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Review

New Perspectives on Dengue Pathogenesis

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Abstract

Dengue viral infections represent a major concern for public health. Yet, the mechanisms of dengue virus (DENV) pathogenesis are not understood very well yet, since there are no suitable animal models for studying the course of disease. The only source of knowledge is limited to clinical studies involving patients, which vary a lot and do not allow for the accurate understanding of the pathological events that occur during viral infection. Nevertheless, several factors seem to be related to DENV pathogenesis: i) viral factors, such as virulence and virus transmissibility and ii) host determinants like the immune response, immune status and genetic characteristics.

In this review we describe the factors that play an important role in dengue pathogenesis in order to have a better understanding of the disease and to allow for a more suitable therapeutic management of patients. Since the current disease classification used for determining risk factors during the course of a dengue infection is no longer congruent with the clinical studies performed, the use of the new dengue disease classification dictated by the World Health Organization (WHO) is suggested.

Keywords: dengue, virus, pathogenesis, severe dengue

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The dengue virus (DENV) has a worldwide distribution in tropical and subtropical regions.[1] it is endemic in many urban or city centers and its presence increases because of housing development in rural areas, which creates the ideal conditions for the reproduction of the main transmitter mosquito, the Aedes aegypti.[2] Worldwide, more than 50 million people are infected each year and 2500 million are at risk of being infected.[3] The DENV belongs to the Flaviviridae and four different serotypes have been defined (DENV-1, DENV-2, DENV-3 and DENV-4). The majority of the infections are subclinical. The most frequent clinical manifestation is classic dengue or dengue fever, which subsides spontaneously. The most severe forms are frequently related to an heterologous inmunopathologic response, or other host or viral factors.

The mechanisms of DENV pathogenesis are not well defined, because of the lack of appropriate animal models to study the course of the disease. Available only is patient data, which are very diverse and do not allow understanding of the pathologic phenomena that occur in the course of the infection. However, various factors[4] are related to DENV pathogenesis: 1) viral factors, such as virulence and viral transmissibility, and 2) host factors, such as immune response, immunologic conditions and its genetic characteristics.

During mid 70´s the World Health Organization (WHO) proposed a dengue severity classification with the purpose of aiding the diagnosis, patient management and monitoring of the disease.[5] The following concepts were defined:

1) **Dengue fever (DF) or classic dengue:** benign disease with an acute phase of 3-7 days of unspecific symptoms, such as high fever, headache, myalgias, arthralgias, maculopapular rash and, in some cases, moderate hemorrhage;

2) **Hemorrhagic dengue (DHF/DSS):** severe disease, with increase in vascular permeability and hemoconcentration, with an increase up to 20% of the normal hematocrit value and a count of less than 100 thousand platelets/mm^3. It was classified in four grades of severity: 1 and 2, without symptoms of circulatory failure, and 3 and 4, characterized by circulatory failure and hypovolemic shock or dengue shock syndrome (DSS). DSS can be

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Abbreviations: ADE, Antibody dependent enhancement; DC, dendritric cell; EC, endothelial cell; DENV, dengue virus; DF, dengue fever; DHF, dengue hemorrhagic fever; HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; CNS, central nervous system; DSS, dengue shock syndrome.

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fatal in 5-15% of the cases.[5] This definition has been questioned thoroughly, because in most countries that have dengue cases and hemorrhagic dengue, the clinical symptoms and the laboratory findings do not agree with the ones defined by the WHO and also, patients present variations due to the infection of different serotypes.[6]

In the present review the factors that could play a fundamental role in the dengue pathogenesis are exposed. Also, the new clinical classification of dengue dictated by the OMS is emphatically presented. This classification will help health professionals have a better understanding of the course of the disease, which will allow an adequate therapeutic approach of patients, and fewer deaths by the more severe forms of the disease.

