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Mid-regional proadrenomedullin

Adrenomedullin: a key marker of endothelial and microcirculatory dysfunction in patients with infection

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The microcirculation – the motor of sepsis

The microcirculation in healthy patients

The microcirculation consists of a vast network of small blood vessels whose main purpose is to provide the surrounding tissues with an adequate supply of oxygen and nutrients, as well as to remove waste products, in order to maintain a constant and uninterrupted organ function[1].

The structure and function of the microcirculation is highly heterogeneous between different organs with the total number of capillaries potentially related to the different functional requirements[2].

The main cell types include endothelial and smooth muscle cells, with involvement of the endothelial cells key in providing a semi-permeable barrier lining the inner surfaces, preventing microorganisms from entering the tissue, and regulating blood flow by controlling the release of mediators and other regulating substances[3].

Endothelial cells are connected by junctions which prevent leukocyte emigration and vascular leak, with a glycocalyx layer on the luminal surface serving as a protective barrier between the flowing blood and vessel wall[4].

Blood flow depends on a pressure gradient along the vascular tree, as well as the amount and distribution of resistance across the microvasculature bed[5], and therefore a key role in maintaining homeostasis and organ function.

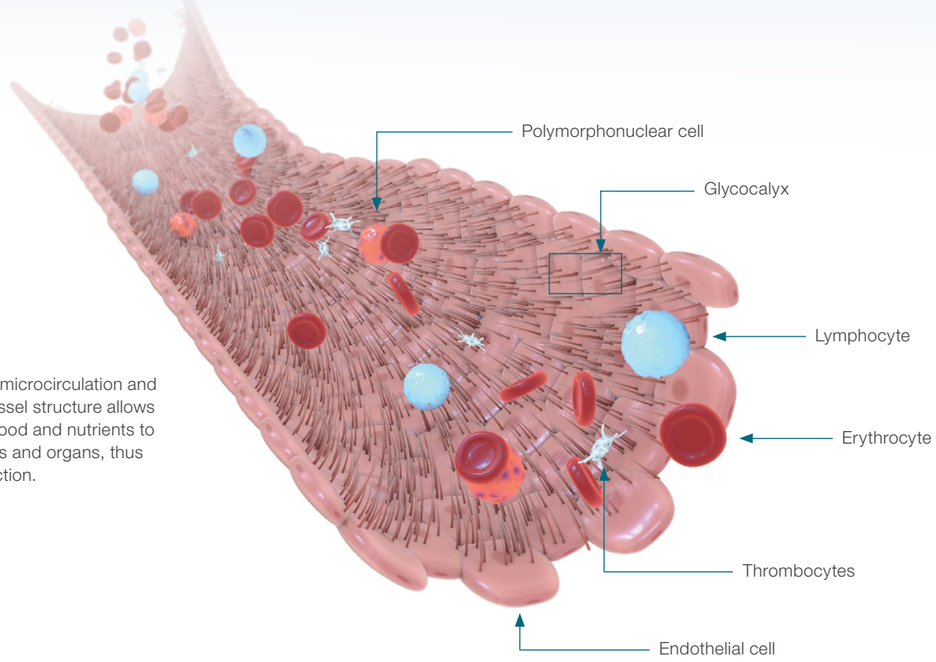


Figure 1: The healthy microcirculation and endothelium. Intact vessel structure allows the constant flow of blood and nutrients to the surrounding tissues and organs, thus maintaining organ function.

