Multicentre prospective study on dengue classification in four South-east Asian and three Latin American countries

Neal Alexander1,2, Angel Balmaseda3, Ivo C. B. Coelho3, Efren Dimaano4, Tran T. Hien5, Nguyen T. Hung6, Thomas Jänisch7, Axel Kroeger8, Lucy C. S. Lum9, Eric Martinez10, Joao B. Siqueira11, Tran T. Thuy12, Iris Villalobos13, Elci Villegas14 and Bridget Wills15 on behalf of the European Union, World Health Organization (WHO-TDR) supported DENC0 Study Group*

1 London School of Tropical Medicine and Hygiene, London, UK
2 Departmento de Virologı´ a, Centro Nacional de Diagnóstico y Referencia, Managua, Nicaragua
3 Universidade Federal de Ceará, Fortaleza, Brazil
4 San Lazaro Hospital, Manila, Philippines
5 Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam
6 Children’s Hospital No.1, Ho Chi Minh City, Vietnam
7 Section of Clinical Tropical Medicine, University Hospital, Heidelberg, Germany
8 Special Programme for Research and Training in Tropical Diseases, TDR-WHO, Geneva, Switzerland
9 Department of Paediatrics, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
10 Instituto Pedro Kouri, La Habana, Cuba
11 Universidade Federal de Goı́as, Goianı́a, Brazil
12 Children’s Hospital No. 2, Ho Chi Minh City, Vietnam
13 Hospital Central de Maracay, Maracay, Venezuela
14 Universidad de los Andes, Nucleo Trujillo, Venezuela
15 Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam
* Author list is alphabetical.

Summary

OBJECTIVE To evaluate the existing WHO dengue classification across all age groups and a wide geographical range and to develop a revised evidence-based classification that would better reflect clinical severity.

METHODS We followed suspected dengue cases daily in seven countries across South-east Asia and Latin America and then categorised them into one of three intervention groups describing disease severity according to the overall level of medical and nursing support required. Using a pre-defined analysis plan, we explored the clinical and laboratory profiles characteristic of these intervention categories and presented the most promising options for a revised classification scheme to an independent group of WHO dengue experts for consideration. Potential warning signs were also evaluated by comparing contemporaneous data of patients who progressed to severe disease with the data of those who did not.

RESULTS A total of 2259 patients were recruited during 2006–2007 and 230 (13%) of the 1734 laboratory-confirmed patients required major intervention. Applying the existing WHO system, 47/210 (22%) of patients with shock did not fulfil all the criteria for dengue haemorrhagic fever. However, no three-tier revision adequately described the different severity groups either. Inclusion of readily discernible complications (shock/severe vascular leakage and/or severe bleeding and/or severe organ...
Dysfunction) was necessary to devise a system that identified patients requiring major intervention with sufficient sensitivity and specificity to be practically useful. Only a small number of subjects (5%) progressed to severe disease while under observation; several warning signs were identified, but much larger studies are necessary to fully characterize features associated with disease progression.

CONCLUSIONS Based on these results, a revised classification system comprised of two entities, ‘Dengue’ and ‘Severe Dengue’, was proposed and has now been incorporated into the new WHO guidelines.

**Keywords** dengue, Asia, Latin America, classification, warning signs, prospective

**Introduction**

Dengue may be caused by any of four virus serotypes, transmitted by *Aedes* mosquitoes (Halstead 2007). It is a major threat to public health with some 40 million symptomatic infections estimated to occur annually, of which around 2 million require hospitalisation (Hales et al. 2002; Kyle & Harris 2008; PDVI 2010). The incidence of infection has increased dramatically since the 1950s, largely because of rapid population growth, uncontrolled urbanisation and geographical spread of the virus and its mosquito vectors (Mackenzie et al. 2004). Neither vaccines nor specific disease-modifying interventions are currently available.

