Diagnostic Criteria and Risk Factors for \textit{Plasmodium ovale} Malaria

Farba B. K. Faye, Andr\'e Spiegel, Adama Tall, Cheikh Sokhna, Didier Fontenille, Christophe Rogier, and Jean-Fran\c{c}ois Trape

\textit{Plasmodium ovale} is a common malaria parasite in Africa, but the epidemiology of \textit{P. ovale} malaria is poorly known. Exposure to malaria, parasitemia, and morbidity were monitored for 6 years among the residents of a village in Senegal. The relationship between the level of \textit{P. ovale} parasitemia and fever risk were analyzed, and diagnostic criteria for clinical \textit{P. ovale} malaria were established. Then the relationships between the occurrence of \textit{P. ovale} clinical malaria and a series of entomological, epidemiological, and genetic factors were investigated. There was no increased risk of fever when the \textit{P. ovale} parasite count was \textless 800 parasites/\(\mu\)L of blood. Of 6,621 episodes of illness, 114 (1.7\%) were attributable to \textit{P. ovale}. Although most clinical episodes occurred during early childhood, a low incidence of the disease persisted among adults. Sickle cell trait carriers had increased susceptibility to the disease.

\textit{Plasmodium ovale} is the least studied of the 4 malaria parasites that infect humans. Many years after its 1922 discovery by Stephens, in the blood of a soldier returning from East Africa [1], it was still frequently confounded with \textit{P. vivax}; it was only in the 1960s that clear pictures of its geographic distribution and prevalence emerged [2–9]. Although \textit{P. ovale} has been reported from all continents, it is only in tropical Africa and New Guinea that it is relatively common, with prevalences frequently reaching 2\%–10\% among children. The patterns of the disease have been described mainly in travelers returning from Africa or in volunteer subjects inoculated with the parasite [3, 6, 10, 11]. \textit{P. ovale} causes a relatively mild form of malaria that is very rarely fatal [12]. Paradoxically, this parasite is rarely reported as a cause of morbidity in tropical Africa. It is not clear whether the rarity of clinical attacks in endemic populations is due to underdiagnosing, rapid acquisition of species-specific immunity, or partial cross-immunity with the much more prevalent \textit{P. falciparum}.

Here, we present an analysis of clinical, parasitological, and entomological data collected for 6 years among the population of a Senegalese village. The objectives of the present study were (1) to identify parasitological criteria for distinguishing \textit{P. ovale} clinical malaria attacks from other causes of fever in populations frequently infected by several malaria species, (2) to assess the incidence of \textit{P. ovale} clinical malaria attacks at the community level in a rural area of tropical Africa, and (3) to investigate the relationship between \textit{P. ovale} morbidity and a series of epidemiological and biological factors.

\textbf{Subjects and Methods}

\textit{Clinical, parasitological, and entomological monitoring}. The study was carried out from 1 June 1990 through 31 May 1996 in Dielmo, Senegal, a village of 250–300 inhabitants in which malaria is highly endemic, with intense perennial transmission [13–15]. The entire population of this village was involved in a prospective study of malaria [13, 16]. To identify all episodes of illness, a field research station with a dispensary was built. The detection of cases was both active and passive. Each villager who volunteered for the study was visited daily at home. The dispensary was open 24 h per day, 7 days per week. Thick blood films were prepared in all cases of fever or related symptoms. In addition, cross-sectional surveys of malaria parasitemia were conducted monthly. During a 4-month period at the beginning of the study, supplementary thick blood films were prepared 2 times a week, and a questionnaire listing the symptoms that had occurred during the previous 48 h was filled out 3 times a week. All thick blood film examinations were standardized, and the ratio of parasites to leukocytes was established separately for each plasmodial species [13]. Parasitemia was calculated with 8000 leukocytes/\(\mu\)L of blood considered to be the average level of leukocytemia. The smears were examined morphologically by a noted expert in malaria microscopy (M. H. Bouganali, Institut de Recherche pour le Développement, Dakar, Senegal), whose diagnostic expertise has been validated by comparison with the results of polymerase chain reaction and by independent

---

Received 17 December 2001; revised 2 May 2002; electronically published 1 August 2002.

