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Published in:
Seminars in Thrombosis and Hemostasis

DOI:
10.1055/s-0030-1262879

2010

Pathophysiology of typical hemolytic uremic syndrome

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ABSTRACT

The typical form of hemolytic uremic syndrome (HUS) is associated with Enterohemorrhagic *Escherichia coli* (EHEC) infection. The disease process is initiated and perpetuated by interactions between the pathogen, its virulence factors and host cells as well as the host response. During EHEC-associated HUS, alterations occurring at the intestinal mucosal barrier and in the circulation, as well as on endothelial cells and other target-organ cells, lead to cell activation and/or cytotoxicity, and trigger a pro-thrombotic state. This review summarizes current knowledge regarding the interactions of the pathogen and its virulence factors with cells in the intestine, bloodstream, kidney and brain. Mechanisms of bacterial colonization, toxin circulation and induction of target organ damage are discussed.

Key words

Hemolytic uremic syndrome, Shiga toxin, enterohemorrhagic *Escherichia coli*, thrombotic microangiopathy, kidney

INTRODUCTION

Hemolytic uremic syndrome (HUS) is diagnosed when the simultaneous features of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure are present. Whilst HUS has a number of underlying etiologies, over 90% of cases in developed countries follow gastrointestinal infection with Enterohemorrhagic *Escherichia coli* (EHEC).[1] This review summarizes current concepts of the pathogenesis of EHEC-associated HUS, or so-called "typical HUS".

EPIDEMIOLOGY

The incidence of EHEC-associated HUS peaks in children under 5 years old, and rises again in the elderly [1]. The incidence rate of EHEC-associated HUS is similar in Europe, North America and Australia,[1,2] but 7-10 times higher in Argentina.[3] EHEC colonize animals, primarily cattle, without causing disease.[4] Transmission to humans occurs by consumption of contaminated meat, milk products, water, fruit and vegetables.[5] Direct contact with animals during visits to farms is increasingly recognized as a risk factor.[6] Approximately 10% of children exposed to EHEC infection develop diarrhea (usually bloody), and 15% of children with diarrhea will develop HUS. The incubation period is usually less than a week, and the interval between onset of diarrhea and diagnosis of HUS is approximately 6-7 days.[7,8]

HISTOLOGY

HUS is characterized by widespread thrombotic microangiopathy (TMA) in renal glomeruli, the gastrointestinal tract, brain and the pancreas.[9] TMA defines a lesion of vessel wall thickening, usually at the arteriolar-capillary junction, with swelling or detachment of the

endothelial cell from the basement membrane, accumulation of amorphous material in the subendothelial space, intraluminal thrombosis and partial or complete obstruction of the vessel lumen.[9] This suggests that microvascular endothelial cell injury is central to the pathogenesis of HUS. In addition, arteriolar thromboses are common at the hilum of glomeruli and are also seen proximally in interlobular arteries. In severe cases, cortical necrosis may be present.[10]

EHEC VIRULENCE FACTORS

EHEC cause a range of clinical manifestations, including diarrhea, hemorrhagic colitis and HUS. HUS is predominantly associated with serotype O157:H7, although other serotypes, including O26:H11, O103:H2, O111:NM, O121:H19, and O145:NM have been reported.[5] EHEC possess certain virulence factors that contribute to the development of HUS. These factors include proteins that promote intestinal colonization and toxins that disseminate within the host resulting in microvascular injury.

The virulence factors that promote intestinal colonization of EHEC are contained within horizontally acquired gene cassettes known as pathogenicity islands (PAI). One such PAI is the locus of enterocyte effacement (LEE),[11] which allows EHEC to attach to the luminal surface of host enterocytes and to cause effacement of the microvilli, resulting in watery diarrhea through loss of absorptive surface. The LEE encodes the adhesin intimin, a type three secretion system (TTSS) and secreted proteins. The TTSS translocates bacterial proteins from EHEC directly into host enterocytes, affecting cellular structure and function.[11,12]

Shiga toxin

Shiga toxin (Stx) is considered the most important factor for the virulence of EHEC. Stx consists of a single enzymatically active A-subunit linked to five B-subunits. EHEC may secrete a number of distinct Stxs (Stx1 and Stx2 and a number of subtypes). Each Stx is encoded by a specific bacteriophage, and EHEC strains with more than one bacteriophage can produce more than one toxin.[13] EHEC responsible for HUS express Stx2 more often than Stx1.[14] Bacteriophages can be induced either spontaneously,[15] or by certain antibiotics, including quinolones,[16] increasing the level of Stx production, which might be an important factor for the pathogenicity of EHEC. The toxin is produced by *Shigella dysenteriae* 1 as well as by EHEC. Stx produced by *Shigella* is almost homologous to Stx1 produced by EHEC.

