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Recent advances in dengue pathogenesis and clinical management

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ABSTRACT

This review describes and commentates on recent advances in the understanding of dengue pathogenesis and immunity, plus clinical research on vaccines and therapeutics. We expand specifically on the role of the dermis in dengue virus infection, the contribution of cellular and humoral immune responses to pathogenesis and immunity, NS1 and mechanisms of virus immune evasion. Additionally we review a series of therapeutic intervention trials for dengue, as well as recent clinical research aimed at improving clinical diagnosis, risk prediction and disease classification.

1. Introduction

The underlying mechanistic causes of the dominant clinical features of severe dengue, i.e. a transient increase in vascular permeability and a hemorrhagic diathesis, remain enigmatic. In principle, acquiring deeper insights into the mechanistic drivers of the clinically important features of dengue should enable improved treatment strategies and uncover novel drug targets. Yet neatly dissecting the pathogenesis of any infectious disease syndrome is never straightforward and dengue is no exception. Host and virus variables shape the clinical outcome of any given dengue virus (DENV) infection. For the host, there is undoubtedly a physiological and immunological component (humoral and cellular) that influences whether infection (or re-infection) has a benign outcome or results in disease that manifests across a gradient of severity. There must also be a virological aspect, such that some viruses are simply better equipped to replicate and reach high titers in a human (or mosquito) host. Neither of these processes work in isolation. Rather, the outcome of exposure to an infectious *Aedes* mosquito will always depend on a constellation of "positive" and "negative" host and viral factors that each influences the overall clinical evolution of the infection. Layered upon this are the benefits that careful clinical management can have in preventing or treating the life-threatening complications that may occur; in many endemic countries improvements in clinical management mean that case fatality rates amongst hospitalized cases have been reduced from 20% to less than 0.5%. Yet there is still room to do better. This might be via development of more effective, evidence-based fluid resuscitation strategies for cases with severe shock, or the arrival of specific anti-viral therapeutics or disease modifiers able to reduce the duration and/or severity of generalized symptoms in the tens of millions of uncomplicated dengue cases that occur globally. Central to all considerations of patient-centered research findings in dengue is an understanding of the temporal "windows" that exist in the evolution of disease. These "windows" are represented schematically in Fig. 1. Beyond all else, they underscore the dynamic nature of disease evolution and that clinical research studies must always consider timing of interventions, observations and sampling in their design and reporting.

Following the bite of an infected mosquito the virus disseminates and infects multiple lymphoid and non-lymphoid tissues. A viremia ensues that is presumed to be a proxy for the underlying severity of tissue infection. The viral burden accumulates to the point that generalized clinical symptoms (fever, headache, myalgia) develop, presumably secondary to a host antiviral state in which interferon expression is abundant. Viremia peaks shortly

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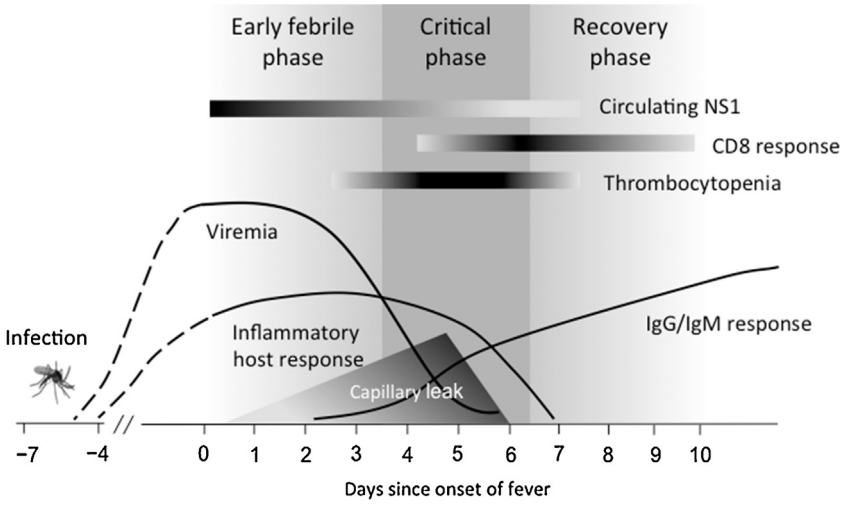

Fig. 1. Schematic showing the clinical phases in the evolution of dengue.

after fever onset (day 0) and then plateaus for 1–2 days before gradually declining, driven by the host adaptive immune response. By the 4–6th day of illness the fever breaks in most patients and symptoms recede. Increased capillary permeability, often subclinical, is measurable in many patients during the critical phase, with likely onset during the febrile phase. The increased permeability, coagulopathy, thrombocytopenia and other laboratory derangements are most pronounced around the time of defervescence, and this is the timepoint where most of the severe complications manifest. During the recovery phase there is gradual normalization of clinical and laboratory features. Activated T cells and increasing concentrations of DENV reactive IgM and IgG are detected toward the end of the febrile phase and during the recovery phase.

2. Pathogenesis

2.1. Initiation of DENV infection: is the dermis an important site of virus infection?

Since the first description of DENV infection of dendritic cells (DCs) in cadaveric human skin explants [1], there has been growing interest in this aspect of the DENV-host interaction. Recent research interest has focused on DENV infection of dendritic cell populations (DCs) that are resident in the skin, plausibly the first anatomic location where DENV interacts with key actors in the human immune system when the virus is delivered via the probing of an infected mosquito. *In vitro*, Cerny et al. [2] challenged single cell suspensions derived from human skin with cultured DENV and detected productive infection of CD14(+) and CD1c(+) DCs, Langerhan cells and dermal macrophages. Of these, Langerhan cells supported the highest virus growth titers. Parallel findings were made in intra-dermally infected mice, where DENV-infected dermis DCs migrated to the skin-draining lymph nodes. In contrast, infection of lymph-node-resident DCs was negligible. These data indicate variability in the range of human skin antigen presenting cells that can be infected with DENV and highlight the potential double-edged sword of infected cells trafficking to the draining lymph nodes; this process enables an adaptive anti-viral immune response but plausibly helps facilitate systemic spread of DENV. Consistent with these previous data, Schmid et al. [3] used a murine model to identify that DENV infection occurs in resident dermis DCs and macrophages, followed by infection of monocytes and moDCs that are recruited from the bloodstream to the dermis, probably via chemoattractant molecules released at the site of infection. Collectively these data help to define the permissiveness of dermis-resident antigen presenting cells to DENV infection, but its uncertain if these artificial experimental systems reflect the natural history of DENV infection of humans. Thus, whilst some level of dermis infection may indeed be a common outcome after successful (or unsuccessful) probing by a female *Aedes* mosquito, there is no evidence that it is a *pre-requisite* for the systemic infection that characterizes dengue. Instead, direct introduction of infectious virions into the lumen of capillaries that are expertly breached by the mosquito proboscis, followed by almost immediate systemic spread of virus and infection of permissive cells in the largest vascularized lymphoid tissue, the spleen, seems a more parsimonious explanation for how successful, systemic DENV infection is initiated in most cases.