The project protocol and the objectives were carefully explained to the assembled village population, and informed consent was obtained individually from all subjects or their parents or guardians. The protocol was approved by the ethics committee of the Pasteur Institute of Dakar and by the Minist\`ere du Plan et de la Coope\'ration and the Minist\`ere de la Recherche Scientifique de Senegal. Each year, the project was reexamined by the Conseil de Perfectionnement of the Pasteur Institute of Dakar and the assembled population of the village; informed consent was individually renewed for all subjects.

Financial support: Minist\`ere de la Coope\'ration (France).

Reprints: Dr. Farba Faye, UR Paludologie-Afrotropicale, Institut de Recherche pour le Développement, B.P. 1386, Dakar, Sénégal (fayef@ird.sn). Correspondence: Dr. Jean-François Trape (trape@ird.sn).

The \textit{Journal of Infectious Diseases} 2002; 186:690–5

\textcopyright\ 2002 by the Infectious Diseases Society of America. All rights reserved.

0022-1899/2002/18605-0014$15.00
observers. Most smears containing P. ovale were also examined and confirmed by an independent observer (J.-F. T.). At baseline, blood samples were obtained from all villagers for hemoglobin electrophoresis, blood-group determination, and tests for glucose-6-phosphate dehydrogenase (G6PD) deficiency. Malaria transmission was monitored during the study period. The methods used for mosquito collection, anopheline identification, and assessment of the sporozoite rate or circumsporozoite protein rate for P. falciparum, P. ovale, and P. malariae are described in detail elsewhere [14, 15].

Establishment of criteria for the diagnosis of P. ovale malaria attacks. In persons living in areas in which malaria is endemic, most P. ovale infections are asymptomatic. To distinguish the episodes of fever caused by P. ovale from those caused by other diseases when this parasite is present by chance, we first investigated the relationship between P. ovale parasitemia and fever using a case-control method. “Case observations” were defined as the biweekly simultaneous measurements of parasitemia and temperature made during the initial 4-month period at the beginning of the study [13]. Owing to the erratic nature of hyperthermia, individual observations were considered to be asymptomatic controls if the temperature was <38°C and if no fever-related symptoms were recorded both 72 h before and 72 h after the collection of thick blood films. We eliminated all case observations that occurred after malaria treatment began.

Analysis of the occurrence of P. ovale malaria attacks. To take into account the interdependence of successive observations in the same individuals, we used a random-effects logistic regression model [17], which is available in the software package EGRET (Statistics and Epidemiology Research). Each month of the survey showed that the risk of fever increased considerably when the level of P. ovale parasitemia was high. There was no increased risk of fever when the P. ovale parasite count was <80 or 80–799 parasites/μL of blood. By contrast, the risk of fever increased 11-fold (for mixed infections) or 93-fold (for P. ovale only) when the P. ovale parasite count was 800–8000 parasites/μL of blood, with <25 days of follow-up was excluded from the analysis. Data collected <2 weeks after malaria treatment were excluded. Two malaria attacks were considered to be distinct if they were separated by >2 weeks.

Because the rate of acquired immunity against P. ovale was presumed to be higher among Dielmo villagers living permanently in the village than among those who had spent a significant part of their life in areas where transmission is generally lower, Dielmo villagers were considered to be either “permanent” or “temporary” residents, on the basis of whether or not they met the following criteria at the beginning of each month of the study period: (1) ≥50% of life since birth spent in Dielmo or an area of high malaria endemicity, (2) ≥2 of the previous 3 years spent in Dielmo or an area of high malaria endemicity, and (3) ≥5 of the previous 6 months spent in Dielmo.

The effect of age, sex, ABO blood group, sickle cell trait, G6PD deficiency, residence status, and entomological inoculation rate (EIR) were tested and taken into account in multivariate analysis. All these variables were included in the initial model, but they remained in the final model only if their effect was significant (P < .05). All possible interactions among the variables remaining in the model were tested. For this analysis, only the first or single P. ovale attack was taken into account, and all subsequent observations were excluded.

