A RETROSPECTIVE EXAMINATION OF ANEMIA DURING INFECTION OF HUMANS WITH PLASMODIUM VIVAX

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Abstract. A retrospective examination was made of archival data on 98 patient episodes of infection with Plasmodium vivax occurring over a period of 4–11 weeks to document changes in hemoglobin (Hb) concentrations associated with continuing parasitemia. The mean percentage change in the Hb concentration for each of the 10 seven-day intervals was −13.4, −10.9, −4.8, 0.12, 0.94, 4.0, 0.69, 11.6, 2.4, and 8.3, respectively. An equilibrium appeared to be established between weeks 4 and 6. Decreases in Hb concentrations were greatest following the first week of parasitemia. Total restoration to preinfection levels did not occur during persistent parasitemia.

INTRODUCTION

The destruction of erythrocytes is a major consequence of infection with Plasmodium spp. Infection with Plasmodium falciparum can produce acute anemia that can be a major cause of morbidity and mortality if parasitemia is allowed to reach a high density and persist for extended periods of time. Jakeman and others1 examined parasitemia and anemia data from patients receiving malarial therapy with P. falciparum for the treatment of neurosyphilis. Based on a mathematical model, an average of 8.5 erythrocytes were destroyed in addition to each erythrocyte observed to become parasitized. They postulated that this was due to the destruction of uninfected erythrocytes through phagocytosis.

As part of our continuing efforts to search archival records on induced infections in patients for the treatment of neurosyphilis between 1940 and 1963, a similar examination was made on the induction of anemia as a result of infection with P. vivax.

Archival data giving daily parasite counts per microliter and weekly determinations of hemoglobin (Hb) concentrations were available. Certain assumptions were necessary to better understand the changes that were recorded.

Plasmodium vivax does not sequester into the deep tissue. It is also well recognized that the parasite has an asexual developmental cycle of approximately 42 hours, depending somewhat on the strain of the parasite.2 This would indicate that four cycles would be completed during a seven-day period, which was the interval between determinations of Hb concentrations. The mean daily parasite count for the seven days × 4 equals the erythrocytes that were infected and subsequently destroyed during each interval between determinations of Hb concentrations. The hemoglobin concentration at the beginning of the seven-day interval divided by the hemoglobin concentration at the end of the seven-day period would equal the percentage reduction (or increase) in the Hb concentration, the available indicator of anemia. The destruction of erythrocytes (and therefore a reduction in the Hb concentration) by parasitic maturation is balanced by the production of new erythrocytes. Additional erythrocyte destruction could be attributable to a number of different activities, including that proposed by Jakeman and others.1

Reported here are the results of a retrospective examination of archival data from 98 induced infections with P. vivax for the treatment of paresis and other mental disorders associated with tertiary syphilis. The goal was to document the extent of changes in Hb concentrations in association with continuing parasitemia and to document, as was observed with infections with P. falciparum, that anemia associated with infection is markedly greater than can be attributed to parasitic destruction of erythrocytes.

MATERIALS AND METHODS

Patient management. Consent for whatever treatments the hospital staff determined necessary was granted by the families of the patients or the courts when patients were admitted to the hospital. The decision to infect a neurosyphilitic patient with a specific species or strain causing malaria was made as part of standard patient care provided by the medical staff of the South Carolina State Hospital. Patient care and evaluation of clinical endpoints (e.g., fever) were the responsibility of the medical staff. As previously reported,3 during infection, the temperature, pulse, and respiration were checked every four hours and hourly during paroxysms (fevers) by hospital personnel. During paroxysms, patients were treated symptomatically. Infections were terminated at the direction of the attending physician. Personnel of the U.S. Public Health Service provided the parasites for inoculation, monitored the daily parasite counts to determine the course of infection, and determined Hb concentrations and white blood counts on a weekly basis. All patients undergoing malarial therapy lived in screened wards of the hospital to prevent possible infection of local anophelines.

Treatment. Patients were frequently allowed to maintain parasitemia for relatively short periods of time, mainly from 2 to 11 weeks. Infections were then terminated by treatment with standard antimalarial drugs. Once treated, the data from these patients were excluded from the analysis.

