Cardiovascular manifestations of the emerging dengue pandemic

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Abstract | Dengue is one of the most important emerging viral diseases globally. The majority of symptomatic infections result in a relatively benign disease course. However, a small proportion of patients develop severe clinical manifestations, including bleeding, organ impairment, and endothelial dysfunction with increased capillary permeability causing hypovolaemic shock that can lead to cardiovascular collapse. Evidence is increasing that dengue can also cause myocardial impairment, arrhythmias and, occasionally, fulminant myocarditis. No antiviral agents or vaccines are licensed for dengue, and treatment remains supportive with judicious fluid replacement for patients with severe disease. Defining the role of cardiac dysfunction in the haemodynamic compromise of severe dengue has potentially important management implications. In this Review, we will outline the current understanding of the cardiovascular manifestations of dengue, including myocardial and vascular involvement, and conclude with a discussion of the available therapeutic options and potential future research directions.

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Introduction

Dengue is currently one of the most important emerging infectious diseases in the world. The dengue virus (DENV), a member of the genus *Flavivirus* in the family *Flaviviridae*, is a single-stranded enveloped RNA virus, of which four distinct, but related, serotypes exist (DENV1–4).[1] The geographic expansion of dengue in the past 3 decades is unprecedented for a vector-borne disease, increasing fourfold during this time.[2] Dengue is transmitted by mosquitoes of the genus *Aedes*, and is now reported in >100 countries, with a particularly high disease burden across South and Southeast Asia, and increasing numbers of cases reported from Latin America.[2] Transmission of dengue has also been recognized in Africa, although the extent of the problem in this continent remains unclear. An estimated 390 million DENV infections occur globally each year, of which 96 million are clinically apparent.[3]

Dengue was previously classified into dengue fever (DF) and dengue haemorrhagic fever (DHF) grades I–IV; DHF grades III and IV together comprised dengue shock syndrome (DSS). In 2009, the WHO revised the classification system owing to difficulties in applying the old system in clinical situations, as well as a number of reports of severe cases that did not fit the criteria for DHF. Patients are currently classified as having dengue with or without warning signs (Box 1), or severe dengue (Box 2).[2] Age seems to influence the clinical phenotype of dengue, with shock occurring more frequently in children, and bleeding and organ impairment being more common in adults.[4] The observation that severe dengue is more common in secondary infections with a different DENV serotype, and that severe manifestations occur late in the disease course when the virus is being cleared from tissues and the peripheral blood, suggest an underlying immunity-driven pathogenesis, although the precise mechanisms remain to be defined.[5]

Although the vast majority of DENV infections are either asymptomatic or result in fairly mild disease, an estimated 1–5% of patients presenting to hospital develop complications, including organ impairment, bleeding, and plasma leakage from the capillaries. In severe cases, leakage can result in potentially fatal cardiovascular collapse; that is, DSS.[6] The clinical course of dengue is divided into three distinct phases: a febrile phase, lasting 3–7 days, during which the patient typically experiences sudden onset high fever, headache, myalgias, vomiting, and malaise; a critical phase, lasting 2–3 days around defervescence, when severe clinical manifestations become apparent in a minority of patients; and a recovery phase, lasting 2–5 days, when clinical improvement occurs in association with resorption of extravascular fluid (Figure 1). The increase in capillary permeability that occurs in some patients, and can cause intravascular hypovolaemia and shock, is the best known cardiovascular complication associated with dengue. Additionally, various specific cardiac manifestations have been described, ranging from rare fulminant myocarditis to more-common associations with functional myocardial impairment and arrhythmias.[7,8] Myocarditis has now been included in the definition of severe dengue adopted in the 2009 WHO revised classification,[2] but the true incidence of myocarditis remains unknown owing to the lack of screening in most countries where DENV is endemic. In the past 2 decades, the critical role of myocardial