The glycosphingolipid receptor for Stx is globotriaosylceramide (Gb3). The distribution of Gb3 has been found to determine the localization of pathological lesions in HUS in humans and other animals (glomerular endothelium, brain, pancreas).[17] Bacteremia is rarely reported in HUS and so it is likely that Stx is transported from the intestine to distant sites. Most studies of the interaction of Stx with human cells have been carried out on endothelial cells. After binding of Stx to Gb3 on endothelial cells, Stx is internalized by receptor-mediated endocytosis. Inside the host cell, the A subunit is proteolytically cleaved to an enzymatically active fragment, which cleaves a residue within the 60S ribosomal subunit. This inhibits protein synthesis, causing cell death.[18] In addition to cytotoxicity, Stx may also exert activating effects on endothelial cells, for example stimulation of interleukin-8 (IL-8) and monocyte chemoattractant protein-1 production and upregulation of adhesion molecule expression.[19] Human umbilical vein endothelial cells (HUVEC) exposed to Stx show an upregulation of genes encoding cytokines, cellular adhesion molecules and transcription

factors.[20] Endothelin-1 is a potent vasoconstrictor produced by endothelial cells. Stx causes upregulation of endothelin-1 (ET-1) mRNA and protein levels.[21] Furthermore, Stx promotes leukocyte dependent inflammation[22] and endothelial cell activation, with a change to a more procoagulant endothelial cell phenotype, in addition to endothelial cell damage[23] which will trigger platelet adhesion to the subendothelium.

Other virulence factors

Since HUS can develop after infection with EHEC strains which do not produce Stx,[24] it is likely that even other virulence factors may play a role in the pathogenesis of HUS. Cytolethal distending toxin V (CDT-V), EHEC hemolysin (EHEC-hly), and subtilase cytotoxin are potential candidates.[25]

Cytolethal distending toxin

About 5% of EHEC 0157:H7 strains investigated carry a gene encoding CDT-V,[26] the cytolethal distending toxin. CDT-V is a cyclomodulin that causes cell cycle arrest.[25] CDT-V may contribute to endothelial injury by causing irreversible G2/M cell cycle arrest, growth inhibition and death of human endothelial cells.[27]

EHEC hemolysin

EHEC-hly is a pore-forming cytolysin encoded on a large 60 MD plasmid and released from EHEC strains associated with HUS. Aldick et al identified Stx gene-negative EHEC O26 strains as the only pathogens in the stools of five patients with HUS and examined the strains

for potential virulence factors and interactions with microvascular endothelial cells.[28] All five isolates possessed the gene encoding EHEC-hly and were cytotoxic to human brain microvascular endothelial cells. Toxicity was significantly reduced in an EHEC-hly-negative strain, and reproduced by introducing recombinant EHEC-hly to EHEC O26, suggesting that EHEC-hly may have cytotoxic properties.

Subtilase cytotoxin

In addition to Stx, a subset of EHEC strains secrete another extremely lethal cytotoxin termed subtilase (SubAB).[29] This toxin is lethal in mice, inducing thrombosis and necrosis in multiple organs, thus mimicking the clinical presentation of HUS. Its mechanism of action is inactivation of the endoplasmic reticulum chaperone BiP (immunoglobulin heavy chain–binding protein) by the serine protease activity of its A subunit.[30] A recent study showed that SubAB prevents secretion of immunoglobulins from B lymphocytes.[31]

PATHOGENESIS OF EHEC-INDUCED DISEASE

EHEC is a non-invasive pathogen.[32,33] Bacterial virulence factors gain access to the circulation after causing intestinal damage, and thereby reach the target organ. In the following section we will review the influence of the intestinal microflora and the host hormonal response on EHEC motility, colonization and Stx production. Intestinal colonization triggers a local inflammatory and innate immune response. Stx and lipopolysaccharide (LPS) are released into the circulation and bind to blood cells thus reaching the renal microcirculation. The consequences of Stx and LPS release into the

circulation, including their interactions with blood cells and endothelium, result in a prothrombotic state within the microvasculature.