3. Recent advances in understanding cellular immune responses to dengue virus infection

3.1. The role of T cells

T cells, and in particular cross-reactive memory T cells recalled during secondary heterotypic infections, have been nominated as contributing to the clinical pathogenesis of dengue. This hypothesis is based on the observation that T cells having surface and functional phenotypes indicative of antigen-driven activation are more abundant in early convalescence in children/adults with severe dengue versus those with milder disease [4–6]. T cell responses cannot however explain the pathogenesis of severe dengue in infants with primary infection and thus there is particular context to the "T cell hypothesis". Stronger evidence, for or against, a mechanistic role for DENV-reactive memory T cells in the severe clinical complications of dengue is needed to help guide therapeutic intervention strategies. Similarly, a better understanding of how T cells might contribute to protection from re-infection is needed for the advancement of vaccine development and identification of immune correlates. Recent advances in understanding the targets of DENV-specific T cell responses, their functional phenotypes and their tissue tropisms goes some way to providing the tools to acquire stronger mechanistic insights into their role in pathogenesis and immunity.

3.2. CD8+ T cell responses: their targets and phenotypes

Substantial new data has been acquired on the targets of T cell responses after natural infection. Rivino et al. [7] confirmed and expanded upon previous work in determining that CD8(+) T cell

epitopes are predominantly located in the nonstructural proteins NS3 and NS5. Similarly Weiskopf et al. [8] identified DENV-reactive CD8+ T cell responses in Sri Lankan blood donors; 408 immunoreactive peptides were identified, two thirds of which were located in NS3, NS5 or NS4b. Finally, following tetravalent vaccination with live attenuated DENV, Weiskopf et al. [9] demonstrated CD8+ T cell responses were universally (99.8%) against non-structural proteins, with 97% directed toward NS3 and NS5. Collectively, these data underscore the importance of DENV non-structural proteins as CD8+ T cell immunogens during natural infection; this remains an important consideration for vaccine strategies that, along with induction of neutralizing antibody, also seek to induce CD8+ T cell memory.

Whether DENV-reactive CD8+ T cell responses contribute mechanistically to vascular leakage, the hallmark of severe dengue, remains unresolved. Based on observations of HLA-A*11-restricted CD8+ T cell responses to the $NS3_{133–142}$ epitope, Mongkolsapaya et al. [10] have suggested original antigenic sin occurs amongst CD8+ T cell populations during secondary heterotypic infection. Yet these conclusions have been drawn from observations made in early convalescence, when CD8+ T cell responses peak, and not during the febrile and critical phase, when vascular leakage commences and is most prominent. Indeed, Dung et al. [11] reported that $NS3_{133–142}$-specific T cells were undetectable until after the development of plasma leakage among infected Vietnamese children. In contrast, Freiberg et al. [12] detected very low frequencies of activated $NS3_{133–142}$-specific CD8+ T cells during the febrile phase in both primary and secondary dengue cases, but found no evidence that these responses correlated with immune status exposure or current disease severity. Although traditionally measured in the blood, Rivino and colleagues [13] also identified highly activated and proliferating $NS3_{133–142}$-specific CD8+ T cells in experimentally induced skin blisters on dengue cases. Whether these dermis-infiltrating CD8+ T cells are relevant to pathogenesis of the clinical and laboratory features of dengue is uncertain.

The next phase of research into CD8+ T cells and pathogenesis needs to take an expansive approach to measuring epitope-specific T cells (e.g. using panels of Class I tetramer reagents, targeting more than one specificity) and investigate responses longitudinally in the three phases of disease; early febrile, critical phase and convalescence, with sample sizes large enough to capture the spectrum of clinical outcomes, from the very mild to the severe. These are challenging studies to perform but are necessary if the field is to advance the understanding of CD8+ T cells in pathogenesis and immunity.

3.3. CD4+ T cells responses: their targets and phenotypes

In contrast to CD8+ T cell responses, DENV-specific CD4+ T cells have been less well characterized. In patients with secondary dengue, Rivino et al. [7] demonstrated that CD4+ T cell epitopes are predominantly located in structural proteins e.g. envelope, capsid, and NS1, which themselves are major targets of the B cell response. Circulating CD4+ T cells during early convalescence had the surface and functional phenotype of follicular helper T cells, suggesting that they are interacting with B cells *in vivo*, presumably to assist antibody production. Whether the size of the acute expansion of the follicular helper T population in blood is predictive of the anti-DENV neutralizing Ab titer deserves investigation as a possible immune correlate of vaccine or infection-driven humoral immune responses. Mangada et al. [14] examined CD4+ cells in 6 donors who had received monovalent live attenuated DENV vaccine 12 months prior. Stimulation with heterologous serotype peptide resulted in more TNFα-producing cells than IFNγ producers relative to stimulation with homologous peptides, suggesting differential functional phenotypes amongst these cell populations that are dependent on the type of antigenic stimulation. This repeats a theme also evident in studies of CD8+ T cells, that partial peptide agonists elicit a diverse range of functional phenotypes in cross-reactive T cells [15]. In a mouse model, Yauch et al. [16] identified that DENV2-specific CD4+ T cells were of a Th1 phenotype and could mediate *in vivo* cytotoxicity and that immunization with dominant CD4+ T cell epitopes led to enhance viral clearance. Whether results in murine models are informative for understanding human immunization or disease remains uncertain, but these results underscore the need for further investigations of CD4+ T cells in dengue pathogenesis.

3.4. Mast cells

Mast cells (MCs), whilst traditionally associated with hypersensitivity responses, express a wide range of Fc receptors and hence are candidates for involvement in dengue pathogenesis. St John et al. demonstrated in a mouse model that MCs are activated *in vivo* during experimental DENV infection and MC-deficient mice had greatly reduced vascular leakage compared to MC-sufficient controls. Treatment of experimentally infected animals with MC-stabilizing drugs also ameliorated vascular leakage [17]. In humans, MC-derived vasoactive products such as chymase, a serine protease, are elevated in the peripheral blood of primary and secondary dengue cases [17,18]. As attractive as MCs might be as "actors" in the pathogenesis of severe dengue, there remain critical questions. For example, to explain the clinical observation that secondary heterotypic DENV infection is associated with greater risk of severe disease, the "MC hypothesis" requires some level of Ab-Ag, (e.g. viral particles or NS1) to trigger Fc receptor aggregation on MCs and their activation/degranulation; this has yet to be shown in a convincing fashion. Moreover, the timing of MC activation would need to occur in a manner temporally consistent with the evolution of vascular leakage, i.e. most pronounced leading up to the time of defervescence in severe cases [19]. Thus, whilst MCs are intriguing, further clinical studies, possibly even drug probe studies, are needed to better understand their contribution to human disease.