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Key points

- The majority of dengue virus infections are mild; only a small proportion of patients develop overt complications, which can include systemic vascular leak syndrome, bleeding, and organ impairment
- Increased capillary permeability in dengue can manifest as pleural effusions, ascites, and narrowing of pulse pressure; in a small proportion of patients, plasma leakage can cause hypovolaemia and cardiovascular collapse
- Dengue can also have specific cardiac manifestations, including functional myocardial impairment, arrhythmias, and myocarditis, which can contribute to the overall severity of the haemodynamic compromise
- Functional myocardial impairment and electrocardiographic abnormalities are observed in many patients hospitalized with dengue, and can be caused by a subclinical myocarditis, myocardial oedema, or circulating myocardial depressant factors
- Fulminant cases of dengue myocarditis are very rare; these patients have evidence of widespread myocyte infection and damage, with marked changes on echocardiography or electrocardiography, accompanied by cardiac biomarker elevation
- Pathogenesis of dengue vasculopathy is likely to be multifactorial, involving activation of complement, upregulation of T cell and proinflammatory cytokine responses, and disruption of the vascular endothelial glycocalyx layer

Box 1 | Dengue warning signs

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleeding
- Lethargy or restlessness
- Liver enlargement >2 cm
- Increase in haematocrit concurrent with rapid decrease in platelet count

Box 2 | Criteria for severe dengue

Severe plasma leakage leading to:
- Shock (dengue shock syndrome)
- Fluid accumulation with respiratory distress

Severe bleeding

Severe organ involvement:
- Liver: AST or ALT level ≥1,000 U/l
- CNS: impaired consciousness
- Heart and other organs

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CNS, central nervous system.

impairment in the development of septic shock has become clear, distinct from cardiovascular compromise caused by reduced preload and systemic vascular resistance.[9] Myocardial impairment is possibly mediated by circulating myocardial depressant factors.[10] By contrast, the contribution of cardiac dysfunction to haemodynamic compromise in DSS remains to be adequately defined.

This Review will cover our current understanding of the cardiovascular manifestations of dengue, focusing on myocardial involvement as well as the more generalized vascular and endothelial dysfunction, and concluding with a discussion of current therapeutic options for dengue and possible future research directions.

Cardiac involvement

Cardiac manifestations of dengue include functional myocardial impairment, arrhythmias, and myocarditis, which can occur through a number of mechanisms (Figure 2). Myocarditis is an inflammatory condition of the myocardium, frequently attributed to viral aetiologies, most commonly enteroviruses, *Parvovirus B19*, adenoviruses, and herpes viruses.[11] The wide clinical presentation of, and difficulties in diagnosing, myocarditis make the incidence difficult to quantify. The signs of myocarditis can vary from a subclinical rise in cardiac biomarkers or detection of asymptomatic electrocardiogram (ECG) abnormalities, through to the more-severe clinical manifestations of dyspnoea, chest pain, and sudden death.[12] Endomyocardial biopsy and cardiac MRI (CMR) can increase the diagnostic accuracy for myocarditis, but are either invasive (biopsy) or not widely available (both procedures) in dengue endemic areas.

Dengue myocarditis has been described, using various diagnostic criteria, in a number of case reports and small case series from endemic areas. However, very few patients with dengue have a formal cardiac assessment, so the frequency of subclinical dengue myocarditis and its relative contribution to the haemodynamic instability in severe dengue remains to be demonstrated.[13–16] The effect of old age (≥65 years) on cardiac involvement has not been studied, but these elderly patients with dengue are more likely to be hospitalized and have a worse prognosis than younger patients.[17] Thus, patients aged >65 years might warrant special consideration, including more-careful assessment and monitoring (Figure 3), owing to the higher frequency of comorbidities (including hypertension, diabetes mellitus, and ischaemic heart disease) in this group. In this section, we review the evidence for the cardiac manifestations of dengue from three perspectives—functional, electrocardiographic, and pathological.

Functional studies

Myocardial dysfunction in acute dengue has been documented in several studies using a variety of techniques (Table 1). Dysfunction is transient, except in a minority of fulminant cases of fatal myocarditis, and most patients have normal cardiac function by the end of their acute illness. No long-term follow-up studies have been conducted, and no definitive evidence of progression to dilated cardiomyopathy exists.