A wide spectrum of disease manifestations is seen, ranging from inapparent infection to severe and fatal disease. Based on pioneering early work from Thailand (Cohen & Halstead 1966; Nimmannitya et al. 1969), a case classification scheme was devised that separates symptomatic infections into two main disease entities, dengue fever (DF), an acute febrile illness usually accompanied by prominent constitutional symptoms, and dengue haemorrhagic fever (DHF), a syndrome characterised by increased vascular permeability and altered haemostasis that may progress to hypovolaemic shock (WHO 1997). Initially introduced into clinical practice in the 1970s (WHO 1975), the classification scheme proved to be invaluable in focusing attention on the urgent need for volume resuscitation for shock. However, as dengue has spread throughout the tropical world and the epidemiological picture has diversified according to the age, ethnicity and immune status of the populations affected, increasing concerns have been voiced regarding the applicability, complexity and usefulness of the system. A number of publications from endemic regions have reported difficulties (Sumarmo et al. 1983; Murgue et al. 1999; Phuong et al. 2004; Balmaseda et al. 2005; Bandypadhyay et al. 2006; Deen et al. 2006; Rigau-Perez 2006). One problem relates to the requirement for four specific criteria to support a diagnosis of DHF; thus, patients with clear evidence of vascular leakage who lack one of the other prerequisites are categorised inappropriately as DF. Secondly, it has become increasingly apparent that clinical syndromes that do not conform to the DF/DHF paradigm do occur and may be severe (Gulati & Maheshwari 2007). As a result, several local modifications have been suggested (Hayes et al. 1988; Kabra et al. 1999; Harris et al. 2000). Surveillance data and case fatality rates are no longer comparable between regions, and research studies are hampered by the lack of a consistent and well-validated endpoint. Finally, many clinicians find that as the existing system is applied retrospectively, its usefulness for triage and patient management is limited.

A consortium of experienced dengue clinicians and scientists therefore set out to address the following main objectives: (i) to evaluate the existing WHO dengue classification across all age groups and a wide geographical range and (ii) to develop a revised evidence-based classification that would better reflect clinical severity. In addition, we hoped to evaluate possible warning signs of likely progression to severe disease.

**Methods**

**Clinical methods and definitions**

We conducted a prospective observational study of patients with clinically suspected dengue recruited at 11 hospitals in 7 countries in South-east Asia and Latin America (See online-only Annex S1 for additional information). Ethical approval was obtained from the Ethics Review Committee of WHO and the review board of each institution. After informed consent by the subject or a parent/guardian, patients older than 6 months with clinically suspected dengue, no symptoms or signs indicative of an alternative diagnosis and fever for less than 7 days were enrolled in the study. Both outpatients and hospitalised cases were eligible for enrolment. Demographic information, clinical history and examination details were recorded on standard case report forms (CRFs), and an initial diagnostic blood sample was obtained. Detailed clinical, laboratory, diagnostic and management information was then recorded
daily. Study physicians were trained to complete the CRFs using a manual of clinical standard operating procedures which included formal definitions for all important entities. WHO-trained clinical monitors ensured that the study was conducted in accordance with GCP guidelines.

Haematocrit and platelet measurements were performed at least once daily, with full blood count, liver and renal function tests at least twice during the episode. Among hospitalised patients, a right lateral decubitus X-ray and/or ultrasound was carried out within 24 h of defervescence. All management decisions were independent of the study, made by the treating clinicians following local policies.

**Day of defervescence/critical period.** The day of reported fever onset was defined as illness day 1. The day of defervescence was defined as the first day when the core temperature dropped to ≤38 °C and remained below this level. Complications are most frequently observed around defervescence (WHO 2009); the critical period for complications was defined as defervescence day ± 1 day. Patients whose final temperature did not fall below 38 °C while under observation were assigned a critical period of day 4–6.

**Laboratory confirmation**

Serological/virological dengue diagnostics were performed in each participating country according to validated local protocols, with support provided by WHO-designated laboratories. According to the specific tests performed, criteria were defined for confirmed and highly suggestive infection (Table 1, online-only Annex S2). Serology results are based on IgM and IgG Capture ELISA of paired specimens, except where indicated.

**Dengue classifications**

**WHO classification.** We classified each individual using the existing formal scheme (WHO 1997; online-only Annex S3). A diagnosis of DHF requires specific evidence of plasma leakage, bleeding and thrombocytopenia in a patient with a febrile illness consistent with dengue. Those who fulfilled all the criteria for DHF and were diagnosed with clinical shock because of vascular leakage were classified as having dengue shock syndrome (DSS). Positive evidence for any of the criteria was accepted at any time, but to ensure that absence of criteria was also evidence based, only patients with a minimum of 3 consecutive days of data, including at least one day within the critical period, were selected for the main analysis.

