Postmortem Characterization of Patients With Clinical Diagnosis of *Plasmodium vivax* Malaria: To What Extent Does This Parasite Kill?

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**Background.** Severe disease attributable to *Plasmodium vivax* infection is already well described worldwide; however, autopsies in these patients are scarce.

**Methods.** From 1996 to 2010, 19 patient deaths with a clinical diagnosis of *P. vivax* infection occurred in a tertiary care center in the Brazilian Amazon. Seventeen of these 19 deaths were fully autopsied. Clinical charts, macroscopic autopsy reports, and stored paraffinized tissue blocks were retrieved. Nested polymerase chain reaction was performed in paraffinized samples of spleen and lung to confirm *P. vivax* monoinfection. Immunohisto-fluorescence was used to detect *P. vivax* parasitized red blood cells (RBCs).

**Results.** Of 17 autopsies, 13 revealed that death could be attributed to *P. vivax* infection; in the remaining 4, acute diseases other than malaria were found to be the cause of death. The primary complication in patients in which malaria contributed to death was acute respiratory distress syndrome (ARDS) and pulmonary edema associated with the accumulation of neutrophils in the interalveolar space (6 cases). Spleen rupture (3 cases) and multiorgan dysfunction syndrome (3 cases) were the second most common complications. One child evolving with coma was also characterized, but no parasite was detected in the brain tissue. In one patient who developed ARDS and presented negative peripheral parasitemia by the time of death, scattered parasitized red blood cells were seen inside pulmonary capillaries, suggesting some sequestration in the lung.

**Conclusions.** In 13 of 17 deceased patients, *P. vivax* infection was the plausible cause of death. However, more studies are needed to understand pathogenesis related to severe disease.

In recent years a paradigm shift has occurred in the widespread belief that *Plasmodium vivax*, the most ubiquitous malaria species, causes only uncomplicated malaria [1]. Using polymerase chain reaction (PCR) to overcome microscopy-related diagnostic uncertainties (excluding *Plasmodium falciparum* coinfection), case series from various malaria endemic areas indisputably show *P. vivax* potential to cause severe disease [2–5]. Although case fatality rates (CFRs) have been consistently described as similar to those of *P. falciparum* [6], the clinical and pathological mechanisms leading to death in *P. vivax*-infected patients have not been properly studied. This deficiency is, in part, due to inadequate exclusion of other possible comorbidities and the lack of full autopsies, a diagnostic gold standard often prevented by cultural and/or operational limitations in endemic regions [7]. Autopsy studies of *P. vivax*-associated deaths are scarce, with only 2
modern case reports [8, 9]. Some of the available data were published more than 50 years ago [10–13]. Here we present a detailed postmortem analysis of 17 fatalities attributed to *P. vivax* infection during a 15-year period at a tertiary referral hospital in the Brazilian Amazon, where *P. vivax* accounts for approximately 85% of malaria cases [14].

**METHODS**

**Ethical Statement**
The study was approved by the ethics committee boards (ERBs) from both the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD), in Manaus, Brazil, and Hospital Clinic, in Barcelona, Spain. A waiver of informed consent was given by the ERBs, justified by the retrospective design of the study. Patient anonymity was preserved throughout the analyses.

**Study Site**
The FMT-HVD is a tertiary care center for infectious diseases in Manaus (Western Brazilian Amazon) and accounts for the diagnosis and treatment of approximately 30% of the cases of malaria in the municipality. Patients can either seek attention directly at the FMT-HVD or be referred for specialized care from the neighboring municipalities. Since the 1990s, there has been a steep decline in the proportion of cases of *P. falciparum* in the Western Brazilian Amazon, with *P. vivax* being responsible for approximately 85% of reported cases in 2010 (unpublished data, Ministry of Health, Brazil, 2011). *Plasmodium vivax* cases are routinely diagnosed by thick blood smear (TBS) and reviewed by experienced microscopists as part of a strict quality control policy. Only patients with a microscopic diagnosis are treated with antimalarials according to the Brazilian Antimalarial Treatment Guidelines [15]. Treatment consists of chloroquine (25 mg/kg in 3 days) and primaquine (0.5 mg/kg/day for 7 days).

