboratory markers alone. Unfortunately, there is the lack of rigorous clinical studies demonstrating the clinical utility of these biomarkers as either a diagnostic or prognostic indicator in sepsis as well as in other infectious conditions. In addition, no biomarker seems to fulfill criteria to be considered an ideal biomarker. Results from the literature suggest that the severity and the prognosis of sepsis are closely associated with a significant increase of serum Ang-2 concentration; importantly, the increase over time of serum Ang-2 in patients with sepsis is predictive of the development of septic shock and mortality. However, further issues should be established before introducing the determination of Ang-1 and Ang-2 in clinical practice: the reliability of analytical methods, including the possibility to measure these markers in emergency, the cut-off level(s), the optimal time of sampling, their clinical added value compared with the clinical value of traditional biomarkers for sepsis. Especially for neonatal sepsis, which is one of the most important causes of morbidity and mortality in preterm babies, these markers have

raised a great expectation in neonatologists, since the onset of neonatal sepsis is often subtle and the diagnosis occurs later, with an increased risk of complications and death. Thus, there is the need to initiate clinical studies to assess the analytical and clinical reliability of Ang-1 and Ang-2; importantly, there is the need to assess specific cut-off limits for clinical decision making in the course of sepsis, severe sepsis, and septic shock. A close cooperation between clinical pathologists and neonatologists is strictly required to reach this goal.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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