

**Unfractionated heparin offers potential advantage over low molecular weight heparin in management of respiratory disease in Covid-19 infection.**

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**Conflicts of interest:**

SM – None

HF - None

The International Society on Thrombosis and Hemostasis interim guidance on recognition and management of coagulopathy in COVID-19 argues for prophylactic use of low molecular weight heparin (LMWH) to treat coagulopathy in this disorder <sup>1</sup>. While LMWH has become established as preferential to unfractionated heparin for routine anticoagulation, Barrett and colleagues have made the case on hematological grounds for using unfractionated heparin instead of LMWH in management of Covid-19 infection <sup>2</sup>. We would support this contention, though on the grounds of the physiological and pathophysiological advantages of unfractionated heparin over LMWH in management of respiratory disease.

### **Pulmonary abnormalities in SARS-CoV-2 infection**

Covid-19 infection can cause significant pulmonary lesions from early in the course of disease <sup>3</sup>. Histology ranges from early proteinaceous exudates in alveolar spaces with interstitial infiltration of mononuclear cells to persistent inflammatory hyaline membrane formation, while a patient who had survived over 50 days showed hyaline membrane remnants and fibrosis <sup>4</sup>.

Hyaline membranes were recognised in neonatal respiratory distress syndrome (RDS), and have important roles in pulmonary pathophysiology, including covering the epithelial surface and sequestering surfactant. They form through intraluminal coagulation of leaked proteins and glycans on a framework of necrotic epithelium <sup>5</sup>. Post-mortem specimens of preterm neonates showed that these membranes incorporate degraded sulphated glycosaminoglycans (GAGs), lost by inflammatory disruption of cell-associated and connective tissue GAGs. Of physiological importance, heparan sulphate proteoglycans (HSPG) were lost from the subepithelial basement membrane and basolateral epithelial surface <sup>5</sup>.

This pattern of GAG disruption is identical to that seen in IBD, where degraded GAGs localise to colonic crypts <sup>6</sup>. Those in the gut would pass distally, whereas in the lung they would require coughing, phagocytosis or reabsorption by pulmonary lymphatics. Aggregation into large hyaline membranes would render the first possibility unlikely.

### **Physiological consequences of lost epithelial heparan sulphate at mucosal surfaces**

These identical findings in gut and lung inflammation have important physiological implications, primarily related to transepithelial protein leak. HSPG line the subepithelial basement membrane and are also anchored to the basolateral epithelial surface in the proteoglycan syndecan-1. In the lung, epithelial syndecan-1 plays a critical role in attenuating lung injury in influenza, through facilitating cytoprotective signalling via c-met to limit epithelial apoptosis <sup>7</sup>. Thus, inflammatory syndecan-1 glycan degradation will worsen epithelial apoptosis and inflammatory lung injury.

Syndecan-1 and its attached HSPG chains are central in regulating permeability of the epithelium to leakage of proteins such as albumin. Children suffer severe protein losing enteropathy (PLE) due to epithelial HSPG deficiency, and epithelial HSPG expression is a critical mediator of albumin restriction in intestinal cell lines and animal models <sup>8-10</sup>. Mice with deficiency of heparan sulphate, syndecan-1 or intestinal-specific heparan sulphate have excess basal protein leakage and are far more susceptible to TNF- $\alpha$  and increased venous pressure. Syndecan-1 loss or haploinsufficiency of Ext1 or Ext2 (limiting GAG chain expression) increases protein loss ex vivo <sup>10</sup>.

Screening of a library of non-anticoagulant heparin like compounds have identified the importance of glycan chain length in regulating this permeability. Low molecular weight

heparin was much less effective than unfractionated heparin <sup>10</sup>. Administration of heparin (100 u/kg) attenuated TNF- $\alpha$  and IFN- $\gamma$  induced intestinal protein leakage by 50%, while 5-fold higher administration completely prevented cytokine induced albumin leak. 2,3-de-O-sulphated heparin was identified to have similar capacity to heparin to reduce transepithelial albumin leakage, but only 10% of its anticoagulant activity <sup>10</sup>.

### **Intestinal and pulmonary albumin leak following Fontan cardiac surgery**

Life-threatening protein leak can occur into both the intestine and the lungs of persons with congenital heart disease following reparative Fontan surgery <sup>11,12</sup>. This leads to chronic PLE in some and a plastic bronchitis in others, where structures composed of leaked materials coalesce to form dense rubbery casts that can fill the entire airway. This often occurs in relation to high venous pressure. Importantly, resolution of both PLE and plastic bronchitis have been achieved by use of systemic unfractionated heparin, with recurrence of intestinal or pulmonary leak whenever discontinued <sup>11,12</sup>. It is important to note that unfractionated heparin is needed and low molecular weight heparin ineffective <sup>11,12</sup>.

Biopsies from patients with Fontan physiology PLE often show distended subepithelial venules due to raised venous pressure, but epithelial HSPG is reduced whether or not these are seen (Figure). Epithelial HSPG is retained in intestinal lymphangiectasia, with pressure the main determinant of leak and heparin ineffective. Intravenous administration of unfractionated heparin restores the subepithelial basement membrane and partially restores basolateral epithelial heparan sulfate expression (Figure). If venous pressure is not excessive this stops protein leakage.