Results

Criteria for diagnosing P. ovale malaria attacks. Table 1 shows that the risk of fever increased considerably when the level of P. ovale parasitemia was high. There was no increased risk of fever when the P. ovale parasite count was <80 or 80–799 parasites/μL of blood. By contrast, the risk of fever increased 11-fold (for mixed infections) or 93-fold (for P. ovale only) when the P. ovale parasite count was 800–8000 parasites/μL of blood,

<p>| Table 1. Relationships between Plasmodium ovale parasitemia and risk of fever in the presence or absence of P. falciparum and/or P. malariae. |
|----------------------------------|------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Type of infection, level of P. ovale parasitemia*</th>
<th>Observations, no. of cases</th>
<th></th>
<th>Odds ratio (95% confidence interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. ovale only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1762</td>
<td>682</td>
<td>2444</td>
<td>1</td>
</tr>
<tr>
<td>&lt;80</td>
<td>31</td>
<td>11</td>
<td>42</td>
<td>0.92 (0.43–1.9)</td>
</tr>
<tr>
<td>80–799</td>
<td>6</td>
<td>5</td>
<td>11</td>
<td>2.2 (0.57–7.9)</td>
</tr>
<tr>
<td>800–7999</td>
<td>1</td>
<td>36</td>
<td>37</td>
<td>93.0 (13.7–1829)</td>
</tr>
<tr>
<td>8000–15,999</td>
<td>0</td>
<td>12</td>
<td>12</td>
<td>∞</td>
</tr>
<tr>
<td>≥16000</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>∞</td>
</tr>
<tr>
<td>Total</td>
<td>1800</td>
<td>751</td>
<td>2551</td>
<td></td>
</tr>
<tr>
<td>Mixed infections</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2862</td>
<td>3624</td>
<td>6486</td>
<td>1</td>
</tr>
<tr>
<td>&lt;80</td>
<td>229</td>
<td>103</td>
<td>332</td>
<td>0.36 (0.28–0.45)</td>
</tr>
<tr>
<td>80–799</td>
<td>39</td>
<td>25</td>
<td>64</td>
<td>0.51 (0.30–0.86)</td>
</tr>
<tr>
<td>800–7999</td>
<td>2</td>
<td>29</td>
<td>31</td>
<td>11.5 (2.7–69.4)</td>
</tr>
<tr>
<td>8000–15,999</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>∞</td>
</tr>
<tr>
<td>≥16000</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>∞</td>
</tr>
<tr>
<td>Total</td>
<td>3132</td>
<td>3798</td>
<td>6930</td>
<td></td>
</tr>
</tbody>
</table>

* Parasites per microliter of blood.

<sup>a</sup> Maximum odds ratio.

<sup>b</sup> Fisher’s exact test. P values shown in boldface are highly associated with an increased risk of fever.
and all subjects with \textit{P. ovale} parasite counts $\geq 8000$ parasites/\(\mu\)L of blood presented with fever.

We further investigated all biological and clinical data available for the 99 fever episodes in which the subject had a \textit{P. ovale} parasite count of $\geq 800$ parasites/\(\mu\)L of blood. For 95 episodes, there was no evidence that a disease other than \textit{P. ovale} malaria could be responsible for the symptoms. For 4 episodes, the level of \textit{P. falciparum} parasitemia was above the age-dependent pyrogenic threshold of this species [18], and the dynamics of the 2 infections suggested that \textit{P. falciparum} was the cause of fever. For further analysis, we considered as \textit{P. ovale} malaria attacks all episodes of fever or fever-related symptoms (headache, vomiting, or subjective sensation of fever) associated with a \textit{P. ovale} parasite count of $\geq 800$ parasites/\(\mu\)L of blood, except when \textit{P. falciparum} parasitemia was higher than the pyrogenic threshold defining \textit{P. falciparum} clinical malaria in this population.

