Pathology and Electron Microscopy of Malaria

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Detailed gross and pathological descriptions of malaria infection in human have been well documented and reviewed during the last 100 years (Marchiafava and Bignami, 1894; Taliaferro and Mulligan, 1937; Spitz, 1946; Thomas, 1971; Connor et al., 1976; Francis and Warrell, 1993; Turner, 1997; Medana and Turner, 2006).

**Brain**: Many, but not all, post mortem brain in cases of fatal malaria has been described as oedematous (Riganti et al., 1990). Cerebral oedema is detected less commonly during life, in two of ten unusually severe cases of cerebral malaria in Thailand, cerebral oedema developed as an agonal phenomenon (Looareesuwan et al., 1983). Macpherson et al. (1985) found minimal evidence of oedema. Oo et al. (1987) found that cerebral oedema was variable in their study.

Common to all pathological descriptions which have been reviewed (Aikawa et al., 1980; 1988; 1990) is the presence of large numbers of parasitized red blood cells (PRBC) in the microvasculature (sequestration) (Figure 1a,b) and to a lesser extent margination of PRBC in the medium and large sized vessels (Figure 2).

![Figure 1: Brain in falciparum malaria. a) A clotted cerebral microvessel is showing numerous parasitized red blood cells margination to the endothelial cells, red blood cells congestion and white blood cells in the center of the lumen (H&E stain, x 400). b) An electron micrograph of a cerebral microvessel. The vessel is tightly packed with parasitized red blood cells (PRBC). Perivascular vacuolation is prominent, suggesting that it was oedema (arrows).](image)
Figure 2: Cerebral malaria. A section of brain tissue showing cerebral capillaries (1) and cerebral venule (2) are packing with parasitized red blood cells (PRBC) (arrows). A small vein (3) is seen with a number of PRBC and aggregation of red blood cells (RBC) in the center of the lumen. Few RBC and PRBC are seen in the lumen of a small artery (4). The PRBC appear attach to the endothelial cells (arrows) (H&E stain, x 200).

Quantitative studies of sequestration in different organs from fatal cases confirmed that the sequestration of PRBC in the cerebral microvasculature was significantly associated with clinical cerebral malaria (Macpherson et al., 1985; Riganti et al., 1990; Pongponratn et al., 2003). Sequestration in the brain of cerebral malaria (CM) patients was higher than in the other organs and also higher than non cerebral malaria (NCM) patients (Pongponratn et al., 1991). Sequestration of PRBC does not seem to be uniform within the brain (Pongponratn et al., 1991; 2003; Silamut et al., 1999).

There are numerous petechial haemorrhages in white matter (Figure 3a,b). Infarction, necrosis and large haemorrhages are however, rare. In many patients focal ring haemorrhages (Figure 3c,d) are seen centered on small subcortical vessels (Oo et al., 1987; Boonpucknavig et al., 1990; Riganti et al., 1990). The reparative process following the ring haemorrhage leads to a small nodule or Dürck’s granuloma, composed of microglial cells aggregated around the area of demyelination associated with the vessel. An inflammatory cell response was generally lacking in cases examined by Macpherson et al. (1985) and Pongponratn et al. (2003). Although fibrin thrombi have been described in the vessels, they are not common and some detailed studies have failed to detect them.
Figure 3a,b: Petechial haemorrhages.  
a) Slicing brain from a fatal case of cerebral malaria shows petechial haemorrhages, mainly in the white matter.  
b) Two small haemorrhages in the white matter, around small vessels (H&E stain, x 400).

Figure 3c,d: Ring haemorrhages.  
c) A ring haemorrhage shows parasitized and non-parasitized red blood cells in the brain white matter, around a small vessel (H&E stain, x 400).  
d) An electron micrograph shows a cerebral capillary (arrow) diapedesis which leading to formation of perivascular haemorrhage. Intravascular content including red blood cells (RBC) and parasitized red blood cells (PRBC) leak into the surrounding brain parenchymal tissue forming ring haemorrhage.