In a study performed in the 1970s, 10 patients with cardiac manifestations who presented days to months after a 'dengue-like' illness were investigated.[18] Although serological tests did reveal past exposure to dengue or chikungunya viruses, conclusively linking the cardiac involvement to these viruses is difficult in endemic areas where background *Flavivirus* seropositivity in the population is high.[18] A study of 81 adults with dengue, conducted in Brazil, showed that 12 patients (15%) had cardiac biomarker elevation (troponin I and pro-B-type natriuretic peptide).[19] Echocardiograms were anomalous in four of the 10 patients who underwent this procedure, with features including myocardial impairment, regional wall abnormalities, and effusions. Myocardial involvement was confirmed using CMR in these patients. The findings were consistent with classical viral myocarditis,

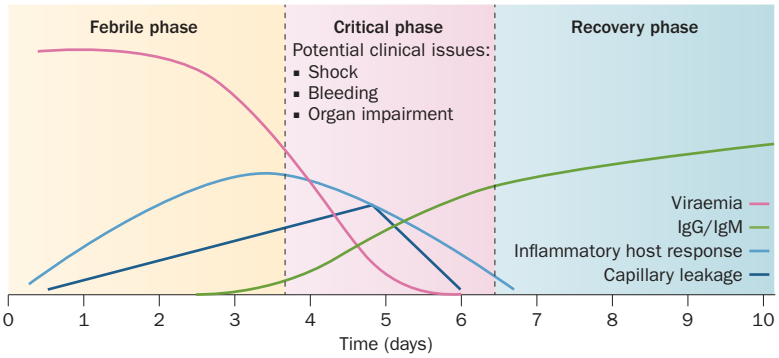

Figure 1 | The clinical course of dengue, showing the three distinct phases. The febrile phase, when the patient develops sudden onset of high fever, is often accompanied by headache, myalgias, and gastrointestinal symptoms. This phase usually lasts 3–7 days, during which time the viraemia peaks. The critical phase is the 2–3 day period around defervescence, when the severe complications can manifest in a small proportion of patients. The final phase is recovery, lasting 2–5 days, when the patient's symptoms resolve and clinical parameters normalize.

including a hyperintense signal on T2-weighted images, as well as early and late gadolinium enhancement.[19] A study in which echocardiography was used to evaluate 54 Indian children hospitalized with dengue demonstrated that nine patients (17%) had evidence of left ventricular (LV) systolic dysfunction (LV ejection fraction [LVEF] <50%), and two (4%) of these patients had substantial impairment (LVEF <35%).[20] These changes were observed equally across the spectrum of dengue severity grades. However, an echocardiographic study of 91 Thai children showed that LV dysfunction was more frequent in severe dengue; 36% of patients with DSS had a LVEF <50%, compared with 6.7% of those with DF, and 13.8% of patients with DHF.[21] Patients with LV dysfunction required more fluids and had more complications of fluid overload than those with LVEF >50%. As LVEF is preload dependent, whether patients with LVEF <50% had true myocardial depression, or whether this dysfunction reflected intravascular hypovolaemia, is unknown. Our group in Vietnam investigated 79 children and young adults (age range 8–46 years) with dengue, using preload-independent parameters with tissue Doppler imaging, and demonstrated that 45% had systolic myocardial impairment and 42% diastolic impairment, predominantly affecting the septal and right ventricular walls.[8] These changes were most frequent and pronounced in patients with severe dengue.[8] In contrast to the Brazilian study, none of the patients in the Vietnamese or Thai studies had a rise in troponin level. This observation is supported by a study of 17 patients with dengue in India, in which ^{99m}Tc-pyrophosphate imaging showed no myocardial necrosis in these individuals.[13]

