ENDOTHELIITIS AND SEPSIS

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In May 2017 the World Health Organisation 70th Assembly agreed on a Resolution to improve the prevention, diagnosis and treatment of sepsis. This recognition of sepsis as a lethal disease to be targeted by health care providers is widely welcomed. The pathophysiology of severe sepsis is a highly complex, co-ordinated host response to an initiating infection that includes the activation of a number of cell types, the production of inflammatory mediators, and dysregulation of hemostatic and hemodynamic physiology. Endothelial cell dysfunction is involved in all these life-threatening disturbances. The endothelial cell membrane and glycocalyx are host to selectins, cell adhesion molecules and coagulation proteins among many others, but I intend to focus on the role of the endothelium and glycocalyx as barrier between the intravascular circulation of blood and the interstitial fluid circulation, *via* lymphatics. Accumulation of parenchymal and interstitial fluid is a feature of worsening systemic sepsis, and recovery is often associated with diuresis and reduction of edema. Hope that capillary permeability might be a life-saving target for therapy is found in research on the control of the vascular endothelial adhesion molecule VE-cadherin, highlighted by Lee and Slutsky in a NEJM article in 2010.

Many of the clinical challenges of critical care are attributed to what we call leaky capillaries. Pushed to elaborate, even the experts may recall 'the reflection coefficient', but few can elaborate further. The equations we are told are Starling's were in fact proposed many years after his death. The most frequently cited equation explains the transvascular volume filtration rate Jv in terms which describe the net hydrostatic pressure difference and net colloid osmotic pressure (COP) difference across the semipermeable microcirculation. Less often taught, but equally important to understanding the pathophysiology, is the equation explaining a solute transfer rate Js as the sum of the mass of that solute carried with the microvascular filtrate (convection) and the mass of that solute that permeates the microcirculation independently of flow (diffusion). In clinical considerations the solute of interest is albumin. If you read the published clinical data, you will find that researchers who measure Js of albumin or another marker molecule in disease states often presume that Jv will be increased with Js and cause edema.

Let's start with Jv. One of the first consequences of inflammation is a fall in the interstitial pressure as integrins change the conformation and hydration of structural collagen fibres. The transendothelial pressure difference and Jv therefore increase, beginning the shift of extracellular fluid balance from the intravascular to the extravascular compartment. NO synthase activation and pre-capillary vasodilation increases the capillary pressure and so further increases Jv. If the haemodynamic reflexes are working, blood pressure is maintained by increased cardiac output (capillary recruitment) which is another factor increasing Jv in the early phase of systemic inflammation. We have accounted for a substantial increase in Jv to the interstitium and so far have not had to appeal to the 'leakiness' of the capillary. Histamine and other autocoids are known to increase the length and number of intercellular junction breaks, especially in the distal part of the capillary and the venules. The surface area for filtration within each capillary and the hydraulic conductivity are thereby increased and further increase the Jv. There is potential for therapeutic manipulation of the adherens junction proteins that zip adjacent endothelial cells together, or unzip them creating the interendothelial junction breaks. Slit proteins occur in the extracellular matrix and, through interaction with Robo receptors on endothelial cells they are involved in angiogenesis, the creation of new blood vessels. One of the stimulating molecules is vascular endothelial growth factor VEGF, which also increases in inflammatory conditions including sepsis. The fact that VEGF was originally termed vascular permeability factor is interesting. The anti-VEGF antibody bevicumab reduces sepsis-induced hyperpermeability in experimental models, but it is not known whether the VEGF response to sepsis is friend or foe. Slit protein interaction with Robo4 receptor stabilizes VE-cadherin and so preserves interendothelial adherens junction integrity. Slit protein does not suppress the cytokine storm of sepsis. `

Tyrosine phosphorylation is associated with weakened adherens junctions and impaired endothelial barrier function. Inducers of tyrosine phosphorylation include histamine, platelet activating factor, tumour necrosis factor alpha, and VEGF. Antagonists of all these molecules are available but have not proven therapeutic in the treatment of sepsis. It always seemed to me that H1 and H2 receptor antagonists do not reverse shock, but could be expected to have some beneficial effect.

