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Dengue conundrums☆

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ABSTRACT

Dengue virus is the most common arboviral infection of humans in the tropical and subtropical regions of the world. This review briefly describes some of the challenges it presents. Dengue is an emerging disease; it is increasing in geographical distribution and severity, despite being significantly underreported. The World Health Organization case definition for the generally more severe manifestation of infection, dengue haemorrhagic fever (DHF), is controversial. The name DHF is something of a misnomer as the disease infrequently results in frank haemorrhage; the hallmark of DHF is actually plasma leakage. The existence of four closely related dengue virus serotypes contributes to difficulties in diagnosis and to original antigenic sin in the serological response to infection. The existence of multiple serotypes can result in more severe disease upon a second infection and complicates vaccine development. Nevertheless, a safe and effective vaccine is the greatest prospect for stemming the tide of dengue.

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1. Introduction

There are four serotypes of dengue virus (DENV1–4) belonging to the Flaviviridae family, Flavivirus genus. DENV is transmitted by *Aedes* spp. mosquitoes, primarily *Ae. aegypti* but also *Ae. albopictus*. Dengue disease is especially prominent in tropical and subtropical areas of the world, where *Ae. aegypti* has been notably successful in occupying an ecological niche alongside humans.

Infection with a dengue virus may be asymptomatic or it can cause a nonspecific viral syndrome, dengue fever (DF) or dengue haemorrhagic fever (DHF). DF is almost always a self-limited but debilitating illness, generally defined by fever with two or more of headache, retro-orbital pain, myalgia, arthralgia, rash, leucopenia and haemorrhagic manifestations (such as petechiae). DHF is defined by fever, haemorrhagic manifestations, thrombocytopenia and plasma leakage (pleural effusion, ascites, haemoconcentration or hypoproteinaemia) [1]. Cases are increasing in number, severity and geographic spread – there are an estimated 36 million cases of DF, 2.1 million cases of DHF and 21 000 deaths annually. Approximately 3.6 billion people (55% of the world's population) in 124 countries are at risk [2].

2. Epidemiology

2.1. World Health Organization (WHO) case definition

The WHO classification scheme for DF and DHF has been in use since 1975 and was revised in 1986 and 1997 [1]. The criteria were originally developed not only for a classification of DF and DHF but also to help distinguish between dengue (or DHF) and other causes of febrile illness. The purpose of the WHO dengue classification scheme was to provide clinicians with clinical criteria to recognize severe dengue illness and a reporting system based on clinical criteria. There have been concerns that the WHO classification is not adequate in the face of the rapidly emerging dengue background; in particular, concerns that some severe disease does not meet all DHF criteria. This can be because of the disease manifestations or the need for testing (complete blood count, chest radiograph), often at multiple time points, in resource-poor areas, or the effect of intervention on test results and disease manifestation [3–6].

One problem is that the current WHO criteria have been interpreted to mean that all DF is mild and all DHF is severe. Although it is true that DHF is generally more severe than DF, the latter can have severe outcomes not included in the WHO case definition. Politically, it may be difficult to muster the resources to address dengue if DF is seen as mild. Another problem is that there is a desire to have a classification system that meets the needs of surveillance, public health planning, early discrimination (of which dengue illness requires hospitalisation), and research studies on dengue pathogenesis. It is probably impractical to meet the objectives of all of these audiences with a single classification system.

Most agree that the name DHF is itself a source of confusion. Although overt haemorrhage is a clinically impressive feature of

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disease in some cases, and evidence of a haemorrhagic tendency is a criterion for DHF, in some patients this tendency is only detectable when elicited. This can be done, for example, with the tourniquet test, a bedside test of capillary fragility in which a blood pressure cuff is applied and inflated to the midpoint between the systolic and diastolic blood pressures for 5 minutes; the test is positive if there are more than 20 petechiae per square inch. In fact, frank haemorrhage is rare in DHF and the hallmark of the disease is vascular leakage. Some have proposed renaming the disease dengue plasma (or capillary) leak syndrome. A new name could help direct and focus clinical attention on plasma leakage and perhaps lead to a reassessment of the best way(s) it can be detected and monitored. The criteria of haemorrhagic tendency and thrombocytopenia could be monitored for prognostic value but would not be required for determination of dengue plasma leak syndrome [7].