Ultrastructural studies of the PRBC in the brain (Macpherson et al., 1985; Pongponratn et al., 1985; 2003) showed PRBC cyoadhered to the cerebral endothelium via knobs (Figure 4). The reduced deformability of the PRBC together with their cyoadherence to endothelium and to the other cells (Figure 5a,b) lead to impairment of blood flow (Dondorp et al., 2000; 2003).

Cerebral involvement appears to be confined to *Plasmodium falciparum* infection. More than 95% of adults or more than 85% of children who recover from cerebral malaria show no persistent neurological sequelae (Warrell, 1983; Brewster et al., 1990) which suggests that much of the pathology must be transient and reversible. Interpretations of pathogenesis of cerebral malaria
have differed, one reason for this is the variation between the findings in different cases, even when clinical criteria are strict. It remains a difficult problem to explain the degree of heterogeneity between different cases.

Figure 4: Sequestration of a parasitized red blood cell (PRBC) to an endothelial cell (Ec) of a cerebral microvessel, is mediated by contacts between electron dense knobs (k) on its surface (arrows).

Figure 5a: A parasitized red blood cell (PRBC) in a congested cerebral capillary. The normal red blood cells (RBC) showing amoeboid movement passing a less deformable PRBC which adheres to the endothelial cell (Ec).

Figure 5b: A congested cerebral venule showing marked sequestration of parasitized red blood cells (PRBC). The venule is tightly packed with PRBC, showing margination to the endothelial cells (Ec), and RBC in the center of the lumen. A vacuolated pericyte (p) is seen.

Kidney: Acute renal failure is one of the major cause of death in human adults with severe falciparum malaria. The presentation and recovery phase is that of acute tubular necrosis (Sitprija et al., 1967; Stone et al., 1972; Sitprija, 1988; Nguansangiam et al., 2007). Renal dysfunction is associated with cerebral involvement, high parasitaemia, jaundice and haemoglobinuria (Canfield et al.,
1968; WHO, 2000), and a high risk of pulmonary oedema. Renal impairment, and ultimately acute tubular necrosis presumably result from reduction in renal microvascular blood flow (i.e. ischaemic nephropathy) (White and Ho, 1992) and linked to localisation of host monocytes in the kidney as well as sequestration of PRBC (Nguansangiam et al., 2007).

Histopathological examination shows PRBC cytoadherence in the glomerular capillaries (Figure 6a,b), but to a lesser degree than in the brain or heart (Macpherson et al., 1985). Several pathological studies (Bhamarapravati et al., 1973; Futrakul et al., 1974; Boonpucknavig and Sitprija, 1979) have also suggested that there is active glomerulonephritis, which would not be surprising in view of the large amounts of malarial and host red cell neoantigen confronting the glomerular filter. Immunofluorescence studies show immunoglobulin, complement and malarial deposition. Mesangial and endothelial proliferation can be seen by electron microscopy. Many cases show malarial pigment in circulating macrophages within the glomerular capillary lumen and some also show PRBC in peritubular capillaries as well as in the glomeruli (Francis and Warrell, 1993).

Figure 6: Falciparum malaria, kidney. a) Parasitized red blood cells with dark brown malarial pigment (arrows) and some mononuclear cells are seen in a renal glomerulus (1) and interstitial blood vessel (2) (H&E stain, x 400). b) An electron micrograph of a kidney glomerulus. The glomerular tuft contains mostly red blood cells (RBC) in the lumen, and few parasitized red blood cells (PRBC). The PRBC are found adhering to the glomerular capillary endothelium (arrows). Mesangial cells (M) show slight basement membrane (Bm) expansion. C = capillary, P = podocyte.

