REVIEW ARTICLE

Dengue: where are we today?

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Abstract

Dengue is considered the main arthropod-borne viral disease of humans. In the last few years, an increasing number of reports of mild and severe cases have been reported. The growing dengue incidence observed in recent years has been accompanied by reports of new observations, findings and global initiatives with an improvement in our understanding of this phenomenon. The epidemiology and new clinical classification of dengue, advances in the diagnostic and pathogenesis knowledge, and vaccine development as well as control methods including new global initiatives are summarised here.

Keywords: *Dengue, review, management, medical sciences*

Introduction

Dengue has been re-emerging in the last decades, with an estimated 50–100 million people infected annually. More than 2.5 billion people live in geographic areas where the infection is endemic, and more than 100 countries are at risk of dengue transmission (1–3). The infection is caused by any of the dengue viruses (DENV-1 to 4), an RNA virus classified as a flavivirus of the family Flaviviridae. The virus is transmitted to humans by the bite of *Aedes* mosquitoes. *Aedes aegypti* is the principal vector, although *Aedes albopictus* is also important in some settings (4,5). Clinical manifestations of the illness varies from asymptomatic infection, observed in most of infected individuals, to the mild illness named Dengue Fever (DF) and to the severe form of the disease called dengue haemorrhagic fever with or without dengue shock syndrome (DHF/DSS) (6). The increase in dengue incidence observed in recent years has been accompanied by reports of new observations, findings and global initiatives with an improvement in our understanding of this phenomenon. Here dengue situation is updated

Current dengue epidemiological situation

The vector and virus expansions throughout tropical and subtropical areas around the world have been favoured by global unplanned urbanisation, population growth, international travel, abundance of non biodegradable containers and basically poor living conditions. Today, more than 70% of dengue cases occur in Asia and the Pacific, followed by the Americas, Africa and the Middle East. While both DF and DHF/DSS were widely recognised in Asia and the Pacific in the 1960s and the 1970s, the expansion to the Americas in the 1980s and 1990s and more recently to the Middle East and Africa has been observed (7), with reports of the four viruses in circulation in endemic areas. A recent report suggests that half of the world's population is at risk of dengue infection (1–3).

The expansion of dengue has been accompanied by the report of epidemics in virgin populations such as the Galapagos Islands and the Easter Islands. An increasing number of epidemics involve more than one viral serotype, and greater dengue activity with epidemics is occurring at shorter intervals (from five to two or three years); there have also been numerous reports of dengue illness in travellers (8,9). The American region has seen a dramatic dengue increase in the last 30 years, with an increasing number of DHF cases,

from 60–80 cases before 1981 to more than 38,000 in 2008. In addition, the co-circulation of several serotypes, the report of over 1 million cases in 2008 worldwide and dengue transmission occurring in over 30 countries reflect the seriousness of the problem (6).

In this context, climate change is expected to worsen the dengue epidemiological situation. The temporal and spatial changes in temperature, precipitation and humidity will affect the biology and ecology of the vectors and consequently the risk of virus transmission. It is estimated that, with the expected temperature increase of 2°C, the mean potential of transmission will rise. If water temperatures climb, the mosquito larvae take a shorter time to mature, and consequently there is a greater capacity to produce more offspring during the transmission period. In warmer conditions, mosquitoes digest blood faster and feed more frequently, thereby increasing dengue transmission. In addition, the extrinsic period of the virus within the vector could be shorter and therefore increase the proportion of infected mosquitoes (10–12).

Two important analyses related to dengue are the estimation of the global epidemiological and economic burden of the illness. Although some studies have been performed, these topics remain priorities of research. The global burden has been recently estimated in DALY (disability adjusted life years) to be 264 DALYs per million people per year for the two billion people living worldwide in areas at risk for dengue. Another study estimated a loss of 420 DALYs per million people per year, which is comparable to the burden of meningitis, twice the burden of hepatitis and one-third of the burden of HIV/AIDS (13). However, few studies focus on the cost of dengue. Since there is no uniform methodology applied, cost variation is observed among reports. Cost per case has been estimated to be 109.16 USD (Thailand 1994), 61.00 USD (Thailand 2005), 125.00 USD (Puerto Rico) and 299.00 USD (Cuba 1981) (14,15).

