Review Article

Endothelium and Systemic Inflammation in Neonates and Children

(endothelium / systemic inflammatory response syndrome / inflammation / neonates / children / multiple organ dysfunction syndrome / biomarkers of inflammation)

PETR JANEC[1,2], JANA DORŇÁKOVÁ[3,4], JAN ŽIVNÝ[2], JAN JANOTA[2,4]

[1]Department of Paediatrics, Neonatology Unit, Hospital Most, Most, Czech Republic
[2]Institute of Pathological Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic
[3]Department of Biomedical Technology, Faculty of Biomedical Engineering, Czech Technical University in Prague, Prague, Czech Republic
[4]Department of Obstetrics and Gynaecology, Neonatal Unit, Motol University Hospital and Second Faculty of Medicine, Charles University, Prague, Czech Republic

Abstract. The endothelium plays a crucial role in maintaining vascular homeostasis. Inflammation is initiated by activation of endothelial cells, which results in endothelial dysfunction. Dysfunction of the endothelium can have significant consequences in neonates and children. It is essential to understand the mechanisms underlying endothelial dysfunction in neonates and paediatric population to develop effective therapy interventions and improve outcomes. The aim of the review is to summarize the recent studies on the endothelium-associated molecules as biomarkers of systemic inflammatory conditions in the neonatal and paediatric population.

Received August 4, 2023. Accepted May 12, 2025.

This study is supported by the Czech Health Research Council Agency (AZV), Ministry of Health of the Czech Republic, grant number NU20-07-00109.

Corresponding author: Jan Živný, Institute of Pathological Physiology, First Faculty of Medicine, Charles University, U Nemocnice 5, Prague 2, 12853, Czech Republic. E-mail: jzivny@lf1.cuni.cz

Abbreviations: BPD – bronchopulmonary dysplasia, DAMPS – damage-associated molecular patterns, EOS – early-onset sepsis, EPCR – endothelial protein C receptor, EVs – extracellular vesicles, IL – interleukin, IUGR – intrauterine growth restriction, LOS – late-onset sepsis, NEC – necrotizing enterocolitis, PAMPS – pathogen-associated molecular patterns, PVL – periventricular leukomalacia, SIRS – systemic inflammatory response syndrome, TEK – endothelial tyrosine kinase, TLR4 – Toll-like receptor 4, TM – thrombomodulin, TNF – tumour necrosis factor, VEGF – vascular endothelial growth factor, VEGFR1/Flt-1 – vascular endothelial growth factor receptor 1, VLBW – very low birth weight, vWF – von Willebrand factor.

Introduction and Background

The endothelium is a thin layer of cells that formats the interior surface of blood vessels, playing a crucial role in vascular homeostasis and regulating vascular tone. Endothelial cells are metabolically active cells that represent the vascular blood-to-tissue barrier to molecules, macromolecules and cells.

The endothelium is considered the primary organ for vascular regulation with exocrine, paracrine and autocrine functions (Burk et al., 2012). Endothelial cells play the triggering role in the entire inflammatory response. An activated endothelium initiates the recruitment of platelets and white blood cells into the inflammatory process through production of cytokines, adhesion molecules, and other regulatory molecules and promotes coagulation (Krishnaswamy et al., 1999). In neonates and children, the endothelium is still developing and has distinct features compared to adults. Systemic inflammation affects the endothelium, leading to endothelial dysfunction, impaired microcirculation and impaired perfusion of tissues and organs (Rajendran et al., 2013). Systemic inflammatory response syndrome (SIRS) is a clinical syndrome characterized by an inflammatory response that spreads throughout the body. SIRS can develop as a result of a number of underlying conditions, including infections, trauma, burns, or surgery. SIRS results in significant morbidity and mortality, even if it is identified and treated promptly (Goldstein et al., 2005). Patients with SIRS exhibit signs of organ dysfunction, including the central nervous system, cardiovascular, respiratory, renal and gastrointestinal systems (Chakraborty and Burns, 2022). Microcirculation abnormalities in SIRS result in organ ischaemia and hypoxia. Endothelial cells, platelets, white blood cells, complement and plasma coagulation systems contribute

to the development of the inflammatory response (Šibíková et al., 2018).

Blood elements such as platelets and leukocytes are recruited into the inflammation either by direct interaction with activated endothelial cells and their products or by molecules associated with pathogenic microorganisms – pathogen-associated molecular patterns (PAMPS) and cell damage – damage-associated molecular patterns (DAMPS), components of activated plasma coagulation systems and complement (Vieira-de-Abreu et al., 2012). Activated platelets interact directly with each other and other cells through receptors on the platelet surface (e.g., CD154, P selectin, E selectin) and indirectly through the release of soluble signalling molecules and platelet-derived extracellular vesicles (EVs) (Burnier et al., 2009; de Stoppelaar et al., 2014; Hally et al., 2020). Platelets are involved in neutrophil guidance and activation, facilitating leukocyte rolling, adhesion and migration to the site of the inflammatory response (Gawaz et al., 1995). Activated leukocytes migrate across the vascular endothelium into the site of inflammatory focus either by squeezing through between adjacent endothelial cells or by migrating across individual endothelial cells (Petri and Bixel, 2006).

An integral part of the inflammatory responses is the activation of complement and plasma coagulation systems, including pro-coagulation, anti-coagulation and fibrinolytic systems. The complement consists of about 30 serum and membrane proteins and is part of the innate humoral immune responses (Greco et al., 2020). The complement system is activated in response to trauma or infection (Rittirsch et al., 2012). In patients with SIRS, plasma levels of complement activation products increase. Complement activation products induce a chain of cellular events in endothelial cells, which lead to the up-regulation of adhesion molecules, release of pro-inflammatory mediators and increase in vascular permeability (Burk et al., 2012). The activation and dysregulation of the components of coagulation systems lead concurrently to an increased risk of bleeding and thrombosis. The progression to disseminated intravascular coagulation with microvascular thrombosis is frequent in patients with severe systemic inflammation (Annane et al., 2005; Gando et al., 2016).