EHEC in the intestine

Very few EHEC colony-forming units are capable of inducing clinical symptoms.[33] The mechanisms by which colonization and expression of virulence factors occur in the intestine have been extensively studied. After ingestion, EHEC reaches the ileum and was detected in the ileocecal valve in one patient sample.[34] EHEC is assumed to initially bind to villi of the terminal ileum and follicle-associated epithelium of Peyer's patches[35,36] followed by colonization of the colon. Cross-talk occurs between EHEC and the commensal intestinal microflora during colonization. EHEC also interact with the host hormonal response. This results in activation of virulence factors, such as those encoded in the LEE,[37] responsible for formation of the attaching and effacing lesion enabling intimate attachment to the intestinal cell, the expression of flagella thereby enhancing mobility, and the induction of Stx.[38,39] These interactions involve bacterial sensing of a molecule termed auto-inducer-3, produced by the intestinal microflora, as well as a response to the host stress hormones epinephrine and norepinephrine, involving the bacterial membrane histidine sensor kinases QseC and QseE. The recent description of the QseC signaling cascade[38] revealed that bacteriaare responsive to host adrenergic signals and this phenomenon would most probably be increased during hemorrhagic colitis as more catecholamines are released from the bloodstream into the intestine.

EHEC strains that lack the LEE pathogenicity island-encoded TTSS and its effector proteins are also capable of colonization and induction of disease in humans.[40] EHEC possess non-

LEE proteins promoting adhesion and virulence.[41-44] For example, the expression of the non-LEE EspI/NleA effector protein by EHEC has been associated with severe disease.[43]

Stx is causally related to severe EHEC-associated disease and the induction of HUS. In a primate model of Shigellosis, the toxin induced dysentery.[45] Enterocytes do not express the Gb3 receptor to which the toxin binds but Stx1 and Stx2 were shown to bind to Gb3-expressing intestinal Paneth cells.[46] Even though intestinal epithelial cells do not express Gb3 they may take up the toxin by actin-dependent macropinocytosis.[34] Stx causes apoptosis of intestinal epithelial cells *in vitro* in human[47,48] and mouse intestinal cells[49] and may translocate across polarized intestinal epithelial cells using a transcellular route,[50] an effect enhanced by neutrophil migration in the opposite direction.[51] Recent studies have shown that Stx production can be induced by quorum sensing signaling[39] but also suppressed by the normal human intestinal microflora.[52] There are several variants of Stx2,[25] some of which exhibit increased virulence to man such as Stx2c and Stx2d(activatable). The latter is activated by intestinal mucus and elastase, is present in EHEC strains that lack the LEE and has been associated with severe disease.[53] It has been assumed that after damaging the mucosal epithelium, Stx may gain access to, and damage, the intestinal vasculature.[54]

EHEC may also secrete SubAB in the intestine. SubAB recognizes a monosaccharide, terminating with the sialic acid N-glycolylneuraminic acid (Neu5Gc), as its receptor on human endothelial cells and intestinal epithelial cells.[55] This glycan is not synthesized in humans but provided in the human diet, specifically in food which may be contaminated with EHEC, such as red meat and milk products,[56] enabling SubAB to cause intestinal damage even in the absence of an inherent receptor.

EHEC infection induces intestinal inflammation, cell death by apoptosis and necrosis as well as an inflammatory response. Stx upregulates the proinflammatory cytokine IL-8, as well as other C-X-C chemokines in the gut.[57-59] Triggering an inflammatory response in the intestine and systemically may upregulate the Stx receptor on endothelial cells.[60] EHEC LPS may also play a role in inducing a mucosal immune response during the initial phase of disease[61,62] which may promote bacterial clearance. A reduced initial response was shown to increase the bacterial burden in mice, allowing more severe disease to proceed both locally and systemically, presumably due to increased secretion of Stx.[62] A recent study reported that EHEC could suppress the intestinal epithelial cytokine response to Stx,[59] an effect that could facilitate bacterial colonization. Host anti-microbial peptides may also be involved in the initial defense against intestinal infection, protecting the mucosal surface from colonization. This has been documented for *Citrobacter rodentium,*[63] a pathogen causing similar attaching and effacing lesions in the gut, and suggested as a plausible protective mechanism in EHEC infection as well.