3.5. Humoral immunity

Antibodies are believed to be critical mediators of resolution of infection, immunity to reinfection and in some circumstances, are believed to be risk factors for severe disease, i.e. antibody dependent enhancement (ADE) of infection. Recent results of phase IIb and III clinical trials of Sanofi Pasteur's recombinant live attenuated tetravalent dengue vaccine (CYD-TDV) have questioned the decades-old assumption that plaque reduction neutralization assays ($PRNT_{50}$) are good predictors of immune status. Specifically, CYD-TDV elicited tetravalent virus neutralizing antibodies after three doses yet offered very low levels of efficacy against DENV-2 and modest efficacy against DENV-1 [20,21]. That the relationship between $PRNT_{50}$ titer and immunity to DENV-2 is complex were reinforced by Buddhari et al. [22] who utilized data from geographic cluster studies in Thailand. They found an overall significant positive association between baseline $PRNT_{50}$ titers to DENV-1, -2 and DENV-4 and immunity from homotypic symptomatic infection during the follow-up period. However the threshold for defining immunity to DENV-2 was unclear and might be higher than for other serotypes. Thus, traditionally accepted $PRNT_{50}$ values (i.e. titer >10) regarded as denoting "immunity" to DENV may not be accurate, especially so for DENV-2. This has provided motivation to the field to explore alternative correlative assays. This is being enabled in part by productive basic research that has identified new, highly potent virus-neutralizing human mAbs elicited by natural infection. Mapping the epitope targets of these mAbs has revealed many target quaternary epitopes on the virus surface [23–25]. Some are serotype specific (e.g. 14C10 targeting DENV-1 [26] and 5J7 that targets DENV-3 [24]) whilst others are

cross-reactive (e.g. the so-called Envelope Dimer Epitope mAbs B7 and C10) [27]. The existence of rare but potently neutralizing, cross-reactive human mAbs elicited by natural infection is provocative. Potentially, such Abs, and their epitope targets, could be deployed for pan-serotype therapeutic indications or vaccine development efforts. Additionally, these mAbs could be used for development and/or validation of 2nd generation virus neutralization assays that replace or augment the $PRNT_{50}$ measurement. For example, 2nd generation assays might compete individual or panels of highly potent virus neutralizing mAbs with polyclonal sera from vaccinees for binding to viral particles or recombinant proteins and thus determine a titer of antibodies in polyclonal sera specific for the critical epitope regions on the virus. Finally, for vaccine development, it is likely that strategies employing DENV envelope protein subunits as immunogens will fail to elicit some of these quaternary epitope binding immune responses; whether this significantly reduces the probability of success of this vaccine strategy is unknown.

3.6. Immune subversion

A recent expert review has reported the latest mechanistic understanding of how DENV manipulates intracellular antiviral responses and directly inhibits cellular signaling cascades in order to favor its own replication [28]. Here, we will focus on the role of lipids, and induction of the autophagy and endoplasmic reticulum (ER) stress pathways during DENV infection. Intracellular replication of DENV occurs on the cytoplasmic side of remodeled ER membranes and requires sufficient lipid resources from the host cell to enable efficient encapsidation and assembly of virions.

Recent evidence suggests lipid droplets and the autophagy pathway positively contribute to the pool of lipid resources that enables DENV genome encapsidation and assembly [29,30]. Hence autophagy, sometimes regarded as a biochemical arm of the innate anti-viral immune response, actually benefits DENV replication. Additionally, cholesterol has been shown to play a pivotal role in the replication cycle of DENV and other flaviviruses, contributing not only to efficient replication but also to immune evasion [31–33]. Although, the mechanism by which dengue regulates lipid homeostasis and recruitment for these purposes is not currently understood, the membrane remodeling protein NS4A appears to be a likely candidate [34,35]. In addition to autophagy it is observed that all flaviviruses modulate the ER stress/unfolded protein response. DENV, JEV and WNV all activate Xbp-1, ATF6 and IRE1 regulatory factors to promote cell survival and immune evasion [36–41]. Although the exact underlying molecular and functional aspects of this induction are not currently understood it is pertinent to highlight that Xbp-1 can promote lipid biosynthesis [42], IRE1 is involved in regulated RNA decay pathway promoting anti-viral defense [43] and ATF6 can regulate activation of innate immune responses and cell death [44]. Thus there appears to be an integrated involvement of autophagy and ER stress/UPR modulation to promote lipid balance, intracellular replication and survival. Given the seemingly central role for these cellular responses in DENV replication key enzymes or by-products within these pathways are attractive candidates for antiviral therapeutics.

3.7. Recent advances in understanding virological determinants of DENV transmission

Whilst humans are dead-end hosts for many *Flaviviruses*, the dynamics of human-to-mosquito transmission of DENV has been central to its successful emergence. Accumulated data from empirical infection studies on human subjects conducted in the first half of the twentieth century showed that humans can be infectious to mosquitoes from 1.5 days prior to the onset of symptoms to around 5 days after the commencement of symptoms [45–49]. Recent studies have quantified the factors shaping transmission to either *Aedes aegypti* or *Ae. albopictus*. In infected humans, the concentration of virus circulating in the blood, and the duration that it circulates, influences the likelihood of a permissive *Aedes* mosquito becoming infected after imbibing a blood meal [50,51]. Nguyet et al. [50], and separately Whitehorn et al. [51] experimentally measured the plasma viremia characteristics in Vietnamese adult dengue cases that led to DENV infection of directly blood-fed *Aedes aegypti* or *Ae. albopictus*. For *Ae. aegypti*, the plasma viremia required to infect 50% of mosquitoes differed between serotypes and was ~10-fold lower for DENV-1 and DENV-2 (6.29 or 6.51 log10 RNA copies/ml) than for DENV-3 and DENV-4 (7.49 or 7.52 log10 RNA copies/ml). For *Ae. albopictus* the 50% mosquito infectious dose was highly similar to that in parallel-fed *Ae. aegypti*, suggesting equal permissiveness between these species for initial infection of midgut tissues. In addition, Nguyet et al. [50] demonstrated that patients with a high early viremia have a longer window of infectiousness to *Ae. aegypti*. Collectively, these findings define the viremia level that interventions such as vaccines and antivirals must target for prevention or amelioration to reduce DENV transmission.

3.8. The evolving story of NS1

More than 40 years ago, a series of papers described a soluble complement fixing (SCF) antigen in dengue virus infected mice and cell culture [52–54]. This SCF antigen was identified as a secreted non-structural viral protein [55] that was later designated NS1, following the sequencing of the first flavivirus genome [56]. It was immediately seen as a potential player in the pathogenesis of severe disease primarily because of the reported association between high levels of complement consumption and dengue shock syndrome (DSS) [57]. These studies lead to an expansion of interest in the role of complement pathway engagement by dengue viruses in infected patients and in particular, the role of immune complexes in potentiating the severity of disease [53,58–61]. However, the underlying *in vivo* mechanism of complement activation and the role of secreted NS1 has remained a matter of conjecture ever since. The development of NS1 capture assays [62,63] and the discovery that high levels of circulating NS1 in patient sera early during the course of infection correlate with progression to severe disease [64,65] has provided further impetus to research in NS1 as a mediator of disease. These studies have lead to a greater understanding of the structure and trafficking of this protein within and from infected cells, its proposed role in viral replication, potential as a vaccine candidate, value in diagnostic applications and its role in pathogenesis *in vivo* through its interaction with an ever increasing number of host cell targets (reviewed in Muller and Young [66]). Not surprisingly, these host cell binding partners have been shown to comprise a number of different complement pathway components in addition to other host cell regulatory proteins. These include the complement regulation protein factor H (fH), complement inhibitory factor clusterin, complement proteins C4 and proC1s/C1s, hnRNP C1/C2, STAT3β, thrombin/prothrombin and has been shown to trigger the generation of C5b-9 and SC5b-9 complexes [66]. The recent publication of the crystal structure of both dengue and WNV NS1 has provided some clues into the structural basis of NS1-host cell protein engagement [67]. Both dimer and hexamer forms of NS1 reveal three distinct structural domains, a hydrophobic β-roll (residues 1–29), an α/β-wing (residues 38–151; comprising a RIG-I like fold) and a central β-ladder (residues 181–352). These are all connected via a 3-stranded β-sheet (residues 30–37 and 152–180). The hydrophobic N-terminal β-roll, along with a hydrophobic loop extension from the connector (residues 159–162) provide a hydrophobic face to the dimeric form, thereby explaining membrane association of