Analysis of endothelium-associated molecules and endothelial extracellular vesicles represents a way to monitor and identify the early pathophysiology mechanisms of the systemic inflammatory response. There is an extensive body of work on the role of endothelium in the inflammatory response in adult population and adult animal models. The neonatal and paediatric population studies are underrepresented and the knowledge on the pathophysiology of inflammatory reactions in children is often extrapolated from studies on adult population. The aim of this review is to evaluate the endothelium and its role in the development of inflammation in neonates and children with the special focus on the utilization of endothelium-related molecules in the diagnosis of specific inflammatory conditions in this population.

Endothelium in Systemic Inflammation in Neonates and Children

The systemic inflammatory response in neonates and children can be initiated by infectious and non-infectious triggers. Typical neonatal conditions that are associated with systemic inflammation and show abnormalities in endothelial cell function include hypoxic-ischaemic encephalopathy, periventricular leukomalacia, neonatal pulmonary hypertension, intraventricular haemorrhage, bronchopulmonary dysplasia and necrotizing enterocolitis (Mezu-Ndubuisi and Maheshwari, 2021). Systemic inflammation associated with infections, such as sepsis and meningitis, can occur both in neonatal and paediatric age. Examples of paediatric systemic inflammatory conditions include paediatric acute respiratory distress syndrome, Kawasaki disease and paediatric multisystem inflammatory syndrome.

Hypoxic-ischaemic encephalopathy

Neonatal hypoxic-ischaemic encephalopathy is a brain injury caused by hypoxia due to maternal (e.g., impaired blood oxygenation and insufficient perfusion of the maternal placenta), placental (e.g., insufficient cord blood flow, abruption of the placenta) or foetal causes (e.g., foetal thrombosis or foetomaternal haemorrhage). Depending on the quality of healthcare, the incidence ranges from approximately 1.5 cases to 20 cases per 1,000 live births (Greco et al., 2020). Elevated levels of inflammatory cytokines such as interleukin 6 (IL-6), tumour necrosis factor (TNF) and interleukin 8 (IL-8) recruit leukocytes to the site of hypoxic brain injury and damage the integrity of endothelial cells (Li et al., 2014). Neuronal cell death is caused by activated inflammatory cells and over-expression of apoptosis-inducing proteins (Borjini et al., 2019).

Periventricular leukomalacia

Premature infants are particularly sensitive to neurologic injury due to the exposure of their immature vascular network to extrauterine abnormalities, such as oxygen tension, blood pressure, acid-base changes and microbiota. Inflammation causing secondary brain injury may result in periventricular leukomalacia. Periventricular leukomalacia (PVL) is a white matter brain injury characterized by necrosis of white matter near the lateral ventricles (Rezaie and Dean, 2002). A high level of IL-8, produced mainly by macrophages, smooth muscle cells and endothelial cells, is associated with white matter injury (Romantsik et al., 2019). IL-6 is elevated in the cord blood of newborns with white matter lesions (Yoon et al., 1996).

Bronchopulmonary dysplasia

Bronchopulmonary dysplasia (BPD) is the most prevalent chronic lung disease in infants and is linked to increased mortality, respiratory morbidity and neurodevelopmental impairment. BPD is characterized by impaired lung parenchymal development and dysregu-

lated lung vascular growth in infants born before 29 weeks of gestation (Gilfillan et al., 2021). Premature delivery stunts lung development, which is frequently exacerbated by prenatal events such as intrauterine growth restriction (IUGR) and inflammation exposure, as well as postnatal events associated with initial resuscitation, oxygen administration, mechanical ventilation and pulmonary and systemic infections, all of which can result in stalled pulmonary vascular and alveolar development (Hwang and Rehan, 2018). The prevalence of BPD increases as gestational age and birth weight decrease. Preterm birth may disrupt lung vascular development during the saccular and alveolar stages of pulmonary development (Berry, 2019). Post-mortem examination of the lungs of infants with BPD revealed low expression of vascular endothelial growth factor (VEGF) and angiogenic receptors vascular endothelial growth factor receptor 1 (VEGFR1/Flt-1) and endothelial tyrosine kinase (TEK or Tie 2) (Bhatt et al., 2001). The BPD severity correlated with increased levels of intercellular adhesion molecule 1 (ICAM-1), angiopoietin 2 (Ang-2) and interleukin 1 beta (IL-1β) and decreased levels of angiopoietin 1 (Ang-1) and monocyte chemoattractant protein 1 (MCP-1) in the blood (Sahni et al., 2020).

Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) remains one of the leading causes of death in premature infants, affecting 5–12 % of neonates born at a very low birth weight (VLBW; < 1, 500 g). It is hypothesized that NEC develops due to an imbalance between excessive pro-inflammatory signalling in the developing gut mucosa, resulting in mucosal damage that is not effectively protected by endogenous repair processes, and impaired mesenteric perfusion, resulting in intestinal ischaemia and disease development. Toll-like receptor 4 (TLR4), which recognizes lipopolysaccharide in gram-negative bacteria, is involved in the maintenance of the equilibrium between injury and repair in the intestine and in the regulation of normal intestinal epithelial development (Hackam and Sodhi, 2018). The gut of premature infants is characterized by elevated expression of TLR4 on the intestinal epithelium. Activation of TLR4 by microbes within the intestinal lumen causes injury to the intestinal epithelial barrier, resulting in translocation of luminal bacteria across the mucosal barrier. Bacteria are subsequently disseminated through the circulation and initiate the systemic inflammatory response, vasoconstriction and intestinal ischaemia (Niño et al., 2016). The effect of vasodilator nitric oxide is diminished in NEC, and the vasoconstrictor endothelin 1 is increased in NEC (Nowicki et al., 2007). The underdevelopment of intestinal microvasculature in premature newborns contributes to the pathogenesis of NEC (Nankervis et al., 2001; Howarth et al., 2022).