At the FMT-HVD, deceased patients who trigger academic or public health interest are systematically considered for full autopsy provided family consent. The pathology department then follows a standard operating procedure, which has not changed considerably over time. All the organs are examined and a macroscopic report is completed before the organs are fixed in 10% buffered formalin. These reports are stored together with the paraffinized tissue blocks in the pathology department. After the appearance of severe vivax disease in the Amazonas State, the Health Surveillance Foundation recommended that a thorough diagnostic investigation be performed in all potential vivax fatalities. A comprehensive investigation includes a full autopsy and adequate exclusion of coinfections (including *P. falciparum*).

**Study Design**
This is a retrospective, descriptive study of the full autopsies performed in *P. vivax*-infected deceased patients at the FMT-HVD from January 1996 through December 2010. Patients with *P. vivax* microscopic diagnosis (TBS stained with Giemsa) on admission or during hospitalization were identified through careful examination of hospital admission and discharge records, as well as the pathology department registry. Once a case was identified, clinical charts, autopsy macroscopic reports, and stored paraffinized blocks were retrieved. The data related to the patient’s clinical manifestations and medical management were taken from the review of medical charts using a structured questionnaire completed by one of the investigators of the study. The macroscopic autopsy reports and paraffin blocks were retrieved from the pathology department archives.

**Laboratory Methods**
Tissue sections measuring 4 μm were created from the paraffinized blocks; stained with hematoxylin and eosin, Masson trichrome, and periodic acid-Schiff; and evaluated by 2 pathologists with experience in malaria. Twenty 10-μm paraffin tissue sections from the spleen and the lung (and from the brain in patient 4) were used for DNA extraction. Nested PCR using 200 ng of DNA was performed as previously described [16] to confirm *P. vivax* infection and exclude *P. falciparum* infection.

Ancillary immunohistochemical (IHC) stains were performed in the liver (anti-CD68 for Kupffer cells, anti-dengue virus antibodies, and anti-hepatitis B virus antibody [HBsAg/anti-HBcAg]), and in the lung (anti-CD15 for neutrophils, anti-CD68 for monocytes/macrophages, anti-CD3 for T lymphocytes, and anti-CD20 for B lymphocytes). In patient 4, IHC was also performed for *Toxoplasma gondii*, herpes simplex virus, cytomegalovirus, and dengue virus in brain sections. IHC was performed on 4-μm sections of formalin-fixed, paraffin-embedded tissue using the automated Autostainer Link 48 immunohistochemical system (Dako, Carpinteria, California) [17]. Sections were incubated with a primary mouse monoclonal or rabbit polyclonal antibodies against human antigens. The IHC technique for the 4 serotypes of dengue virus used a pool of monoclonal antibodies from mouse hyperimmune ascitic fluid.

Immunohistofluorescence (IHF) for variant VIR proteins located at the surface of *P. vivax*-infected reticulocytes was used to detect parasitized red blood cells (RBCs) in lung from cases in which nested PCR was positive in this organ, and no other pulmonary comorbidity was identified. After blocking, sections were incubated at 4°C overnight with anti-Vir antibodies produced against long, synthetic peptides representing conserved Vir motifs [18].
Definitions
After thorough analysis of the clinical information and pathological findings, the cases were classified as follows: (1) *P. vivax* as probable direct cause of death, if *P. vivax* alone justified the clinical and pathological alterations leading to patient’s death, and no other comorbidity could be detected; (2) *P. vivax* contributing to death, if the patient had a concomitant clinical condition (acute or chronic) that along with *P. vivax* infection acted synergistically to cause death; or (3) *P. vivax* probably as an incidental finding, if there were no severe alterations associated with malarial infection and a more likely cause of death could be established. Patients were allocated in each category based on a panel (3 clinicians and 1 pathologist) consensus. Clinical syndromes considered to be severe malaria were identified using the World Health Organization (WHO) criteria originally intended for *P. falciparum* disease [19] because they also seem to discriminate severe vivax disease, except for spleen rupture and hyperparasitemia [20].