The pathogenic mechanisms underlying functional myocardial impairment remain to be elucidated, but as the majority of the patients do not have evidence of myocardial damage, direct viral invasion of cardiomyocytes is unlikely. Other postulated mechanisms include myocardial oedema from local capillary leakage, presence of a circulating myocardial depressant factor (for example, one or more proinflammatory mediators), coronary hypoperfusion, altered calcium homeostasis,[22] or a combination of these factors. By contrast, in fulminant dengue myocarditis, evidence exists of widespread myocyte damage with substantial increases in levels of cardiac biomarkers, ST-segment changes on ECG mimicking acute myocardial infarction, and cardiac-specific symptoms, and clear signs of functional impairment.[7,23] Like fulminant myocarditis of other aetiologies, dengue myocarditis is associated with high mortality.[7,15,19,24]

Whether the minor degrees of myocardial impairment observed in many patients with dengue are the result of subclinical myocarditis with fulminant cases representing the severe end of this spectrum, or whether separate disease entities exist, is not evident from the available literature. Varying degrees of myocardial depression could occur in response to a number of mechanisms noted

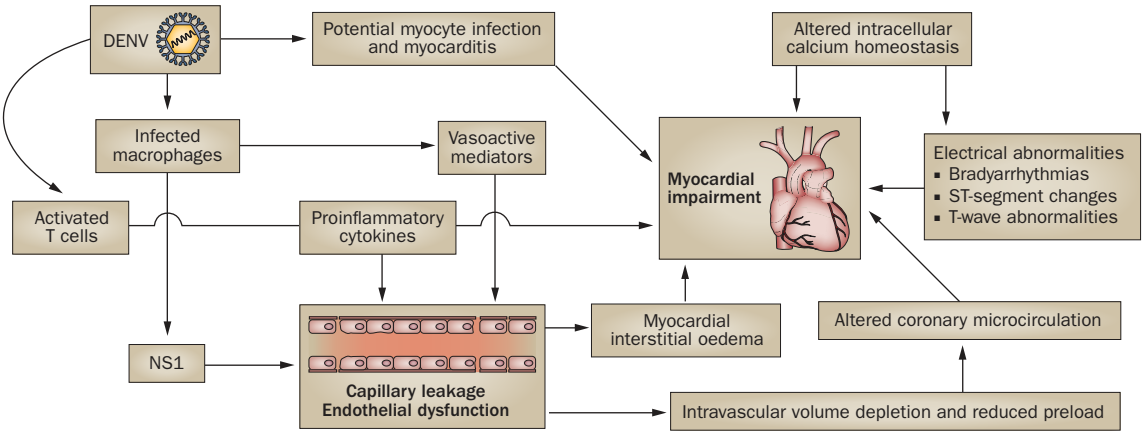

Figure 2 | Proposed viral and immune mechanisms involved in the cardiac and vascular manifestations of dengue. DENV is taken up into macrophages with the resulting T-cell activation and release of vasoactive and proinflammatory cytokines implicated in the capillary leak and possibly also in myocardial impairment. The interaction between the NS1 and the glycocalyx layer of the vascular endothelium is thought to increase capillary permeability. The resulting plasma leakage can contribute to the cardiac dysfunction in the form of reduced preload, altered coronary microcirculation, and myocardial interstitial oedema. Altered intracellular calcium homeostasis has also been demonstrated in dengue infected myotubes. Abbreviations: DENV, dengue virus; NS1, nonstructural protein 1.

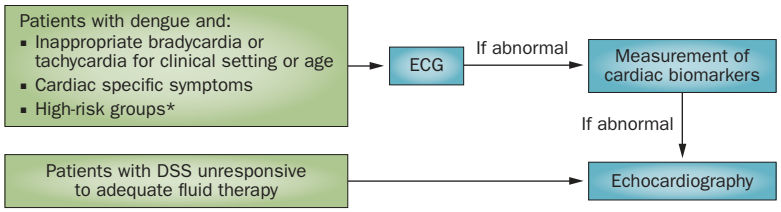

Figure 3 | Proposed algorithm for cardiac tests in patients with dengue. *Elderly patients with known cardiovascular disease or risk factors for cardiovascular disease. Abbreviations: ECG, electrocardiography; DSS, dengue shock syndrome.