Sepsis

The definitions of neonatal sepsis differ from those of adults and children because the diagnosis frequently relies on microbiological results rather than organ dysfunction. Neonatal sepsis is a systemic infection in infants up to 28 days of age and is a significant cause of newborn morbidity and mortality. Early-onset sepsis (EOS) is most commonly defined as occurring in the first three days of the life of neonates. It is caused by bacterial pathogens transmitted vertically from mother to infant before or during delivery. Late-onset sepsis (LOS) is sepsis occurring after 72 h and may be caused by vertically or horizontally acquired pathogens (Simonsen et al., 2014). The incidence of early-onset neonatal sepsis with positive cultures in newborns is approximately 0.98 per 1,000 live births and is higher in infants with very low birth weight (VLBW) (Schrag et al., 2016; Janec et al., 2023). In VLBW infants, the incidence of LOS can be as high as 30 % (Greenberg et al., 2017). The pathogenesis of neonatal sepsis is linked to a dysregulated immune response. Most deaths associated with gram-negative sepsis occur in the acute phase, within the first three days after the onset of sepsis (Stoll et al., 2011). During inflammation, the vascular endothelium expresses an abundance of cytokines accompanied by a local chemotactic gradient that recruits leukocytes into surrounding tissues. These recruitment responses in neonates may be weaker in most organs compared to adults (Karenberg et al., 2016). Due to impaired endothelial expression of adhesion molecules such as E-selectin, ICAM-1 and P-selectin, inflammation of vascular endothelium during sepsis alters chemotaxis and leukocyte transmigration (Nussbaum et al., 2013). Endocan also shows promising potential as a biomarker of neonatal sepsis (Mwesigye et al., 2021). IL-6 is a reliable marker of systemic inflammatory response and could be utilized to rule out EOS within the first twenty-four hours (Berka et al., 2022). New biomarkers are emerging, such as soluble pattern recognition receptor pentraxin 3 (PTX3) expressed by monocytes/macrophages and dendritic cells, endothelial cells and neutrophils. Microbe detection, complement activation and opsonization are among its primary functions. In response to stimuli such as pathogens and inflammatory cytokines, PTX3 is released into the extracellular space and localized in neutrophil extracellular traps (NETs).

Meningitis

Meningitis is a potentially fatal disease that affects 0.1–0.4 neonates per 1,000 live births, with a higher incidence in premature and chronically hospitalized babies (Okike et al., 2014). Approximately 10 % of affected infants die, and 20–50 % of survivors develop seizures, cognitive deficiencies, motor abnormalities, hearing and visual impairments, and other complications. The traditional gold standard for bacterial meningitis diagnosis is cerebrospinal fluid (CSF) culture. Meningitis is estimated to occur in 1–2 % of suspected sepsis cases (Ray et al., 2006). To form bacteraemia, meningitis pathogens must enter the peripheral blood and cross the blood-brain barrier (BBB), which anatomically has a tight junction of brain microvascular endo-

thelial cells. Polymorphonuclear leukocyte (PMN) transendothelial migration across the BBB is another significant feature of bacterial meningitis that occurs following the entry of bacteria into the cerebrospinal fluid. Several paediatric and neonatal meningitis studies have looked at CSF cytokine levels and other potential biomarkers. IL-6 produced the best results (Srinivasan et al., 2016).

Paediatric acute respiratory distress syndrome

Sepsis, severe pneumonia, trauma and inhalation of harmful substances are the most common causes of paediatric acute respiratory distress syndrome (PARDS) (Orloff et al., 2019). Endothelial biomarkers include Ang-2 and von Willebrand factor (vWF), as well as endothelin and soluble endothelin. Morbidity and mortality have been linked to E-selectin and soluble thrombomodulin in both paediatric and adult ARDS (Goldenberg and Kuebler, 2015). The pathophysiology of PARDS includes injury to lung, bronchial and alveolar epithelial and endothelial cells. Plasma intercellular adhesion molecule-1 (sICAM-1) levels were significantly higher in non-survivors and those who required extended mechanical ventilation during the first days of acute lung injury (Flori et al., 2003).

Kawasaki disease

Kawasaki disease (KD), also known as mucocutaneous lymph node syndrome, is a febrile vasculitis disorder that is usually identified by a cluster of symptoms, signs and laboratory findings. It is a multisystem disorder that affects young children, with a preference for small and organ arteries of medium size, particularly coronary arteries. It can lead to various complications such as coronary artery aneurysm, thrombosis, stenosis and sudden death (Guo et al., 2015). Cardiovascular complications are common in acute phase KD, including valvulitis, myocarditis, pericarditis and KD shock syndrome. The subacute to convalescent phase is the most common for coronary artery aneurysms and dilatation. The majority of cases show signs of systemic inflammation during the acute phase (Takahashi et al., 2014).

Multisystem inflammatory syndrome in children

Multisystem inflammatory syndrome in children (MIS-C) and neonates (MIS-N), also referred to as paediatric multisystem inflammatory syndrome (PMIS), paediatric hyperinflammatory syndrome, or paediatric hyperinflammatory shock, is a novel hyperinflammatory disorder in children associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infection, which was first reported in April 2020 (Carter et al., 2020). These patients displayed severe Kawasaki disease-like symptoms (Patel, 2022). Inflammatory cytokines play a role in the pathogenesis of this disease, with the IL-1 pathway activated and levels of pro-inflammatory cytokines such as TNF elevated. Patients have been found to have elevated levels of IL-6, IL-8, IL-18, TNF and interferon γ (IFN-γ) (McMurray et al., 2020). This inflammatory condition is much more common in the paediatric than neonatal population (Molloy et al., 2023).

Markers of Endothelial Dysfunction

Endothelial activation is a key initial event of inflammatory responses and is associated with aberrant expression of endothelium-specific molecules. The endothelial activation leads to endothelial dysfunction, which is associated with the loss of the protective function of endothelium. The endothelial cells progressively change their phenotype to become pro-coagulant and pro-adhesive. They change the pattern of the produced soluble molecules affecting vasoactivity, inflammation and haemostasis. Decreased production of endothelium-derived nitric oxide (NO) results in a diminished vasodilator response and in pro-thrombotic and pro-inflammatory activation of the endothelium. The following markers associated with endothelial dysfunction have been extensively studied in the inflammatory response in neonates and children (Medina-Leyte et al., 2021).

Endothelium-derived vasoactive peptides and their receptors

Endothelins are a group of three peptides, ET-1, ET-2 and ET-3, with 21 amino acids that have potent vasoconstrictor properties. They are essential for normal embryonic and neonatal development, renal homeostasis and basal vascular tone maintenance. The endothelin system plays a role in the development of atherosclerosis and pulmonary hypertension, as well as mediating cardiac remodelling in heart failure (Davenport et al., 2016). They play a role in the closure of the ductus arteriosus in newborns. Endothelin is a potent vasoconstrictor, one of the mediators in the cause of persistent pulmonary hypertension in newborns (PPHN) (Hansmann et al., 2021).