Current hypothesis about dengue pathogenesis

Antibody dependent enhancement (ADE)

In vitro essays and epidemiologic studies link the secondary infection due to to DENV heterologous serotype (different to the one of the first infection) with severe disease.[7-11] In studies performed in Thailand incidence of DHF/DSS was observed to present mostly in two groups of children:[8-12] the first group constituted by neonates between 6-9 months of age, infected by a different serotype than the one that infected their mothers, and those in which the maternal antibodies had descended to subneutralizing levels; and the other group formed by children that were previously infected by a DENV serotype and then by a different one. These observations allowed to conclude that a subsequent infection in preimmunized people with a heterologous serotype could, through preexisting antibodies, exacerbate, instead of mitigating the disease. This phenomena is called antibody dependant enhancement (ADE),[8] based on the fact that in a primary infection neutralizing antibodies against the infective serotype are generated, but non neutralizing antibodies that react against heterologous serotypes are generated as well. The latter antibodies maximize a subsequent infection with an heterologous serotype, by enhancing the entry of the virus with Fc-γ receptors in monocytes and macrophages, achieving not only a larger number of infected cells, but also an increase in the viral replication in its target cell and, as a consequence, increasing vascular permeability.[11, 13-18]

An alternate or complementary hypothesis states that the viral entry to its target cell by means of Fc-γ receptors inhibits the antiviral immune response through the production of IL-6 and IL-10, and the transcriptional inhibition of the production of IL-12, TNF-α and IFN-γ, and as a consequence, an ideal environment that promotes viral replication is created.[19-20] Despite the clinical studies related to the ADE phenomenon, evidence is still circumstantial. Evidence that proofs otherwise has been observed in other dengue outbreaks. In 1972 a DENV-2 epidemic happened in a remote South-Pacific island, where no evidence of previous outbreaks were found.[21] Some of the infected adjusted to the definition of DHF and 12 fatal cases were observed; clearly, it was a primary infection in absence of preexisting antibodies against the virus that enhance the infection. Similar observations were made during an epidemic in Fiji in 1975, were no significant difference was found between the incidence of hemorrhage and other symptoms in primary infections, versus secondary infections.[22] Because of these data, Rosen questions the justification of dividing dengue in two clinical entities (benign and severe), as well as the validity of the WHO definitions of DHF.[23]

It is proposed that, as well as most infections, DENV behaves epidemiologically under the "concept of the iceberg",[6] that states that the presentation can go from an asymptomatic infection or a mild disease, in most of the cases, being this the base of the iceberg, to a severe disease and in some occasions a fatal disease, in a much lower percentage, being this the tip of the iceberg (figure 1). Similarly, it is postulated that the hemorrhage and the shock syndrome are not necessarily linked to the same pathogenic mechanism.[22] Another study that refutes the ADE phenomenon was made in Tahiti,[24] where risk factors for DHF were examined, after two consecutive epidemics by DENV-3 in 1989 and DENV-2 in 1996. The study based on the behavior of 401 hospitalized children (with variable ages) that had dengue. Ten of these cases were fatal. Using the WHO classification and other clinical and biologic criteria, 50 of the most severe cases were selected. Seventeen (34%) did not fulfill the requirements for DHF, because increase in vascular permeability was not detected. Of these patients, 6 died from hepatic disorders (elevated up to 20 times transaminases), severe thrombocytopenia (less than 20000 cells/mm^3), severe hemorrhage and shock. The study demonstrated that many cases of infection with DENV do not fulfill WHO´s definition for DHF, and a significant percentage of DHF cases are caused by primary infections.

The study of viral structure and the immunogenicity of DENV has demonstrated a relationship between the maturation state of the virion, the infectivity and the recognition by the antibodies.[25;26] It is suggested that various populations of functionally different virus coexist: a) a population of virions with high density of membrane precursor protein (PrMEM, viral protein that contains a precursor peptide, which needs to be sliced for the virion to mature appropriately), that represents immature viral particles and non-infectious, except if they are opsonized by anti-PrMEM antibodies, b) a population with intermediate density of this protein, which is infectious, but can be neutralized by anti-PrMEM antibodies, and c) a population of mature virions which can´t be neutralized with anti-PrMEM antibodies, but can be neutralized with antibodies against the viral envelope (E).[27] The study suggests that viral immature particles also play an important part in the enhancement and can also contribute to the possible development of the severe forms, because in a secondary infection they are capable of infecting target cells in the presence of non neutralizing antibodies.