2.2. Increasing disease

Over the last three decades a 4.6-fold increase in dengue cases was reported in the Americas, and DHF cases increased 8.3-fold [8]. Despite already extremely high rates of dengue, data from the Southeast Asia Region of the WHO show that cases over the 5-year period 2002–2006 increased by 67% from 1985 to 1989 [9]. Dengue is more poorly documented in Africa than in Asia and the Americas. Other infections such as HIV and malaria dominate in Africa and there is an assumption that dengue may not be a significant health threat [10]. DHF is rarely reported and this may be the result of relative resistance of Africans to severe dengue disease or differences in African and Asian viruses [11]. Still not known is how much dengue is diagnosed and treated as other fevers such as malaria [10].

2.3. Underreporting

Subclinical dengue infections are common and are important to disease transmission. In addition, the burden of clinical disease is underestimated. Based on work in Puerto Rico in the 1990s, it has been estimated that dengue was underreported by a factor of 10 for 0–15-year-olds and by a factor of 27 for those over 15 years of age [12]. We compared the incidence of dengue disease in cohort studies amongst patients less than 20 years of age in Thailand and Cambodia with national surveillance data from the same provinces and years. The average underestimation of total and inpatient dengue cases was 8.7- and 2.6-fold in Thailand, and 9.6- and 1.4-fold in Cambodia, respectively (unpublished data). These data indicate that although dengue is regularly reported in many countries, national surveillance data are a gross underestimate of the true burden of disease.

2.4. Desert disease

Because of the role of *Aedes* spp. mosquitoes in the transmission of dengue it may seem surprising to find dengue in arid regions. *Ae. aegypti* has been notably successful in an ecological niche alongside humans. Urbanisation fosters increased *Ae. aegypti* through rapid human population growth that overwhelms or exceeds sanitation capabilities, resulting in breeding sites in garbage areas and water storage vessels. Increasing numbers of dengue outbreaks and circulating serotypes have been seen in normally arid regions such as Saudi Arabia [13,14] and parts of India [15,16]. Ironically, in Australia there is concern that the reintroduction of *Ae. aegypti* to more southern locations will occur in the midst of a drought because of the human response of installing large domestic water storage tanks. Modelling of habitats in the dry regions of southeast Australia has suggested that the water storage tanks could result in an expansion of the range of the vector and increase the risk of dengue transmission in these regions [17].

2.5. Limits of vector control

Vector control has had limited success in controlling dengue [18,19]. The situation in Singapore illustrates that such control may not be sustainable [20]. Singapore has dedicated substantial resources for over 40 years to dengue prevention through *Aedes* control. The *Aedes* premises index (percentage of premises positive for *Aedes* breeding) has been only 1–2% since the 1980s [21]. This resulted in a decline in the transmission of dengue and DHF. However, it ultimately resulted in dramatically lowered herd immunity, meaning that less force of transmission is needed for disease outbreaks to occur. Ongoing virus introduction from Malaysia and other locations has resulted in epidemics of DF, particularly amongst adults, who are more likely than young children to present with symptomatic disease on first dengue infections [22]. Disease incidence increased from 4.9 cases/100 000 in 1985 to 322.5 cases/100 000 in 2005 [23]. Ironically, the strict vector control, whilst preventing DHF in the young, is leading to more DF in the older populations.

2.6. Travellers

In a recent report of 30 GeoSentinel sites on six continents, dengue was the second most common cause of systemic febrile illness (excluding diarrhoeal diseases) in returning travellers, and was the most common cause identified in travellers returning from the Caribbean, South America, South Central Asia and Southeast Asia [24]. Dengue occurred more frequently than malaria in all regions except Africa and Central America. In another study, dengue accounted for 6% of febrile illnesses in returning travellers, with 29% of these cases hospitalized [25]. Dengue appears to be being increasingly diagnosed in travellers, with frequencies increasing from 2% in the 1990s to 16% in the last decade [26]. In addition, these numbers invariably reflect underreporting, since the incubation period of dengue is usually only 4–5 days and travellers may become ill during travel within the endemic country, where the disease is often not reported.