Endothelium-specific adhesion molecules

The activation of endothelium through specific receptors, such as pattern recognition receptors and cytokine receptors, leads to increased expression of cell adhesion molecules (CAMs). In sepsis, the increased blood level of soluble adhesion molecules, namely E-selectin, L-selectin, P-selectin, sICAM-1 and vascular cell adhesion molecule-1 (sVCAM-1), has been documented (Zonneveld et al., 2014). Increased endothelial expression of adhesion molecules results in increased recruitment of leukocytes to the marginal leukocyte pool and facilitates the migration of leukocytes to the site of inflammation. In systemic inflammation, increased expression of endothelial adhesion molecules and increased production and activity of proteolytic enzymes result in the release of soluble forms of adhesion molecules into the bloodstream. Elevated levels of soluble adhesive molecules correlate well with sepsis in both children and adults (Amalakuhan et al., 2016).

Haemostasis-associated endothelial molecules

Thrombomodulin

Thrombomodulin (TM) is a transmembrane glycoprotein expressed primarily on endothelial cell glycocalyx surfaces. In the processes of haemostasis and inflammation, thrombomodulin, as a receptor for thrombin, is involved in the activation of protein C and deactivation of thrombin. Endothelial injury with glycocalyx degradation contributes to thrombophilia (Kornacki et al., 2021). Another function of TM may be to maintain the glomerular filtration barrier. In children with acute respiratory failure, plasma sTM is associated with mortality, severity of the hypoxic respiratory failure and worsening extrapulmonary multiorgan failure (Monteiro et al., 2021).

Endothelial protein C receptor (EPCR)

EPCR (CD201) is a type I transmembrane protein localized on the endothelial cells of large blood vessels. The soluble form of EPCR (sEPCR) is released to the human plasma by metalloproteinase-mediated cleavage (Laszik et al., 1997; Neyrinck et al., 2009). The release of sEPCR is constitutive and is augmented by thrombin, IL-1β and many other inflammatory mediators, suggesting that the concentration of sEPCR should be increased in patients with sepsis and systemic inflammation (Liaw et al., 2000). In an animal model of systemic endotoxin-induced inflammation, the sEPCR plasma concentration significantly increased in a thrombin-dependent manner (Gu et al., 2000). In the adult population, controversial values were published in terms of sEPCR levels in septic patients (Kurosawa et al., 1998; Faust et al., 2001; Liaw et al., 2004; Borgel et al., 2007). The only clinical study that focused on the sEPCR plasma concentration in paediatric sepsis did not show significant changes (Kendirli et al., 2009).

Von Willebrand factor

Endothelial cells and megakaryocytes produce the glycoprotein von Willebrand factor (vWF). It is essential in vascular haemostasis because it promotes platelet adhesion to the endothelium and stabilizes factor VIII. vWF is also an acute-phase protein with multiple roles in vascular inflammation, secreted in large amounts from Weibel-Palade bodies upon endothelial cell activation (Kiouptsi and Reinhardt, 2020). The association of elevated vWF levels with inflammation and endothelial damage in glomerulonephritis, arteritis, diabetes and sepsis led to vWF's early classification as an acute-phase reactant (Pottinger et al., 1989). Elevated plasma vWF levels have been linked to acute respiratory distress syndrome and sepsis and have been shown to correlate with mortality independently (Ware et al., 2001). vWf and its cleaving protease, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS-13), are required for haemostasis. These plasma proteins have also been linked to a variety of diseases, including those that affect children (Katneni et al., 2019). Spontaneous or unprovoked thrombotic events, such as deep venous thrombosis, pulmonary embolism and stroke, in healthy neonates are rare (Chalmers, 2000). Indwelling vascular catheters, inherited thrombophilia, sepsis, perinatal hypoxia, systemic viral infections, congenital heart disease, polycythemia and low birth weight are all known risk factors for neonatal thromboembolisms (Veldman et al., 2008). A growing body of evidence suggests that abnormal vWF/ADAMTS-13 interactions contribute to pathologic clot formation in other common diseases (Nguyen et al., 2007). Data from studies that measured vWF in different age groups suggest that vWF levels in infants reach adult levels by six months of age; other studies providing contrasting findings reported higher vWF antigen levels in preterm infants and higher values in full-term infants (Strauss et al., 2017). Stress conditions during labour were shown to affect vWF levels during the perinatal period (Kulkarni et al., 2013).

Endothelium-produced cytokines and soluble receptors

Inflammatory cytokines are produced during inflammation by various cell types, including activated endothelial cells. Pro-inflammatory cytokines such as TNF, IL-6, IL-8 and IL-18 are essential inflammatory mediators (Medina-Leyte et al., 2021; Mojzisek et al., 2023).

TNF causes endothelial cells to express and release a variety of inflammatory cytokines, chemokines and adhesion molecules (Zhang et al., 2009). TNF is a cytokine that plays a critical role in inflammation and immune function. It induces an inflammatory cascade, which is essential in the host's response to infections and local injury. Elevated levels of TNF can be found in neonates, infants and children under several conditions, often linked to the immune system response, inflammation and certain diseases. TNF levels typically rise during bacterial, viral, or fungal infections as part of the body's immune response. The increase in TNF helps stimulate the immune system to fight off the invading pathogen (Parameswaran and Patial, 2010). Elevated TNF levels have been linked to NEC due to the inflammatory response (Hackam and Caplan, 2018). Autoimmune diseases such as juvenile idiopathic arthritis or inflammatory bowel disease can cause high TNF levels. TNF inhibitors are increasingly being administered to children and adolescents with juvenile idiopathic arthritis (JIA) or paediatric inflammatory bowel disease (Toussi et al., 2013). IL-6 is promptly and transiently produced in response to infection and tissue injury and contributes to the host defence by stimulating acute phase responses, haematopoiesis and immune reactions (Tanaka et al., 2014). IL-6, combined with other diagnostic markers, is often used to diagnose neonatal sepsis and plays a great role in the diagnosis of early-onset neonatal sepsis (Cortés et al., 2021). IL-8 is a cytokine secreted by numerous cell types. IL-8 is a potent chemoattractant for neutrophils and T lymphocytes. IL-8 in

neonatal sepsis is moderately elevated (Simonsen et al., 2014). VEGF, VEGFR-1 and VEGFR-2 are required for the development function of vasculature. VEGF serum concentrations were significantly lower in peripheral blood of critically ill newborns on extracorporeal membrane oxygenation (ECMO) (Vítková et al., 2018a). VEGF was also found to be significantly lower in bronchoalveolar lavage of patients with BPD compared to controls (Been et al., 2010).