\textbf{Incidence of \textit{P. ovale} malaria attacks.} Over a 6-year period, there were 6621 episodes of fever or fever-related symptoms during 502,217 person-days of clinical monitoring of the study population. Of 11,246 thick blood films made during these episodes, 490 were positive for \textit{P. ovale} parasites. There were 334 episodes of fever or fever-related symptoms for which $\geq 1$ thick blood film was found to be positive for \textit{P. ovale} alone or in association with other malaria species. On the basis of the level of parasitemia, 114 of these episodes were considered to be clinical malaria attributable to \textit{P. ovale}, during which fever was documented in 97 cases and fever-related symptoms only were documented in 17 cases. The youngest person who presented with \textit{P. ovale} clinical malaria was a 3-month-old infant, and the oldest was a 67-year-old man. Figure 1 shows that most attacks (57%) occurred in children 0–7 years old and that 11% of cases occurred in adults $\geq 30$ years old. The highest parasitemia level recorded was 36,000 parasites/\(\mu\)L of blood in a 3-year-old child.

\textit{P. ovale} attacks were observed in 86 villagers (1 episode each for 61 villagers, 2 episodes each for 22 villagers, and 3 episodes each for 3 villagers). The time interval between 2 episodes (relapse or reinfection) ranged from 28 days to 26 months (median, 5 months). Table 2 shows the incidence density of \textit{P. ovale} clinical episodes according to age. Among permanent residents of the village, it increased rapidly with age, reaching a maximum of 24.7 attacks/100 person-years among children 3–4 years old, then decreasing to low values in older children and a min-

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Residence status, age (years) & No. of person-months & No. of attacks & Days of follow-up & Incidence of attacks \tabularnewline & & & & \tabularnewline \hline
Permanent & & & & \tabularnewline <1 & 722 & 6 & 21779 & 10.1 \tabularnewline 1–2 & 1134 & 14 & 34342 & 14.9 \tabularnewline 3–4 & 1022 & 21 & 31023 & 24.7 \tabularnewline 5–6 & 901 & 10 & 27368 & 13.3 \tabularnewline 7–14 & 2690 & 9 & 81758 & 4.0 \tabularnewline $\geq$15 & 5656 & 11 & 170991 & 2.3 \tabularnewline Total & 12125 & 71 & 367261 & 7.1 \tabularnewline \hline
Temporary & & & & \tabularnewline <1 & 7 & 0 & 212 & 0.0 \tabularnewline 1–2 & 166 & 4 & 5008 & 0.0 \tabularnewline 3–4 & 209 & 4 & 6306 & 23.2 \tabularnewline 5–6 & 217 & 5 & 6568 & 27.8 \tabularnewline 7–14 & 871 & 14 & 26368 & 19.4 \tabularnewline $\geq$15 & 3008 & 20 & 90494 & 8.1 \tabularnewline Total & 4478 & 43 & 134956 & 11.6 \tabularnewline \hline
\end{tabular}
\caption{Incidence density per 100 person-years.}
\end{table}

\textit{P. ovale} malaria clinical episodes, by residence status and age.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Residence status, age (years) & No. of person-months & No. of attacks & Days of follow-up & Incidence of attacks \tabularnewline & & & & \tabularnewline \hline
Permanent & & & & \tabularnewline <1 & 722 & 6 & 21779 & 10.1 \tabularnewline 1–2 & 1134 & 14 & 34342 & 14.9 \tabularnewline 3–4 & 1022 & 21 & 31023 & 24.7 \tabularnewline 5–6 & 901 & 10 & 27368 & 13.3 \tabularnewline 7–14 & 2690 & 9 & 81758 & 4.0 \tabularnewline $\geq$15 & 5656 & 11 & 170991 & 2.3 \tabularnewline Total & 12125 & 71 & 367261 & 7.1 \tabularnewline \hline
Temporary & & & & \tabularnewline <1 & 7 & 0 & 212 & 0.0 \tabularnewline 1–2 & 166 & 4 & 5008 & 0.0 \tabularnewline 3–4 & 209 & 4 & 6306 & 23.2 \tabularnewline 5–6 & 217 & 5 & 6568 & 27.8 \tabularnewline 7–14 & 871 & 14 & 26368 & 19.4 \tabularnewline $\geq$15 & 3008 & 20 & 90494 & 8.1 \tabularnewline Total & 4478 & 43 & 134956 & 11.6 \tabularnewline \hline
\end{tabular}
\caption{Incidence density per 100 person-years.}
\end{table}
imum of 2.3 attacks/100 person-years among adults. The highest incidence of *P. ovale* clinical episodes was observed among temporary residents, with a maximum of 27.8 attacks/100 person-years among children 5–6 years old, and high values persisted in older children and adults. *P. ovale* attacks were significantly more frequent in sickle cell trait carriers (who have hemoglobin type AS) than in individuals with hemoglobin type AA. Differences according to sex were not significant, nor were those according to G6PD status.