Deviant lymphocyte T response

The reactivation of memory T cells that react with heterologous serotypes, can provide partial immunity. Nevertheless, it also may be the cause of immunopathology.[28] The pathologic finding of tissue damage as a result of cytolysis or inflammation, induced by an elevated number of effector T cells, is possible in DENV infections.[29-30] During the acute

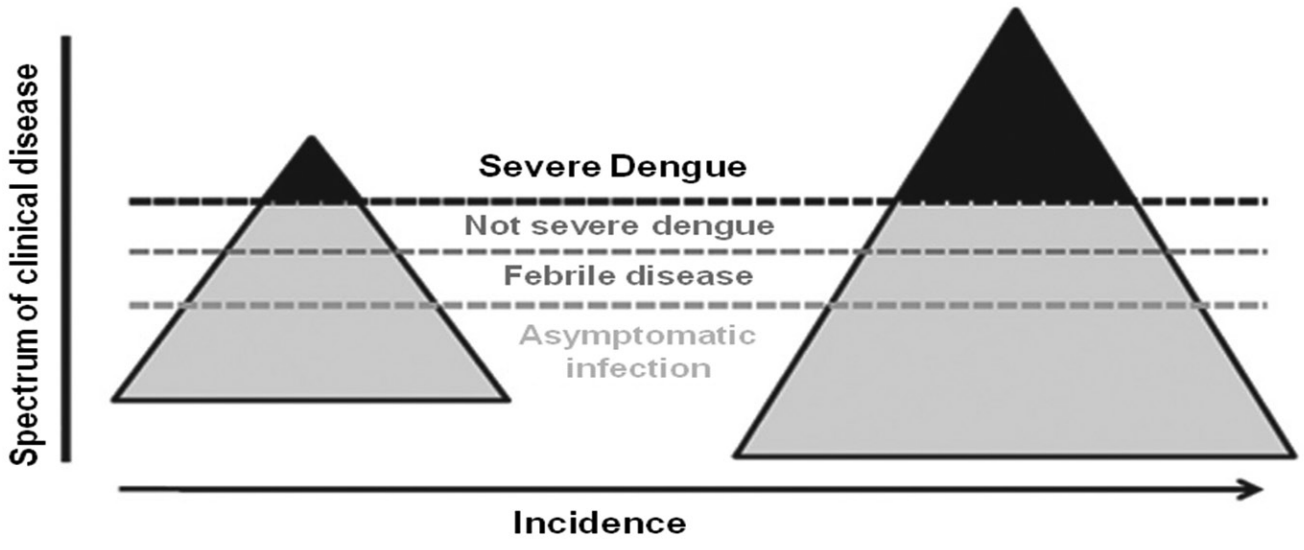

Figure 1. Concept of "iceberg" in dengue infections. The grave form of the disease represents the tip that emerges from the water level (in black). As the incidence increases (from primary to secondary infection), the cases of severe and mortal disease increase as well. (adapted from Desk Encyclopedia of Human and Medical Virology By Brian W. J. Mahy, Marc H. V. van Regenmortel)

phase of a secondary infection by an heterologous serotype, hyperactive CD8+ clones activate, which can produce an elevated concentration of pro and anti-inflammatory cytokines, like IFN-γ, TNF-α and IL-13 with low levels of IL-10.[31-32] a prolonged activation of CD8+ cells is maintained with elevated production of TNF-α, IL-6 and other soluble factors that affect the vascular permeability (read ahead in tropism and endothelial cells). These T cells react differently against heterologous serotypes and against homologous epitopes.[33] Also, they lose their cytolytic capacity, which would explain the delay in the viral elimination during a secondary infection. Nevertheless, it is possible that during a DENV heterologous infection, only a small subpopulation of T cells are sero-crossed, and this combined with the fact that every human being has a repertoire of T cell specific receptors, could explain la great variability in the presentation of the disease after a secondary infection.[4]