above (Figure 2), whereas fulminant dengue myocarditis might represent a separate phenomenon in which host genetics or increased cardiotropism of the virus allows widespread myocyte infection and damage. Reports of dengue epidemics with a high incidence of cardiac manifestations have raised the possibility of certain serotypes or genotypes of DENV having increased cardiotropism. Although DENV serotypes 1–3 have been identified in patients with cardiac involvement, DENV-4 has not been reported to date, which could reflect a lack of serotype reporting in the majority of studies. Cardiac involvement has been reported in patients with primary, and in those with secondary, DENV infections. Variations in the availability and methods of cardiac screening during dengue epidemics might account for some of these differences in serotype reporting, and insufficient evidence currently exists to support a specific serotype or genotype predominance in dengue-associated cardiac involvement.

The pericardium can also be affected by dengue, but less frequently than the myocardium, with very few reports of isolated dengue pericarditis.[25] However, pericardial effusions are observed occasionally, particularly in severe dengue and are likely to be related to the severity of systemic plasma leakage from the capillaries. Large, clinically relevant pericardial effusions are extremely rare, but have been documented.[19]

Electrocardiographic studies

ECG alterations reported in dengue are often transient and nonspecific, including sinus bradycardia, atrioventricular block, T wave, and ST-segment abnormalities.[26,27] An example of a marked bradycardia is shown in Figure 4. The association between dengue and bradyarrhythmias was highlighted in a study from Singapore, in which the relationship between heart-rate and body temperature was investigated among adults with various febrile illnesses.[28] The heart rate response at peak body temperature was consistently lower in dengue compared with other febrile illnesses.[28]

Classically, such rhythm disturbances were thought to occur primarily in the recovery phase. In a 24 h Holter monitoring study of 35 children in the recovery phase of dengue, 29% were identified as having ECG abnormalities,[27] primarily bradyarrhythmias such as first and second degree heart block, as well as atrial and ventricular ectopic beats. Tachyarrhythmias, including atrial fibrillation, have also been documented in patients with dengue, but are much less common than bradyarrhythmias.[29,30] However, ECG abnormalities are now known to occur during any phase of the disease and are fairly common, being observed in 30–44% of patients hospitalized with dengue.[8,13] These arrhythmias tend to be self-limiting and benign, and the ECG changes might be the only sign of cardiac involvement, with normal biomarker levels and echocardiograms often documented.[26] However, rhythm disturbances have also been noted in association with more-severe disease. For example, in one paediatric case report, a junctional bradycardia associated with hypotension was noted 1 day after recovery from DHF.[14] In another case report, a 10-year-old girl with sinoatrial exit node block and atrioventricular dissociation had an associated cardiac biomarker rise and profound bradycardia with a pulse of 45 bpm.[31] An unusually high proportion of patients (62%) admitted with dengue during an outbreak of this infection in Sri Lanka in 2005 had ECG abnormalities, predominantly bradyarrhythmias, and T wave and ST-segment changes.[32] These patients were more likely to develop hypotension than those with a normal ECG.[32]

The underlying mechanisms for these electrical abnormalities have not been adequately explored. Possibilities include altered autonomic tone, electrolyte and calcium derangements, or subclinical myocarditis. The clinical relevance of ECG alterations in dengue remains speculative, but a bradyarrhythmia starting in the critical phase when hypovolaemia is also present is an obvious concern, as inability to mount an appropriate heart rate response to maintain cardiac output will potentially add to haemodynamic instability. Very careful attention to fluid balance and haemodynamic monitoring is warranted in these patients. Figure 3 shows a suggested algorithm for cardiac testing in patients with dengue.