Endothelial cell-derived extracellular vesicles

Extracellular vesicles (EVs) are released by eukaryotic cells. The blood contains vesicles derived from cell membranes of various cell and tissue origins, including platelets, endothelial cells, erythrocytes, leukocytes, tumour cells and other cell types (van der Pol et al., 2012; Colombo et al., 2014; Hromada et al., 2017). The mechanisms that would be responsible for the regulation of EV release, maintenance and function in health and disease are still not fully understood. In neonates, a dramatic increase in the concentration of small and large EVs of platelet and endothelial origin was observed in the group of preterm infants during the adaptation period at day 3 compared to healthy full-term controls (Murphy et al., 2025). Large EVs are a diverse subgroup of cell-membrane vesicular bodies that range in size from 100 to 1,000 nm (Šibíková et al., 2018). Large EV surface antigens reflect their cell origin and allow cell-specific EVs to be identified (Inzhutova et al., 2012). The large EVs are released to the circulation during pathological processes that cause cell injury (Burger et al., 2013). Large EV analysis and measurement methods include flow cytometry, enzyme-linked immunosorbent assay (ELISA), electron microscopy, dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) (Aupeix et al., 1997; Gelderman and Simak, 2008; Dragovic et al., 2011; Yuana et al., 2011). Endothelial large EVs may be utilized as targets, mediators and vectors in the treatment of inflammatory diseases (Vítková, et al., 2018b). Increased plasma concentrations of large endothelial EVs have been found in critically ill newborns with multiple organ dysfunction and systemic inflammatory response syndrome (Vítková, et al., 2018b). Endothelial EVs were increased in umbilical cord blood after spontaneous vaginal delivery compared to elective caesarean section. Activating neonatal innate immunity and postnatal cardiovascular transition can explain this. It can also be assumed that labour is associated with an inflammatory response that the type of labour may modify (Sibikova et al., 2020).

Endothelial glycocalyx

Endothelial glycocalyx is the primary outer layer of the endothelial cell containing various proteoglycans, glycoproteins, glycolipids and glycosaminoglycans. The endothelial glycocalyx is present early in foetal development and is important in vessel formation and maturation. The components of endothelial glycocalyx are involved in maintaining the vessel wall integrity, controlling leukocyte and platelet adhesion and antithrombotic activity. Serum concentrations of soluble endothelial glycocalyx components may reflect the degree of endothelial damage. Endocan 1 (ESM-1), hyaluronan (HA) and syndecan 1 (SDC-1) are the most reliable markers of endothelial glycocalyx degradation (Dogné et al., 2018). Endocan is a biomarker studied in terms of late-onset sepsis in neonates. Endothelial glycocalyx shedding has been demonstrated in infants and children with acute inflammatory conditions and chronic diseases with childhood onset (Mwesigye et al., 2021).

Conclusion

The endothelium is a crucial layer of cells in blood vessels that maintains the vascular function. Systemic inflammation is associated with the activation of endothelium, leading to its dysfunction and impairment of tissue perfusion. Neonatal and paediatric inflammatory conditions are accompanied by production of various substances and cell-derived vesicles. These are released by the activated endothelium, which is in the centre of the local and systemic inflammatory reaction. The products of the activated endothelium, tissue damage-associated pattern molecules and pathogen-associated molecules are responsible for perpetuation of the inflammatory response. Molecules and extracellular vesicles produced by the activated endothelium may provide an insight into the pathogenesis of systemic inflammatory response syndrome in the paediatric population. Highly sensitive and specific biomarkers of endothelial activation would aid early detection, risk stratification and monitoring of inflammatory processes in neonatal and paediatric patients. Targeting of endothelium-derived pro-inflammatory molecules might interfere with the progression of diseases of this specific fragile population and possibly positively influence the outcome. Overall, future research should aim to deepen our understanding of the complex interplay between the endothelium and other tissues during systemic inflammation in neonates and children.

References

Amalakuhan, B., Habib, S. A., Mangat, M. et al. (2016) Endothelial adhesion molecules and multiple organ failure in patients with severe sepsis. *Cytokine* **88**, 267-273.

Annane, D., Bellissant, E., Cavaillon, J. M. (2005) Septic shock. *Lancet* **365**, 63-78.

Aupeix, K., Hugel, B., Martin, T. et al. (1997) The significance of shed membrane particles during programmed cell death in vitro, and in vivo, in HIV-1 infection. *J. Clin. Invest.* **99**, 1546-1554.

Been, J. V., Debeer, A., van Iwaarden, J. F. et al. (2010) Early alterations of growth factor patterns in bronchoalveolar lavage fluid from preterm infants developing bronchopulmonary dysplasia. *Pediatr. Res.* **67**, 83-89.

Berka, I., Korček, P., Straňák, Z. (2022) Serial measurement of interleukin-6 enhances chance to exclude early-onset

sepsis in very preterm infants. *Clin. Pediatr. (Phila)* **62**, 288-294.

Berry, M. J. (2019) The heart of the matter: a vascular hypothesis for bronchopulmonary dysplasia. *J. Physiol.* **597**, 991-992.

Bhatt, A. J., Pryhuber, G. S., Huyck, H. et al. (2001) Disrupted pulmonary vasculature and decreased vascular endothelial growth factor, Flt-1, and TIE-2 in human infants dying with bronchopulmonary dysplasia. *Am. J. Respir. Crit. Care Med.* **164 (10 Pt 1)**, 1971-1980.

Borgel, D., Bornstain, C., Reitsma, P. H. et al. (2007) A comparative study of the protein C pathway in septic and non-septic patients with organ failure. *Am. J. Respir. Crit. Care Med.* **176**, 878-885.

Borjini, N., Sivilia, S., Giuliani, A. et al. (2019) Potential biomarkers for neuroinflammation and neurodegeneration at short and long term after neonatal hypoxic-ischemic insult in rat. *J. Neuroinflammation* **16**, 194.