Little is known about the response of CD4+ T cells during a DENV infection and the involvement of HIV coinfection. It has been reported that during an acute dengue infection, the HIV viral load in an infected patient decreases.[34] This obeys to the expression of NS5 (one of the non structural proteins of dengue virus), that decreases the expression of CD4 on T cell surface, hence, inhibits the infection and replication of HIV.[35,36] The decrease of CD4 on the T cell surface could also damage the helper function of this cells and, potentially, delay or avoid the development of an effective adaptative response. Similarly, HIV triggered pathogenesis could be diminished, decreasing the signal necessary to activate the T cell receptor and inhibiting cell proliferation.[36] Also, during a sequential infection with different DENV serotypes, there is evidence of alteration in the response of CD4+ produced cytokines that sero-cross, inducing high levels of proinflamatory cytokines,[37] which jointly with CD8+ cell cytokine response, can cause adverse effects in the immune response.

DENV Tropism

Target cells and DENV tropism play an important part in the outcome of dengue infection. There is no concluding data about which are the target organs in vivo. However, in vitro data and some autopsies suggest that three systems have a fundamental role in DHF/DSS:

a) **Immune system:** DENV infection occurs because of the bite of a mosquito through the epidermis and dermis. In this way, infected cells are immature Langerhans cells (epidermal dendritic cells) and keratinocytes.[38,39] Infected cells migrate from the infection site to the lymph nodes, were macrophages and monocytes are recruited, both becoming the target of the infection. The virus spreads through the lymphatic system. As a result of the first viremia, a population of mononuclear descent cells are obtained, such as monocytes, myeloid dendritic cells (DC) and infected liver and spleen macrophages.[40-44] Furthermore, during secondary infections with heterologous DENV, a high concentration of a complex between the new virus and immunoglobulin G (IgG) is observed. These immunocomplexes are phagocytized by mononuclear cells. Most of these cells die by apoptosis,[45-46] while the nearest DC are stimulated and produce most of the mediators related with the inflamatory[44,45,47-49] and hemostatic[50-52] host response. The quantity of infected cells and, therefore, the viremia level, could be the determinants of pro-inflammatory and anti-inflammatory cytokine relation, as well as the level of chemokines and other mediators.[41]

b) **Liver:** cases of hepatitis with necrosis, steatosis and Councilman bodies (probably apoptotic cells) have been reported in association with DENV.[53,54] Also, the tendency towards graveness because of DENV has been related to the elevation of liver enzymes.[55,56] Although DENV has been detected in a significant population of hepatocytes and Kupffer

cells, there is no evidence of inflammation in the liver. This suggests that the apoptosis and necrosis observed are caused directly by the virus and not by inflammatory mediators. The prevalence of apoptosis is higher than necrosis and this could explain la small amount of inflammation observed in the area. Nevertheless, the role of the hepatic damage in regard to the coagulopathy and the severity of the disease, has yet to be well established.

c) **Endothelial cells (EC)** that line capillary vessels: the integrity of cellular epithelium is regulated by many factors that also play an important role in the coagulation response in cases of severe inflammation. Tropism of DENV towards EC in vivo is still very controversial. Some preliminary studies of skin biopsies indicate that the microvasculature of the dermis is the most affected site, although no DENV antigen has been detected in the EC that surround the microvasculature.[57,58] On the contrary, there is evidence of DENV antigen in the lung´s endothelial vasculature,[43] despite that this does not necessarily means active viral replication. Contrary to mononuclear cells, EC do not have Fc-γ receptors and immune complexes are not internalized. And so, the presence of virus in these cells could only be explained by pinocytosis. Replication of the four DENV serotypes has been demonstrated in vitro in EC, and the consequence of this infection tends to generate more functional damage than morphologic damage.[59,60] There is no evidence that viral susceptibility varies between vascular systems, but it is proposed that the coagulation response in a severe EC inflammation varies in different parts of the organism.[61] Similarly, DENV´s infection pattern in microvasculature cells is different, which suggests that different tissues have different activation patterns.[62] It has been demonstrated that the increase in peripheral microvasculature permeability occurs in patients with DHF as well as DSS.[63] Therefore, pulmonary and abdominal EC could react specifically to a DENV infection,[64] explaining the vascular effusion syndrome characteristic of DHF/DSS. Studies suggest that the damage or vascular dysfunction is fundamental in the pathogenesis of these severe forms of DENV infection.[65-68] There is selective apoptosis of endothelial cells of the microvasculature in lung and abdominal tissue, especially in the fatal cases,[69] explaining the intense vascular effusion in the pleura and peritoneal cavities. It is interesting to emphasize that the non-structural protein 1 (NS1) of DENV unites preferably to EC in the lung and liver.[70] The union of NS1 to its specific antibody could contribute to the selective effusion in the lung.