The microcirculation during infection

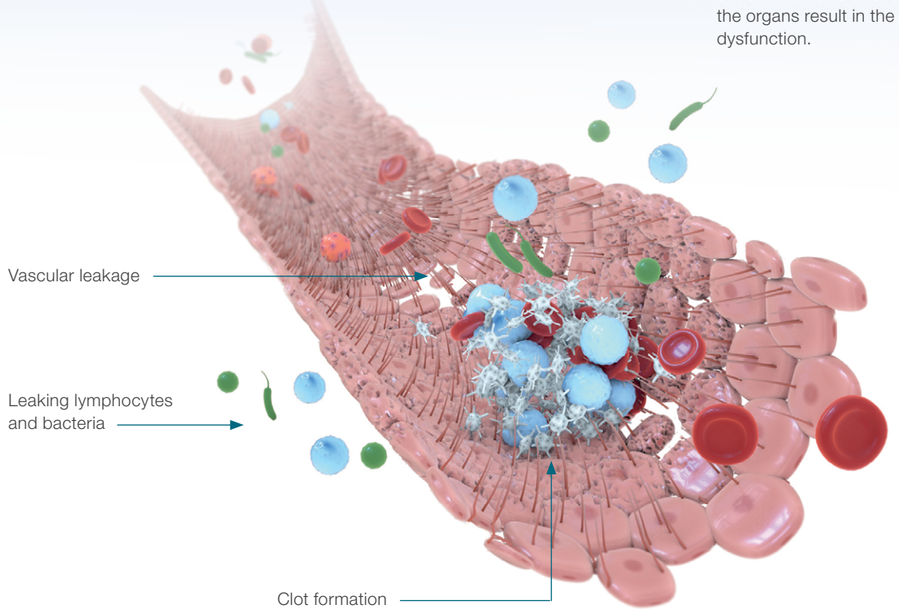
Endothelial dysfunction with vascular leak and tissue edema followed by disruption of the microcirculation is an early, central event in the initial stages of sepsis development[4].

Bacterial endotoxins, pathogen-derived products and pro-inflammatory factors immediately alter the structure of the endothelial cells, with associated shedding of the glycocalyx, resulting in a subsequent loss in structural integrity, sensitivity and tone[6-8]. The structural integrity of the endothelial barrier becomes weaker causing endothelial junctions to break down[9,10], leading to the movement of fluid from the vessels into the extracellular spaces[11-14].

Such processes can result in the development of oedema, local tissue hypoxia, the initial stages of organ dysfunction, and ultimately, organ failure.

Increased endothelial dysfunction and structural damage can lead to a significant decrease in vessel density and in the proportion of perfused vessels, with the greatest changes found in the most severely ill patients[15,16]. Conversely, microcirculatory function improves more rapidly in surviving patients and those responding to therapy, despite global haemodynamic variables remaining similar irrespective of treatment response or survival outcome[17].

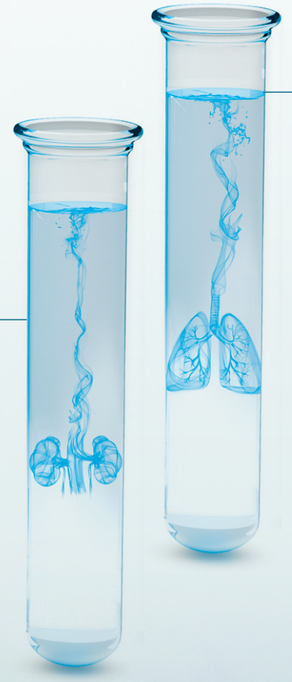
Figure 2: The microcirculation after microbial invasion. Structural changes to the endothelium and a reduced blood flow to the organs result in the early stages of organ dysfunction.



Organ dysfunction and Sepsis Development

The early detection of endothelial damage, microcirculatory dysfunction and tissue hypoxia may allow corrective measures to be taken in order to limit potential organ dysfunction and improve outcome. However, tissue hypoxia is difficult to assess due to non-specific clinical signs and a lack of accurate diagnostic tests. In addition, current measurements of hemodynamic and oxygen-derived parameters do not accurately assess the microcirculation where oxygen delivery is most crucial[2].

An early identification of endothelial damage and microcirculatory dysfunction is therefore key in preventing sepsis development and progression.