Cyclic AMP elevating drugs are known to enhance barrier function. Hence there has been interest in phosphodiesterase IV inhibitors such as rolipram. Phosphokinase A and RAP1 have received attention. RAP1 in particular is a ubiquitous Ras-related GTPase involved in the physiological regulation of capillary permeability.

Ascorbate (Vitamin C for humans) is considered an important cellular anti-oxidant and free radical scavenger, but there is also evidence for a specific tightening of endothelial permeability, associated with acute reduction in ascorbate transfer and a longer effect on basal collagen deposition. Sepsis is just one of many disease states in which plasma ascorbate levels are reduced, and a strong case can be made for supplementation.

Glucocorticoids enter endothelial cells to interact with cytosolic glucocorticoid receptors. Dexamethasone increases VE-cadherin levels in endothelial cells, strengthening the adherens junctions, and also increases the production of tight junction proteins such as occludins and claudins. Glucocorticoid therapy of sepsis has been disappointing, but the possibility of a synergistic effect with other permeability-focused treatment or treatments remains intriguing.

Now let's consider the endothelial glycocalyx (EG). It is easily compressed in volume by almost anything we do to our patients. It entraps and retains albumin molecules, in part by electrostatic means, restricting their free passage through it and so behaving as an imperfect filter. It has been claimed that it is too fragile to have any clinical relevance, but we only need a few glycoprotein and proteoglycan fibres supporting some hyaluronan and heparan sulphate on the luminal aspect of our endothelial junction breaks to perform the filter function. With extensive endothelial injury our patient would probably have succumbed to massive intravascular coagulation long before she ran out of fibrematrix caps over the increasing junction breaks. Inflammation and capillary hypertension damage the EG and so reduce the reflection coefficient sigma for albumin, with EG components like syndecan-1, heparan sulfate and hyaluronan appearing in blood samples. Sigma is an index of the effective as opposed to measured COP effect on Jv. As such, a reduced sigma limits the COP opposition to Jv and gives free rein to the hydrostatic pressure filtration driver. However, even when sigma approaches zero the impedance to fluid filtration remains substantial. Glucocorticoid therapy appears to reduce the disintegration of EG.