Lack of an animal model that mimics completely the severe disease of dengue, has allowed the physiopathology to be inferred mostly from in vitro studies, using mostly endothelial cell lines, like the HUVEC.[71] These cells can be infected by the dengue virus, but in culture their trans-endothelial barrier function is compromised, making them an inappropriate model to study vascular permeability induced by the virus. HMEC-1 cells, derived from human dermic microvasculature, have been used for this purpose because they maintain their endothelial functions and barrier functions in culture, because of the stability of the proteic complexes that form their tight junctions.[72] During infection with dengue virus, a loss of continuity in the localization of occludine (protein of tight junctions), which coincides with an increase of permeability to diverse molecules of different sizes. Also, the interaction of the actin cytoskeleton with the components of the tight junctions works as a significant modulator of endothelial permeability.[73] During dengue infection, disorganization and fragmentation of the actin fibers is observed, increasing the endothelial permeability. On the other hand, cytokines modulate the organization of the cytoskeleton and of the proteins that form intercellular unions. Dengue produces IL-8 secretion in HMEC-1, which agonistically united to other factors, causes the reorganization of the cytoskeleton. In summary, the direct effect of the dengue virus on the tight junctions and the cytoskeleton, together with IL-8 release, induce enough structural modifications that could be important in the alteration of the endothelial permeability[72] and responsible of the plasmatic extravasation.

Virulence

During the 70´s, Rosen and Gubler performed epidemiologic and entomologic studies in the Asian South Pacific, and described for the first time differences in the virulence of dengue.[74,75] They noticed that some outbreaks on this region had less or no cases of DHF, considering the virus transmitted as a low virulence virus. Other outbreaks observed had many cases of DHF after a primary infection, and so these viruses were considered highly virulent. The development of DNA sequencing methods leading to copying of viral RNA and the generation of phylogenetic trees, has demonstrated that some groups of variants or genotypes are related more frequently with more severe disease.[76-80]

The most important reason for this genetic variability and its clinical outcome is exemplified by the American genotype of DENV-2 (for example the strain Trinidad/53), that is not associated to the severe forms of the disease.[81] These facts imply that it constitutes a low virulence genotype.[80] For example, during the 1995 epidemic in Iquitos (Peru), close to 50000 secondary infections presented. Based on previous projections (with projection models of severe clinical presentations of the disease in Thailand), the severe cases in Iquitos should have been from 900 to 10000, however, no severe case was reported.[82] On the other hand, the first epidemic of DHF in America occurred in 1981 in Cuba, and it coincided with the entrance of the most virulent DENV-2 genotype of Southasian origin.[79;80;83] The American genotypes present different characteristics from the southasian strain in their capacity of causing severe forms of the disease.[84] These viruses differ in the aminoacid E-390 (a virulence determinant), in the ability of replicating in macrophages[85] and in the sequence and secondary structure o RNA in the region 3´UTR.[86] It has been proposed that the American strains are less capable of replication in A. aegypti, than the asian virus, making them less transmissible.[87]