EHEC virulence factors and blood cells

Damage to the intestinal epithelium allows bacterial virulence factors to enter the circulation. Blood cells from patients with HUS are coated with Stx and LPS.[64,65] During HUS, Stx circulates bound to platelets, monocytes and neutrophils as well as to platelet-monocytes and platelet-neutrophils in complex.[64,66] Stx may bind to blood cells via the Gb3 receptor as well as other glycolipid receptors.[67-69] LPS binds to blood cells via Toll-like receptor 4, which, on platelets, is in complex with CD62 (P-selectin).[65]

Stx induces cell death by blocking protein synthesis[70] or by apoptosis.[54] Neutrophils, monocytes and IgM-producing B lymphocytes exhibit resistance to Stx's cytotoxic effect.[69,71-73] In macrophage-like THP-1 cells, both apoptotic and cell survival signaling pathways were activated after exposure to Stx1.[74] Thus, most leukocytes encountering Stx will not undergo cell death, allowing the toxin to circulate bound to their cell membrane.

Leukocytosis and high IL-8 levels at HUS presentation are associated with poor outcome.[75,76] Neutrophils demonstrate prolonged survival during severe forms of HUS.[77] Interestingly, Stx has been shown to prolong the life-span of neutrophils,[71] and impair neutrophil migration in mice.[78] *E. coli* O157 also secrete StcE, a protease shown to increase the neutrophil oxidative burst and adhesion, thus impairing neutrophil migration[79] which could explain increased tissue destruction at sites of neutrophil influx in HUS patients.[80] Neutrophil activation during HUS and in experimental models was recently reviewed.[81]

The role of monocytes during HUS may be related to toxin transport[64,82] although the transfer of Stx from monocytes to its target cells has not been conclusively documented. The toxin is, however, capable of stimulating the human monocytic cell line THP-1 to secrete cytokines.[69,83] Guessous et al showed that THP-1 cells stimulated with Stx2 released the chemokines IL-8, macrophage-derived chemokine (MDC), and Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES) and this effect was enhanced in the presence of LPS.[84] The released chemokines activated platelets, indicating an interaction between these blood cells. Stimulation of THP-1 cells with Stx1 also led to upregulation of tissue factor.[85] We have recently shown that monocytic microparticles bearing tissue factor

are released during HUS and these microparticles may contribute to the prothrombotic state[64] as described below.

During HUS, platelets are deposited on injured endothelial cells. Multiple microthrombi lead to thrombocytopenia. Many studies have addressed the pro-thrombotic state occurring during EHEC infection and resulting in TMA (reviewed in[86]). Platelets are activated by a direct interaction with LPS, Stx,[65,87,88] chemokines[84] and by factors released from damaged endothelium. Coagulation factors are, however, not consumed during this process. Mice inoculated with *E. coli* O157:H7 developed thrombocytopenia.[62] Likewise, mice treated with Stx2 and LPS developed thrombocytopenia and platelet clumping in the kidneys.[89]

During the acute phase of HUS, patients were shown to have elevated levels of tissue factor,[90] circulating tissue factor-bearing platelet-monocyte complexes as well as tissue factor-expressing microparticles, mainly derived from platelets, but also from monocytes.[64] These tissue factor expressing-complexes and microparticles decreased considerably after the patients' recovery. Stimulation of whole blood with Stx2 induced the formation of platelet-monocyte complexes, and, to a lesser degree, platelet-neutrophil complexes. The effect was enhanced when blood cells were co-stimulated with Stx2 and LPS, and O157LPS was more potent than other LPS serogroups. The formation of platelet-leukocyte complexes was further enhanced by application of high shear stress, mimicking the capillary shear stress present in glomeruli.[64]