this otherwise hydrophilic protein as well as its ability to assemble as a hexameric lipoparticle that carries a lipid cargo. The RIG-I like wing domain is intriguing as it suggests that NS1 may act as an RNA sensor, interacting with dsRNA recognition systems of the innate immune response. However RIG-I is located in the cytoplasm of cells, on the opposite side of the ER membrane to NS1 and so how it could operate in this way is unknown. Akey et al. [68] have speculated that the wing domain may interfere with RLR-dsRNA signaling. Perhaps the most revealing structural insight has come from comparisons with crystal structures of complement components bound to other pathogen proteins [68]. A common feature of these structures is the association of anti-parallel β-sheets from the pathogen proteins with the conserved complement control protein (CCP) domain of their complement partners. The CCP domain is found in factor H, C1s, C4 and C4 binding proteins, all demonstrated binding partners of NS1. The β-roll and β-ladder are clearly candidates for the NS1 domains responsible for complement protein binding. Mutagenesis and binding studies should quickly reveal the potential *in vivo* role for these interactions.

3.9. Clinical management

Until such time as safe and effective vaccines are available and routinely deployed in endemic countries, ensuring good clinical outcomes for individuals with symptomatic dengue must rely on a combination of early case detection and prompt and appropriate case management, allied with close follow-up to identify and treat severe manifestations as soon as they occur. However, since dengue shares clinical features with a wide variety of illnesses especially during the early febrile phase, large numbers of patients with possible dengue are often admitted to healthcare facilities in endemic areas, primarily for observation, and seasonal epidemics may overwhelm health service capacity. Improvements in clinical diagnosis of dengue and risk prediction for severe disease are urgently needed, especially in settings with a high case burden where appropriate allocation of limited resources is crucial to outcome.

3.9.1. Improving clinical diagnosis and risk prediction

As noted earlier the clinical presentation of dengue varies widely. While the majority of symptomatic patients recover after a short illness, a small proportion progress to more severe disease, typically manifesting as a vasculopathy characterized by plasma leakage and a hemorrhagic diathesis. Plasma leakage may be profound, particularly in children, sometimes resulting in life-threatening dengue shock syndrome (DSS). Other severe complications do occur, particularly severe liver and/or neurological involvement, but are less frequent. The 2009 World Health Organization (WHO) revised classification system defines two major entities, dengue and severe dengue, in place of the more complex dengue fever/dengue hemorrhagic fever categories previously recommended [69] Although the revised classification is more easily applicable for clinical and epidemiological purposes, debate continues regarding the utility of both systems for pathogenesis research [70–72]; however efforts are on-going within the international dengue research community to develop agreed standards for the detailed discrimination of dengue clinical phenotypes for use in pathogenesis studies and/or therapeutic intervention trials.

Given the protean manifestations of clinical dengue, it is clear that observational studies need to be very large to allow meaningful interpretation of the relative importance of different clinical features. In one recent study that enrolled more than 5000 Vietnamese children presenting within 72 h of fever onset with clinically suspected dengue [73] of whom 1692 were subsequently confirmed to have dengue, a diagnostic algorithm using the patient's age, total white cell count and platelet count at presentation, resulted in sensitivity and specificity of around 75% for diagnosis of dengue. Inclusion of additional clinical and routine laboratory data did not improve significantly on the performance characteristics of the Early Dengue Classifier, but use in conjunction with an NS1 rapid test improved the sensitivity to over 90%. However it is notable that this classifier would only be relevant in similar epidemiological settings with a high burden of pediatric dengue cases. A second major prospective dataset describing the clinical features and management for over 1700 Vietnamese children with DSS admitted to a single hospital [19], has also been published recently; clinical signs and symptoms were generally consistent with empirical descriptions of DSS, although at presentation 9% of cases were still febrile and almost one-third had no bleeding during the entire illness episode. Only 8 patients died, confirming that with prompt, assiduous clinical care by experienced staff the outcome of this potentially fatal condition can be excellent. These data were also used to develop a prognostic model to identify patients at risk of developing profound or recurrent shock [74]. Earlier presentation with DSS, and more severe hemodynamic compromise at presentation, were also identified as important risk factors for recurrent shock in the only other published study to examine this issue [75], which used data collected from 444 children managed at two other healthcare facilities in southern Vietnam. The other risk factors identified differed between the two studies, highlighting the fact that elements of study design including study definitions, timing of observations in a disease with a rapidly evolving natural history, sample size, etc., can affect the comparability and generalizability of research findings in the wider context.

The ability to identify, during the febrile phase, patients at high risk of progression, i.e. those likely to benefit from admission for close observation and early intervention with supportive therapy, has become the focus of intense research effort in recent years. The 2009 classification encompasses a set of 'warning signs', derived in part from a dataset describing almost 2000 patients with dengue recruited across Asia and Latin America [76]. However, a major limitation of this and all other related research published to date has been the limited number of patients who have developed severe disease while under detailed observation. Recognizing this limitation, and the importance of providing a clear evidence-base for any future refinement of the WHO guidelines, a major clinical study is presently underway, coordinated by one of the three large EU funded consortia that are currently working on dengue research themes [77]. The study aims to recruit 8–10,000 outpatients of all ages, presenting with possible dengue at sites across 9 endemic countries within the first 3 days of fever (ClinicalTrials.govID: NCT01550016. www.idams.eu). Participants are followed daily to identify readily available clinical and laboratory parameters, and/or viral and immunological markers, that differentiate between dengue and other common febrile illness, and secondly to identify any features that predict likely progression to a more severe disease course. Almost 6000 participants have been recruited to date, and the study is expected to report toward the end of 2016. It is to be hoped that with this very large patient cohort, clinical and/or laboratory warning signs with high predictive power can be identified. In addition, while previous efforts have always used data collected at a single time-point to assess risk, this dataset makes it possible to evaluate the evolution of particular symptoms or laboratory values over time for their utility in risk prediction.

3.9.2. Therapeutic interventions

Current management strategies continue to focus on supportive care [78]. Individual case-management relies largely on careful monitoring to recognize vascular leakage and provide judicious fluid replacement, combined with prompt volume resuscitation for patients who do develop DSS. Regrettably no formal research to establish an evidence-base to support these fluid resuscitation

recommendations has taken place for 10 years [79], likely reflecting the practical difficulties inherent in such trials, as well as the very large sample sizes that would be required and the financial cost. However, given the on-going controversy surrounding fluid resuscitation in critical care generally [80,81], there is a clear need to address these difficulties [82]. On the other hand, in the face of the ever-increasing burden of dengue globally [83], the development of specific therapeutic interventions directed toward symptomatic dengue, rather than DSS specifically, has become a major focus of current research efforts.