Pathological studies

Histopathological studies of the heart in patients with dengue are lacking, with only a limited number of autopsy studies published, and no reports of endomyocardial biopsies from patients with suspected dengue myocarditis. Autopsies of five patients from Sri Lanka showed predominantly interstitial oedema, inflammation, and myocardial fibre necrosis (Figure 5).[24] These individuals had clinical syndromes that would be consistent with fulminant myocarditis, with associated ECG, echocardiographic, and cardiac biomarker abnormalities. Similar findings were demonstrated on necroscopy specimens from two Brazilian patients with dengue who died of cardiogenic shock, with widespread oedema and inflammation, and predominantly mononuclear cell infiltrates.[19]

Viral antigens, including dengue capsid protein, nonstructural protein 1 (NS1) and viral RNA, have been identified using reverse transcription polymerase chain reaction on cardiac specimens (as well as other tissues, such as the liver, lung, spleen, and lymph nodes) from a small number of patients who died of dengue.[33,34] Dengue capsid protein was demonstrated by immunohistochemistry to be present in several cardiac cell types in a Columbian patient who died of dengue.[22] These cells included cardiomyocytes, myocardial interstitial cells, and myoblasts, often in a perinuclear location.[22] In addition, expression of the

Table 1 | Studies of myocardial dysfunction in acute dengue

Reference	Study population	*n*	Age	Country	Methods of cardiac assessment	Main findings
Yacoub *et al.* (2012)[8]	Hospitalized adults and children: 22 dengue, 42 dengue with warning signs, 15 severe dengue	79	Median 20 years (range 8–46 years)	Vietnam	Echocardiography including tissue Doppler imaging, biomarker assessment (17 patients), ECG (51 patients)	Systolic impairment in 45% of patients, diastolic impairment in 42%. Septal and right ventricular walls predominantly affected, worse in severe cases. ECG abnormalities in 35% of 51 patients assessed. One patient had borderline troponin elevation, biomarker levels in the other 16 patients were normal.
Wali *et al.* (1998)[13]	Hospitalized older children and adults: nine DHF, eight DSS	17	Mean 29.7 years (range 14–58 years)	India	Echocardiography, radionucleotide ventriculography, Tc-pyrophosphate imaging (four patients), ECG (17 patients)	Mean LVEF was 41.7% using radionucleotide ventriculography, and 47.1% using echocardiography. Global hypokinesia detected in 12 patients (70.59%). Mean LVEF in patients with DSS was 39.63%. No myocardial necrosis detected in four patients using ^{99m}Tc-pyrophosphate imaging. ECG showed ST and T wave changes in 29.4% of 17 patients.
Khongphatthanayothin *et al.* (2003)[16]	Hospitalized children: four DHF grade I, 10 DHF grade II, 10 DSS	24	Mean 10.8 years (±2.8 years)	Thailand	Echocardiography (VCFc/ESS)	Lower LVEF, VCFc/ESS, CI, EDV and higher SVR during critical phase versus recovery.
Miranda *et al.* (2013)[18]	Hospitalized children and adults: 54 DF, 26 DHF grades I and II, one DSS	81	Mean 32 years (range 4 months to 81 years)	Brazil	Echocardiography (10 patients), biomarker assessment, CMR (four patients)	Raised biomarker levels in 15% of patients. 10 patients underwent echocardiography, four of whom had abnormalities, including functional and regional wall abnormalities. CMR showing myocardial enhancement in four patients.
Kabra *et al.* (1998)[19]	Hospitalized children	54	<12 years	India	Echocardiography	LVEF was <50% in 16.7% of patients, and <35% in 3.7% of patients. No difference between severity grades.
Khongphatthanayothin *et al.* (2007)[20]	Hospitalized children: 30 DF, 36 DHF, 25 DSS	91	Mean 10.5 years	Thailand	Echocardiography, biomarker assessment (11 patients)	Reduced LVEF (<50%) was found in 6.7%, 13.8%, and 36% of patients with DF, DHF, and DSS during the critical phase, respectively. Biomarker levels were normal in all 11 patients tested.
La-Orkhun*et al.* (2011)[26]	Hospitalized children	35	Mean 11.7 years (±2.3 years)	Thailand	ECG 24 h Holter monitoring	During recovery phase, 29% of patients had ECG abnormalities including, sinus arrhythmias, first-degree and Mobitz type I second-degree AV block, and atrial and ventricular ectopic beats. No difference in severity between the groups.
Kularatne *et al.* (2007)[31]	Hospitalized older children and adults	120	Median 34 years (range 13–76 years)	Sri Lanka	ECG, biomarker assessment (17 patients)	ECG abnormalities, including sinus bradycardia, T wave and ST-segment changes and right bundle branch block, in 62.5% of patients. Increased troponin levels in 29.4% of 17 patients tested.
Salgado *et al.* (2010)[34]	Hospitalized children: 23 DF, 79 DHF	102	Mean 6 years (range 13 months to 10 years)	Colombia	Clinical assessment, echocardiography (seven patients), ECG (11 patients)	Myocarditis diagnosed clinically in 13.9% of 79 patients with DHF. ECG showed sinus bradycardia in 81.8% of 11 patients, tachycardia in 18.2% of 11 patients, and T wave inversion in seven of 11 patients. Echocardiography showed pericardial effusions in 71.4% of seven patients and diastolic dysfunction (parameters not specified) in 28.3% of seven patients.