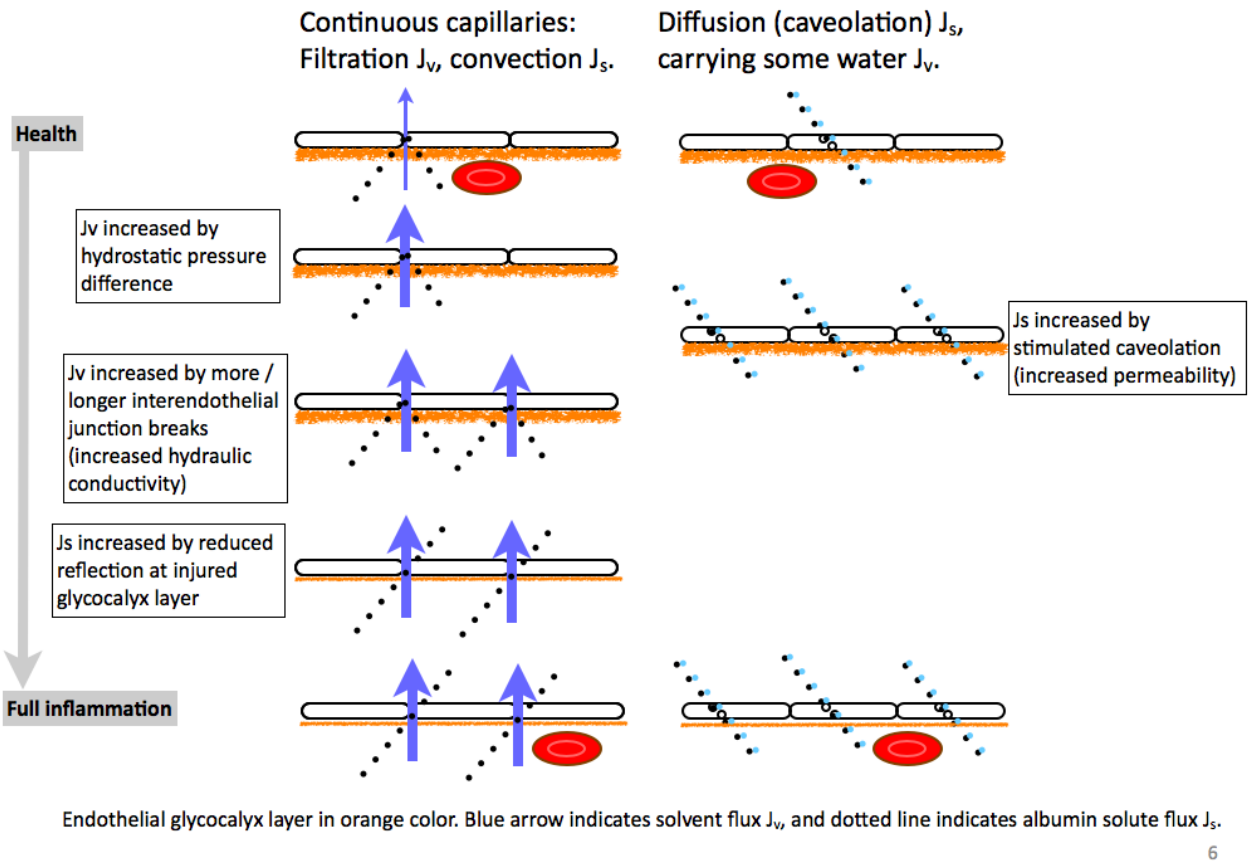

Figure 1: increased solute and solvent flux with increasing inflammatory state.

Only finally will we consider the permeability of capillaries to albumin. Pore theory speaks of large pores which pass albumin molecules, and small pores which retain them. But what are the anatomic equivalents of these biophysically defined concepts? Permeability as it appears in the equation for Js is an index of how readily albumin appears to diffuse across a capillary if it were a simple semipermeable membrane dividing static fluid spaces. It is not. Albumin is actively transported across continuous capillaries via a membrane-associated protein that has been called gp60 and is now referred to as caveolin. Caveolin deficiency is incompatible with life. The rate of transfer of albumin, and other proteins having their own transport system, to the interstitium will appear to be a change in the number of large pores. Caveolae will carry some water with the albumin, but the convective inter-endothelial pathways predominate. Places where the fibrematrix covering a junction break is thinned will also behave like more large pores.

My revised Starling equation and glycocalyx model recognises that the microvasculature is not a passive biophysical barrier separating the vascular and interstitial compartments of the extracellular fluid's circulation. The collecting (afferent) lymph vessels have barrier properties comparable to to venules and carry filtered tissue fluid to the lymph nodes whose capillaries are diaphragm fenestrated and capable of fluid absorption. As much as 50% of the fluid arriving at a lymph node is reabsorbed to the blood circulation there, so the lymph in the efferent lymphatics has a high protein concentration and is pumped to the thoracic duct. It is thought that most of the efferent lymph re-enters the venous system via the thoracic duct, but other lymphatic - venous collaterals can be recruited if the duct is tied off. Radiation ablation of lymph nodes predisposes to oedema, a clear practical demonstration that non-fenestrated capillaries outside the lymph nodes are not capable of significant absorption of tissue fluid. The spontaneous contractility of the lymphatics is enhanced by adrenergic agents and suppressed by inflammatory mediators. It's worth recalling that 25% of the cardiac output goes to the discontinuous capillary circulations of the liver, spleen and bone marrow, where sigma for albumin and any other large molecule is very low, and that more than 50% of the high-protein lymph in the thoracic duct originates from the liver. In resuscitated hyperdynamic sepsis the proportion of blood going to the liver rises to as much as 50% so that higher molecular weight molecules will be easily lost from the blood stream.