## **The case for early use of unfractionated heparin in SARS-CoV-2 respiratory disease**

These data suggest that persons with significant respiratory disease due to Covid-19 infection might benefit from unfractionated full molecular weight heparin in a way that cannot be achieved using LMWH. Unfractionated heparin cannot be targeted towards the lung epithelium alone and will have impact on all endothelial surfaces and basement membranes. Additional effects of unfractionated heparin in experimental septic lung injury included augmenting tight junction integrity to reduce vascular leak and reducing concentrations of IL-6 in lung lavage fluid <sup>13</sup>.

Though it is not the primary target of this intervention, it is important to note that parenteral heparin will augment basement membrane barrier function at the blood brain barrier, as at other basement membranes. Blood brain barrier integrity is centrally dependent on the proteoglycans agrin, within basement membrane, and perlecan, allowing cell to cell integration by binding to endothelial cells and astrocytes <sup>14</sup>. Glycan chains on these proteoglycans will be degraded in severe infection, similar to vascular endothelium and other basement membranes. Heparan sulfate degradation fragments, released in sepsis from damaged vascular endothelium, penetrate the disrupted hippocampal blood brain barrier and contribute to cognitive dysfunction <sup>15</sup>. This is mediated through inhibition of brain-derived neurotropic factor signalling within the hippocampus. Increased circulating levels of heparan sulfate fragments at admission to ICU, enriched in 2-O -and N-sulfated disaccharides, predicted persistent cognitive impairment 14 days after discharge <sup>15</sup>. Augmentation of blood brain barrier integrity by unfractionated heparin may thus have potential neuroprotective consequences.

## **Use of systemic unfractionated heparin**

Continuous intravenous infusion of unfractionated heparin at standard anticoagulant rate would allow termination if problems were to ensue. In places where intravenous treatment may be difficult, subcutaneous heparin could be considered, providing that initial assessment of parenteral unfractionated heparin shows benefit.

Proteinaceous alveolar leak occurs in the very early symptomatic stage of SARS-Cov-2 infection<sup>3</sup>. This suggests that early introduction of unfractionated heparin has the potential to retard the development of hyaline membranes through reduced protein leak.

Administered unfractionated heparin will leak into the lumen as it does in post-Fontan surgery bronchitis<sup>12</sup>. In clearance of plastic bronchitis, heparin does not dissolve the casts, but appears to work as a charge-based lubricant, freeing the cast to allow clearance by coughing or ventilatory manoeuvres<sup>12,16,17,18</sup>. The casts of plastic bronchitis provide a much more extensive luminal plug than do hyaline membranes, but their composition appears similar, though acellular. Persons with more advanced respiratory symptoms of Covid-19 will already have hyaline membranes<sup>4</sup>. They would benefit from reduction of albumin leak, and may additionally be able to clear established membranes by coughing, as occurs with pulmonary casts in post-Fontan plastic bronchitis.

## **Clinical caveats**

Any implementation of unfractionated heparin would clearly need initial close assessment by both Haematologists and Intensivists. It may rapidly become apparent that this approach needs to be revised. There may be the potential for over-anticoagulation or the development of complications such as heparin-induced thrombocytopenia on prolonged use. Initial adopters of such an approach should do so in centres with the skill and resources to make a rapid and dispassionate assessment of the potential benefits and hazards of such an approach, and thus guide others. If helpful, this approach is likely to allow benefit in management of ARDS more generally.

We emphasise that this approach has potential limited to impact on respiratory status and barrier function in addition to anticoagulation, and is most unlikely to ameliorate any of the immunological aspects of severe SARS-CoV-2 infection.

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## Figure

Sulphated GAG expression within small intestine in acquired protein losing enteropathy, demonstrating that unfractionated heparin restores basement membrane and epithelial HSPG expression <sup>19</sup>.

Upper left: Lymphangiectasia showing strong HSPG expression on basement membrane and enterocytes, with multiple lymphocytes leaking into the lumen.

Upper right: Sulfated GAG expression in a case of Post-Fontan PLE, showing extensive loss of epithelial HSPG. The cause of this loss remains unknown.

Middle left: Villus in lymphangiectasia showing strong basolateral HSPG expression. Heparin treatment is ineffective and pressure dominates the protein leak.

Middle right: Villus from Post-Fontan PLE showing reduced basolateral HSPG expression together with gross dilatation of both villous core lymphatic and subepithelial venules due to pressure. As in lymphangiectasia, restoration of the epithelial barrier would be ineffective in this case as pressure is the main determinant of protein leak.

Lower left: Post-Fontan PLE on heparin therapy, showing restored staining of epithelial basement membrane and enterocytes.

Lower right: Villus in heparin-treated Post-Fontan PLE showing that treatment has restored HSPG on subepithelial basement membrane and on the basolateral epithelial surfaces.