First, there is considerable interest in the potential utility of antiviral agents. An effective and safe antiviral therapy, if given orally to outpatients with dengue, might be expected not only to decrease the burden on healthcare services in endemic areas, but potentially might also reduce on-going transmission of the virus to mosquitoes and new human hosts. Compounds that target a number of possible virus and host proteins are in development, but only a few agents have so far been assessed in formal clinical trials in dengue-infected humans [84]. These include chloroquine, balapiravir (a polymerase inhibitor developed for treatment of the related virus, hepatitis C) and celgosivir (a cellular glucosidase inhibitor) [85–87]. Unfortunately, although the safety assessments were generally satisfactory, there was no evidence in any of these trials of a benefit in reducing plasma viremia or in preventing the development of complications. Subsequent investigation of the lack of response to balapiravir has indicated that dengue virus infection likely limits conversion of the prodrug to the active moiety [88]. It is also noteworthy that all three trials were conducted in southeast Asian adults, a population at relatively low risk for complications; to power any subsequent trials in similar populations to a robust clinical efficacy endpoint would likely require enrolment of several thousand patients, so the development of reliable algorithms to identify high-risk patients could greatly facilitate the conduct of such trials in the future.

An alternative intervention strategy involves suppression of the host immune response. A number of small clinical trials have examined the efficacy of corticosteroids in patients with DSS, but with inconsistent results. However most of the work was carried out over 25 years ago, and the studies were underpowered and lacked stringent randomization or allocation concealment [89]. Notably also, the steroids were administered after onset of shock, which is likely to be too late in the disease evolution to exert a beneficial effect. An alternative strategy involves early intervention during the febrile phase in an attempt to prevent or attenuate severe disease. One randomized, blinded, placebo-controlled clinical trial of early prednisolone was recently conducted in Vietnam in 225 children with confirmed dengue, with the primary goal of assessing safety during the phase of active viral replication [90]. Other than a trend to hyperglycaemia in high-dose steroid recipients the trial showed no evidence of harm with early prednisolone use compared to placebo, but there was also no evidence of efficacy in preventing DSS. Prednisolone conferred only a small change in the whole blood gene expression profile, with only 81 transcripts from 64 genes differentially abundant between high-dose prednisolone and placebo recipients [91]. Secondly, no attenuation of early-convalescent T cell responses or plasma cytokine levels was observed. Overall the influence of prednisolone on immune response parameters in dengue patients was minimal, in line with the trial evidence showing lack of impact on clinical laboratory endpoints or the clinical phenotype. One possible explanation could be that even commencing prednisolone therapy within the first 72 h is too late to attenuate the infection-driven processes that lead to the development of complications. Use of higher dose therapy might be considered as an option, but given the trend to hyperglycemia among participants receiving the 2 mg/kg prednisolone dose, it is unlikely that clinicians would consider this an acceptable risk to take with a treatment likely to be administered to large numbers of patients in the community. Interestingly, the failure of prednisolone to show a benefit in either clinical or laboratory outcomes for dengue patients leaves the door open for the hypothesis that mast cell mediators play a role in dengue pathogenesis as corticosteroids have no effect on mast cell degranulation [92].

Another agent that is under active investigation as a therapeutic for dengue is lovastatin, one of the statin group of lipid-lowering drugs. Additional to their well-established role in cardiovascular risk modification [93], statins are recognized to have anti-inflammatory and endothelial-stabilising properties. Retrospective studies have suggested potential benefit from adjunctive statin therapy in severe sepsis [94], although in more recent formal randomized controlled trials statin use did not improve clinical outcomes in patients with sepsis-associated Acute Respiratory Distress Syndrome or reduce exacerbations of chronic obstructive pulmonary disease [95–97]. Given that endothelial dysfunction is one of the defining features of severe dengue, and that, in addition to its immunomodulatory properties, lovastatin has been shown to inhibit dengue virus replication in vitro [98], a randomized placebo-controlled trial to evaluate the safety and tolerability of early lovastatin therapy in 300 Vietnamese adults with dengue was initiated in 2011 (ISRCTN03147572) [99]. The trial is expected to report shortly.

Finally, the results of the Adult Dengue Platelet Study (ADEPT, ClinicalTrials.gov: NCT01030211), a prospective randomized open-label trial to examine the safety and efficacy of prophylactic platelet transfusion in Singaporean adults with severe dengue-related thrombocytopenia (platelet count below 20,000/μl) but no bleeding, are also likely to be published shortly. Thrombocytopenia is frequently observed in patients with dengue, but is rarely accompanied by clinically relevant bleeding. Prophylactic transfusion of platelets is common however [100–102], despite a lack of evidence of benefit, significant cost, and the acknowledged risks of fluid overload, allergic reactions and transmission of blood–borne pathogens. A recent publication from Pakistan describing a randomized open-label study of 87 patients with dengue and a platelet count below 30,000/μl [103], concluded that administration of platelets did not prevent development of severe bleeding or shorten the time to cessation of bleeding, but was associated with significant harm. If the results of the ADEPT trial, with a larger sample size and a more stringent platelet cutoff, prove to be similar, it is to be hoped that this evidence will be rapidly incorporated into international and local management guidelines, so as to inform rational decision-making on this important aspect of supportive care for dengue patients.

4. Conclusions

Unquestionably, the phase III vaccine clinical trial results of CYD-TDV have reminded the research community that there is still much to learn with regards to understanding induction and expression of immunity to DENV. Further, there remain knowledge gaps in clinical pathogenesis that preclude a wider, evidence-based spectrum of therapeutic strategies for improved case management. Focused research efforts on these critical bottlenecks will hopefully lead to improvements in disease prevention and management in the next decade.

References

[1] Wu SJ, Grouard-Vogel G, Sun W, Mascola JR, Brachtel E, Putvatana R, et al. Human skin Langerhans cells are targets of dengue virus infection. Nat Med 2000;6:816–20.
[2] Cerny D, Haniffa M, Shin A, Bigliardi P, Tan BK, Lee B, et al. Selective susceptibility of human skin antigen presenting cells to productive dengue virus infection. PLoS Pathog 2014;10:e1004548.