3. The complicating factor of multiple viral serotypes

3.1. Antibody-dependent enhancement of disease

The four DENV serotypes are antigenically distinct but cause very similar disease in humans. There is substantial evidence that DHF is associated with secondary infection with a serotype different from that to which an individual has already been exposed [27–32]. In addition, DHF in infants has been associated with waning levels of maternal antibodies [33–35]. The most widely accepted hypothesis for the pathogenesis of DHF in these settings is antibody-dependent enhancement (ADE), in which anti-DENV IgG, either actively or passively acquired, enhances DENV infection of Fc receptor-bearing cells, resulting in an increased infected cell mass and triggering an immunological wave [34]. In addition, there is evidence in secondary infections that dengue-specific memory T cells may be reactivated by the heterologous serotype dengue infection. Expanding to high levels, T-cell effector mechanisms, including cytokine production and lysis of infected cells, could result in direct and indirect effects causing increased vascular permeability [36]. However, this latter mechanism would not contribute to the occurrence of DHF in primary infection of infants. There is also evidence that ADE may play a role not just in causing DHF but in worsening a spectrum of dengue illness [37]. ADE has been shown in vitro for

a number of virus families. Amongst human pathogens, in vivo evidence for ADE has been most prominent in dengue and respiratory syncytial virus (RSV) infections [38].

In endemic regions the risk for DHF appears to be highest in infants, as described above, and during a second dengue infection; however, in an endemic region the DHF risk with a third or fourth infection appears low [39]. Studies from Cuba, where there are intervals free of dengue, suggest that a longer interval between infections may increase the risk for DHF [40]. There is interest in the relative risk of DHF occurring after infection with specific sequences of serotypes. Infection with all sequences of serotype has been shown to result in DHF, except DENV-4 followed by DENV-1 and DENV-3 [39]. It is noteworthy that, in children, DENV-2 and DENV-4 are much less likely than DENV-1 and DENV-3 to present as symptomatic primary infections [41,42]; this suggests that DENV-2 and DENV-4 often require some enhancement to be virulent.

3.2. Antigenic sin

Infection with one DENV serotype provides long-term protection only against the same serotype, but induces antibodies cross-reactive to heterologous DENV serotypes. Over 20 years ago the phenomenon of original antigenic sin in the neutralising antibody response to DENV was described, in which a secondary DENV infection could induce a higher titre of antibodies against the previous infecting serotype than the current infecting serotype [43–45]. We found that our serology tests for secondary infection only identified the infecting serotype 26% of the time (range 5–50%, depending on serotype). In 50% of cases (range 0–84%, depending on serotype) the serology test results appeared to match the likely prior infecting serotype (unpublished data).

3.3. Vaccine

The effort to develop a dengue vaccine dates back to the 1920s [46]. Vaccines in development include live vaccines (classically attenuated vaccines, site-directed mutagenesis vaccines, dengue and dengue–yellow fever chimeras), inactivated vaccines (recombinant E protein subunit and purified inactivated virus), and DNA vaccines [47]. The front-runners are entering phase 3 trials. Because pre-existing heterotypic immunity is a risk factor for DHF, a successful vaccine must protect against all four serotypes. Vaccine-induced enhancement has been a major stumbling block in the development of flavivirus, coronavirus, paramyxovirus and lentivirus vaccines [48]. There is evidence that ADE played a role in the failure of an inactivated experimental RSV vaccine in the 1960s [38,49,50].

Immunological interference has been documented for several multitypic vaccines dating back to the 1950s [51–53]. Analogous to the dengue situation is that of the oral polio vaccine, which contains three different serotypes of polio virus. It was found that seroconversion rates, which had exceeded 90% for monovalent polio vaccines, were significantly reduced when the serotypes were combined in trivalent formulations. Adjusting the dose of each serotype tended to increase seroconversion rates to some degree, but in an unpredictable manner. The imbalance in seroconversion was finally overcome by the administration of three doses of the multivalent vaccine [54].

Similar difficulties have been encountered in the development of dengue vaccines. The monovalent vaccines had higher neutralising antibody responses than when they were combined in various multivalent formulations [55,56]. As for the oral polio vaccine, it has been anticipated that multiple doses of the vaccine will be necessary to achieve seroconversion to three or four DENV serotypes.

4. Conclusion

Dengue has been worsening in geographical scope and severity since World War II. All indications, with the exception of progress in vaccine development, are that this discouraging trend will continue. Dengue nomenclature and case classifications are under debate. It should be emphasised that plasma leakage is the defining pathology of DHF and the most common mechanism of shock. The existence of multiple cross-reactive serotypes complicates dengue through ADE and original antigenic sin. ADE in particular, along with viral interference, has made vaccine development challenging. The prospect of a safe and effective vaccine leads to the hope that the tide of dengue can be stemmed.

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