Analysis of genotype or individual populations has not confirmed that the viral isolates from patients with the severe form of the disease are different from the ones taken of patients with dengue fever.[88,89] However, genotype IV of DENV-3 has been associated with moderate forms of the disease;[90] certain strains associated with severe forms have demonstrated higher infectivity in monocytes[91] and also, certain strains of DENV-2 differ in their capacity of infecting different types of human cells.[92]

It has also been proposed that during the 1981 Cuban epidemic, the virus evolved to more virulent genotypes responsible for the severe disease with the transmission between hosts, and for this reason, the mortality rate increased at the end of the epidemic.[83] A similar situation occurred in 1992 during a DENV epidemic in Australia,[93]and again in Cuba in 1997.[14,94] Analysis of genomes of DENV genotypes have demonstrated that these could evolve during an epidemic,[95,96] but more studies are needed to prove that some virus evolve to more virulent forms.

The sequence of infection with the different serotypes has been proposed as an important factor in the severity of the disease by dengue virus. Epidemics with a high incidence of DHF have been related with a primary DENV infection, followed by an infection of DENV-2 or DENV-3.[97,98] Such studies also proved that the longer the time interval between a primary and the secondary infection, the higher the risk of developing the severe disease.

Age has also been postulated as a risk factor in a secondary infection with heterologous DENV.[15] It has been demonstrated that DENV in different geographic areas varies in its ability to infect different types of cells in vitro, or causing severe disease in humans.[18,92] Despite this, the observation that DHF/DSS presents in a relatively low percentage in secondary infections and much less in primary infections (although the infection is because of virulent strains), suggests that the host factors are determinant and critical in the development of the severe disease.

Activation of the complement system

One of the fundamental components of the innate immune humoral response is the complement system, which interacts with the homeostatic system to provide the first line of defense against pathogen infection. In DENV infection it has been reported that during the period of fever decline, when vascular permeability increases, elevated plasma levels of products of complement activation (C3a and C5a) are detected, followed by hypocomplememtemia in patients with DF and DSS.[99,100] Therefore, it is postulated that complement activation has a fundamental role in the pathogenesis of dengue. Also, studies about genic expression in mononuclear cells of peripheral blood (of patients with DF and DHF/DSS) suggest their connection with the severity of the disease.[101] During DENV´s RNA replication, one of the non-structural proteins encoded by the virus is released: NS1. It is postulated that this protein not only activates directly the complement system,[102] but also activates the immune complexes formed between NS1 protein and the non-homotypic antibodies, that activate the classical pathway of the complement.[103] Complement activation produces the complex C5b-C9, that triggers cellular reactions and stimulates pro-inflammatory cytokine production, associated to the development of DHF/DSS. This attack membrane complex can activate other local and systemic mechanisms involved with disseminated intravascular coagulation (DIC).[104]

Temporary autoimmunity

It has been shown that during a DENV infection, there is production of antibodies that can sero-cross with some host antigens. Although, it is not clear if this phenomenon occurs only during a secondary infection or also during the primary infection. Some antibodies that recognize a linear epitope in the viral E protein of the envelope can also unite to human plasminogen and inhibit plasmin activity.[105-107] Anti-NS1 antibodies that sero-cross with EC can trigger nitric oxide (NO) production and hence induce apoptosis.[108] Although it has been demonstrated that NO inhibits DENV replication,[109] its excessive production causes cellular damage. Anti-NS1 antibodies can also stimulate expression of IL-6, IL-8 and intracellular adhesion molecule 1 (ICAM-1).[110] More studies are necessary to prove that the crossed reaction between anti-NS1 with EC leads to an increase in vascular permeability, characteristic of DSS. Also, it was reported that anti-NS1 can sero-cross with platelets and cause temporary thrombocytopenia and hemorrhage,[111] which demonstrates that these anti-platelets antibodies are pathogenic.