RESULTS

From 1996 to 2010, 36,854 cases of *P. falciparum* malaria (12 deaths reported; CFR = 0.032%), and 170,286 *P. vivax* cases (19 deaths reported; CFR = 0.011%) were diagnosed at the FMT-HVD (outpatients and inpatients). All patients were from Manaus or surrounding municipalities. No patient with the diagnosis of *P. falciparum* was autopsied in the period. Seventeen of the 19 deaths in patients with a clinical diagnosis of *P. vivax* were fully autopsied. Table 1 summarizes the clinical and laboratory data available regarding the 17 autopsies performed in the patients aged 1–88 years. Peripheral parasitemia on admission was not shown in the table due to the fact that most of the patients arrived at the institution already in treatment with chloroquine and/or primaquine, and therefore, with altered parasitemias. In patients 2 and 10, PCR was also performed in the peripheral blood because these patients were enrolled in ancillary clinical studies, in which this procedure was standard.

The most common severe clinical syndromes diagnosed prior to death were respiratory distress (15 of 17), jaundice (7 of 17), acute renal failure (6 of 17), severe anemia (5 of 17), and coma (3 of 17). With the exception of patient 3, all patients fulfilled at least 1 of the WHO criteria for severe malaria infection [19]. As seen in Table 1, 4 patients were found to have most likely died from complications of *P. vivax* infection, including spleen rupture and/or pulmonary edema and/or coma. In patient 4, who developed neurological involvement, a computed tomographic scan of the brain showed diffuse cerebral edema, and cerebral spinal fluid (CSF) analysis revealed 21 mononuclear cells/mm$^3$, normal CSF glucose and protein, and no bacteria detected in the Gram stain or in the culture.

IHC against *T. gondii*, herpes simplex virus, cytomegalovirus, and dengue virus in brain sections were all negative. PCR for *Plasmodium* species was also negative. The other 9 patients were classified as having *P. vivax* as an important factor contributing to their deaths (Table 1). In the remaining 4 patients, *P. vivax* seemed to be an incidental finding, because more probable causes of death were found (Table 1).

Patients 11, 12, and 13 did not have *P. vivax* confirmed by PCR in tissue samples, and the diagnosis was based solely on light microscopy. In these patients, all of whom presented to the institution after 4 days of effective antimalarial treatment, there was complete clearance of peripheral parasitemia. In 2 cases the cause of death was directly related to hemolysis triggered by primaquine in the presence of G6PD deficiency (diagnosis based on qualitative evaluation of the enzyme activity).

With exception of patient 10, who received intravenous artesunate, all the other patients were treated with chloroquine/primaquine, following the Brazilian Antimalarial Treatment Guidelines [15] at the time. Of note, by the time of death, all patients presented a negative TBS for at least 2 days. Coinfection with *P. falciparum* was excluded in all cases by nested PCR. Supplementary Table 1 presents findings in the lung, where alveolar edema (with simultaneous interstitial neutrophilic infiltrate) was the most frequent finding. Supplementary Table 2 summarizes pathological findings in the liver microscopy. The most common finding was Kupffer cell hyperplasia with intracellular hemozoin deposits. Dengue fever and hepatitis B coinfections were also ruled out. No uniform pathological finding was seen in either the lung or liver. Figures 1–3 illustrate diverse macroscopic and histological findings of some of the patients. IHF using anti-VIR antibodies was performed in patients 2 and 9, and a few scattered parasitized RBCs were seen inside capillaries of patient 9 (Figure 4).

DISCUSSION

This autopsy case series confirms that *P. vivax* may be a fatal disease in Latin America. In 13 of 17 studied patients, *P. vivax* was considered to be the direct cause of death, or contributed considerably to the decompensation of a preexisting condition, leading to death.