Ultrastructurally, pathological alterations of human renal glomeruli have been reported. Bhamarapravati et al., (1973) described granular deposits of P. falciparum antigen in the mesangium. This finding supports the theory that immune complex glomerulonephritis is due to P. falciparum antigens and their corresponding antibodies. El-Shoura (1994) reported that glomerular capillaries were occasionally occluded with enlarged endothelial cells. Subendothelial, hump-like deposits, similar to previous described (Bhamarapravati et al., 1973) in immune complex nephritis, were detected in most cases. This study
concluded that kidney glomerular damage is mediated by immunopathological mechanisms.

**Black water fever** is an acute hemolytic condition associated with fever, anaemia, jaundice and hemoglobinuria and is usually associated with *P. falciparum* infection. Rosen *et al.*, (1968) reported partial hyalinization and segmental fibrosis of glomeruli in a biopsy specimen. The main histological changes are various degrees of degenerative alterations ranging to necrosis, mainly in the loop of Henle and in the distal convoluted tubules. Epithelial, hyaline, or granular casts as well as heme casts are often seen in the tubular lumen (Figure 7). In addition, a diffuse or focal mononuclear cell infiltration can be seen in the interstitium.

![Figure 7: Black water fever, kidney.](image)

**Figure 7: Black water fever, kidney.** Within the degenerated renal tubules, observe the haemoglobin cast as well as the frank red blood cells. These are the result of massive hemolysis. Note dark brown pigments in the interstitial blood vessels (H&E stain, x 400).

**Lung:** Pulmonary oedema was a universal finding at autopsy. In addition to alveolar oedema, the alveoli are filled with PRBC, RBC, neutrophils and pigment-laden macrophages (Figure 8a,b). In many fatal cases, alveoli are lined with a laminate periodic acid-schiff (PAS) positive membrane which eventually destroys and incorporated the alveolar wall within it. This is associated with abundant oedema fluid and may have a marked inflammatory infiltrate. Hyaline membrane formation in the alveoli suggests leakage of proteinaceous fluid (Charoenpan *et al.*, 1990).

On average, 51% of blood vessels showed PRBC sequestration in the septal capillaries and small blood vessels in the lung of the CM patients Figure 8a), which is significantly higher than the NCM patients (5%). Mononuclear cells, pigment-laden macrophages were always seen admix with PRBC in the microvessels of alveolar septa (Pongponratn *et al.*, 1991). It has been suggested that pulmonary capillary damage might be caused by release of toxic mediators from these adherent mononuclear cells.
Figure 8: Lungs in falciparum malaria. a) In addition to oedema fluid, the alveoli are filled with PRBC, RBC, neutrophils and pigment-laden macrophages. Parasitized red blood cells (PRBC) sequester in the septal capillaries and small blood vessels in the lung (H&E stain, x 200). b) A good number of pigment-laden alveolar macrophages are always seen (H&E stain, x 400).

Heart: In patients who died of malaria, the heart showed little macroscopic abnormality. Petechial haemorrhage of epicardial surfaces may be found (Figure 9a). Most striking findings are the congestion of myocardial microvessels with PRBC (Figure 9b), pigment laden macrophages, lymphocytes and plasma cells. The percentage of PRBC sequestration in the myocardial vessels is about 56% in the CM patients which is higher than in the NCM patients (19%) (Pongponratn et al., 1991).

Figure 9: Heart in falciparum malaria. a) Petechial haemorrhage of epicardial surface. b) Congestion of myocardial microvessels with PRBC, RBC, pigment laden macrophages, lymphocytes and plasma cells (H&E stain, x 200).

Liver: The liver is enlarge and oedematous, and coloured brown, grey or even black (Figure 10a) as a result of deposition of malaria pigment. Hepatic sinusoids are dilated and congested with hypertrophied Küpffer’s cells, variable mononuclear cells, and PRBC that may obstruct the circulation. There is phagocytosis of PRBC and RBC by Küpffer’s cells, endothelial cells and sinusoidal macrophages. PRBC also sequester in the portal and hepatic...
vasculature (Figure 10b). There may be centrizonal necrosis in some cases (Spitz, 1946).