Of interest is the report of possible dengue transmission associated with blood transfusion. Recently, Mohammed et al. found that 1 in 1000 blood donations in Puerto Rico contained DENV RNA. On the other hand, Linnen et al. found variable evidence of dengue viraemia among asymptomatic blood donors (from 0.30% in Honduras to 0.04% in Brazil). These new findings raise concerns regarding transfusion-transmitted DENV (16,17).

Clinical illness

According to the World Health Organization (WHO) classification, the presence of fever, bleeding, thrombocytopenia (<100,000/mm^3) and haemoconcentration (including pleural effusion, ascitis etc) allows the classification of a patient as DHF/DSS (18). The increasing number of patients, variation in clinical manifestation and the extension of the transmission to new areas of the world have been accompanied by difficulties in the application of this WHO classification (19,20). Significant numbers of severe dengue patients do not meet all of the criteria outlined by the WHO.

Not surprisingly, there is a wide request for a classification that is useful for the management of the acute case. Under the leadership of the TDR/WHO and as part of a multi-centre study, the WHO classification was recently reviewed. As a consequence of this study, a new clinical classification was proposed for validation in several countries of the American and Asian regions. Accordingly, cases will be classified as dengue or severe dengue. The presence of warning signs, such as persistent vomiting and intense abdominal pain, in non-severe cases will alert clinicians about a bad prognosis. Severe dengue includes not only the former DHF/DSS cases but also patients with severe plasma leakage, severe bleeding and severe organ impairment. In recent years, an increasing number of unusual manifestations of dengue such as neurological disorders and myocarditis have been reported. The new classification also considers these aspects of dengue (21). In the Bolivian dengue epidemic of 2009, the new proposed classification was applied with very good acceptance by clinicians and epidemiologists (Martinez E. and Castro O., personal communication). Interestingly, in 1981 a similar dengue classification had been successfully applied in Cuba, during the first DHF/DSS epidemic in the American region (22,23). It is expected that once this new classification is validated, a favourable impact on mortality and better case management will be observed.

Diagnosis

Dengue diagnosis is based on the isolation of the virus, the detection of the viral antigen or RNA in serum or tissues or the detection of specific antibodies in the patient's serum. Virus isolation (mainly from mosquito cell lines such as *A. albopictus*), the identification by immunofluorescence assay using specific dengue monoclonal antibodies and the genomic detection by RT-PCR or real-time RT-PCR confirm the

infection (24,25). Serum is the sample of choice for dengue diagnosis, although tissue samples (liver, spleen, lymphatic nodes, etc.) in fatal cases can be employed for virus isolation and genomic (RT-PCR) or antigen (immunohistochemistry) detection (26-28). Serum samples collected in the first five days of fever are useful for virus detection.

IgM detection by ELISA in samples collected after day 5 of illness is routinely used for dengue diagnosis. IgG seroconversion or a fourfold increase in paired serum samples is also among the criteria for dengue diagnosis (29,30). One of the current research priorities is the evaluation of diagnostic assays and commercial kits. Recently, TDR and the Pediatric Dengue Vaccine Initiative (PDVI) identified a network of laboratories in Latin America and Asia to perform diagnostic evaluations. As part of this initiative, nine IgM commercial kits based on ELISA and rapid test formats were evaluated. Good sensitivity (95–99%) and specificity (79.9–86.6%) were demonstrated in three of them (the PanBio, Standard Diagnostic and Focus ELISA tests) (31).

There is still a need for early dengue diagnosis. During viral replication, NS1 non-structural protein is secreted in the blood, appearing as early as day 1 of fever and declining after days 6–7. Considering the characteristics of the protein, several studies focus on NS1 detection as an early marker of dengue infection (32,33). Sensitivity ranges of 60.4–87.4 % and specificity ranges of 97.9–100% have been found using ELISA and rapid tests. The highest percentage of NS1-positive samples has been observed in individuals with a primary dengue infection. The presence of anti-dengue immune complexes could be an explanation of the lower sensitivity observed in samples collected from individuals with a secondary infection (33). As part of the multicentre DENCO project conducted by TDR, NS1 detection was evaluated in serum samples collected from confirmed dengue cases during the acute phase of illness. Preliminary results support the usefulness of this marker for early dengue diagnosis, but, much more importantly, these results suggest the need for a new dengue diagnostic algorithm where NS1 and IgM detection complement each other to achieve higher sensitivity.