Figure 2 shows the monthly distribution of *P. ovale* attacks over a 6-year period. There were marked yearly variations. Multivariate analysis indicated that the presence of the sickle cell trait increased the risk of *P. ovale* attacks by 2-fold. Other significant factors were age, residence status, year of study, and crude EIR (table 3).

**Discussion**

Only episodes of high *P. ovale* parasitemia are associated with an increased risk of fever in this community that is continuously exposed to intense malaria transmission. The existence of a threshold effect in the relationship between the level of parasitemia and the occurrence of fever has never been investigated for *P. ovale* before this study but was previously documented for *P. falciparum* in the same community [18] and in other populations residing in areas in which malaria is highly endemic [19]. As a result of this threshold effect, the parasite-density measurements make it possible to distinguish *P. ovale* clinical attacks from other causes of fever.

*P. ovale* was a common cause of morbidity in the community. Although the highest incidence was observed among children 0–7 years old, clinical attacks were observed in all age groups. Among permanent residents of the village, they were ~8-fold more frequent in young children than in adults. However, even in children, most *P. ovale* infections were asymptomatic. We have previously reported the high incidence and recovery rates of *P. ovale* patent infections in the study population, with each child presenting with a new patent infection every 44–116 days and those infections lasting an average of 7–11 days [20]. By contrast, the maximum number of *P. ovale* attacks per individual during the 6 years of the study was only 3 attacks, and

![Figure 2. Monthly distribution of *Plasmodium ovale* malaria attacks](image)

**Table 3.** Final model of risk estimation of exposition factors.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Relative risk</th>
<th>95% Confidence interval</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>2.87</td>
<td>1.07–7.66</td>
<td>.035</td>
</tr>
<tr>
<td>1–2</td>
<td>4.16</td>
<td>2.11–8.17</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>3–4</td>
<td>5.61</td>
<td>2.95–10.65</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>5–6</td>
<td>4.69</td>
<td>2.24–9.82</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>7–14</td>
<td>1.69</td>
<td>0.90–3.14</td>
<td>.100</td>
</tr>
<tr>
<td>≥15</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin type, AS vs. others</td>
<td>1.88</td>
<td>1.01–3.52</td>
<td>.047</td>
</tr>
<tr>
<td>Residence, permanent vs. temporary</td>
<td>0.56</td>
<td>0.35–0.88</td>
<td>.012</td>
</tr>
<tr>
<td>Season, rainy vs. dry</td>
<td>0.54</td>
<td>0.32–0.89</td>
<td>.015</td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990–1991</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1992–1993</td>
<td>1.49</td>
<td>0.55–4.08</td>
<td>.433</td>
</tr>
<tr>
<td>1993–1994</td>
<td>1.50</td>
<td>0.52–4.35</td>
<td>.456</td>
</tr>
<tr>
<td>1994–1995</td>
<td>5.35</td>
<td>2.20–13.00</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>1995–1996</td>
<td>2.13</td>
<td>0.83–5.96</td>
<td>.113</td>
</tr>
</tbody>
</table>

**EIR, Plasmodium species**

2.59 1.41–4.77  .002

---

*a* Wald’s test. *P* values shown in boldface refer to categorical variables.

