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Original contribution

Pulmonary pathology in pediatric cerebral malaria☆,☆☆

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Summary Respiratory signs are common in African children where malaria is highly endemic, and thus, parsing the role of pulmonary pathology in illness is challenging. We examined the lungs of 100 children from an autopsy series in Blantyre, Malawi, many of whom death was attributed to *Plasmodium falciparum* malaria. Our aim was to describe the pathologic manifestations of fatal malaria; to understand the role of parasites, pigment, and macrophages; and to catalog comorbidities. From available patients, which included 55 patients with cerebral malaria and 45 controls, we obtained 4 cores of lung tissue for immunohistochemistry and morphological evaluation. We found that, in patients with cerebral malaria, large numbers of malaria parasites were present in pulmonary alveolar capillaries, together with extensive deposits of malaria pigment (hemozoin). The number of pulmonary macrophages in this vascular bed did not differ between patients with cerebral malaria, noncerebral malaria, and nonmalarial diagnoses. Comorbidities found in some cerebral malaria patients included pneumonia, pulmonary edema, hemorrhage, and systemic activation of coagulation. We conclude that the respiratory distress seen in patients with cerebral malaria does not appear to be anatomic in origin but that increasing malaria pigment is strongly associated with cerebral malaria at autopsy.

1. Introduction

Diseases affecting the respiratory system are a major challenge in many tropical settings where human immunodeficiency virus (HIV), tuberculosis, pneumonia, and malaria are common. In pediatric malaria, metabolic acidosis causing deep or labored breathing is a frequent manifestation [1-6]. More importantly, acidosis is an independent risk factor for mortality among children with severe pediatric *Plasmodium falciparum*

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malaria and has been associated with case fatality rates of 24.2% to 31% [1,2,5,7-9]. Among the many perturbations to the human host during acute infection with malaria, macrophages are systemically activated in response to the large antigen load and play an important role in the phagocytosis of parasitized red blood cells. In other disease states characterized by systemic macrophage stimulation, such as transfusion-related lung injury, macrophages accumulate in the lungs, causing hypoxia and extensive radiographic opacification [10]. Disseminated intravascular coagulation is a recognized feature of severe malaria, and autopsy studies have demonstrated microthrombi in many tissues [11].

As part of an ongoing clinicopathologic study of severe malaria, we evaluated the pathology of the lung in fatal malaria. In particular, we aimed to identify whether local pulmonary pathology, with accumulation of activated macrophages and microthrombi might contribute to the pathogenesis of respiratory distress in children with severe malaria infections. Anecdotally, we had subjectively noticed variable amounts of macrophage and pulmonary pigment present in this data set and attempted to quantitatively define the subjective histologic findings using detailed immunological and morphological tissue counts. An additional aim was to identify pulmonary comorbidities that might be associated with fatal malaria.

2. Materials and methods

2.1. Patients

The clinicopathologic correlation study of severe and fatal malaria in the Queen Elizabeth Central Teaching Hospital, Blantyre, Malawi, commenced in 1996. Clinical management, laboratory investigations, and treatment protocols have been previously described [12]. Blood cultures were sporadically performed at admission but were not available in all patients. In the event of death, a Malawian clinician or nurse met with key family members to consent for an autopsy. Clinical diagnoses were determined before each autopsy, as previously described [12]. If permission was granted, the postmortem was performed as quickly as possible in the mortuary at the Queen Elizabeth Central Hospital. Microbiological cultures were not performed at autopsy. In analysis, we compared histologic findings between patients without antemortem respiratory signs and those with a collection of respiratory signs (indicating some form of respiratory distress) including nasal flaring, intercostal recession, deep breathing, grunting, irregular breathing, or abnormal chest sounds on auscultation. We also specifically noted the prevalence of anatomical abnormalities in all patients and compared patient groups by the final anatomical diagnosis. The research ethics committees at the University of Malawi College of Medicine, Michigan State University, the University of Liverpool, and the Brigham & Women's Hospital have approved all or appropriate portions of this study.

2.2. Autopsy procedures

Gross examination, documentation, and histologic assessment of the brains and other organs were performed, as previously described [12]. Cases were classified after autopsy examination as one of the following final anatomical diagnoses: cerebral malaria (CM) with intracerebral histologic findings limited to parasite sequestration only (CM1); CM with both sequestration and extravascular pathology in the brain (CM2); clinically diagnosed CM with no sequestration in the brain and with another anatomical cause of death (CM3); other patients enrolled in the study without clinical or pathologic evidence of CM (Other)—these included parasitemic and aparasitemic patients. Segments of right lung were inflated via the bronchus with formalin and allowed to fix before slicing to obtain optimal morphological preservation. Segments of the left lung were cut directly and placed in formalin.

2.3. Histology

Sections of hematoxylin and eosin (H&E)–stained brain tissue were examined and quantified for total parasites, as previously described [12]. Standard sections of H&E stained lung tissue were used to make histologic assessments except for immunohistochemistry quantification, which was performed on tissue microarray (see below). As part of the review of cases for final anatomical diagnosis, increased amounts of apparent macrophages and/or pigment globules were noted in many cases, and thus, a subjective scale for the progressive apparent increase in these elements was used to categorize patients by histologic appearance only. Briefly, the low- to medium-power appearance of the lung histology was graded, blinded to final anatomical diagnosis, by R. W. as "normal" (graded 0) or as mild, moderate, severe (grades 1-3) by assessing the relative amounts of intravascular accumulation of macrophages and/or pigment and the absence of other types of pathology (ie, intra-alveolar inflammatory cell accumulation, interstitial fibrosis or inflammatory cell accumulation, and pneumocyte atypia). Sections of representative grades of lung from each case were photographed using polarized light microscopy, as previously described [13].

2.4. Tissue microarray construction

Histologic sections of lung tissue were examined and marked for areas of pure alveolar tissue (ie, avoiding bronchus, lymphoid tissue, large vessels, and tissue of the hilum). Using the corresponding formalin-fixed, paraffin-embedded tissue blocks for 88 sequential autopsies, 4 cores, each 0.6 mm in diameter, were selected from each case from the marked areas of the slide and embedded into a master tissue array block (Supplementary Figure 1). Once constructed, the array included sections of stock normal liver as