ORIGINAL ARTICLE

Predictive Value of Thrombocytopaenia in the Diagnosis of Dengue Infection in Outpatient Settings

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SUMMARY
Thrombocytopaenia is often relied upon as an important criterion for the diagnosis of dengue infection among patients presenting with an acute non-specific febrile illness. This study was aimed to assess usefulness of thrombocytopaenia in the diagnosis of acute dengue virus infection. This was a clinic based prospective cohort study from May to November 2003. Consecutive patients presenting with acute non-specific febrile illness of less than two weeks were selected from two urban primary care centres. We did full blood count examination (FBC) on the day of visit and dengue serology on day five of illness for all patients enrolled. We repeated the FBC examination for patients who had initial normal platelet counts. Thrombocytopaenia was defined as platelet count <150X10^9/L Eighty-seven patients enrolled in the study. Complete data was available for 73 patients. The prevalence of acute dengue virus infection was 27.6%. The sensitivity and specificity were 88% and 71% respectively. The likelihood of acute dengue infection in the presence of thrombocytopaenia was 2.52 and likelihood of not having dengue infection in normal platelet count patients was 5.22. Thrombocytopaenia has fair predictive value in diagnosing acute dengue virus infection. It was more useful to exclude than to diagnose dengue infection.

KEY WORDS:
Dengue infection, Thrombocytopaenia, Likelihood ratio, Sensitivity, Specificity

INTRODUCTION
Dengue infection is the commonest arboviral disease in the world. It carries a significant mortality if the diagnosis and treatment are delayed. Full blood count assessment is often done for early detection if there is clinical suspicion of acute dengue infection in an outpatient setting. Many clinicians rely on the presence of thrombocytopaenia to diagnose acute dengue virus infection. This is because office dengue serology test kit is not widely available in all primary care clinics. Presence of thrombocytopaenia is also the main reason for hospital admission. However, dengue infection is only one of the many causes of acute febrile thrombocytopaenia. Other causes of febrile thrombocytopaenia include scrub typhus, chikungunya fever, infectious mononucleosis, malaria, typhoid fever, leptospirosis and acute human immunodeficiency virus conversion disease.

Although thrombocytopaenia is an important finding in acute dengue infection among adults, the rate of its occurrence varies widely. It ranges from 40%-97% Since the thrombocytopaenia is given so much emphasis in the diagnosis of acute dengue virus infection, it is of interest to know how valid thrombocytopaenia is, in diagnosing acute dengue virus infection in an outpatient setting. The objective of this study was to determine the validity of thrombocytopaenia in diagnosing acute dengue virus infection among patients with acute non-specific febrile illness presenting to the outpatient clinics.

MATERIALS AND METHODS
We conducted a prospective cohort study of patients attending Primary Care Centre of Hospital Universiti Kebangsaan Malaysia (HUKM) and Batu 9 Health Clinic Hulu Langat, from May to November 2003. We invited consecutive patients who were age 12 years and above with a history of fever (oral temperature of > 37.5°C) for less than two weeks to participate in the study. Patients were excluded if there was an apparent localized source of infection e.g. urinary tract infection, acute abdomen, or any illness with pathognomonic clinical features e.g. varicella infection, measles, rubella, scarlet fever and obvious dengue hemorrhagic manifestations. Ethics approval was obtained from the research and ethics committee of the Medical Research Unit, National University of Malaysia (UKM). Consent was taken from all eligible patients before enrolling them into the study.

Full blood count examination was done for every patient. Patients were assumed to have acute dengue virus infection if thrombocytopaenia was detected and were managed by protocol for acute dengue virus infection. All the other patients with normal platelet counts were called back on day 5 of illness for a repeat full blood count so as not to miss any patient with thrombocytopaenia. We considered patients to have thrombocytopaenia if any of the platelet count result was < 150X10^9/L. We did dengue serology for all patients on day 5 of illness. If the patients were admitted, data was then collected from hospital records.