Strains of P. vivax. Of the 98 patient episodes for whom weekly hemoglobin concentrations were recorded, 85 were infected with the St. Elizabeth strain of P. vivax, 11 with the Chesson strain, and 2 with the Korean strain.

Parasitemia. Patients were infected by the intravenous inoculation of parasitized erythrocytes or via sporozoite inoculation. Thick and thin peripheral blood films were made daily by the method of Earle and Perez,4 stained with Giemsa, and examined microscopically for the presence of parasites. The threshold of detection was approximately 10 parasites/µL. Asexual and sexual parasites were recorded per microliter of blood. Infections often persisted for many weeks. The number of patients decreased weekly as their infections were terminated; only 14 of the patients had continuing parasitemia.
counts/patient S-1126 infected with Plasmodium vivax. The daily parasite related to the intervening accumulated daily parasite counts calculated upto the time of the first determination of the Hb concentration before treatment. The significance level was set at alpha = 0.05. All statistics were calculated using SAS version 6.0 (SAS Institute, Inc., Cary, NC).

Data presentation. To monitor the well being of the patients, Hb concentrations were measured weekly, beginning some time during the first seven days of patent parasitemia. This was continued to the week the patient received antimalarial drug treatment. Accumulated parasite counts were calculated up to the time of the first determination of the Hb concentration (week 1); subsequent Hb concentrations were related to the intervening accumulated daily parasite counts through 11 weeks of patent parasitemia. The daily parasite count × 4 = erythrocytes destroyed as attributable to parasite maturation.

Statistical analysis. Piecewise linear regression was used to estimate the decrease in Hb concentrations from week 1 to 4 and the subsequent rate of increase from week 5 to 11 controlling for parasitemia. A paired t-test was used to compare mean differences in the initial Hb concentration with the last mean difference in the initial Hb concentration with the last value determined at seven-day intervals for patient S-1126 infected with Plasmodium vivax. Solid line = parasite counts/µL; dash line = hemoglobin values.

for 11 weeks. The analysis used weekly Hb concentrations and daily parasite counts for those patients still infected through each of 10-weekly intervals.

### TABLE 1

<table>
<thead>
<tr>
<th>Weeks</th>
<th>No. of patients</th>
<th>Change from</th>
<th>Change from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Initial (%)</td>
</tr>
<tr>
<td>Week 1</td>
<td>98</td>
<td>886</td>
<td>12.51</td>
</tr>
<tr>
<td>Week 1–2</td>
<td>98</td>
<td>33,751</td>
<td>10.80</td>
</tr>
<tr>
<td>Week 2–3</td>
<td>97</td>
<td>21,104</td>
<td>9.61</td>
</tr>
<tr>
<td>Week 3–4</td>
<td>91</td>
<td>10,384</td>
<td>9.03</td>
</tr>
<tr>
<td>Week 4–5</td>
<td>85</td>
<td>7,827</td>
<td>8.98</td>
</tr>
<tr>
<td>Week 5–6</td>
<td>61</td>
<td>5,975</td>
<td>9.26</td>
</tr>
<tr>
<td>Week 6–7</td>
<td>41</td>
<td>5,408</td>
<td>9.55</td>
</tr>
<tr>
<td>Week 7–8</td>
<td>28</td>
<td>4,241</td>
<td>9.68</td>
</tr>
<tr>
<td>Week 8–9</td>
<td>23</td>
<td>3,613</td>
<td>10.52</td>
</tr>
<tr>
<td>Week 9–10</td>
<td>17</td>
<td>2,235</td>
<td>10.50</td>
</tr>
<tr>
<td>Week 10–11</td>
<td>14</td>
<td>2,160</td>
<td>11.00</td>
</tr>
</tbody>
</table>

*Based on a 42-hour developmental cycle for P. vivax, there would be 4 generations of parasites each week; mean accumulated parasite count divided by 7 (days) × 4 (generations) = erythrocytes/µL infected by parasites during a 7-day period.