Burger, D., Schock, S., Thompson, C. S. et al. (2013) Microparticles: biomarkers and beyond. *Clin. Sci. (Lond)* **124**, 423-441.

Burk, A. M., Martin, M., Flierl, M. A. et al. (2012) Early complementopathy after multiple injuries in humans. *Shock* **37**, 348-354.

Burnier, L., Fontana, P., Kwak, B. R. et al. (2009) Cell-derived microparticles in haemostasis and vascular medicine. *Thromb. Haemost.* **101**, 439-451.

Carter, M. J., Fish, M., Jennings, A. et al. (2020) Peripheral immunophenotypes in children with multisystem inflammatory syndrome associated with SARS-CoV-2 infection. *Nat. Med.* **26**, 1701-1707.

Chakraborty, R. K., Burns, B. (2022) Systemic inflammatory response syndrome. In: *StatPearls [Internet]*, Treasure Island (FL): StatPearls Publishing.

Chalmers, E. A. (2000) Neonatal thrombosis. *J. Clin. Pathol.* **53**, 419-423.

Colombo, M., Raposo, G., Théry, C. (2014) Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu. Rev. Cell Dev. Biol.* **30**, 255-289.

Cortés, J. S., Losada, P. X., Fernández, L. X. et al. (2021) Interleukin-6 as a biomarker of early-onset neonatal sepsis. *Am. J. Perinatol.* **38**, e338-e346.

Davenport, A. P., Hyndman, K. A., Dhaun, N. et al. (2016) Endothelin. *Pharmacol. Rev.* **68**, 357-418.

de Stoppelaar, S. F., van 't Veer, C., van der Poll, T. (2014) The role of platelets in sepsis. *Thromb. Haemost.* **112**, 666-677.

Dogné, S., Flamion, B., Caron, N. (2018) Endothelial glycocalyx as a shield against diabetic vascular complications: involvement of hyaluronan and hyaluronidases. *Arterioscler. Thromb. Vasc. Biol.* **38**, 1427-1439.

Dragovic, R. A., Gardiner, C., Brooks, A. S. et al. (2011) Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis. *Nanomedicine* **7**, 780-788.

Faust, S. N., Levin, M., Harrison, O. B. et al. (2001) Dysfunction of endothelial protein C activation in severe meningococcal sepsis. *N. Engl. J. Med.* **345**, 408-416.

Flori, H. R., Ware, L. B., Glidden, D. et al. (2003) Early elevation of plasma soluble intercellular adhesion molecule-1 in pediatric acute lung injury identifies patients at increased risk of death and prolonged mechanical ventilation. *Pediatr. Crit. Care Med.* **4**, 315-321.

Gando, S., Levi, M., Toh, C. H. (2016) Disseminated intravascular coagulation. *Nat. Rev. Dis. Primers* **2**, 16037.

Gawaz, M., Fateh-Moghadam, S., Pilz, G. et al. (1995) Platelet activation and interaction with leucocytes in patients with sepsis or multiple organ failure. *Eur. J. Clin. Invest.* **25**, 843-851.

Gelderman, M. P., Simak, J. (2008) Flow cytometric analysis of cell membrane microparticles. *Methods Mol. Biol.* **484**, 79-93.

Gilfillan, M., Bhandari, A., Bhandari, V. (2021) Diagnosis and management of bronchopulmonary dysplasia. *BMJ* **375**, n1974.

Goldenberg, N. M., Kuebler, W. M. (2015) Endothelial cell regulation of pulmonary vascular tone, inflammation, and coagulation. *Compr. Physiol.* **5**, 531-559.

Goldstein, B., Giroir, B., Randolph, A. (2005) International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatr. Crit. Care Med.* **6**, 2-8.

Greco, P., Nencini, G., Piva, I. et al. (2020) Pathophysiology of hypoxic-ischemic encephalopathy: a review of the past and a view on the future. *Acta Neurol. Belg.* **120**, 277-288.

Greenberg, R. G., Kandefer, S., Do, B. T. et al. (2017) Late-onset sepsis in extremely premature infants: 2000-2011. *Pediatr. Infect. Dis. J.* **36**, 774-779.

Gu, J. M., Katsuura, Y., Ferrell, G. L. et al. (2000) Endotoxin and thrombin elevate rodent endothelial cell protein C receptor mRNA levels and increase receptor shedding in vivo. *Blood* **95**, 1687-1693.

Guo, M. M., Tseng, W. N., Ko, C. H. et al. (2015) Th17- and Treg-related cytokine and mRNA expression are associated with acute and resolving Kawasaki disease. *Allergy* **70**, 310-318.

Hackam, D., Caplan, M. (2018) Necrotizing enterocolitis: pathophysiology from a historical context. *Semin. Pediatr. Surg.* **27**, 11-18.

Hackam, D. J., Sodhi, C. P. (2018) Toll-like receptor-mediated intestinal inflammatory imbalance in the pathogenesis of necrotizing enterocolitis. *Cell. Mol. Gastroenterol. Hepatol.* **6**, 229-238.e221.

Hally, K., Fauteux-Daniel, S., Hamzeh-Cognasse, H. et al. (2020) Revisiting platelets and Toll-like receptors (TLRs): at the interface of vascular immunity and thrombosis. *Int. J. Mol. Sci.* **21**, 6150.

Hansmann, G., Sallmon, H., Roehr, C. C. et al. (2021) Pulmonary hypertension in bronchopulmonary dysplasia. *Pediatr. Res.* **89**, 446-455.

Howarth, C., Banerjee, J., Eaton, S. et al. (2022) Biomarkers of gut injury in neonates - where are we in predicting necrotising enterocolitis? *Front. Pediatr.* **10**, 1048322.

Hromada, C., Mühleder, S., Grillari, J. et al. (2017) Endothelial extracellular vesicles-promises and challenges. *Front. Physiol.* **8**, 275.

Hwang, J. S., Rehan, V. K. (2018) Recent advances in bronchopulmonary dysplasia: pathophysiology, prevention, and treatment. *Lung* **196**, 129-138.

Inzhutova, A. I., Larionov, A. A., Petrova, M. M. et al. (2012) Theory of intercellular communication in the development of endothelial dysfunction. *Bull. Exp. Biol. Med.* **153**, 201-205.