The evaluation of available RT-PCR and real time RT-PCR protocols, the development of a single test combining antigen and antibody detection and new diagnostic tools combining high sensitivity and specificity, low cost, simplicity and, ideally, high prognostic capacity for disease severity are still priorities for dengue diagnosis (24,34).

Pathogenesis

Two exclusive hypotheses, the secondary infection by a different dengue serotype and the viral virulence, were proposed early on to explain DHF/DSS (35,36). Observations in the last 50 years support an integrated view of the problem, since the secondary infection is needed for severity (37,38). Age (a higher risk is observed in children), chronic diseases such as bronchial asthma, diabetes mellitus and sickle cell anaemia, ethnicity (a higher risk is observed in whites compared to blacks) and genetic factors (26,38–42) have been reported as the main host risk factors for DHF/DSS. In this context, the virus serotype, the sequence of infecting viruses and the virus genotype are also of importance. Genotypes of DENV-2 and 3 from Asia have been associated with DHF epidemics (43–46). The report of quasi-species and recombinant viruses adds more complexity to the problem and demonstrates the genetic diversity of dengue viruses (47–49).

The increase in vascular permeability (clinically expressed as haemoconcentration, pleural effusion, ascites, and cardiovascular hypotension after fever deffervescence) characterises the severe syndrome. Molecular mechanisms involved in this syndrome are not well understood. Severe dengue has been associated with a second heterotypic dengue infection even after a long interval after primary infection (50,51). Although several sero-epidemiological studies support this observation (52), probably the “unique” epidemiological Cuban dengue situation best exemplifies the important role of the secondary infection as a risk factor for severity. In three dengue epidemics (DENV-2 in 1981 and 1997, and DENV-3 in 2001), severe cases occurred in individuals previously infected by DENV-1 in the 1977 epidemic who then had a second infection (53–56). Children suffering their primary infection during the 1997 and 2001 epidemics developed only a mild disease.

The phenomenon of antibody-dependent enhancement (ADE), whereby dengue antibodies at sub-neutralising concentrations enhance DENV infections in Fc receptor-bearing cells, was first proposed to offer a unifying basis to explain clinical, serological and epidemiological observations (36). After an initial period of cross-protection, cross-reactive antibodies waned to non-neutralising levels. These non-neutralising antibodies could mediate an increased uptake of viral particles through virus-antibody complexes, leading to increased viral replication and immune activation accompanied by cytokine release (57). Cytokines may play a direct role on the immunopathogenesis

of dengue. Their proinflammatory effects on vascular endothelial cells could lead to leaky junction and, consequently, to the increase of vascular permeability. Of interest is the association of higher viraemia to severe disease supported by several clinical studies (58–61). A complementary hypothesis explaining DHF/DSS involves the reactivation of cross-reactive memory T cells specific for the previous infecting virus resulting in a delayed viral clearance and an increase of cytokine production (62,63). A "tsunami of cytokines and chemical mediators" released from T cells, monocytes and endothelial cells has been associated with severe illness, with high levels of IL-10, TNF-alpha, IL-8, IL-12, IFN-γ and other cytokines found in the sera of patients (57,64–67).

Activation of complement is also involved in DHF pathogenesis since high levels of circulating C3a and C5a are observed in the plasma of severe patients. Although the mechanism of complement activation is not well known, it is assumed that complement is activated by the circulating immune complexes reported in patients. High levels of secreted NS1 and pre-existing cross reactive antibodies may mediate complement activation. Furthermore, infected monocytes and endothelial cells could activate complement via classical and alternative pathways (57,68,69).

Although the high cytokine production as a consequence of ADE and T cell activation could explain the vascular endothelial leakage and the increased capillary permeability observed during DHF/DSS, severe disease reported in infants with dengue-immune mothers cannot be explained by T cell involvement when infants suffer their primary dengue infection (70,71). Relatively recently, an autoimmune mechanism has been proposed. Some studies suggest that anti-NS1 antibodies cross react with platelets and endothelial cells, resulting in endothelial dysfunction and cytokine and complement activation (72,73).