The preceding account allows me to offer some practical guidelines for the management of the septic patient. Some will argue that my suggestions have yet to be confirmed by randomized controlled clinical trials to increase survival, but I would respond that we give many drugs for their known pharmacology without demanding evidence that they are life-saving. We cannot save every patient, but we can maximize the number of patients we save.

It should no longer presumed that all septic patients need acute intravenous fluid resuscitation, and old standards like the 20 or 30 ml/ kg crystalloid bolus were probably harmful. Observing the hemodynamic response to smaller aliquots (perhaps 3 ml/ kg) and restricting fluid once non-response has been confirmed makes much more sense. DO NOT target high CVP (8 mmHg+) to increase stroke volume; in this range, right heart failure due to fluid overload is likely.

Weigh the patient at least daily and keep accurate fluid balance accounts; expect a small increase in body weight/ water (up to 10%) in the first 48 hours, and be prepared to reverse that during the recovery phase.

DO NOT use albumin solutions for volume expansion or for the treatment of hypoalbuminaemia, they will worsen refractory edema. The only conceivable indication is massive loss of albumin from the body.

Early use of norepinephrine (or other alpha agonist) in low dose to offset arteriolar vasodilation and capillary hypertension makes sense. Jv would be minimized, opposing the interstitial accumulation of circulating extracellular fluid. Theoretically this could be achieved even before severe arterial hypotension demands pressor support. Note also that norepinephrine increases the venous excess and so supports the hyperdynamic circulation of sepsis. Norepinephrine increases the contractions of lymphatic vessels and thereby assists return of fluid to the bloodstream. DO NOT use alpha agonists to drive mean arterial pressure above 70 mmHg in the futile hope you can squeeze blood though underperfused capillaries. Gangrene will result.

Continued alpha receptor responsiveness requires glucocorticoid receptor stimulation, and so a dose of glucocorticoid that guarantees maximal stress should be given by infusion. Steroid therapy limits the shedding of EG in response to tumor necrosis factor alpha. Similarly, a dose of ascorbate to ensure optimal (high normal range) plasma levels makes good sense for preservation of the endothelial barrier. An H1 receptor antagonist will protect the patient from further histamine-release circulatory insults and offer a degree of sedation, while an H2 receptor antagonist will protect the gastric mucosa.

References:

Dejana E, Orsenigo F, Lampugnani MG. The role of adherens junctions and VE-cadherin in the control of vascular permeability. Journal of Cell Science 2008;121:2115-2122. http://jcs.biologists.org/content/121/13/2115.full

Chelazzi C, Villa G, Mancinelli P, De Gaudio AR, Adembri C. Glycocalyx and sepsis-induced alterations in vascular permeability. Crit Care 2015;19(1):26.

Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome Blood 2003;101:3765-3777.

May JM, Harrison FE. Role of vitamin C in the function of the vascular endothelium. Antioxid Redox Signal. 2013 Dec 10;19(17):2068-83.

Salvador E, Shityakov S, and Förster C. Glucocorticoids and endothelial cell barrier function. Cell Tissue Res. 2014; 355(3): 597–605.

Huxley VH, Scallan J. Lymphatic fluid: exchange mechanisms and regulation. J Physiol. 2011 Jun 15; 589(Pt 12): 2935–2943.

Nelson A, Johansson J, Tydén J, Bodelsson M. Circulating syndecans during critical illness. APMIS. 2017;125(5):468-475.

Puskarich MA, Cornelius DC, Tharp J, Nandi U, Jones AE. Plasma syndecan-1 levels identify a cohort of patients with severe sepsis at high risk for intubation after large-volume intravenous fluid resuscitation. J Crit Care. 2016 Dec;36:125-129.

Anand D, Ray S, Srivastava LM, Bhargava S. Evolution of serum hyaluronan and syndecan levels in prognosis of sepsis patients. Clin Biochem. 2016 Jul;49(10-11):768-76.

Gao SL, Zhang Y, Zhang SY, Liang ZY, Yu WQ, Liang TB. The hydrocortisone protection of glycocalyx on the intestinal capillary endothelium during severe acute pancreatitis. Shock. 2015 May;43(5):512-7.

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