### RESULTS

Patient S-1126 (Figure 1) was infected with the St. Elizabeth strain of P. vivax. A Hb concentration of 14.0 g/dL was determined on day 3 of patent parasitemia. The accumulated parasite count up to and including day 3 was 89/µL. This was arbitrarily designated as week 1. Following the next seven-day interval (week 1–2), the Hb value was 13 g/dL (a decrease of 1.0 g); the accumulated parasite count for the interval was 26,612/µL (divided by 7 then multiplied by 4 = 15,209 erythrocytes destroyed/µL of blood during this period). This pattern of data collection was continued until the infection was terminated. Each patient infection from initiation to treatment was considered an episode.

The Hb values and the geometric mean number of erythrocytes infected with parasites per microliter during the seven-day interval were then tabulated for each of 98 patient episodes (Table 1 and Figure 2).

All but two episodes had Hb values ≥10 g/dL during the first week of infection. Changes in Hb values were often marked. Between week 1 and week 2, nine patient episodes showed no change in Hb values and four increased. The other 85 patient episodes had decreases in Hb values during this period. Throughout the 11-week period of parasitemia, there were additional reductions in Hb concentrations, coinciding with more frequent instances of increases in value, indicating an apparent replenishment of erythrocytes (Figure 3). During the interval between weeks 10 and 11, only two of 14 patients (14%) had decreases in Hb concentrations.

In the absence of erythrocyte counts per microliter, it is not possible to accurately determine the percentage of erythrocytes destroyed or the percentage of cells replaced. However, during weekly interval 1–2, 19,286 erythrocytes/µL were infected and subsequently destroyed. Assuming an erythrocyte concentration of 5,000,000/µL, this would indicate that only 0.39% of the 13.37% reduction in hemoglobin concentration was due to parasitic erythrocyte destruction. The difference between the Hb value at the end of the seven-day interval and the initial Hb value was used to determine the percentage reduction or increase in the Hb concentration. The mean percentage change in Hb concentration for each of the 10 seven-day intervals beginning with week 2 was −13.4, −10.9, −4.8, 0.12, 0.94, 4.0, 0.69, 11.6, 2.4, and 8.3, respectively. The Hb
concentration decreased an estimated 0.78 g/dL (95% confidence limit [CL] = −0.86, −0.70) through week 4, then increased an average of 0.34 g/dL (95% CL = 0.13, 0.56) from week 5 to 11. An equilibrium appeared to be established between weeks 4 and 5. Restoration to preinfection Hb levels did not occur at any time during the 11 weeks of persistent parasitemia; rather, the last Hb value before treatment was significantly lower than the initial value (P < 0.0001).

DISCUSSION

Early in these infections with *P. vivax*, the decreases in Hb concentrations were far greater than can be accounted for by parasitic activity; later in the infection, Hb values increased somewhat in the continued presence of parasitemia (Table 1). The destruction of erythrocytes (as shown by the reduced concentrations of Hb) was most marked during the first three weeks, and the percentage change from week to week increased at a significant average rate of 0.34 g/dL. It is therefore difficult to explain why the Hb level did not return to near preinfection levels. Certainly, the continued destruction of erythrocytes by parasitic activity had stabilized, and there was evidence that the percentage change in the value had also stabilized.

Why did the Hb concentrations remain on average greater than 20% below preinfection values? *Plasmodium vivax* primarily invades reticulocytes. It is postulated that the continued parasitemia may have been sufficient to infect and destroy most of the new reticulocytes, thus preventing restoration of Hb levels to preinfection levels. Such an explanation may account for the marked reduction in Hb concentration and the occurrence of anemia in infections with *P. vivax*. It is also possible that the reticulocytes were destroyed by the parasites as they developed, and thereby prevented the restoration of Hb levels to preinfection levels. Chronic infections with this parasite could have a debilitating effect on the patient.

Generally, one would expect the immune response of the patient to control parasite density and persistence. However, recrudescence, reinfection, and relapse of parasite populations could result in continued parasitemia and anemia in patients infected with *P. vivax*. Studies on the dynamics of infections with *P. vivax* and related species of *Plasmodium*, such as *P. cynomolgi* in nonhuman primates, may provide a better understanding of the role of chronic and persistent infection on anemia caused by this human-infecting parasite.

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REFERENCES