If clinical signs of plasma leakage are not detectable, the existing WHO scheme for DHF relies on an increase in haematocrit by 20% or more relative to baseline. The peak haematocrit was taken as the maximum value observed. The baseline haematocrit was defined as the minimum value recorded before day 3 or after day 9, to minimise effects of fluid shifts occurring during the expected period for leakage and re-absorption. For patients without

<table>
<thead>
<tr>
<th>Country</th>
<th>Confirmed dengue case (one of the following)</th>
<th>Highly suggestive dengue case (one of the following)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All countries</td>
<td>RT-PCR positive or virus isolation positive</td>
<td>IgM 'seroconversion' (from below 40 to ≥40 units) regardless of IgG results</td>
</tr>
<tr>
<td>Thailand, The Philippines (according to AFRIMS protocol)</td>
<td>IgM ≥ 40 units (acute or convalescent sample or both) and IgG titre increase to above 100 units (paired samples)</td>
<td>IgM ≥ 40 units (acute or convalescent sample or both) and IgG titre above 100 units in convalescent sample, but no IgG increase between acute and convalescent sample</td>
</tr>
<tr>
<td>Malaysia, Nicaragua, Venezuela, Vietnam, Brazil</td>
<td>Twofold IgG titre increase (paired samples) with a titre ≥100 units in the convalescent sample</td>
<td>IgM positive in a single sample or no increase in paired samples</td>
</tr>
<tr>
<td></td>
<td>IgM seroconversion (paired samples) or IgM positive in both samples with rising levels of ELISA units (≥20% rise in 2nd sample)</td>
<td>IgG positive in a single sample, titre ≥1280 by HI†</td>
</tr>
<tr>
<td></td>
<td>IgG seroconversion (paired samples) or fourfold or greater increase in titre (paired samples)*</td>
<td>IgG positive (paired samples) with rising levels of ELISA units (≥20% rise in 2nd sample and 2nd sample ≥200 ELISA units)</td>
</tr>
</tbody>
</table>

Note that acute samples were collected at enrolment, with convalescent samples obtained after the seventh day of illness and at least 48 hours after the first sample.

*In Nicaragua, inhibition ELISA (equivalent to HI) was performed instead of IgG Capture ELISA.
†HI, hemagglutination inhibition antibody titre was only carried out in Malaysia.
baseline values, the WHO guidelines recommend use of population reference data. Because we were unable to identify published reference data for any of the participating countries, we collated available unpublished haematocrit data from healthy individuals and created a reference table stratified by continent, age and sex.

**Intervention category.** We classified each patient into one of three groups (standard, intermediate or major) based on the highest level of medical and nursing interventions that he or she required (Table 2). This approach is similar to the grading of clinical trial adverse events (ICH 1995) or to the approach that has been used to evaluate respiratory rate thresholds in acute respiratory infections (Lanata et al. 2004). The system was structured to take into account site-specific variations in management policies.

**Statistical methods and data analysis**
Sample size was determined according to the hypothesis that the revised classification would result in 10% higher sensitivity (90–95% vs. 80–85%) to detect severe disease compared with the existing classification, assuming 10% of severe cases among a total of 3000 patients (power 90%, two-sided significance level 0.05). However, given the lack of robust baseline information on which to base such calculations, we planned the study pragmatically, aiming to run for one dengue season with each site encouraged to recruit all possible patients during this time period.

Data were double-entered and checked at facilities in Guatemala and Thailand. Analysis was performed using STATA versions 9.2 and 10 (STATA Corporation, College Station, TX, USA) and JAVA (Sun Microsystems, Santa Clara, CA, USA).

**Evaluation of the existing WHO classification.** We used the need for major intervention as the gold standard for severity. First, sensitivity was defined as the proportion of those requiring major intervention who fulfilled the DSS criteria, with specificity defined as the proportion of those not requiring major intervention who did not fulfil the DSS criteria. Subsequently, a similar analysis was performed using the combined classification of DHF/DSS instead of DSS alone.