[3] Schmid MA, Harris E. Monocyte recruitment to the dermis and differentiation to dendritic cells increases the targets for dengue virus replication. PLoS Pathog 2014;10:e1004541.
[4] Duangchinda T, Dejnirattisai W, Vasanawathana S, Limpitikul W, Tangthawornchaikul N, Malasit P, et al. Immunodominant T-cell responses to dengue virus NS3 are associated with DHF. Proc Natl Acad Sci USA 2010;107:16922–7.
[5] Mongkolsapaya J, Duangchinda T, Dejnirattisai W, Vasanawathana S, Avirutnan P, Jairungsri A, et al. T cell responses in dengue hemorrhagic fever: are cross-reactive T cells suboptimal. J Immunol 2006;176:3821–9.
[6] Zivna I, Green S, Vaughn DW, Kalayanarooj S, Stephens HA, Chandanayingyong D, et al. T cell responses to an HLA-B*07-restricted epitope on the dengue NS3 protein correlate with disease severity. J Immunol 2002;168:5959–65.
[7] Rivino L, Kumaran EA, Jovanovic V, Nadua K, Teo EW, Pang SW, et al. Differential targeting of viral components by CD4+ versus CD8+ T lymphocytes in dengue virus infection. J Virol 2013;87:2693–706.
[8] Weiskopf D, Angelo MA, de Azeredo EL, Sidney J, Greenbaum JA, Fernando AN, et al. Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for CD8+ T cells. Proc Natl Acad Sci USA 2013;110:E2046–53.
[9] Weiskopf D, Angelo MA, Bangs DJ, Sidney J, Paul S, Peters B, et al. The human CD8+ T cell responses induced by a live attenuated tetravalent dengue vaccine are directed against highly conserved epitopes. J Virol 2015;89:120–8.
[10] Mongkolsapaya J, Dejnirattisai W, Xu XN, Vasanawathana S, Tangthawornchaikul N, Chairunsri A, et al. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. Nat Med 2003;9:921–7.
[11] Dung NT, Duyen HT, Thuy NT, Ngoc TV, Chau NV, Hien TT, et al. Timing of CD8+ T cell responses in relation to commencement of capillary leakage in children with dengue. J Immunol 2010;184:7281–7.
[12] Friberg H, Bashyam H, Toyosaki-Maeda T, Potts JA, Greenough T, Kalayanarooj S, et al. Cross-reactivity and expansion of dengue-specific T cells during acute primary and secondary infections in humans. Sci Rep 2011;1:51.
[13] Rivino L, Kumaran EA, Thein TL, Too CT, Gan VC, Hanson BJ, et al. Virus-specific T lymphocytes home to the skin during natural dengue infection. Sci Transl Med 2015;7:278ra35.
[14] Mangada MM, Rothman AL. Altered cytokine responses of dengue-specific CD4+ T cells to heterologous serotypes. J Immunol 2005;175:2676–83.
[15] Bashyam HS, Green S, Rothman AL. Dengue virus-reactive CD8+ T cells display quantitative and qualitative differences in their response to variant epitopes of heterologous viral serotypes. J Immunol 2006;176:2817–24.
[16] Yauch LE, Prestwood TR, May MM, Morar MM, Zellweger RM, Peters B, et al. CD4+ T cells are not required for the induction of dengue virus-specific CD8+ T cell or antibody responses but contribute to protection after vaccination. J Immunol 2010;185:5405–16.
[17] St John AL, Rathore AP, Raghavan B, Ng ML, Abraham SN. Contributions of mast cells and vasoactive products, leukotrienes and chymase, to dengue virus-induced vascular leakage. Elife 2013;2:e00481.
[18] Furuta T, Murao LA, Lan NT, Huy NT, Huong VT, Thuy TT, et al. Association of mast cell-derived VEGF and proteases in Dengue shock syndrome. PLoS Negl Trop Dis 2012;6:e1505.
[19] Lam PK, Tam DT, Diet TV, Tam CT, Tien NT, Kieu NT, et al. Clinical characteristics of Dengue shock syndrome in Vietnamese children: a 10-year prospective study in a single hospital. Clin Infect Dis 2013;57:1577–86.
[20] Sabchareon A, Wallace D, Sirivichayakul C, Limkittikul K, Chanthavanich P, Suvannadabba S, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. Lancet 2012;380:1559–67.
[21] Capeding MR, Tran NH, Hadinegoro SRS, Ismail HIHJM, Chotpitayasunondh T, Chua MN, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. Lancet 2014;384:1358–65.
[22] Buddhari D, Aldstadt J, Endy TP, Srikiatkhachorn A, Thaisomboonsuk B, Klungthong C, et al. Dengue virus neutralizing antibody levels associated with protection from infection in thai cluster studies. PLoS Negl Trop Dis 2014;8:e3230.
[23] Smith SA, de Alwis AR, Kose N, Jadi RS, de Silva AM, Crowe Jr JE. Isolation of dengue virus-specific memory B cells with live virus antigen from human subjects following natural infection reveals the presence of diverse novel functional groups of antibody clones. J Virol 2014;88:12233–41.
[24] Fibriansah G, Tan JL, Smith SA, de Alwis R, Ng TS, Kostyuchenko VA, et al. A highly potent human antibody neutralizes dengue virus serotype 3 by binding across three surface proteins. Nat Commun 2015;6:6341.
[25] Dejnirattisai W, Wongwiwat W, Supasa S, Zhang X, Dai X, Rouvinsky A, et al. A new class of highly potent, broadly neutralizing antibodies isolated from viremic patients infected with dengue virus. Nat Immunol 2015;16:170–7.
[26] Teoh EP, Kukkaro P, Teo EW, Lim AP, Tan TT, Yip A, et al. The structural basis for serotype-specific neutralization of dengue virus by a human antibody. Sci Transl Med 2012;4:139ra83.
[27] Rouvinski A, Guardado-Calvo P, Barba-Spaeth G, Duquerroy S, Vaney MC, Kikuti CM, et al. Recognition determinants of broadly neutralizing human antibodies against dengue viruses. Nature 2015;520:109–13.
[28] Green AM, Beatty PR, Hadjilaou A, Harris E. Innate immunity to dengue virus infection and subversion of antiviral responses. J Mol Biol 2014;426:1148–60.