Dengue serology tests were done using PanBio Rapid Immunochromatography method in the virology laboratory, HUKM. Primary acute dengue virus infection is defined as a positive serology test of IgM alone. Secondary infection is defined as a positive IgG with or without a positive IgM. Collectively, both primary and secondary infections constitute acute dengue infection.
Statistical analysis
Results were analysed as intention to treat with SPSS version 11. Sensitivity, specificity and likelihood ratio of the test (thrombocytopaenia) in predicting the disease (acute dengue infection) was calculated. Likelihood of disease for a positive result, which was denoted by (+)LRd, calculated as [Sensitivity/(1-Specificity)] and Likelihood of no disease for a negative result, which was denoted by (-)LRn, calculated as [Specificity/(1-sensitivity)]^9.

RESULTS
We screened 153 patients, of which 49 did not give consent for the study and 17 were excluded for not fulfilling the criteria. Fourteen patients defaulted follow up as they claimed to be well and refused reassessment. Hence, complete data was available for 73 patients. However, we have included all 87 patients (including 14 defaulters) enrolled in our data analysis. (Fig.1) Mean age of the patients in this study was 27.3 years (27.5, SD±12.0); and fifty-seven (65.3%) were males; Malay (73.6%) was the main ethnic group, followed by Chinese (11.5%), Indian (9.2%) and others (5.7%). Patients presented to our clinics at different days of fever. They ranged from 2 to 10 days of fever. There were 37 patients who had thrombocytopaenia on their first blood taking of which 15 presented with less than 5 days of illness (Fig. 2). On second blood taking which was on day 5 of illness, 5 more patients developed thrombocytopaenia from their initial normal platelet count. These made a total of 42 (48.3%) patients had thrombocytopaenia.

There were 24 patients (27.6%) with positive dengue serology testing. Among these patients with dengue infection, 21(87.5%) had thrombocytopaenia. Fourteen patients who defaulted did not have dengue serology performed and they were assumed as not having dengue infection. Among the 14 defaulters, four patients had thrombocytopaenia and 10 had normal platelet count.

We first cross tabulated and analysed on complete data from 73 patients. (Table I) The sensitivity and specificity of thrombocytopaenia in diagnosing acute dengue virus infection were 88% and 65% respectively. The likelihood ratio for not having acute dengue virus infection, when there was normal platelet count, was two times higher than the likelihood ratio for having acute dengue virus infection, when there was thrombocytopaenia. (Table II) In order to study how precise this estimate of likelihood ratios could be, we further analysed the data, firstly assuming all defaulted patients had acute dengue virus infection and secondly, assuming they did not have dengue infection. The likelihood ratios were lower when we assumed the defaulters had acute dengue virus infection but the ratios did not change much if we assumed that they did not have dengue infection. (Table II)

DISCUSSION
Thrombocytopaenia occurred in 87.5% of patients with acute dengue infection. The finding of high percentage of dengue patients with thrombocytopaenia in our study was also noted in other studies involving adult dengue patients^6,10,11. It ranges from 67.9% to 97%. Studies that defined thrombocytopaenia as less than 100X10^9/L11 had lower percentage of dengue patients with thrombocytopaenia compared to studies which define thrombocytopaenia as less than 140 X10^9/L^2. This is logical as the higher the level of thrombocytopaenia used in its definition, the higher percentage of dengue patients with thrombocytopaenia.

Even though there was high percentage of dengue patients with thrombocytopaenia, we should still exercise caution in relying on the presence of thrombocytopaenia in diagnosing a patient with dengue infection as it has low sensitivity and specificity (both were <90%) in diagnosing dengue infection. From our study, both the (+)LRd and (-)LRn were less than 10. Hence it reiterates that thrombocytopaenia is not an ideal screening test to either rule in or rule out dengue infection 9,10. However, presence of thrombocytopaenia among patients with acute non-specific febrile illness increases the odds of dengue by three times and absence of thrombocytopaenia decreases the odds by almost six times among patients with acute non-specific febrile illness. This shows that thrombocytopaenia is more useful as the criterion in ruling out acute dengue infection virus than diagnosing dengue infection. This is easily understood as only 8.57% of patients with normal platelet count had acute dengue virus infection, while there were as many as 44.7% of patients with thrombocytopaenia who did not have dengue infection. However, in the absence of thrombocytopaenia, we should consider other clinical features of dengue infection like nausea, vomiting, abdominal pain, duration of fever more