**Development of the revised classification.** Two methods were used to develop a revised system intended to better reflect clinical severity. First, logical algorithms were used to evaluate the performance of a systematically generated series of permutations of signs, symptoms or laboratory variables considered potentially important in distinguishing between severe and non-severe disease (i.e. the major intervention group versus the intermediate and standard therapy groups combined) and subsequently between the intermediate and standard groups alone. Because interventions are likely to accord with clinical presentation, we limited the variables investigated to those not directly linked to the interventions used to define the outcome. A range of possible permutations of cut-offs for the continuous variables, together with all combinations of the categorical variables, was tested, aiming for the highest sensitivity and specificity with respect to the calibration

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Characteristics used to define the three intervention categories. Patients were classified daily, and the highest level of intervention required during the illness episode defined the final category</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Category 1 (Standard)</strong></td>
<td><strong>Category 2 (Intermediate)</strong></td>
</tr>
<tr>
<td>Nursing care*</td>
<td>Level 1 and no intervention</td>
</tr>
<tr>
<td>Fluid therapy</td>
<td>No IV fluids</td>
</tr>
<tr>
<td>Blood products</td>
<td>No blood products</td>
</tr>
<tr>
<td>Additional interventions</td>
<td>No additional interventions</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Nursing care levels, customised to reflect differing practices between sites: 1: in- or outpatient, free to walk around, standard observation protocol – e.g. 6 hourly; 2: hospitalised with more stringent observation protocol – e.g. 2–4 hourly; 3: bed rest with ICU level observation protocol (even if managed outside an ICU) – e.g. hourly.
†At some sites, these blood products were given in response to abnormal laboratory results rather than for clinical reasons. We classified such interventions in the intermediate category.
tool, i.e. the intervention category, in addition to improved performance over the existing WHO scheme.

Secondly, the existing WHO classification scheme was modified in a stepwise fashion, with the sensitivity and specificity of each iteration compared with the major intervention category. As one of the main criticisms of the existing classification is its rigidity, the logical connection between the individual criteria was converted from ‘and’ to ‘or’, resulting in considerable improvement in sensitivity but a major loss of specificity. Subsequently, particular features inherent in the existing scheme were removed or added to the model in turn, again aiming for the most favourable sensitivity/specificity profile. The most favourable permutations identified were then presented to an independent group of specialists at an Expert Meeting convened by WHO (September 2008; 37 dengue experts from 5 continents, 5 dengue WHO regional advisers from 4 WHO Regional offices and 21 experts from WHO Headquarters and TDR). The feedback from the expert meeting was used to decide between different candidate options.

To assess the robustness of the estimates arising from these analytical pathways, the results were recalculated using a jackknife-like procedure in which each of the study sites was dropped in turn (Armitage et al. 2001b). A similar analysis was performed on 10 further subsets of the data generated by omitting a random 10% of patients.

**Case–control study to evaluate warning signs.** We used an unmatched case–control approach where the warning signs present 1 day before requirement for major intervention (cases) were evaluated by logistic regression models using robust sandwich standard errors with patient as the cluster (Armitage et al. 2001a). The analysis was stratified by day of illness so that, for example, day 4 data from cases who progressed to severe disease on day 5 were compared with day 4 data of controls (i.e. those who did not subsequently require intermediate or major intervention). The analysis was carried out for days 4–7 of illness, the period when major interventions were most likely to be needed. All controls with data in this period contributed to the analysis, but only to one stratum, i.e. only one day of their data was used. Where several days’ data were available, the day with the lowest proportion of missing data was selected, although selecting the day at random gave very similar results. The ratio of cases to controls ranged from 1:6 to 1:10 depending on the day of illness. Age group, day of illness and continent (Asia or Latin America) were included in these models as potential confounders. The potential warning signs analyzed can be grouped into those related to plasma leakage, platelets and bleeding, liver disease, and neurology (footnote to Table 3).