**Figure 10: Falciparum malaria, liver.**  
(a) The liver is enlarged and oedematous, and coloured brown, as a result of deposition of malaria pigment.  
(b) Hepatic sinusoids are dilated and congested with hypertrophied Küpffer’s cells-laden malaria pigment, variable mononuclear cells, and PRBC (H&E stain, x 400).

**Gastrointestinal tract:** Gastrointestinal disturbances and dyspepsia (abdominal pain, nausea and vomiting) are common in falciparum malaria. Comatose patients may aspirate stomach contents into the lungs with fatal consequences. In cerebral malaria the vomitus often contains altered blood, indicating gastric or duodenal bleeding.

PRBC sequestration and cytoadherence have been seen in the small and large bowel, predominantly within the lamina propria capillaries (Figure 11a) but also in larger submucosal vessels. The percentage of vessels showing PRBC sequestration in the microvasculature of the small intestine of fatal malaria in CM patients is significantly higher (61%) than in NCM patients (15%) (Pongponratn et al., 1991). In CM patients submucosal haemorrhages were found in two cases out of twenty-four (Figure 11b). These patients had a high percentage of PRBC sequestration in mucosal villi vessels (90% and 95%). These findings may explain in part pathophysiologic mechanisms responsible for malabsorption (Karney and Tong, 1972; Molyneux et al., 1989) and algid malaria (Pongponratn et al., 1991).

**Figure 11: Small intestine in malaria.**  
(a) PRBC sequestration are seen in the small intestine, predominantly within the lamina propria capillaries (H&E stain, x 200).  
(b) Submucosal haemorrhages (H&E stain, x 100).
Spleen: In acute falciparum infection the spleen is enlarge and soft, varying from dark red to dark or slate gray, depending on duration of infection and the amount of pigment present (Figure 12a). There is congestion with hyperplasia of red and white pulp (Figure 12b). Splenic cord and sinuses are filled with monocytes and macrophages containing pigment, infected and non-infected red cells (Figure 12c).

There have been very few detailed electron microscopic studies of the spleen in human falciparum malaria. In one case report (Pongponratn et al. 1987), numerous PRBC were seen adhered to macrophages and endothelial cells via their surface knobs. Erythro-phagocytosis by macrophages, endothelial cells and littoral cells were seen. The part of PRBC containing the parasite may be cut off during migration through the splenic cords (Figure 12d), a process known as ‘pitting’ (Schnitzer et al., 1972). Falciparum rosettes were commonly seen in the spleen (Pongponratn et al., 1987; 1989; Francis and Warrell, 1993).

Figure 12: Spleen in falciparum malaria. a) The spleen is enlarge, coloured dark red. b) There is congestion with hyperplasia of red and white pulp (H&E stain, x100). c) Splenic cord and sinuses are filled with monocytes and macrophages containing pigment, parasitized and non-parasitized red blood cells (H&E stain, x 400). d) An electron micrograph of spleen shows two PRBC are being cut off during migration through the splenic cords.
**Placenta:** Pathologically, there is heavy parasitism of red blood cells in the maternal sinuses (Figure 13) accompanied by monocytosis containing haemozoin pigment. Transplacental infection (congenital malaria) is uncommon.

![Image of Placenta with red blood cells and haemozoin pigment](image)

**Figure 13: Placenta, falciparum malaria (H&E stain, x 400).**

Recent studies documented occurrence of endothelial apoptosis in the cytoadherence of *P. falciparum* (Pino et al., 2003a, Pino et al., 2003b, Viriyavejakul et al. (manuscript in preperation). The findings were supported by the real time single cell study using confocal laser scanning microscope and confirmed by caspase-8 and -9 activities.

![Image of fluorescence imaging of apoptotic human umbilical vein endothelial cells (HUVEC) with attached PRBC](image)

**Figure 14 (a & b):** Floreescence images of apoptotic human umbilical vein endothelial cells (HUVEC) with attached PRBC. HUVEC displays cellular blebbing and annexin V staining (green). Background shows adherent HUVEC in monolayer.
REFERENCES


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