Acute renal failure associated to paroxysmal nocturnal haemoglobinuria leads to intratubular haemosiderin accumulation and CD163 expression

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Abstract
Decreased renal function has been observed in diseases with intravascular haemolysis, including paroxysmal nocturnal haemoglobinuria (PNH). However, the mechanisms via which haemoglobin enhances renal damage in this pathology are not fully known. We report a case of acute renal failure associated to PNH and extensive haemosiderin deposits in tubular cells. Renal biopsy also revealed a strong immunostaining of CD163 (a haemoglobin scavenger receptor expressed in macrophages) and oxidative stress markers (NADPH-p22 phox and haeme oxigenase-1) in areas with deposits of iron. This fact provides evidence for a pathogenic role for free haemoglobin in tubulointerstitial renal injury in human PNH disease.

Keywords: CD163; haemosiderin; macrophages; paroxysmal nocturnal haemoglobinuria

Background
Reversible acute renal failure due to haemolysis-induced severe tubular damage is a recognized complication of paroxysmal nocturnal haemoglobinuria (PNH). It has been proposed that acute tubular necrosis and acute renal failure associated to PNH results from tubular haemoglobin (Hb)-mediated toxicity due to haemolysis [1]. However, there is little information on the mechanisms via which Hb promotes renal damage in PNH from human renal biopsies. We now show a case of acute renal failure associated to PNH with haemosiderin deposition in renal tubules. In this context, we identified the expression of several proteins associated to Hb oxidative damage in the tubular interstitium and inflammatory infiltrate, suggesting the involvement of Hb in renal injury in PNH.

Case report
A 24-year-old man was referred to our hospital in July 2009 because of nausea, vomiting, diarrhoea, constipation and bloodstained urine. He had a history of intermittent passage of dark-coloured urine (June 2007 and January 2009).

At presentation, he was normotensive. Investigations revealed a normochromic normocytic anaemia (haemoglobin 9.7 g/dL), mild elevation of liver enzymes (cholestatic and hepatocellular), indirect hyperbilirubinaemia, elevated levels of lactate dehydrogenase (up to 2583 IU/L) and decreased haptoglobin. A peripheral blood smear showed no fragmented red blood cells (RBCs), and a direct Coombs
test was negative. Serum creatinine was 92 μmol/L. Diuresis volume was 400 mL on the first day and the urine volume increased with fluid administration up to 1200 mL on the second day. Urine examination revealed trace proteinuria and microscopic haematuria (>10 RBCs/HPF). Abdominal ultrasonographic examination showed normal-sized kidneys with normal echogenicity and corticomedullary differentiation. Renal function (creatinine 633 μmol/L) and haemoglobin (8.4 g/dL) decreased at Day 3.

The patient’s granulocytes were found to be deficient in CD59, CD55 and CD16 by flow cytometry with a clonality of 70%. In addition, 50% of the erythrocytes were deficient in CD59, CD55 and CD16, showing a similar clone. A diagnosis of PNH was therefore made.

Light microscopy of the kidney biopsy revealed 12 unremarkable glomeruli. The tubules showed diffuse deposition of brown-coloured pigment, which gave a positive blue-coloured reaction on Perls stain, consistent with deposition of ferric ions (haemosiderin) (Figure 1). There were structural changes indicative of tubular damage, such as distention of tubules and cell debris in the lumen. There was neither interstitial fibrosis nor vascular changes. Immunofluorescence (anti-IgG, anti-IgA, anti-IgM, anti-C3 and anti-C1q) was negative. Interstitial macrophages were present, as determined by CD68 staining (Figure 2). Interestingly, a marked infiltration of CD163-expressing macrophages was observed in the renal interstitium surrounding areas with iron deposits. There was high expression of p22 phox, a subunit of NADPH oxidase, and haeme oxygenase-1 (HO-1) in tubular and macrophage cells from haemosiderin-rich areas of the cortex and outer medulla of kidney (Figure 3).

Treatment with eculizumab was started. The dark colour of the urine disappeared progressively and there was a rapid and clear improvement in haematologic values and serum creatinine declined to 78 μmol/L over the next 2 months.

**Discussion**

PNH is an acquired clonal disorder associated with somatic mutations of the X-linked phosphatidylinositol glycan anchor biosynthesis class A gene in haematopoietic stem cells, which results in the absence of the phosphatidylinositol-linked proteins necessary to protect cells from complement-mediated lysis [2]. The primary clinical manifestations of PNH include intravascular haemolytic anaemia, thrombosis in vessels and bone marrow failure, which can cause pancytopenia. Renal involvement in PNH is usually not clinically apparent but essentially all patients with classic PNH report gross haemoglobinuria at some point during the course of their illness. However, when renal disease is significant, it usually manifests as acute kidney injury (AKI) and rarely as chronic kidney disease (CKD) [3].

Treatment of PNH has been largely symptomatic but as the haemolysis of PNH is a consequence of complement-mediated cytolysis, inhibition of complement is a logical approach to therapy. Eculizumab is a humanized monoclonal antibody that targets complement protein C5, thereby preventing production of the pro-inflammatory mediator C5a and the assembly of the membrane attack complex. Data from treated patients demonstrated a reduction in haemolysis and a reduction in thrombotic events. Administration of eculizumab to patients with renal dysfunction was well tolerated and usually associated with clinical improvement [4, 5].