Thrombocytopenia and bleeding also accompany the severe illness; however, the mechanisms involved are not well defined. Early bone-marrow suppression with peripheral platelet destruction has been postulated to explain the former (50). Recent studies support the key role of innate immunity in determining disease outcome. High levels of IFN-α and IFN-γ in response to DENV infection have been suggested to be associated with a protective host response. In addition, high levels of NK cells and activated NK have been related to mild illness (68,74).

A better understanding of dengue pathogenesis is needed for implications in drug and vaccine development. In particular, research on the innate and adaptive immune response in vivo and the molecular mechanisms associated with plasma leakage and bleeding (67,75) is important.

Vaccine development

The development of a safe and effective dengue vaccine is one of the public health priorities defined by the WHO (76). The development of a dengue vaccine involves several complexities such as the need to develop a vaccine against all four viruses, to avoid the ADE phenomenon, the poor understanding of the protective dengue immunity and disease pathogenesis and the lack of an animal model for vaccine evaluation. However, significant advancements have been observed in the last ten years. Currently, several vaccine candidates are in phase I and II clinical studies, and others are in advanced preclinical phase.

The main applied strategies include the traditionally and molecularly attenuated vaccines, chimeric live virus vaccines and DNA and recombinant subunit vaccines (77). The main concerns for live vaccines include the potential risk of enhanced illness after vaccination (if an adequate immune response to the four viruses is not simultaneously achieved) and the enhanced vaccine reactogenicity in persons with pre-existing anti-flavivirus antibody. On the other hand, subunit vaccines will probably require booster immunisations to maintain high levels of immunity.

The dengue vaccine pipeline appears to be sufficiently advanced (76). In preparation are population-based efficacy trials in exposed populations both in Asia and the Americas as the vaccine should be evaluated under different patterns of dengue transmission and circulating dengue viruses. Vaccine developers and the PDVI are working together to establish field sites for vaccine evaluation. Although protective immunity against dengue viruses is not completely defined, it is accepted that neutralising antibodies play an important role in protection against the viral infection. However, the role of the cellular immune response in the protection and recovery is not well known. Limited information is available to correlate immune response with disease outcome, so the definition of the correlates of protection is still a priority of research (57,77,78). As no animal model is available, it is urgent to define correlates of protection to allow the establishment of the efficacy of a vaccine candidate.

Control

Vector control is the only available method to control epidemics and prevent transmission, but a range of control strategies is needed to face the varying situations. However, until now, sustainability is the main problem. It is recommended that the application of integrated vector control strategies, including tools for reducing larvae and adult mosquito, be complemented with strong community and intersectoral participation.

At the end of the 1990's, the WHO established the Global Strategy for Dengue Prevention and Control (79), comprising five aspects: integrated vector control based on the community and intersectoral participation, active dengue surveillance, emergency preparedness, capacity building and vector control research. New tools for vector control include integrated vector management, the eco-health approach for dengue control and prevention (to improve community health) and the integrated management strategy for dengue prevention and control, *Estrategia De Gestion Integrada,* (EGI)/Dengue, with the objective to achieve a sustainable national strategy allowing a functional integration of actions. Additional control strategies include the Communication of Behavioral Impact (COMBI), the application of geographic information systems (GIS) to epidemiological and entomological studies, and others. More research on the development and evaluation of vector control tools and strategies and surveillance and response is needed (80,81).

Conclusions

Today, dengue is considered the most rapidly expanding arboviral disease in the tropics and subtropics and is now a serious public health concern. The re-emergence of Yellow fever, West Nile Fever and Chikungunya worsen the epidemiological situation (82). The last decade are marked by major advances and the implementation of several international initiatives (PDVI, The Innovative Vector Control Consortium, Asia-Pacific Dengue Partnership, DENFRAME and DENCO projects, others); however, more research is needed to improve the dengue situation (9).
Recognising the severity of this situation, the global dengue research agenda, discussed by the dengue expert group convened by the TDR/WHO at the end of 2006, provides a strategic plan for reducing dengue morbidity and mortality and its negative socioeconomic impact (Scientific Working Group. World Health Organization. Report on dengue 1-5 October 2006, Geneva, Switzerland. TDR/SWG/08) (21). It is expected that the integrated actions among countries based on the application of more advanced knowledge will positively impact dengue control and prevention.

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Author's contributions

All authors have contributed equally to drafting of the article and the critical revision.

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