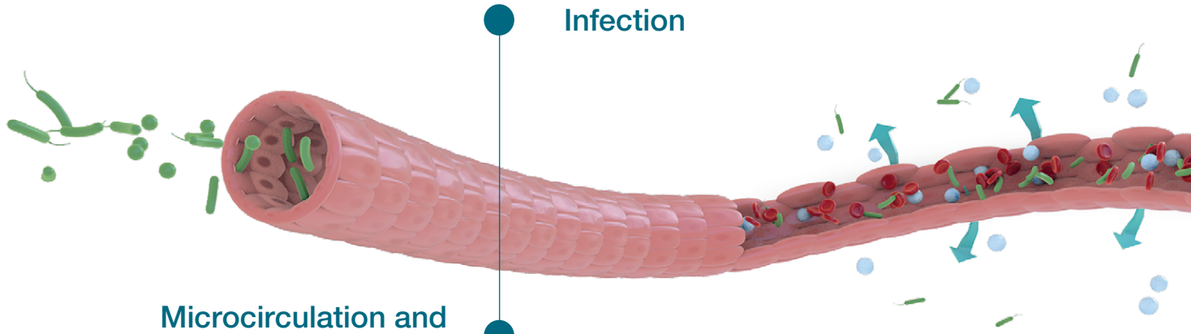


Multiple mechanisms are evident in the microcirculation during sepsis, which include:

- Redistribution of blood flow to more critical body areas
- Endothelial cell barrier and transduction dysregulation
- Increased microvascular permeability and capillary leakage
- Capillary blockage and obstruction
- Disruption of structural and cellular interactions
- Impaired smooth muscle tone

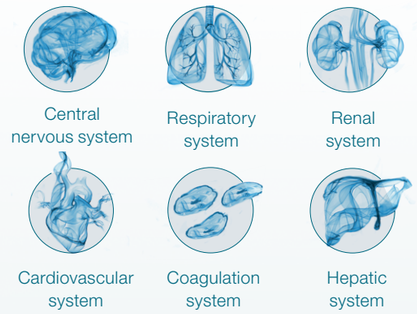
Disruption of these mechanisms can subsequently contribute to:

- A reduction in perfused capillaries and functional density
- The development of heterogeneous abnormalities in microcirculatory blood flow
- A loss of intrinsic vasoregulation in vascular beds
- The initial stages of organ dysfunction and impairment
- Progression towards organ failure



Infection

Microcirculation and Endothelial damage



Organ Dysfunction

⊕ Sepsis-related Organ Failure Assessment (SOFA) score ≥ 2

Sepsis — Life-threatening organ dysfunction caused by a dysregulated host response to infection

Septic Shock — A subset of sepsis in which particularly profound circulatory, cellular and metabolic anomalies are associated with a greater risk of mortality than with sepsis alone

Multiple Organ Failure

Figure 3: Overview of disease progression from an initial infection to sepsis and septic shock.

Adrenomedullin – the key to the microcirculation

Adrenomedullin has been shown to play a significant role in many physiological processes, including cellular growth, development, chemotaxis and migration[18-23]. It also has potent anti-microbial activity, eliciting its response through membrane channel formation and lysis, as well as anti-apoptotic actions[24-27]. However, two main properties are of significant interest in sepsis:

Microcirculation and vascular endothelial barrier function

Increasing evidence suggests that Adrenomedullin is produced in order to reduce vascular permeability and increase the stability of the microcirculation. Recent studies have shown that Adrenomedullin may be a good surrogate marker for predicting sodium and extracellular fluid overload in a variety of critically ill intensive care patients[28], with a strong correlation to the severity of plasma leakage in patients with Dengue Hemorrhagic Fever/Dengue Shock Syndrome (DHF/DSS)[29].

Further experimental evidence has found that Adrenomedullin reduces vascular hyperpermeability[30] and stabilises endothelial barrier fuction[31], and can redistribute blood flow back to sites which have experienced a lack of perfusion and oxygenation due to alpha-toxin infusion[32].

Adrenomedullin may therefore accurately reflect the developing pathophysiological changes occurring in the microcirculation and microvasculature during disease onset. An early assessment of Adrenomedullin levels could be useful in evaluating disease progression or assessing the efficacy of therapeutic interventions on the host response.

- Protects against endothelial permeability and consequent organ damage[3,30,34]
- Protective effects in organs in response to bacterial induced shock[35,36]
- Stabilization of microcirculation in inflammation - a hallmark of organ failure[32]
- Restoration of endothelial stability in infected organs due to prevention of undesired inflammatory decompartmentalization[37]
- Increased in the early stages of organ dysfunction[33]

Vasodilation

Perhaps one of the most well-known features of Adrenomedullin are its vasodilatory properties. Increased circulating concentrations have been shown to result in a potent and sustained hypotension, mainly due to the generation of Nitric Oxide, with transient concentration surges contributing to the dramatic hypotension and vascular collapse found in patients with acute episodes of systemic capillary leak syndrome[38].