In addition to its effect on platelets and monocytes, Stx induces tissue factor expression on endothelial cells.[91-93] Tissue factor is the receptor for coagulation factor VII, thus converting factor X to Xa in the extrinsic pathway. Tissue factor expression will trigger thrombin generation resulting in clot formation and further platelet activation. Thrombin's role in TMA was implied by its increased generation in mice injected with Stx2 and LPS[94] as well as by the inhibitory effect of lepirudin, a thrombin inhibitor, on Stx-mediated injury in the dog.[95]

The mechanism by which hemolysis occurs during HUS has not been elucidated. Red blood cell fragmentation is noted and has been mimicked in animal models of EHEC infection using the whole bacterium[61] or Stx alone.[95,96] It has been assumed that fragmentation is the result of mechanical breakdown in occluded vessels but oxidative damage has also been proposed as a mechanism of hemolysis.[97] Regardless of the cause, the products of hemolysis may have a cytotoxic effect. Bitzan et al showed that heme and Stx induced an additive cytotoxic effect on renal tubular epithelial cells and microvascular endothelial cells.[98]

Renal damage in HUS

The main target organ affected during HUS is the kidney, and in severe cases also the brain, as well as other organs.[99] Stx, with or without LPS, has been studied as the major virulence factor affecting target organs. In order to exert its cytotoxic effect, the toxin binds to its receptor, and kidney cell vulnerability is predicted by the presence of the Gb3 receptor.[23,100-102] Human kidneys exhibit both glomerular and tubular damage during HUS with extensive apoptosis of renal cortical cells.[103,104] It is, however, unclear if the initial toxin insult occurs at the glomerular or tubular cell level.

Stx exerts a cytotoxic and apoptotic effect on glomerular endothelial[105,106] and epithelial cells.[107,108] The cytotoxic effect is enhanced in the presence of TNF-α[60] as well as IL-1, LPS and butyrate.[108] Stx1 was shown to upregulate cytokine production in glomerular epithelial cells[109] and tubular epithelial cells,[110] which would in turn enhance toxicity. Furthermore, Stx induced expression of the chemokine fractalkine on glomerular endothelial cells promoting leukocyte adhesion to the endothelium,[111] an effect verified *in vivo* in mice and suggested to contribute to severity of disease in humans.[112]

Studies have demonstrated that tubular cells are affected during HUS.[103] Tubular cell damage was indicated by an increase of neutrophil gelatinase-associated lipocalcin in patient urine.[113] The beneficial effect of intravenous volume expansion during the early stages of disease[114] could also indicate an initial reversible tubular cell injury. EHEC infection in mice and rats induced acute tubular injury[103,115 62,116] and Stx, in particular, triggered tubular cell apoptosis in mice,[103] affecting primarily the cortical tubular cells. Silberstein et al showed that Stx2 could inhibit water absorption in primary human proximal tubular cells[117] and others have shown increased urine volume in vivo in rats[116,118] and mice[119] attributed to collecting duct injury. Thus both proximal tubular as well as collecting duct cells appear to be vulnerable to Stx. Interestingly, human proximal tubular cells exposed to Stx1 exhibited increased tissue factor expression[120] suggesting that these cells may trigger the coagulation system upon Stx stimulation. Tissue factor is expressed in glomerular capillary endothelial cells and tubular epithelial cells during EHEC-associated HUS (Figure 1).

Podocytes are highly specialized glomerular epithelial cells. Using murine podocytes Morigi et al showed that Stx2 increased endothelin-1 mRNA and protein expression affecting cytoskeleton remodeling.[121] A similar affect of Stx1 and Stx2 on preproendothelin-1 expression was previously reported in vascular endothelial cells.[122] Thus, by affecting endothelin-1 expression, Stx will possibly modulate vascular tone and glomerular permeability.