In the Indonesian Papua, high and equivalent CFRs have been observed for both *P. vivax* and *P. falciparum* infection (1.6% and 2.2%, respectively) [6]. These CFRs are significantly higher than those observed in our region, where *P. vivax* CFRs were low (0.011%) but not dissimilar from those reported for *P. falciparum* (0.032%). Such low incidences parallel the evolution of malaria control in Brazil, which has achieved considerable progress in reducing morbidity and mortality through rapid diagnosis and treatment [21]. Public health
improvements, such as bed nets, screens, and education on vector control have contributed to improvements in malaria-associated morbidity and mortality. These public health measures are particularly effective in reducing exposure in the pediatric population, which partially explains the lower CFRs and older age of cases in our region relative to endemic locales [6].

In virtually all the patients, WHO criteria for severe malaria (originally proposed for *P. falciparum*) were fulfilled, which suggests that these criteria could also be useful in the screening of suspected severe vivax infections, added to the peculiar complication of spleen rupture, which is not so rare among deceased vivax patients.

Our observation of respiratory distress as a primary cause of death in severe vivax infections is consistent with a recent case report of a young woman from India who developed similar clinical manifestations of disease. On histopathological analysis of her lung tissue, however, she demonstrated a predominantly mononuclear rather than neutrophilic infiltrate as seen in the present study. Regardless of cellular mechanism, acute lung injury has been described as one of the most frequent complications of *P. vivax* infection [22, 23]. There is reasonable evidence that it may be triggered or exacerbated by the initiation of treatment [24], which could be the case in most of our patients, most of whom presented to our reference institution having started standard therapy.

In the liver, Kupffer cell hyperplasia, malarial pigment within these cells, portal inflammation, and steatosis were frequent observations, which is consistent with existing data [25, 26].

<table>
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<tr>
<th>Patient</th>
<th>Age (y)/Sex</th>
<th>RD</th>
<th>NS</th>
<th>ARF</th>
<th>SA</th>
<th>Leukocytes/ mm³</th>
<th>Jaundice</th>
<th>AST/ALT (U/L)</th>
<th>Comorbidities</th>
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<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
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<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td>9 500</td>
<td></td>
<td></td>
<td>Lung edema &amp; coma</td>
<td>L</td>
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<td></td>
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<td>Yes</td>
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<td>Yes</td>
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<td>7 500</td>
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<td>HIV</td>
<td>Pneumonia</td>
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Abbreviations: ALT, alanine aminotransferase; ARDS, acute respiratory distress syndrome; ARF, acute renal failure; ASH, alcoholic steatohepatitis; AST, aspartate aminotransferase; F, female; G6PD, glucose-6-phosphate dehydrogenase; HIV, human immunodeficiency virus; L, lung; M, male; MODS, multiorgan dysfunction syndrome; NS, neurological symptoms; PB, peripheral blood; PCR, polymerase chain reaction; RD, respiratory distress; S, spleen; SA, severe anemia.
with jaundice; however, they all had alternative causes of necrosis, supporting the fact that jaundice per se may not necessarily be a severity criterion in either vivax or falciparum malaria alone [27]. Severe anemia, which is a manifestation more associated with pediatric disease, was infrequent (5 cases in this series), likely owing to the older age of our patients. Spleen rupture was confirmed in 3 patients, and should be included in the differential diagnosis of shock in malaria endemic areas [28]. Traditionally described as a complication of malaria [29], a recent systematic review of published cases

**Figure 1.** Major macroscopic and microscopic findings in patients in whom *Plasmodium vivax* infection was the probable direct cause of death. A, Ruptured hematoma in the anterior border of the spleen from patient 2, who evolved with hypovolemic shock. B, Kupffer cell hyperplasia in the liver (anti-CD68+ in patient 2. C, Presence of hemozoin (dots of malarial pigment) within Kupffer cells in patient 3. D, Lung microscopy with severe alveolar edema and congestion with interstitial infiltrate (hematoxylin and eosin [H&E] stain, ×400) in patient 2. E, Lung with interstitial neutrophilic infiltrate (anti-CD15+) in patient 2. F, Brain microscopy of patient 4, evidencing edema and perivascular mononuclear infiltrate (H&E, ×400).