Acute or chronic increases in Adrenomedullin can also result in a significant decrease in total peripheral resistance, accompanied by a fall in blood pressure, as well as an increased heart rate, cardiac output and stroke volume.

Increases or decreases in Adrenomedullin levels, therefore, can have a significant effect on the volume of blood supply to organs in specific disease settings.

- Key mediator of vascular tone regulation resulting in an intense, prolonged vasorelaxation and hypotension[39-44]
- Widespread production helps maintain blood supply to individual organs[39,41,45]
- Localized cellular production and release to meet specific perfusion requirements of individual organs[46,47]
- Significant role in hemorrhagic and endotoxic shock[48-50], pulmonary hypertension[51], hypertrophy[52,53], hypoxia[54-59], oxidative stress[60], ischaemic myocardial injury[61-64] and ischaemic injury and organ failure[65,66]

Mid-regional proadrenomedullin

Reliable measurement of Adrenomedullin is complicated by a number of issues, such as a short half-life, a fast metabolism, low concentrations, a rapid degradation by proteases, and binding to compliment factor H. Accordingly, levels of Adrenomedullin can therefore be typically underestimated. The measurement of mid-regional proadrenomedullin provides a solution to these problems.

Biosynthesis of MR-proADM

MR-proADM is a fragment of 48 amino acids which splits from the proADM molecule in a ratio of 1:1 with Adrenomedullin, and proportionally represents the levels and activity of Adrenomedullin. Its biological inactivity means that it is not involved in the binding to vessel walls and surfaces found with Adrenomedullin. Its longer half-life of several hours and biological inactivity results in a more accurate estimation of plasma concentration levels than using ADM. Levels are not influenced by food or water intake nor gender[69].

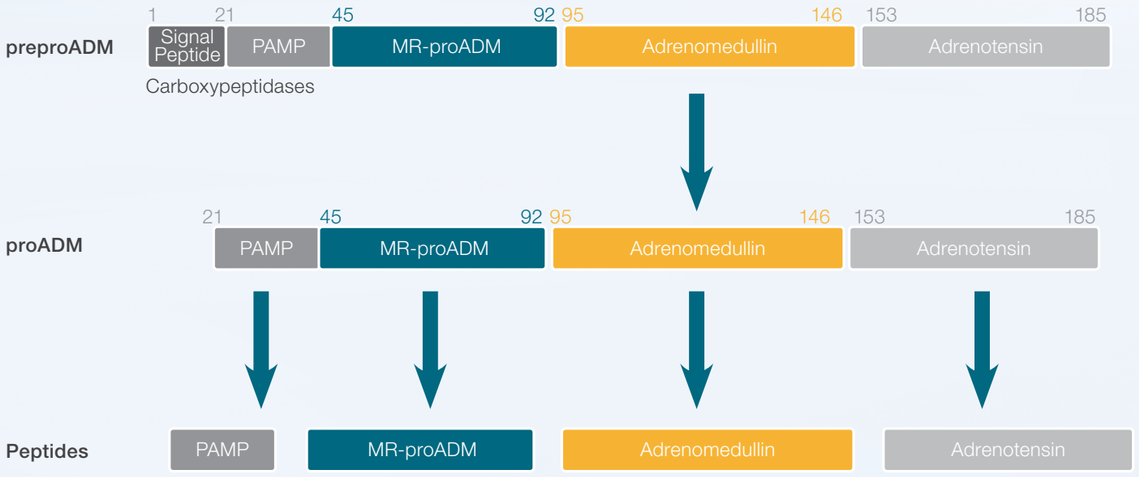


Figure 4: Structure of the preproADM molecule and generation of the related MR-proADM and Adrenomedullin peptides

Thermo Scientific™ B·R·A·H·M·S™ MR-proADM KRYPTOR™ kinetics and features

MR-proADM levels have been shown to be rapidly elevated immediately after the administration of LPS, reaching significantly increased levels after only 2 hours[67,68] and almost doubling in concentration[68]. Concentrations were found to subsequently peak at 4 hours[67,68] before decreasing.