[29] Samsa MM, Mondotte JA, Iglesias NG, Assunção-Miranda I, Barbosa-Lima G, Da Poian AT, et al. Dengue virus capsid protein usurps lipid droplets for viral particle formation. PLoS Pathog 2009;5:e1000632.
[30] Mateo R, Nagamine CM, Spagnolo J, Méndez E, Rahe M, Gale M, et al. Inhibition of cellular autophagy deranges dengue virion maturation. J Virol 2013;87:1312–21.
[31] Mackenzie JM, Khromykh AA, Parton RG. Cholesterol manipulation by West Nile virus perturbs the cellular immune response. Cell Host Microbe 2007;2:229–39.
[32] Soto-Acosta R, Mosso C, Cervantes-Salazar M, Puerta-Guardo H, Medina F, Favari L, et al. The increase in cholesterol levels at early stages after dengue virus infection correlates with an augment in LDL particle uptake and HMG-CoA reductase activity. Virology 2013;442:132–47.
[33] Rothwell C, Lebreton A, Young Ng C, Lim JY, Liu W, Vasudevan S, et al. Cholesterol biosynthesis modulation regulates dengue viral replication. Virology 2009;389:8–19.
[34] Miller S, Kastner S, Krijnse-Locker J, Buhler S, Bartenschlager R. Non-structural protein 4A of Dengue virus is an integral membrane protein inducing membrane alterations in a 2K-regulated manner. J Biol Chem 2007;282:8873–82.
[35] Roosendaal J, Westaway EG, Khromykh A, Mackenzie JM. Regulated cleavages at the West Nile virus NS4A-2K-NS4B junctions play a major role in rearranging cytoplasmic membranes and Golgi trafficking of the NS4A protein. J Virol 2006;80:4623–32.
[36] Peña J, Harris E. Early dengue virus protein synthesis induces extensive rearrangement of the endoplasmic reticulum independent of the UPR and SREBP-2 pathway. PLoS ONE 2012;7:e38202.
[37] Ambrose RL, Mackenzie JM. ATF6 signaling is required for efficient West Nile virus replication by promoting cell survival and inhibition of innate immune responses. J Virol 2013;87:2206–14.
[38] Ambrose RL, Mackenzie JM. West Nile virus differentially modulates the unfolded protein response to facilitate replication and immune evasion. J Virol 2011;85:2723–32.
[39] Yu CY, Hsu YW, Liao CL, Lin YL. Flavivirus infection activates the XBP1 pathway of the unfolded protein response to cope with endoplasmic reticulum stress. J Virol 2006;80:11868–80.
[40] Yu C, Achazi K, Niedrig M. Tick-borne encephalitis virus triggers inositol-requiring enzyme 1 (IRE1) and transcription factor 6 (ATF6) pathways of unfolded protein response. Virus Res 2013;178:471–7.
[41] Medigeshi GR, Lancaster AM, Hirsch AJ, Briese T, Lipkin WI, Defilippis V, et al. West Nile virus infection activates the unfolded protein response, leading to CHOP induction and apoptosis. J Virol 2007;81:10849–60.
[42] Sriburi R, Jackowski S, Mori K, Brewer JW. XBP1: a link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum. J Cell Biol 2004;167:35–41.
[43] Cho Jin A, Lee A-H, Platzer B, Cross Benedict CS, Gardner Brooke M, De Luca H, et al. The unfolded protein response element IRE1α senses bacterial proteins invading the ER to activate RIG-I and innate immune signaling. Cell Host Microbe 2013;13:558–69.
[44] Wu J, Rutkowski DT, Dubois M, Swathirajan J, Saunders T, Wang J, et al. ATF6alpha optimizes long-term endoplasmic reticulum function to protect cells from chronic stress. Dev Cell 2007;13:351–64.
[45] Cleland JB, Bradley B. Dengue fever in Australia: its history and clinical course, its experimental transmission by Stegomyia fasciata, and the results of Inoculation and other Experiments. J Hyg (London) 1918;16:317–418.
[46] Cleland JB, Bradley B, Macdonald W. Further experiments in the etiology of dengue fever. J Hyg (London) 1919;18:217–54.
[47] Chandler ACR L. Observations on the etiology of dengue fever. Am J Trop Med 1923;3:233–62.
[48] Siler JFH, Hitchens MWAP. Results obtained in the transmission of dengue fever. J Am Med Assoc 1925;84:1163–72.
[49] Siler JF, Hall MW, Hitchens AP. Dengue: its history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity and prevention. Philipp. J. Sci 1926.
[50] Nguyet MN, Duong TH, Trung VT, Nguyen TH, Tran CN, Long VT, et al. Host and viral features of human dengue cases shape the population of infected and infectious *Aedes aegypti* mosquitoes. Proc Natl Acad Sci USA 2013;110:9072–7.
[51] Whitehorn J, Kien DT, Nguyen NM, Nguyen HL, Kyrylos PP, Carrington LB, et al. Comparative susceptibility of *Aedes albopictus* and *Aedes aegypti* to dengue virus infection after feeding on blood of viremic humans: implications for public health. J Infect Dis 2015.
[52] Brandt WE, Chiewslip D, Harris DL, Russell PK. Partial purification and characterization of a dengue virus soluble complement-fixing antigen. J Immunol 1970;105:1565–8.
[53] Russell PK, Chiewsilp D, Brandt WE. Immunoprecipitation analysis of soluble complement-fixing antigens of dengue viruses. J Immunol 1970;105:838–45.
[54] Smith TJ, Brandt WE, Swanson JL, McCown JM, Buescher EL. Physical and biological properties of dengue-2 virus and associated antigens. J Virol 1970;5:524–32.
[55] Cardiff RD, Brandt WE, McCloud TG, Shapiro D, Russell PK. Immunological and biophysical separation of dengue-2 antigens. J Virol 1971;7:15–23.
[56] Rice CM, Lenches EM, Eddy SR, Shin SJ, Sheets RL, Strauss JH. Nucleotide sequence of yellow fever virus: implications for flavivirus gene expression and evolution. Science 1985;229:726–33.
[57] Russell PK, Intavivat A, Kanchanapilant S. Anti-dengue immunoglobulins and serum beta 1 c-a globulin levels in dengue shock syndrome. J Immunol 1969;102:412–20.