Abbreviations: AV, atrioventricular; CI, cardiac index; CMR, cardiac magnetic resonance; DF, dengue fever; DHF, dengue haemorrhagic fever; DSS, dengue shock syndrome; ECG, electrocardiography; EDV, end diastolic volume; LVEF, left ventricular ejection fraction; SVR, systemic vascular resistance; VCFc/ESS, velocity of circumferential fibre shortening/end-systolic wall stress.

inflammatory markers monocyte chemotactic protein 1 (also known as C-C motif chemokine 2) and major histocompatibility complex class II was observed in the endothelium of small myocardial vessels, interstitial cells, and myoblasts. In another fatal case of clinically suspected dengue with serological confirmation, clusters of viral particles were demonstrated inside cardiomyocytes using electron microscopy.[30] These viral clusters were associated with myocyte necrosis and widespread interstitial oedema; however, molecular tests were not performed to confirm the identity of the virus.

Although DENV RNA has been found occasionally in myocytes, evidence of active viral replication using *in situ* hybridization has yet to be demonstrated in cardiac-specific cells.[35] In studies from Europe and North America, viral genomes have only been identified in cardiac specimens in 10–20% of patients with active viral myocarditis.[36] Thus, failure to demonstrate the presence of DENV

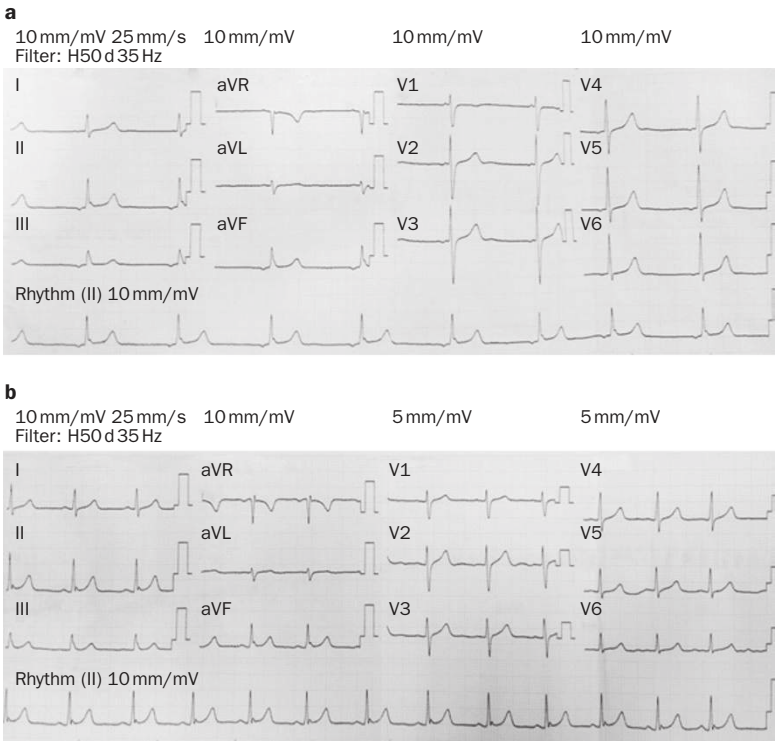

Figure 4 | Bradycardia in dengue. ECGs from a 14-year-old boy with dengue, who developed symptomatic bradycardia with presyncope on the day of defervescence. The patient's heart rate was 53 bpm. **a** | ECG on day of defervescence, showing junctional bradycardia with inverted P waves and a short PR interval (0.1 s). **b** | Follow-up ECG 10 days after recovery, showing resolution of the initial ECG abnormalities. Abbreviation: ECG: electrocardiogram.