### Results

Between August 2006 and May 2007, a total of 2259 patients were recruited, of whom 1734 were diagnosed with dengue, 1568 (90%) confirmed and 166 (10%) highly suggestive, and fulfilled the minimum data set required for the main analysis (Figure 1). One-hundred and forty-nine (9%) were managed wholly as outpatients, while the remaining 1585 (91%) were admitted for varying time periods. Only inpatients were recruited in Thailand and Vietnam, where suspected dengue cases are usually admitted for observation. In the other Asian countries, approximately 14% of patients were recruited as outpatients, while in Latin American countries, this proportion varied between 20% and 50%. Clinical disease profiles were similar between countries, except that children generally received higher levels of intervention than adults, as did patients recruited at Asian sites rather than Latin American sites (Annex S4). Two patients died – both children presenting with profound shock – but all other subjects recovered fully. In 78/1734 (4%) patients, a critical period of day 4-6 was assigned as the temperature did not drop below 38 °C by discharge.

**Existing WHO classification**

The existing system could not be applied in 770/1734 patients (44%) without use of population haematocrit data, but a baseline haematocrit was inferred from the reference database for all but 32 cases. Three-hundred and six patients (18%) remained unclassifiable in a strict sense, in most cases owing to missing or inappropriately timed but negative radiology (237 cases) or missing tourniquet tests (37 cases). However, as the existing scheme classifies all patients in whom the requisite four criteria are not demonstrated as having DF by default, we adopted this principle for the subsequent analysis. Figure 2 presents the sensitivity and specificity of the WHO classification in relation to the calibration tool. Considering DSS, we found a sensitivity of 70% and specificity of 100% with respect to major intervention, while for DHF/DSS, the figures were 76% and 54%, respectively. Shock is the most easily identifiable complication of dengue and requires very specific intervention. Among the 210 patients who received shock resuscitation, 27 were classified as DF with a further 20 unclassifiable in a strict sense, giving a total of 47/210 (22%) who did not fulfil all the criteria necessary for DHF, with a number of patients failing on more than one criterion. However, 4 of these 47 cases experienced significant bleeding necessitating blood transfusion as well as fluid resuscitation, thereby precluding differentiation between shock owing solely to vascular leakage and shock...
owing to blood loss. Applying the existing WHO classification to all 2259 suspected dengue patients enrolled in the study, the sensitivity for DSS with respect to major intervention was 73%, with specificity maintained at 100%, while for DHF, the sensitivity was 79% with specificity of 53%.

Revised classification

The best candidate classification from the range of systematically generated permutations had a sensitivity of 87% and specificity of 53% with respect to requirement for major intervention (relative to the other two intervention