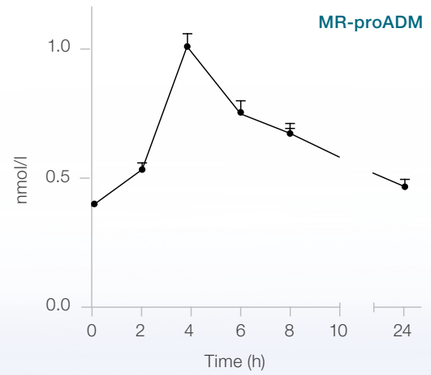


Figure 5: Significant increases in MR-proADM levels can be observed 2 hours after a single LPS injection (4ng/kg IV) (adapted from de Kruif et al. 2008)[68].

Clinical utility of MR-proADM as a biomarker

- Indicator of microcirculatory status and organ dysfunction
- Rapid release in response to infection and inflammation
- Stable molecule, equimolar to native ADM
- Stability of up to 24 hours in EDTA plasma at room temperature and over four freeze/thaw cycles[69]
- Rapidly available to aid timely clinical decision making using the KRYPTOR™ platform*

* assay incubation time 29 mins and small sample volume (26 µL in EDTA plasma)

MR proADM is a valuable biomarker for early detection of organ dysfunction and outcome

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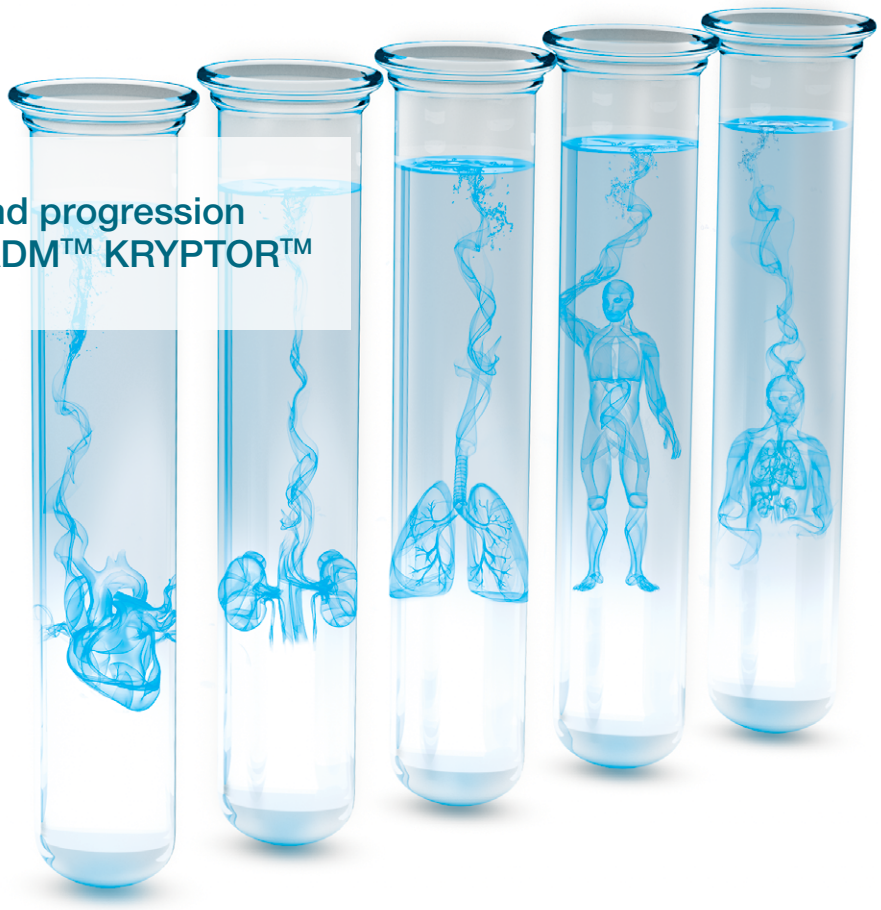

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