*b* The effect of transmission was taken into account through the decimal logarithm of the rate of inoculation of *Plasmodium* species transmission from mosquitoes to humans (i.e., the entomological inoculation rate [EIR]). This logarithmic transformation significantly improved the adjustment of data by the regression models.
a majority of individuals suffered no attack or only 1 attack. Temporary residents were at greater risk of developing \textit{P. ovale} clinical malaria than were permanent residents, and differences between children and adults were less marked among temporary residents than among permanent residents. These observations are consistent with a rapid acquisition of clinical immunity under conditions of high exposure to \textit{P. ovale} (and/or \textit{P. ovale} plus \textit{P. falciparum}), because most temporary residents traveled to urban areas where malaria transmission was much lower than in the village. However, 3 \textit{P. ovale} attacks were documented in adults >50 years old living permanently in the village who were presumed to be fully protected against such attacks.

The incidence of \textit{P. ovale} attacks was related to the crude EIR (any \textit{Plasmodium} species), but its relationship with \textit{P. ovale}–specific EIR was not significant. Because the proportion of \textit{P. ovale}–infected mosquitoes was very low, a huge number of mosquito collections and tests each month would have been needed for a detailed investigation of the relationship between the fluctuations in malaria transmission and the incidence of \textit{P. ovale} attacks.

Multivariate analysis indicated that sickle cell trait carriers were at significantly greater risk of \textit{P. ovale} clinical attacks than were individuals with AA hemoglobin. This is a striking observation, because hemoglobin type AS is known to confer relative protection against \textit{P. falciparum} malaria [21–23]. Furthermore, we have recently shown in the same study population that \textit{P. falciparum} attacks were ~2-fold more frequent among AA individuals than among sickle cell trait carriers [24]. \textit{P. falciparum} is much more prevalent than \textit{P. ovale} in Dielmo children (80%–92% vs. 8%–14%, according to age) [13], and most children suffer several \textit{P. falciparum} clinical attacks every year [24]. The increased susceptibility to \textit{P. ovale} of sickle cell trait carriers could be related to their relative protection against \textit{P. falciparum} through interactions between these 2 species. It has long been known that infection with one malaria species can affect susceptibility to the others, although these interactions have never been well understood [25]. Immunosuppression or cross-stimulation might influence the risk that a given malaria infection will become symptomatic and, hence, might affect morbidity patterns. There is evidence in the literature that \textit{P. ovale} may be suppressed by \textit{P. falciparum} [6], and it has been suggested that \textit{P. malariae} suppresses \textit{P. falciparum} clinical attacks [26]. Our data suggest that the relative risk of \textit{P. ovale} attack is higher in the absence of coinfection with other malaria species. Furthermore, the analysis of the dynamics of the first \textit{P. ovale} infections among the children of this village, who were monitored since birth, indicated that \textit{P. ovale} parasitemia remained at low level in case of coinfection with \textit{P. falciparum} but always reached high levels and was symptomatic when infection was with this species alone (authors’ unpublished data).

Among travelers returning from Africa to Europe or North America, \textit{P. ovale} is frequently diagnosed, and this species may represent up to 15% of malaria attacks [27]. By contrast, \textit{P. ovale} is almost never mentioned in the registers of outpatient clinics and health statistics in African countries. Most cases of illness are treated without microscopic examination of blood. Furthermore, \textit{P. ovale} may be easily confounded with other malaria species, because parasitemia is lower than that in \textit{P. falciparum} infection, and identification of the most common forms of the parasite necessitates special training. Our data suggest that underdiagnosing is the main cause of the rarity of reports of \textit{P. ovale} clinical malaria in tropical Africa.

Acknowledgments

We are grateful to the villagers of Dielmo for their active participation and continuing collaboration in the project. We thank all field doctors, nurses, technicians, and field workers who were involved in health care, data collection, and laboratory tests, with special mention of M. H. Bouganali, who did thick blood film examinations.

References