[58] Bokisch VA, Top Jr FH, Russell PK, Dixon FJ, Muller-Eberhard HJ. The potential pathogenic role of complement in dengue hemorrhagic shock syndrome. N Engl J Med 1973;289:996–1000.
[59] Ruangjirachuporn W, Boonpucknavig S, Nimmanitya S. Circulating immune complexes in serum from patients with dengue haemorrhagic fever. Clin Exp Immunol 1979;36:46–53.
[60] Sobel AT, Bokisch VA, Muller-Eberhard HJ. C1q deviation test for the detection of immune complexes, aggregates of IgG, and bacterial products in human serum. J Exp Med 1975;142:139–50.
[61] Theofilopoulos AN, Wilson CB, Dixon FJ. The Raji cell radioimmune assay for detecting immune complexes in human sera. J Clin Invest 1976;57:169–82.
[62] Young PR, Hilditch PA, Bletchly C, Halloran W. An antigen capture enzyme-linked immunosorbent assay reveals high levels of the dengue virus protein NS1 in the sera of infected patients. J Clin Microbiol 2000;38:1053–7.
[63] Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M. Enzyme-linked immunosorbent assay specific to Dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. J Clin Microbiol 2002;40:376–81.
[64] Libraty DH, Young PR, Pickering D, Endy TP, Kalayanarooj S, Green S, et al. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. J Infect Dis 2002;186:1165–8.
[65] Avirutnan P, Punyadee N, Noisakran S, Komoltri C, Thiemmeca S, Auethavornanan K, et al. Vascular leakage in severe dengue virus infections: a potential role for the nonstructural viral protein NS1 and complement. J Infect Dis 2006;193:1078–88.
[66] Muller DA, Young PR. The flavivirus NS1 protein: molecular and structural biology, immunology, role in pathogenesis and application as a diagnostic biomarker. Antiviral Res 2013;98:192–208.
[67] Akey DL, Brown WC, Dutta S, Konwerski J, Jose J, Jurkiw TJ, et al. Flavivirus NS1 structures reveal surfaces for associations with membranes and the immune system. Science 2014;343:881–5.
[68] Akey DL, Brown WC, Jose J, Kuhn RJ, Smith JL. Structure-guided insights on the role of NS1 in flavivirus infection. Bioessays 2015;37:489–94.
[69] Organization WH. Dengue: guidelines for diagnosis, treatment, prevention and control – New edition. Geneva: World Health Organization; 2009.
[70] Horstick O, Jaenisch T, Martinez E, Kroeger A, See LL, Farrar J, et al. Comparing the usefulness of the 1997 and 2009 WHO dengue case classification: a systematic literature review. Am J Trop Med Hyg 2014;91:621–34.
[71] Farrar JJ, Hien TT, Horstick O, Hung NT, Jaenisch T, Junghanns T, et al. Dogma in classifying dengue disease. Am J Trop Med Hyg 2013;89:198–201.
[72] Halstead SB. Dengue: the syndromic basis to pathogenesis research Inutility of the 2009 WHO case definition. Am J Trop Med Hyg 2013;88:212–5.
[73] Tuan NM, Nhan HT, Chau NV, Hung NT, Tuan HM, Tram TV, et al. Sensitivity and specificity of a novel classifier for the early diagnosis of dengue. PLoS Negl Trop Dis 2015;9:e0003638.
[74] Lam PK, Hoai Tam DT, Dung NM, Hanh Tien NT, Thanh Kieu NT, Simmons C, et al. A Prognostic model for development of profound shock among children presenting with dengue shock syndrome. PLOS ONE 2015;10:e0126134.
[75] Huy NT, Thao NT, Ha TT, Lan NT, Nga PT, Thuy TT, et al. Development of clinical decision rules to predict recurrent shock in dengue. Crit Care 2013;17:R280.
[76] Alexander N, Balmaseda A, Coelho IC, Dimaano E, Hien TT, Hung NT, et al. Multicentre prospective study on dengue classification in four South-east Asian and three Latin American countries. Trop Med Int Health 2011;16:936–48.
[77] Jaenisch T, Sakuntabhai A, Wilder-Smith A. Dengue research funded by the European Commission-scientific strategies of three European dengue research consortia. PLoS Negl Trop Dis 2013;7:e2320.
[78] Organization WH. Handbook for clinical management of dengue; 2012.
[79] Wills BA, Nguyen MD, Ha TL, Dong TH, Tran TN, Le TT, et al. Comparison of three fluid solutions for resuscitation in dengue shock syndrome. N Engl J Med 2005;353:877–89.
[80] Myburgh JA, Finfer S, Bellomo R, Billot L, Cass A, Gattas D, et al. Hydroxyethyl starch or saline for fluid resuscitation in intensive care. N Engl J Med 2012;367:1901–11.
[81] Myburgh JA, Mythen MG. Resuscitation fluids. N Engl J Med 2013;369:1243–51.
[82] Huynh TT, Nguyen VC, Wills BA. Resuscitation fluids. N Engl J Med 2013;369:2461.
[83] Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature 2013;496:504–7.
[84] Whitehorn J, Yacoub S, Anders KL, Macareo LR, Cassetti MC, Nguyen Van VC, et al. Dengue therapeutics, chemoprophylaxis, and allied tools: state of the art and future directions. PLoS Negl Trop Dis 2014;8:e3025.
[85] Tricou V, Minh NN, Van TP, Lee SJ, Farrar J, Wills B, et al. A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. PLoS Negl Trop Dis 2010;4:e785.
[86] Low JG, Sung C, Wijaya L, Wei Y, Rathore AP, Watanabe S, et al. Efficacy and safety of celgosivir in patients with dengue fever (CELADEN): a phase 1b, randomised, double-blind, placebo-controlled, proof-of-concept trial. Lancet Infect Dis 2014;14:706–15.
[87] Nguyen NM, Tran CN, Phung LK, Duong KT, Huynh Hle A, Farrar J, et al. A randomized, double-blind placebo controlled trial of balapiravir, a polymerase inhibitor, in adult dengue patients. J Infect Dis 2013;207:1442–50.
[88] Chen YL, Abdul Ghafar N, Karuna R, Fu Y, Lim SP, Schul W, et al. Activation of peripheral blood mononuclear cells by dengue virus infection depotentiates balapiravir. J Virol 2014;88:1740–7.
[89] Panpanich R, Sornchai P, Kanjanaratanakorn K. Corticosteroids for treating dengue shock syndrome. Cochrane Database Syst Rev 2006:CD003488.
[90] Tam DT, Ngoc TV, Tien NT, Kieu NT, Thuy TT, Thanh LT, et al. Effects of short-course oral corticosteroid therapy in early dengue infection in Vietnamese patients: a randomized, placebo-controlled trial. Clin Infect Dis 2012;55:1216–24.
[91] Nguyen TH, Nguyen TH, Vu TT, Farrar J, Hoang TL, Dong TH, et al. Corticosteroids for dengue – why don't they work? PLoS Negl Trop Dis 2013;7:e2592.
[92] Cohan VL, Undem BJ, Fox CC, Adkinson Jr NF, Lichtenstein LM, Schleimer RP. Dexamethasone does not inhibit the release of mediators from human mast cells residing in airway, intestine, or skin. Am Rev Respir Dis 1989;140:951–4.
[93] Taylor F, Huffman MD, Macedo AF, Moore TH, Burke M, Davey Smith G, et al. Statins for the primary prevention of cardiovascular disease. Cochrane Database Syst Rev 2013;1:CD004816.
[94] Bruyere R, Vigneron C, Prin S, Pechinot A, Quenot JP, Aho S, et al. Impact of prior statin therapy on the outcome of patients with suspected ventilator-associated pneumonia: an observational study. Crit Care 2014;18:R83.
[95] Papazian L, Roch A, Charles PE, Penot-Ragon C, Perrin G, Roulier P, et al. Effect of statin therapy on mortality in patients with ventilator-associated pneumonia: a randomized clinical trial. JAMA 2013;310:1692–700.
[96] Truwit JD, Bernard GR, Steingrub J, Matthay MA, Liu KD, Albertson TE, et al. Rosuvastatin for sepsis-associated acute respiratory distress syndrome. N Engl J Med 2014;370:2191–200.
[97] Criner GJ, Connett JE, Aaron SD, Albert RK, Bailey WC, Casaburi R, et al. Simvastatin for the prevention of exacerbations in moderate-to-severe COPD. N Engl J Med 2014;370:2201–10.
[98] Martinez-Gutierrez M, Castellanos JE, Gallego-Gomez JC. Statins reduce dengue virus production via decreased virion assembly. Intervirology 2011;54:202–16.
[99] Whitehorn J, Van Vinh Chau N, Truong NT, Tai LT, Van Hao N, Hien TT, et al. Lovastatin for adult patients with dengue: protocol for a randomised controlled trial. Trials 2012;13:203.
[100] Whitehorn J, Rodriguez Roche R, Guzman MG, Martinez E, Gomez WV, Nainggolan L, et al. Prophylactic platelets in dengue: survey responses highlight lack of an evidence base. PLoS Negl Trop Dis 2012;6:e1716.
[101] Lye DC, Lee VJ, Sun Y, Leo YS. Lack of efficacy of prophylactic platelet transfusion for severe thrombocytopenia in adults with acute uncomplicated dengue infection. Clin Infect Dis 2009;48:1262–5.
[102] Tomashek KM, Biggerstaff BJ, Ramos MM, Perez-Guerra CL, Garcia Rivera EJ, Sun W. Physician survey to determine how dengue is diagnosed, treated and reported in puerto rico. PLoS Negl Trop Dis 2014;8:e3192.
[103] Khan Assir MZ, Kamran U, Ahmad HI, Bashir S, Mansoor H, Anees SB, et al. Effectiveness of platelet transfusion in dengue fever: a randomized controlled trial. Transfus Med Hemother 2013;40:362–8.