Janec, P., Mojzisek, M., Panek, M. et al. (2023) Early-onset neonatal sepsis: inflammatory biomarkers and microRNA as potential diagnostic tools in preterm newborns. *Folia Biol. (Praha)* **69**, 173-180.

Karenberg, K., Hudalla, H., Frommhold, D. (2016) Leukocyte recruitment in preterm and term infants. *Mol. Cell. Pediatr.* **3**, 35.

Katneni, U. K., Ibla, J. C., Hunt, R. et al. (2019) von Willebrand factor/ADAMTS-13 interactions at birth: implications for thrombosis in the neonatal period. *J. Thromb. Haemost.* **17**, 429-440.

Kendirli, T., Ince, E., Ciftci, E. et al. (2009) Soluble endothelial protein C receptor level in children with sepsis. *Pediatr. Hematol. Oncol.* **26**, 432-438.

Kiouptsi, K., Reinhardt, C. (2020) Physiological roles of the von Willebrand factor-factor VIII interaction. *Subcell. Biochem.* **94**, 437-464.

Kornacki, J., Gutaj, P., Kalantarova, A. et al. (2021) Endothelial dysfunction in pregnancy complications. *Biomedicines* **9**, 1756.

Krishnaswamy, G., Kelley, J., Yerra, L.et al. (1999) Human endothelium as a source of multifunctional cytokines: molecular regulation and possible role in human disease. *J. Interferon Cytokine Res.* **19**, 91-104.

Kulkarni, A. A., Osmond, M., Bapir, M. et al. (2013) The effect of labour on the coagulation system in the term neonate. *Haemophilia* **19**, 533-538.

Kurosawa, S., Stearns-Kurosawa, D. J., Carson, C. W. et al. (1998) Plasma levels of endothelial cell protein C receptor are elevated in patients with sepsis and systemic lupus erythematosus: lack of correlation with thrombomodulin suggests involvement of different pathological processes. *Blood* **91**, 725-727.

Laszik, Z., Mitro, A., Taylor, F. B. et al. (1997) Human protein C receptor is present primarily on endothelium of large blood vessels - implications for the control of the protein C pathway. *Circulation* **96**, 3633-3640.

Li, S. J., Liu, W., Wang, J. L. et al. (2014) The role of TNF-α, IL-6, IL-10, and GDNF in neuronal apoptosis in neonatal rat with hypoxic-ischemic encephalopathy. *Eur. Rev. Med. Pharmacol. Sci.* **18**, 905-909.

Liaw, P. C. Y., Esmon, C. T., Kahnamoui, K., et al. (2004) Patients with severe sepsis vary markedly in their ability to generate activated protein C. *Blood* **104**, 3958-3964.

Liaw, P. C. Y., Mather, T., Oganesyan, N. et al. (2000) Identification of the protein C/activated protein C binding domain on the endothelial cell protein C receptor: implications for a novel mode of ligand recognition by an MHC class 1-type receptor. *Blood,* **96**, 813a.

McMurray, J. C., May, J. W., Cunningham, M. W. et al. (2020) Multisystem inflammatory syndrome in children (MIS-C), a post-viral myocarditis and systemic vasculitis - a critical review of its pathogenesis and treatment. *Front. Pediatr.* **8**, 626182.

Medina-Leyte, D. J., Zepeda-García, O., Domínguez-Pérez, M. et al. (2021) Endothelial dysfunction, inflammation and coronary artery disease: potential biomarkers and promising therapeutical approaches. *Int. J. Mol. Sci.* **22**, 3850.

Mezu-Ndubuisi, O. J., Maheshwari, A. (2021) The role of integrins in inflammation and angiogenesis. *Pediatr. Res.* **89**, 1619-1626.

Mojzisek, M., Sibikova, M., Panek, M. et al. (2023) Delivery-associated changes in the levels of inflammatory molecules in newborns. *Folia Biol. (Praha)* **69**, 1-5.

Molloy, E. J., Nakra, N., Gale, C. et al. (2023) Multisystem inflammatory syndrome in children (MIS-C) and neonates (MIS-N) associated with COVID-19: optimizing definition and management. *Pediatr. Res.* **93**, 1499-1508.

Monteiro, A. C. C., Flori, H., Dahmer, M. K. et al. (2021) Thrombomodulin is associated with increased mortality and organ failure in mechanically ventilated children with acute respiratory failure: biomarker analysis from a multicenter randomized controlled trial. *Crit. Care* **25**, 271.

Murphy, C. A., O'Reilly, D., Weiss, L. et al. (2025) Unique patterns of circulating extracellular vesicles in preterm infants during adaptation to extra-uterine life. *J. Extracell. Vesicles* **14**, e70064.

Mwesigye, P., Rizwan, F., Alassaf, N. et al. (2021) The role and validity of diagnostic biomarkers in late-onset neonatal sepsis. *Cureus* **13**, e17065.

Nankervis, C. A., Reber, K. M., Nowicki, P. T. (2001) Age-dependent changes in the postnatal intestinal microcirculation. *Microcirculation* **8**, 377-387.

Neyrinck, A. P., Liu, K. D., Howard, J. P. et al. (2009) Protective mechanisms of activated protein C in severe inflammatory disorders. *Br. J. Pharmacol.* **158**, 1034-1047.

Nguyen, T. C., Liu, A., Liu, L. et al. (2007) Acquired ADAMTS-13 deficiency in pediatric patients with severe sepsis. *Haematologica* **92**, 121-124.

Niño, D. F., Sodhi, C. P., Hackam, D. J. (2016) Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. *Nat. Rev. Gastroenterol. Hepatol.* **13**, 590-600.

Nowicki, P. T., Caniano, D. A., Hammond, S. et al. (2007) Endothelial nitric oxide synthase in human intestine resected for necrotizing enterocolitis. *J. Pediatr.* **150**, 40-45.

Nussbaum, C., Gloning, A., Pruenster, M. et al. (2013) Neutrophil and endothelial adhesive function during human fetal ontogeny. *J. Leukoc. Biol.* **93**, 175-184.

Okike, I. O., Johnson, A. P., Henderson, K. L. et al. (2014) Incidence, etiology, and outcome of bacterial meningitis in infants aged <90 days in the United Kingdom and Republic of Ireland: prospective, enhanced, national population-based surveillance. *Clin. Infect. Dis.* **59**, e150-e157.