### Table 3 Warning signs associated with disease progression.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>N Cases (%) or mean (95% CI)</th>
<th>N Controls (%) or mean (95% CI)</th>
<th>Univariate OR (P-value) 95% CI</th>
<th>Multivariate OR (P-value) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15 years</td>
<td>56 (70.9)</td>
<td>435 (63.0)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>≥15 years</td>
<td>23 (29.1)</td>
<td>256 (37.0)</td>
<td>0.70 (0.167) 0.42–1.16</td>
<td>0.34 (0.002) 0.17–0.68</td>
</tr>
<tr>
<td>Continent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE Asia</td>
<td>67 (84.8)</td>
<td>579 (83.9)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>L America</td>
<td>12 (15.2)</td>
<td>112 (1.62)</td>
<td>0.93 (0.816) 0.48–1.77</td>
<td>2.83† (0.013) 1.24–6.47</td>
</tr>
<tr>
<td>Day of illness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9 (11.4)</td>
<td>101 (14.8)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>5</td>
<td>25 (31.6)</td>
<td>197 (28.9)</td>
<td>1.42 (0.386) 0.64–3.17</td>
<td>1.05 (0.917) 0.45–2.42</td>
</tr>
<tr>
<td>6</td>
<td>27 (34.2)</td>
<td>228 (33.5)</td>
<td>1.33 (0.481) 0.60–2.93</td>
<td>0.63 (0.283) 0.27–1.47</td>
</tr>
<tr>
<td>7</td>
<td>18 (22.8)</td>
<td>155 (22.8)</td>
<td>1.22 (0.636) 0.53–2.83</td>
<td>0.58 (0.237) 0.23–1.44</td>
</tr>
<tr>
<td>Abdominal pain and/or tenderness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>29 (36.7)</td>
<td>528 (76.4)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Present</td>
<td>30 (63.3)</td>
<td>156 (22.6)</td>
<td>5.84 (&lt;0.001) 3.57–9.54</td>
<td>3.53 (&lt;0.001) 2.09–5.96</td>
</tr>
<tr>
<td>Lethargy§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>69 (87.3)</td>
<td>672 (97.2)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Present</td>
<td>10 (12.7)</td>
<td>15 (2.2)</td>
<td>6.49 (&lt;0.001) 2.81–15.01</td>
<td>10.69† (&lt;0.001) 3.17–36.09</td>
</tr>
<tr>
<td>Mucosal bleeding**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>58 (73.4)</td>
<td>618 (89.6)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Present</td>
<td>21 (26.6)</td>
<td>72 (10.4)</td>
<td>3.11 (&lt;0.001) 1.78–5.42</td>
<td>2.87 (0.002) 1.49–5.53</td>
</tr>
<tr>
<td>Haematocrit increase†** (per 1% increase)</td>
<td>42.3 (40.8–43.7)</td>
<td>41.8 (41.5–42.1)</td>
<td>1.02 (0.561) 0.95–1.10</td>
<td>1.00 (0.983) 0.93–1.07</td>
</tr>
<tr>
<td>Platelet decrease†‡ (per 10 000/µl)</td>
<td>70 000 (57 000–82 000)</td>
<td>104 000 (100 000–109 000)</td>
<td>1.16 (&lt;0.001) 1.07–1.25</td>
<td>1.18 (&lt;0.001) 1.08–1.29</td>
</tr>
</tbody>
</table>

±Other variables tested as warning signs, but not included in the final model were: ALT, AST, liver enlarged, jaundice, bilirubin, albumin, systolic BP, fainting, oral intake, coma scale, restlessness, any bleeding, tourniquet test, WBC, atypical lymphocytes, rash, skin flush, chest pain, persistent vomiting, watery stools.

†The elevated multivariate OR for Latin America can be explained by the fact that the average platelet count is lower for cases as well as for controls in Asia compared with Latin America, which is probably due to the fact that the frequency of severe disease is higher in Asia compared with Latin America (see also Annex S4, Table S5).

‡Information on abdominal pain/tenderness missing for seven controls.

§Information on lethargy missing in four controls.

†The increase in the multivariate OR for lethargy when compared with the univariate OR is explained by the fact that the univariate OR differs between the two age groups (OR = 1.6 for below 15 years and OR = 15.8 for above 15 years) and that adjustment for age changes the weighting between these two groups.

++Information on mucosal bleeding missing in one control.

††Information on hematocrit increase missing in one case and five controls.

‡‡Information on platelets missing in one case and six controls.
levels combined). For three-level classifications, discrimination between the intermediate and standard intervention groups gave slightly inferior operating characteristics – depending on the criteria and cut-offs chosen, sensitivity varied between 60% and 80%, with specificity of 45% and 70%. For the stepwise modification of the existing system (Figure 3), we first assessed the sensitivity and specificity of combining fever with any two of the three necessary criteria, but saw no improvement (Revisions 1a–c). In subsequent revisions, the best scheme that could be identified using general clinical and simple laboratory variables (Revision 7) gave a sensitivity of 62% and specificity of 69%.

Although several of these schemes differentiated between severity levels with greater sensitivity and specificity than the existing classification, the clinical experts did not consider any of the alternatives robust enough for general clinical application. A simple and practical system that clearly identified the major complications of dengue was felt to be the first priority – thus we focused on describing the major syndromes seen in the 230/1734 patients (13%) who required major intervention. Fluid resuscitation for clinical shock was the most frequent reason for inclusion (210 cases) – a further seven patients had severe bleeding, six had severe plasma leakage without overt shock (defined as pleural effusions/ascites causing respiratory distress without cardiovascular compromise), one patient required treatment for liver failure and six patients had a combination of these factors without shock. Incorporating the following features (Annex S3, Table S4) – vascular leakage resulting in shock or respiratory distress, and/or severe bleeding, and/or severe organ dysfunction – into the pathways described above identified 271 (16%) of all