Host genetic factors

Significant differences have been observed, individually and among populations, with the gravity of a DENV infection. Epidemiologic research indicates that some genetic factors can be important components of infection susceptibility. Some human alleles of HLA class I and II[112-116] have been related with the development of DHF as well as polymorphism in genes that encode for TNF-α,[117] Fc-γ receptors[118], vitamin D receptor,[118] amongst others. Some variants of glucose-6-phosphate dehydrogenase (G6PD) also contribute to an enhanced monocyte replication.[119] The risk of developing a more severe disease could be determined, however, by a combination of host genetic factors and not by individual polymorphisms.[120] Studies in patients that develop DHF or DSS, could help to identify more polymorphisms or defects in unique genes that could predispose to the development of more severe diseases.

Modulation of the interpheron´s response

Dengue virus is detected by cells with Toll-like receptors (TLR) and the intracellular receptors, producing a response mediated by INF-α and INF-γ.[44,121] IFN works with infected and non-infected cells stimulating JAK-STAT signaling cascade, causing the activation of specific genes that establish an antiviral status.

Some non-structural viral proteins are capable of modulating interpheron response[122] against dengue virus. The importance of this process lies in that the modulating of the response translates to elevated viremia levels or exacerbated viral propagation, despite the existence of a proper early immune rersponse.

New criteria for the classification of dengue caused disease

Independently of the classification of the disease caused by dengue virus, severe dengue associates to certain manifestations that include hemorrhage or,[123-126] hepatic alterations,[127,128] CNS manifestations[128,129] and shock syndrome.[123,124,126] Although hypovolemic shock associated to an increase in vascular permeability is an obvious manifestation of severe disease,