Orloff, K. E., Turner, D. A., Rehder, K. J. (2019) The current state of pediatric acute respiratory distress syndrome. *Pediatr. Allergy Immunol. Pulmonol.* **32**, 35-44.

Parameswaran, N., Patial, S. (2010) Tumor necrosis factor-α signaling in macrophages. *Crit. Rev. Eukaryot. Gene Expr.* **20**, 87-103.

Patel, J. M. (2022) Multisystem inflammatory syndrome in children (MIS-C). *Curr. Allergy Asthma Rep.* **22**, 53-60.

Petri, B., Bixel, M. G. (2006) Molecular events during leukocyte diapedesis. *FEBS J.* **273**, 4399-4407.

Pottinger, B. E., Read, R. C., Paleolog, E. M. et al. (1989) von Willebrand factor is an acute phase reactant in man. *Thromb. Res.* **53**, 387-394.

Rajendran, P., Rengarajan, T., Thangavel, J. et al. (2013) The vascular endothelium and human diseases. *Int. J. Biol. Sci.* **9**, 1057-1069.

Ray, B., Mangalore, J., Harikumar, C. et al. (2006) Is lumbar puncture necessary for evaluation of early neonatal sepsis? *Arch. Dis. Child.* **91**, 1033-1035.

Rezaie, P., Dean, A. (2002) Periventricular leukomalacia, inflammation and white matter lesions within the developing nervous system. *Neuropathology* **22**, 106-132.

Rittirsch, D., Redl, H., Huber-Lang, M. (2012) Role of complement in multiorgan failure. *Clin. Dev. Immunol.* **2012**, 962927.

Romantsik, O., Bruschettini, M., Ley, D. (2019) Intraventricular hemorrhage and white matter injury in preclinical and clinical studies. *Neoreviews* **20**, e636-e652.

Sahni, M., Yeboah, B., Das, P. et al. (2020) Novel biomarkers of bronchopulmonary dysplasia and bronchopulmonary dysplasia-associated pulmonary hypertension. *J. Perinatol.* **40**, 1634-1643.

Schrag, S. J., Farley, M. M., Petit, S. et al. (2016) Epidemiology of invasive early-onset neonatal sepsis, 2005 to 2014. *Pediatrics* **138**, e20162013.

Sibikova, M., Vitkova, V., Jamrichova, L. et al. (2020) Spontaneous delivery is associated with increased endothelial activity in cord blood compared to elective cesarean section. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **251**, 229-234.

Šibíková, M., Živný, J., Janota, J. (2018) Cell membrane-derived microvesicles in systemic inflammatory response. *Folia Biol. (Praha)* **64**, 113-124.

Simonsen, K. A., Anderson-Berry, A. L., Delair, S. F. et al. (2014) Early-onset neonatal sepsis. *Clin. Microbiol. Rev.* **27**, 21-47.

Srinivasan, L., Kilpatrick, L., Shah, S. S. et al. (2016) Cerebrospinal fluid cytokines in the diagnosis of bacterial meningitis in infants. *Pediatr. Res.* **80**, 566-572.

Stoll, B. J., Hansen, N. I., Sánchez, P. J. et al. (2011) Early onset neonatal sepsis: the burden of group B streptococcal and E. coli disease continues. *Pediatrics* **127**, 817-826.

Strauss, T., Elisha, N., Ravid, B. et al. (2017) Activity of von Willebrand factor and levels of VWF-cleaving protease (ADAMTS13) in preterm and full term neonates. *Blood Cells Mol. Dis.* **67**, 14-17.

Takahashi, K., Oharaseki, T., Yokouchi, Y. (2014) Update on etio and immunopathogenesis of Kawasaki disease. *Curr. Opin. Rheumatol.* **26**, 31-36.

Tanaka, T., Narazaki, M., Kishimoto, T. (2014) IL-6 in inflammation, immunity, and disease. *Cold Spring Harb. Perspect. Biol.* **6**, a016295.

Toussi, S. S., Pan, N., Walters, H. M. et al. (2013) Infections in children and adolescents with juvenile idiopathic arthritis and inflammatory bowel disease treated with tumor necrosis factor-α inhibitors: systematic review of the literature. *Clin. Infect. Dis.* **57**, 1318-1330.

van der Pol, E., Böing, A. N., Harrison, P. et al. (2012) Classification, functions, and clinical relevance of extracellular vesicles. *Pharmacol. Rev.* **64**, 676-705.

Veldman, A., Nold, M. F., Michel-Behnke, I. (2008) Thrombosis in the critically ill neonate: incidence, diagnosis, and management. *Vasc. Health Risk Manag.* **4**, 1337-1348.

Vieira-de-Abreu, A., Campbell, R. A., Weyrich, A. S. et al. (2012) Platelets: versatile effector cells in hemostasis, inflammation, and the immune continuum. *Semin. Immunopathol.* **34**, 5-30.

Vítková, V., Pánek, M., Janec, P. et al. (2018a). Endothelial microvesicles and soluble markers of endothelial injury in critically ill newborns. *Mediators Inflamm.* **2018**, 1975056.

Vítková, V., Živný, J., Janota, J. (2018b) Endothelial cell-derived microvesicles: potential mediators and biomarkers of pathologic processes. *Biomark. Med.* **12**, 161-175.

Ware, L. B., Conner, E. R., Matthay, M. A. (2001) von Willebrand factor antigen is an independent marker of poor outcome in patients with early acute lung injury. *Crit. Care Med.* **29**, 2325-2331.

Yoon, B. H., Romero, R., Yang, S. H. et al. (1996) Interleukin-6 concentrations in umbilical cord plasma are elevated in neonates with white matter lesions associated with periventricular leukomalacia. *Am. J. Obstet. Gynecol.* **174**, 1433-1440.

Yuana, Y., Bertina, R. M., Osanto, S. (2011) Pre-analytical and analytical issues in the analysis of blood microparticles. *Thromb. Haemost.* **105**, 396-408.

Zhang, H., Park, Y., Wu, J. et al. (2009) Role of TNF-α in vascular dysfunction. *Clin. Sci. (Lond.)* **116**, 219-230.

Zonneveld, R., Martinelli, R., Shapiro, N. I. et al. (2014) Soluble adhesion molecules as markers for sepsis and the potential pathophysiological discrepancy in neonates, children and adults. *Crit. Care* **18**, 204.