Mesangial expansion, necrosis and mesangiolysis have been described in renal samples obtained during HUS although some of the cases reported were presumably not EHEC-associated.[123] Mesangial cells also possess Gb3 receptors which are upregulated by TNF-α[124] enabling Stx to inhibit protein synthesis and exert a cytotoxic effect, after prolonged incubation,[107,125] as well as reduce nitric oxide production.[126] Stx did not, however, stimulate the release of cytokines or chemokines from mesangial cells.[127]

Brain damage in HUS

A subset of HUS patients will develop central nervous system involvement. Symptoms may vary from mild irritability to coma. Human brain expresses the Gb3 receptor in neurons and endothelium.[128] *In vitro* experiments have shown that human brain microvascular endothelial cells undergo apoptosis after exposure to Stx2;[129] the effect was enhanced by TNF-α which sensitized cells to Stx1-induced apoptosis.[130] This is due to upregulation of the Gb3 receptor.[131] In the mouse Gb3 was demonstrated in CNS neurons.[128] Mice injected with Stx develop convulsions with brain edema[101] and hind-limb paralysis.[128] Gb3-null mutant mice were protected[101] indicating the importance of Gb3 expression for targeting of Stx-induced damage. Intracerebroventricular injection of Stx2 in rats led to neuron apoptosis and glial

affection with reactive astrocytes.[132,133] Of note, stimulation of human brain endothelial cells with Stx1 and TNF-α was cytotoxic but also induced cytokine synthesis and release.[134] The cellular signaling events that dictate if the toxin will induce a cytotoxic or stimulatory response are yet to be elucidated.

The emerging role of complement

Of those cases of HUS not associated with EHEC infection, inherited disorders of complement regulation are the most frequent underlying cause.[8] In these cases of atypical HUS, TMA is thought to result from complement-mediated endothelial damage and platelet activation.[135,136] Limited evidence suggests that complement activation may play a role in the pathogenesis of EHEC-associated HUS. Patients with EHEC-associated HUS had elevated levels of complement factors Bb and sC5b-9 at presentation, indicating activation of complement through the alternative pathway.[137] Stx activates complement in human serum via the alternative pathway.[138] In addition, Stx binds to the cell binding domains of complement factor H, and appears to inhibit the regulatory function of factor H on cell surfaces.[138] Complement activation may be a secondary phenomenon, which could, nonetheless, exacerbate renal injury.

The chain of events from EHEC ingestion to the development of HUS

EHEC will, after ingestion, colonize the terminal ileum and follicle-associated epithelium of Peyer's patches.[35] In the gut the bacterial virulence and adhesion will be activated by interkingdom signaling,[139] allowing colonization to proceed with release of virulence factors such as Stx and LPS. It is, as yet, unclear which host factors increase susceptibility to develop HUS. Bacterial virulence factors migrate across the intestinal epithelium,[51] gain access to the

blood, circulating bound to platelets, monocytes and neutrophils as well as platelet-leukocyte aggregates.[64] Hypothetically, larger amounts of circulating Stx and LPS could increase the risk to will develop HUS. In line with this assumption, and using a limited number of patient samples, Ståhl et al showed that during EHEC infection, only the platelets of patients who later developed HUS carried Stx and LPS on their surface.[65] Thrombin generation and fibrinolysis inhibition also precede the development of HUS.[140]

Stx exerts a stimulatory effect on cells, triggering cytokine release and/or tissue factor expression, as well as a cytotoxic effect inhibiting protein synthesis and inducing apoptosis. These effects may occur in the circulation, affecting blood cells, as well as when reaching Gb3-expressing target cells in the kidney, brain and other organs. In the presence of high shear, as in the renal glomerular microcirculation, the effects of Stx and LPS may be enhanced,[22,64] resulting in leukocyte adhesion and the formation of microthrombi.

CONCLUSION

This review has summarized many of the known mechanisms by which EHEC colonize the intestine and induce disease affecting the intestine, kidney and brain, as well as other organs, in infected individuals. As EHEC is a non-invasive organism the bacteria triggers lesions in the host by the interaction of its factors with host cells, firstly at the mucosal level, followed by binding to blood cells and transfer to target organs. Ultimately this will lead to endothelial cell injury and platelet activation resulting in a prothrombotic state. Advances in understanding the complex events leading to EHEC-induced thrombotic microangiopathy will hopefully facilitate the development of specific therapeutics agents in the future.

Acknowledgments

Diana Karpman is supported by grants from The Swedish Research Council (K2010-65X-14008-10-3), The Torsten and Ragnar Söderberg Foundation, Konung Gustav Vs 80-årsfond, Crown Princess Lovisa's Society for Child Care, Fanny Ekdahl foundation and is the recipient of a clinical-experimental research fellowship from the Royal Swedish Academy of Sciences. Lisa Sartz is supported a grant from the Queen Silvia Jubilee Fund.