In certain cases of severe haemolysis with volume depletion, like during a gastrointestinal illness, as in the case of our patient, the concentration of haemoglobin in the tubular filtrate becomes sufficiently high to impair renal function and acute renal failure results. In these patients, conservative treatment, which includes fluid administration and urine alkalization, improves renal function [3, 6, 7]. Corticosteroids, attenuating acute haemolytic exacerbations, may reduce the severity and duration of the crisis [8]. However, their efficacy is limited and is not recommended for long-term treatment.

The repetitive exposure of the kidney to cell-free haemoglobin can cause renal haemosiderin accumulation, tubule-interstitial inflammation and chronic kidney damage. In
theses cases, eculizumab, preventing the cascade of events that leads to recurrent and chronic haemolysis, may improve CKD. In a recent study designed to evaluate the efficacy and safety of eculizumab in 29 Japanese patients with PNH, CKD was common (66%) and eculizumab treatment improved CKD in 41% of patients at 12 weeks [9]. This protective effect of eculizumab has been also confirmed recently in 25 Spanish patients with PNH [10].

Reversible AKI in PNH is thought to depend on renal epithelial haemoglobin-mediated toxicity due to haemolysis, contraction of renal blood vessels and intratubular obstruction [6, 7]. Intravascular haemolysis releases Hb into plasma, where Hb is bound quickly to haptoglobin (Hp), forming a Hp–Hb complex [11]. Under normal conditions, this large complex is not filtered by glomerulus and is further degraded by the liver, spleen and bone marrow and degraded. In persistent intravascular haemolysis, plasma haptoglobin is consumed and free Hb accumulates in plasma and dissociates from its usual tetrameric form to dimeric Hb. Dimeric Hb is filtered more easily by the glomerulus and incorporated into proximal tubules, leading to accumulation of ferric ions (haemosiderin) in these cells [11]. The renal biopsy showed haemosiderin deposits in tubular cells, most prominent in the proximal tubules. Although haemosiderin accumulates quite rapidly in tubules, its role in AKI remains controversial since intense renal haemosiderosis can be found in PNH patients with normal renal function [6].

Cytotoxic effect of Hb includes generation of oxygen reactive species, apoptosis and inflammation [1]. To avoid these injurious effects, free Hb is bound to Hp and the Hp–Hb complex is exclusively cleared via CD163, a membrane protein present on the surface of tissue macrophages [12]. The expression of CD163 is up-regulated by haemoglobin [12]. To our knowledge, this is the first report showing the presence of CD163-expressing macrophages in acute renal failure due to PNH, suggesting a possible role of this Hb scavenger receptor in response to massive haemolysis. Recently, we have reported that the presence of CD163-positive macrophages in a patient with IgA nephropathy, macroscopic haematuria and AKI with extensive interstitial and intratubular RBCs extravasation and interstitial haemosiderin deposits [13]. AKI occurring in the course of acute haemolysis following PNH differs from glomerular bleeding-associated AKI because RBCs are absent. However, it may provide pathophysiologic clues to the molecular mechanism of kidney injury and the role of free haeme-containing molecules.

Fig. 2. Interstitial infiltrate in renal biopsy. Immunohistochemistry of CD68 (black triangles) and CD163 (white triangles) macrophages. We observed mainly CD163-macrophages that were mainly related to areas lining tubules containing haemosiderin deposits. Original magnification ×400.

Fig. 3. Oxidative stress in PNH. Immunohistochemistry of NADPH-p22 phox subunit and HO-1. Oxidative stress markers, such as NADPH-p22 phox and HO-1, were highly expressed in interstitial macrophages (white triangles) and tubular epithelial cells, respectively (black triangles). Original magnification ×400.
Uptake of Hb by CD163 not only attenuates toxic effects of cell-free Hb but also induces several anti-inflammatory responses, including interleukin-10 release and HO-1 synthesis [14]. HO-1 catalyses the conversion of haeme to biliverdin, decreasing the production of reactive oxygen species (ROS) [15]. HO-1 synthesis results as an adaptative cell response to different deleterious stimuli, including Hb [15]. This fact may explain the increased HO-1 expression by tubular and phagocytic cells surrounding haemosiderin-rich areas observed in our case. HO-1 expression and its anti-inflammatory and anti-apoptotic effects have already been observed in patients with PNH [16] and in experimental models of haeme-induced damage [17]. On the other hand, several studies have reported that NADPH oxidase is associated to renal damage via production of superoxide anion [18]. NADPH oxidase consists of several units, including p22 phox, which correlates with the amount of ROS and severity of kidney injury [15]. The elevated p22 phox and HO-1 observed in this case illustrates, by the first time, the relationship between acute haemolysis following PNH and oxidative stress.

In conclusion, acute renal failure due to PNH was associated with tubular haemosiderin deposits, local inflammation, oxidative stress and activation of the anti-inflammatory response mediated by CD163-positive macrophages, which may contribute to restoration of tissue integrity and amelioration of renal function.

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