**Table I: Platelet count results and their dengue serology status among 73 patients with complete data**

<table>
<thead>
<tr>
<th>Platelet count status</th>
<th>Dengue serology</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopaenia (&lt;150 X 10^9/L)</td>
<td>21</td>
<td>38</td>
</tr>
<tr>
<td>Normal platelet count</td>
<td>3</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>73</td>
</tr>
</tbody>
</table>

**Table II: Comparing validity and predictive values of thrombocytopaenia in diagnosing acute dengue virus infection among different categories of analysis**

<table>
<thead>
<tr>
<th>Category of analysis</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>(+)LRd</th>
<th>(-)LRn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among 73 patients with complete data</td>
<td>88</td>
<td>65</td>
<td>2.52</td>
<td>5.22</td>
</tr>
<tr>
<td>Assuming defaulted patients had dengue infection</td>
<td>66</td>
<td>71</td>
<td>1.90</td>
<td>1.91</td>
</tr>
<tr>
<td>Assuming defaulted patients did not have dengue infection</td>
<td>88</td>
<td>67</td>
<td>2.63</td>
<td>5.33</td>
</tr>
</tbody>
</table>

* the likelihood ratio for disease for a positive result
† the likelihood ratio for no disease for a negative result
Fig. 1: Patient enrolment in the study.

Fig. 2: Occurrence of thrombocytopenia with respect to days of illness at presentation.
then three days\textsuperscript{12} and leukopaenia\textsuperscript{13} before dismissing a case of dengue infection. These clinical features are significantly associated with acute dengue virus infection.

In the analysis of intention to treat, the likelihood ratios were reduced when all defaulters were assumed to have acute dengue virus infection but they remained unchanged when we assumed them not to have acute dengue virus infection. The later situation was more likely as all defaulters claimed to be well and refused reassessment. In this study a third of the patients had acute dengue virus infection. Assuming a third of the defaulters had acute dengue virus infection, the +LR(d) and –LR(n) improved to 3.10 and 6.64 respectively. This means thrombocytopenia is even a better criterion in excluding than diagnosing acute dengue virus infection. Hence, likelihood ratios estimated using the only available complete data would represent the true situation.

However, we have to be cautious in interpreting this data. Panbio Rapid Immunochromatography test kit was used as the diagnostic tool in this study. This test kit is routinely used in the microbiology laboratory of Hospital Universiti Kebangsaan Malaysia for the diagnosis of acute dengue virus infection. Serology results from this test kit are not the gold standard in the diagnosis of acute dengue virus infection. Antigen detection by polymerase chain reaction, viral culture or paired serology should be used instead\textsuperscript{14}. Therefore, even though Panbio Rapid Immunochromatography test kit has very good sensitivity and specificity, false positive and negative test results do occur. In reference to a local study by Lam \textit{et al.}, the false negative rate of this Panbio Rapid test for acute dengue virus infection was 0\% (sensitivity of 100\%) and the false positive rate was 11\% (specificity of 89\%)\textsuperscript{15}. Whereas, Vaughn \textit{et al.} demonstrated that 80\% of dengue patients would show a diagnostic level of secondary dengue infection by day 1 of defervescence using haemagglutination-inhibition test (HAI, in detecting IgG) which gave a false negative of 20\%. Day of defervescence was defined as the day when the temperature dropped below 38\textdegree C which commonly occurs around day 5 to 7 of illness\textsuperscript{16}. In the present study, dengue serology was done on day 5 of illness. Hence, by considering all the above factors, we estimated the diagnostic error from Panbio Rapid Immunochromatography test kit to be 11 to 20\%.

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