Figure 1 Flowchart of patients enrolled in the study. In the boxes at the bottom of the flowchart, the numbers indicate the number of patients per country/hospital centre confirmed to have dengue and included in the final dataset, as a proportion of the total number enrolled, with the median age (90% range) and sex distribution. Note that some hospitals are paediatric centres and only recruited children (P).
patients as clinically severe, with sensitivity of 96% and specificity of 97% relative to the intervention category status (Figure 3, final revision). The reliability of this finding (±1%) was confirmed on all re-sampling analyses that we performed. Applying this revised system to all 2259 suspected dengue patients in the study, the sensitivity (95%) and specificity (97%) were virtually unchanged.

These results support the idea of a revised system incorporating two main categories – a major category consisting of patients diagnosed with dengue and a smaller group comprising those with major complications. Comparing the existing and revised systems directly demonstrates that 873/1734 (50%) of the study patients would be classified as DHF or DSS, while 271/1734 (16%) would be classified as severe dengue (Figure 4). Almost all patients who required major intervention (221/230, 96%) were identified as severe dengue, but of the nine patients missed, six were picked up as DHF. An additional 50 patients were classified as severe dengue but were managed with intermediate or standard support only. By comparison, 55/230 patients (24%) requiring major intervention were classified as DF rather than DHF, and an additional 13 patients with DHF but without shock required major intervention. With regard to requirement for intermediate support versus standard support (i.e. observation only), there was little difference between the different systems; thus among the remaining 710 DHF cases, 421 (59%) required intermediate support, slightly more than the 392/861 (46%) DF cases, and similar to the 813/1463 (56%) cases classified as dengue.

**Warning signs for progression to severe disease**

Among the 1734 patients, 147 required major intervention on enrolment, leaving 1587 at risk of progression. Overall, 83/1587 (5%, 95% CI 4–6%) patients who required standard (41 cases) or intermediate (42 cases) level
Figure 3 Sensitivity and specificity calculated for each step of a gradual modification of the current WHO classification into a revised classification. The sensitivities and specificities were calculated with reference to requirement for major intervention.
intervention at presentation went on to require major intervention. Also, $177/868$ (20%) of patients on standard management at enrolment progressed to require intermediate care. Most patients who became severe ($79/83$) did so between days 4 and 7 of illness. Table 3 shows the risk factors for progression, with their respective crude and adjusted odds ratios. In the multivariable model, abdominal pain or tenderness, lethargy, mucosal bleeding and a decrease in platelet count were associated with a significantly increased risk for severe disease. Age group, continent and day of illness, all potential confounders, were adjusted for in the model.

**Discussion**

Using a large data set of laboratory-confirmed cases recruited prospectively across a range of dengue-endemic countries, we attempted to develop a revised dengue classification scheme that would be simpler and more practical than the previous WHO system (WHO 1997). It proved necessary to include readily discernible complications (shock/severe vascular leakage, severe bleeding and severe organ dysfunction) to achieve a scheme with sufficient sensitivity and specificity to be considered useful by the clinical experts, reflecting the experience of many that the features of dengue infection are typically nonspecific until or unless complications develop.

With respect to triage and clinical management, the revised scheme has the advantage of classifying by severity at any time during the evolution of the disease. A formal DHF diagnosis, by contrast, is often only possible late in the evolution of the infection, encouraging practising clinicians to develop a variety of loose and incommensurable interpretations to allow prompt institution of appropriate management. Using this revised system, any single serious manifestation classifies the patient immediately as severe dengue, while at the end of the illness episode, the final worst classification level can be used for epidemiological purposes thereby facilitating collection of standardised surveillance data.

With respect to intervention studies, a binary classification system providing a well-defined endpoint should prove useful, especially for vaccine studies. However, for pathogenesis studies focused on vascular leakage, further development is required. Although the proposed system would allow all cases with severe leakage to be identified, cases previously classified as DHF without shock would no longer be identifiable directly. However, it is apparent that DHF itself is a highly specific but relatively insensitive diagnosis, and there is increasing evidence for
the existence of a continuous spectrum of dengue disease rather than two separate disease entities (Libraty et al. 2002; Phuong et al. 2004). Further work is undoubtedly needed to develop robust and practical clinical tools to identify and quantify vascular leakage for research purposes.