**Figure 2.** Major macroscopic and microscopic findings in patients in whom *Plasmodium vivax* infection probably contributed to death. A, Subdural hematoma (hemorrhagic stroke) in patient 13 with chronic systemic arterial hypertension, who presented initially with neurological symptoms and was transferred to the intensive care unit evolving with multiorgan dysfunction syndrome. B, Liver microscopy of patient 5, evidencing portal fibrosis suggestive of cirrhosis (of unknown etiology), who evolved with lung edema and spleen rupture (Masson trichrome stain, ×1000). C, Liver microscopy of patient 8, evidencing severe diffuse necrosis (Masson trichrome stain, ×400). D, Kidney microscopy with tubular hemoglobin casts from the glucose-6-phosphate dehydrogenase (G6PD)–deficient patient 12, who developed severe hemolysis with acute renal failure after the use of primaquine (hematoxylin and eosin stain, ×1000). E, Kidney microscopy evidencing acute tubular necrosis in the same patient 12, with G6PD deficiency and primaquine-triggered hemolysis (periodic acid-Schiff stain, ×1000). F, Lung microscopy of patient 6, evidencing panacinar emphysema, which evolved with acute respiratory distress syndrome (Masson trichrome stain, ×400).
points out that the frequency of spleen rupture may not differ between species [30]. This is difficult to interpret, though, because of likely underdiagnosis, which makes the real incidence of spleen rupture in malaria cases inaccurate. In the 8-year-old patient 4 presenting with impaired consciousness and coma, most causes of encephalitis could be ruled out through IHC. Therefore, it is not possible to exclude cerebral malaria related to \textit{P. vivax} infection as the cause of encephalitis. This theory is supported by recent, well-characterized reports of \textit{P. vivax}-associated cerebral malaria in children from India [31, 32] and Indonesia [33]. The absence of \textit{P. vivax} DNA in post-mortem brain samples from 3 children with mixed infection (\textit{P. vivax}/\textit{P. falciparum}) also corroborates our data, showing that \textit{P. vivax} sequestration in the brain may not be a relevant mechanism in cerebral malaria related to this species [34].

Even without the results of positive blood cultures for aerobes in the clinical charts, the presence of leukocytosis in some patients points to the possibility of sepsis as a coexisting diagnosis contributing to death. Multiorgan dysfunction syndrome, including its associated acute renal failure, and acute respiratory distress syndrome could both be related to sepsis as well. In fact, the coexistence of \textit{falciparum} malaria and invasive bacterial infections is a frequent and life-threatening condition in children from many endemic African settings [35].

In 4 deaths, however, the presence of \textit{P. vivax} infection was probably an incidental finding, and other more plausible causes of death could be found, such as pneumonia (related or unrelated to human immunodeficiency virus), meningitis, or even yellow fever. There was a high prevalence of comorbidities, as can be seen in Table 1. The perception that \textit{vivax} malaria could have a worse prognosis in the presence of comorbidities was first made in the era when malaria chemotherapy was used to treat neurosyphilis, with CFRs as high as 30.3% [36]. This problem is certainly more noticeable in areas of unstable transmission, where older adults suffering from chronic conditions such as hypertension, diabetes, or cirrhosis are more prone to present malarial disease. The fatalities occurring in patients with G6PD deficiency and sickle cell disease, hereditary disorders that are particularly frequent in malaria-endemic areas, highlight that \textit{P. vivax} infection can be especially harmful in these individuals. The prevalence of G6PD deficiency among men in the Amazon is estimated to be approximately 3%, mostly the African genotype of the

![Figure 3](image1.png)