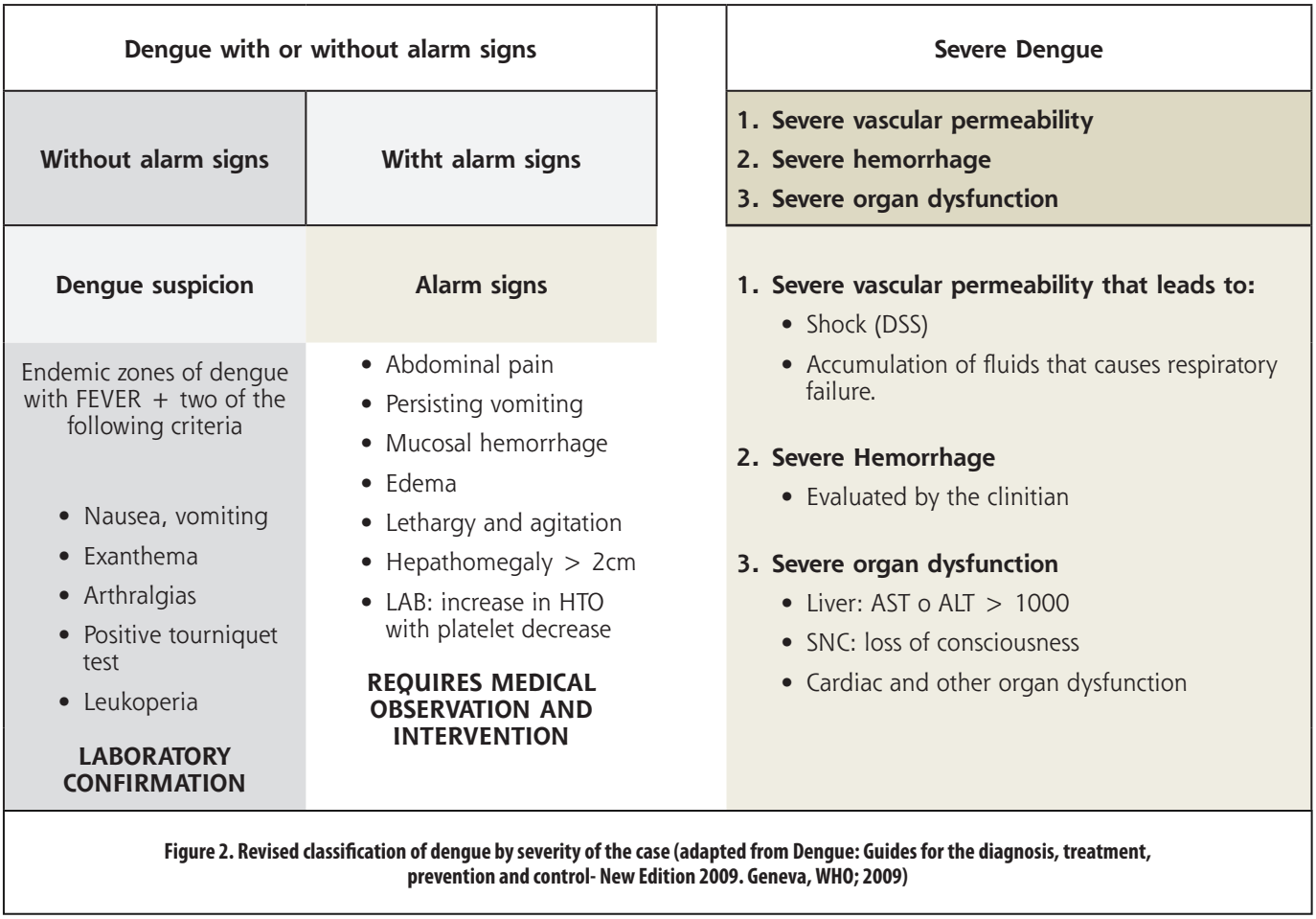

Figure 2. Revised classification of dengue by severity of the case (adapted from Dengue: Guides for the diagnosis, treatment, prevention and control- New Edition 2009. Geneva, WHO; 2009)

this phenomenon can occur without thrombocytopenia and hemorrhage.[123,126] Many studies show that the clinical determination of plasma leakage during the acute phase of the disease is very difficult, when symptoms of effusion are absent and the value of hematocrit is normal.[125] As a consequence and using the previous classification by the WHO, clinicians tend to classify all severe cases as DHF. This leads to confusing estimates of the incidence and even erroneous concepts about the pathogenesis of the disease. For these reasons it is suggested that the previous definitions by the WHO for DF and DHF are both inadequate and disorientating.[130,131] It is suggested that an alternative approach for the classification of the cases could be "severe" vs "not severe", and that the severe cases should include the whole range of manifestations including shock with or without evidence of plasmatic effusion, manifestations of liver failure, CNS, hemorrhage and thrombocytopenia. So, the definition of shock due to plasma loss would be simple and practical for the diagnosis and management of the patient, independently from thrombocytopenia and hemorrhage, and very adequate in countries were dengue is endemic or epidemic.

New clinical multicenter prospective studies have been performed by the WHO, with the purpose of redefining the categories of the disease to standardize the clinical guidelines to follow.[3,132]

Therefore, this disease is being classified with levels of severity: dengue with or without the presence of alarm signals and severe dengue (figure 2), based on clinical and laboratory data. It must be remembered that even patients with dengue without alarm signals can develop severe symptoms. This classification with levels of severity has a high potential to help clinicians make decisions as to where and how intensely a patient has to be observed and treated, and has demonstrated to be more effective than the DF/DHF/DSS for a rapid recognition of severe disease.[132] However, training is required, spreading of information and more investigating on the clinical manifestations of the alarm signals and the definition of the clinical cases in absence of laboratory data.

Consequences for prevention and control

Although dengue has been attempted to be controlled and prevented via vector vigilance, better medical management, better education and better information; the main prevention method would be a vaccine. However, a secure and effective vaccine against this disease becomes difficult because of the important role of the immune-pathogenesis. An essential requisite of the vaccine would be to induce prolonged protection against the four serotypes of dengue, as incomplete immunity could influence the development of severe disease in vaccinated people. Another source of concern is the lack of clear protective determinants against the disease and the absence of a robust experimental model that mimics observed pathogenesis in humans. However, a more appropriate definition of the causes of dengue´s pathogenicity would assist the formulation

of effective candidates for the vaccine and the development of antiviral agents.

Note: the authors of the present article don't have any conflicts of interest.

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