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Figure legend

Figure 1: Tissue factor expression in the renal cortex of a child with *E. coli* O157:H7-associated HUS

Kidney tissue from a 14 year-old boy with *E. coli* O157-associated HUS (A) and from an adult control whose kidney was removed due to renal cancer, showing an area unaffected by cancer (B).[141] Tissue factor was detected by immunohistochemistry using monoclonal mouse anti-human tissue factor antibody (0.7µg/ml, American Diagnostica Inc, Stamford, CT, USA). Signal was detected using an EnVision System goat-α-mouse:HRP (Dako Cytomation, Glostrup, Denmark) secondary antibody as described.[141] Tissue factor was detected in glomerular capillaries (arrow) and proximal tubular cells (asterix) as well as in the Bowman´s capsule. Mouse IgG1 (Dako Cytomation) was used as the isotype control and did not show staining (not shown). The study was approved by the Ethics Committee of the Medical Faculty at Lund University and biopsies were taken with the informed consent of the patient and control.

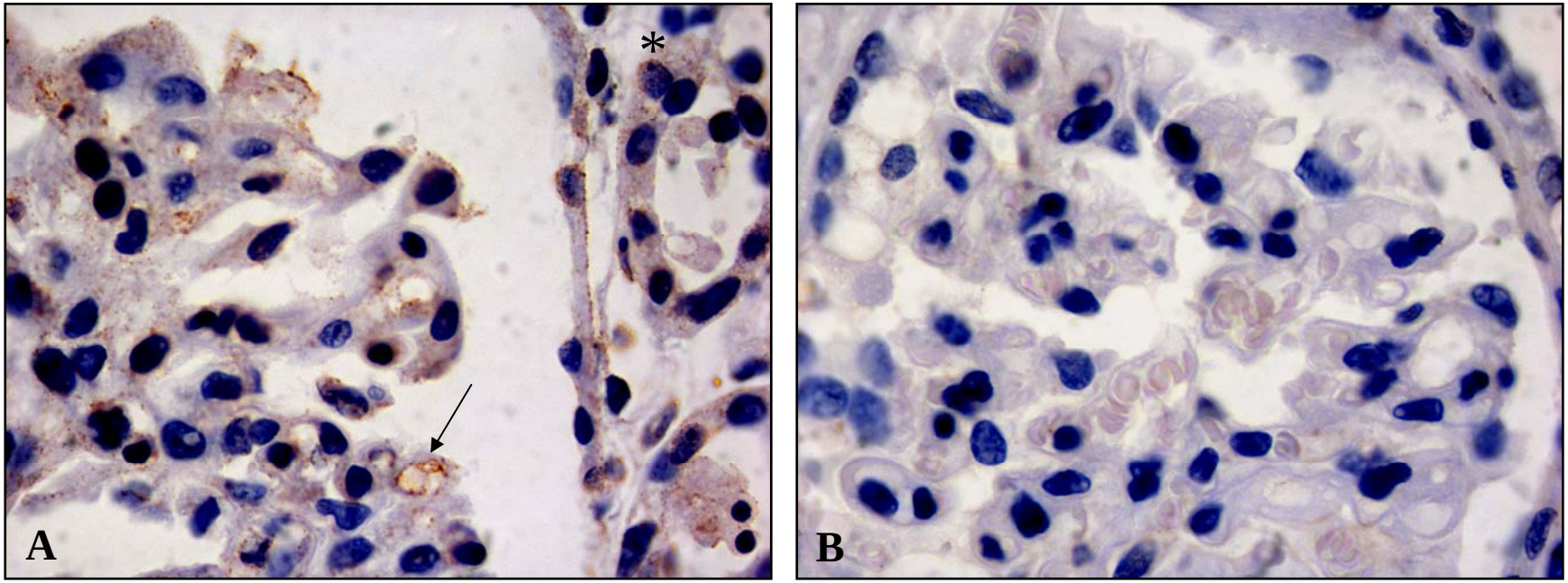

Figure 1