We identified certain warning signs for disease progression (Table 3); in particular, the importance of abdominal pain was confirmed with a threefold increase in risk for severe disease demonstrated in our model (Guzman et al. 1999; Rigau-Perez & Laufer 2006). Lethargy, decreasing platelet count and mucosal bleeding were also associated with significantly increased risk, although it is possible that the decision to admit a given patient to the study was influenced by the presence of these ‘established’ warning signs. In any event, because only a small proportion of patients developed severe disease, these results must be regarded as preliminary findings.

Another limitation of our study is the inevitable difference in laboratory diagnostic methods used, as well as local interpretation of some clinical definitions, particularly the care level, which may have resulted in between-site variation. Also, despite all the efforts made, some patients had fewer than three days of data collected and were excluded from analysis.

As our major objective was to develop an evidence-based classification system, we deliberately focused on classification in laboratory-confirmed cases. We did not attempt to address the issue of initial diagnosis – i.e. differentiating dengue from other febrile illnesses. In a recent smaller study from a single centre, the general results were similar, with limited concordance between the WHO classification and physician diagnosis, and the observation that severe clinical features occurred in cases that were not classified as DHF (Srikiatkhachorn et al. 2010). In addition, however, while DHF/DF demonstrated excellent specificity (99%) in differentiating dengue from other febrile illnesses, the sensitivity was poor at 36%. This emphasises the considerable heterogeneity of dengue illness and highlights the need for identification of better clinical disease markers as well as development of reliable and affordable diagnostics for use in endemic areas.

In summary, the classification system for dengue presented here has significant advantages over the previous system, both for case management and for disease surveillance. At the WHO-sponsored expert review meeting, there was general agreement that a new system based on these results and comprising two entities, ‘Dengue’ and ‘Severe Dengue’, should be incorporated into the new WHO guidelines (WHO 2009). Although the link between development of specific complications and use of particular interventions was recognised as contributing to the high sensitivity/specificity of the final model, the scheme was considered likely to prove effective and practical. However, further refinements remain necessary with respect to risk prediction and for application in specific areas of pathogenesis research. Similar binary systems are in use for other diseases (Mulholland et al. 1992; WHO 2006; Puimalainen et al. 2008), but to our knowledge, this is the first time that such a scheme has been developed based on research evidence rather than expert opinion. The upheaval inherent in changing the existing classification must not be underestimated however, and it is crucial that the new system be subject to ongoing evaluation and refinement in the light of future research.

**Acknowledgements**

We thank the medical, nursing and laboratory staff at all the sites, who worked very hard to care for the patients. We also offer sincere thanks to Albrecht Jahn, scientific officer at the European Union in Brussels, and to all the administrative staff who made the study possible, in particular Judit Barniol, the administrator at the coordinating institution, the University Hospital of Heidelberg. Furthermore, we want to thank the Ministries of Health, particularly Brazil, who were involved in the study.

The study was supported by the European Union 6th Framework Program, TDR-WHO and the Wellcome Trust. WHO-TDR was involved in the quality assurance of the study through TDR trained external monitors. Other than that, none of these agencies played any role in the design or execution of the study.

**References**


Cohen SN & Halstead SB (1966) Shock associated with dengue infection. I. Clinical and physiologic manifestations of dengue


**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

Annex S1. Clinical methods and definitions.

Annex S2. Laboratory confirmation.

Annex S3. Dengue Classifications.


Table S1. Definitions used according to the clinical SOP.
Table S2. List of laboratories performing diagnostic testing for the patients enrolled in the DENCO study.

Table S3. Criteria for classification according to the current WHO system – all patients had fever.

Table S4. Clinical definitions used in the classification of severe dengue.

Table S5. Highest intervention category per patient by country.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Corresponding Author Thomas Jaenisch, Heidelberg University Hospital, Heidelberg, Germany. E-mail: thomas.jaenisch@ urz.uni-heidelberg.de