**Figure 3.** Major microscopic findings in patients in whom \textit{Plasmodium vivax} infection was probably an incidental finding. \textit{A}, Lung microscopy from patient 14, evidencing massive intra-alveolar polymorphonuclear infiltration suggesting bacterial lobar pneumonia (hematoxylin and eosin [H&E] stain, ×400). \textit{B}, Lung from the same patient 14, with neutrophilic (anti-CD15+) infiltration. \textit{C}, Lung microscopy from human immunodeficiency virus (HIV)-positive 1-year-old child (patient 17), evidencing massive intra-alveolar polymorphonuclear infiltration suggesting bacterial bronchoalveolar pneumonia (H&E, ×400). \textit{D}, Lung from the same HIV-positive patient 17, with neutrophilic (anti-CD15+) bronchoalveolar infiltration.

![Figure 4](image2.png)

**Figure 4.** Are these \textit{Plasmodium vivax}-parasitized red blood cells (RBCs) cytoadhered to the lung endothelium? Immunohistochemistry from a tissue section of the lung of patient 9 (with negative peripheral parasitemia by the day of death after antimalarial treatment, and positive nested polymerase chain reaction for \textit{P. vivax} in the lung tissue). The figure shows specific staining of \textit{P. vivax}-infected RBCs (green), nuclei (blue), tissue autofluorescence (red), and bright field image (gray). \textit{A}, Negative control without using anti-Vir antibody. \textit{B}, Immunohistochemistry using anti-Vir antibody for specific detection of \textit{P. vivax}-infected RBCs. \textit{C}, Blow-up of \textit{B}, suggesting sequestration of a \textit{P. vivax}-infected RBC in a vessel (arrow).
deficiency [37]. The use of primaquine in G6PD-deficient population entails a risk that should not be neglected, as previously demonstrated in a series of patients suffering from this complication in the Brazilian Amazon [38]. The consequences of inappropriate drug choice underscores the need of accessible, rapid tests for these conditions, as well as the importance of developing alternative treatments to eradicate hypnozoites [39, 40].

From the clinical spectrum of vivax-associated disease presented here, it is probable that this species shares some common pathophysiologic mechanisms with P. falciparum. Moreover, the observation of a few infected RBCs inside lung microvasculature (Figure 4) in an apasitemic patient by the time of death supports the hypothesis that P. vivax could also undergo mild sequestration secondary to cytoadhesion. Sequestration in P. vivax has already been demonstrated ex vivo, albeit perhaps in a lower magnitude as compared with the cytoadherence seen in P. falciparum [41]. In vivo data, coming from an autopsy performed in a patient with cerebral vivax malaria, demonstrating infected RBCs in the retinal and choroidal microvasculature further support this hypothesis [9]. Furthermore, the observation of neutrophils in lung samples in these autopsies coincide with in vitro evidence of leukocyte aggregation during the malarial paroxysm [42]. Due to the small numbers, however, the extent and intensity of these phenomena, as well as the clinical implications, remain a matter of debate.

Areas that report severe vivax disease are the same that demonstrate P. vivax chloroquine resistance, as suggested in the pivotal study from Indonesia, in 2008 [6]. In Brazil, similarly, chloroquine resistance [43] was reported in parallel with increase in vivax severe infections. As a result, vivax severity should be routinely treated with artemisinin derivatives, according to WHO guidelines [44].

In conclusion, P. vivax may be an important contributing factor to deaths in malaria patients with preexisting illnesses but also independently accounts for mortality, as evidenced in our case series. Here multiple mechanisms of severe disease are proposed based on our histopathology results, but the scientific knowledge base for interpreting our observations in a small study is insufficient. What appears clear, however, is a correlation between malaria-associated mortality and the presence of comorbid medical conditions. Thus, studies attributing severity to P. vivax without adequate exclusion of comorbidities [45] may overestimate the independent effect of vivax on mortality. This effect was suggested in studies of P. falciparum [35, 46, 47] and, more recently, P. vivax–attributed cerebral malaria [33]. Therefore, autopsy studies are critical to understanding the discrepancies between pathology and clinical findings in severe vivax malaria [48], enabling the connection between basic science and clinical research.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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