in cardiac specimens does not rule out viral induction of pathogenesis.[36]

Vascular involvement

The critical determinant of disease severity in the majority of patients with dengue is hypovolaemia secondary to increased systemic vascular permeability and plasma leakage. A particular pattern of haemostatic abnormalities typically evolves in parallel with the plasma leakage, although the coagulopathy is not always accompanied by clinical manifestations of bleeding. Although these features indicate a disturbance in the permeability and thromboregulatory properties of the microvasculature, only minor nonspecific vascular changes have been shown in the limited histological studies published to date. Endothelial cells can be infected with DENV *in vitro*, but infection and viral replication have not been demonstrated in endothelial cells *in vivo*.[37] Morphologically, endothelial cells appear grossly normal, with electron microscopy of capillaries from skin biopsies showing preservation of intercellular junctions and some evidence of single endothelial cell swelling.[38] Endothelial activation has been demonstrated *in vitro* through increased expression of endothelial cell surface adhesion molecules.[39] In addition, studies *in vivo* have shown increased plasma levels of a number of endothelial activation markers, including soluble thrombomodulin, intercellular adhesion molecule 1, vascular cell adhesion protein 1, and E-selectin, correlating with disease severity in some studies.[40–42] Lack of an appropriate animal model, and the difficulty in studying human endothelial cells *in vivo*, has hampered our understanding of the mechanisms underlying dengue vasculopathy.

Clinical features of vasculopathy

Vascular permeability

Increased vascular permeability is recognized clinically in only a small proportion of patients with dengue predominantly, but not exclusively, children and young adults.[43] Although profound plasma losses can occur during the critical phase and result in DSS, lesser degrees of plasma leakage are hard to identify clinically, and general information on the onset, evolution, and duration of the leakage process is limited.

Direct evidence for increased vascular permeability has been obtained using strain gauge plethysmography, a noninvasive technique to assess filtration capacity (Kf), an overall measure of microvascular permeability. A study in Vietnamese children with DSS or DHF without shock, showed increased Kf in both groups compared with healthy control individuals.[44] No significant difference was found between the group with and the group without shock, but patient numbers were small and patients with DSS were studied after initial volume resuscitation, rather than at the onset of shock.[44] Thus, although permeability was clearly increased in all study participants, without continuous measurements of Kf fluctuations the intensity of leakage could not be assessed. The results also suggest that an individual's compensatory reserve, that is their ability to upregulate homeostatic mechanisms to balance the intravascular volume loss, is important in determining whether they will develop shock. Strain gauge plethysmography has also been used to demonstrate that healthy children have higher Kf than healthy adults, possibly owing to the higher number of developing capillaries in children, which are more vulnerable to leakage than mature capillaries.[45] This phenomenon could explain why children are more susceptible to DSS than adults.

In addition, evidence from serial ultrasound studies indicates that the transient increase in capillary permeability commences in the febrile phase, with signs of minor leakage detectable as early as day 2–3 of fever.[46] Although capillary leakage leading to DSS is more common in secondary infections, more than half of volunteers with artificially induced primary dengue infection have been shown to have ultrasonographic evidence of subclinical fluid accumulation,[47] and rare cases of DSS (severe plasma leakage) associated with primary dengue infection have been reported.[48–50] Additionally, in one report, ultrasound assessment of travellers with dengue showed evidence of capillary leakage in a similar proportion of patients with primary infections and those with secondary infections (32% and 40%, respectively; $P = 0.69$).[51] A number of other ultrasound studies have confirmed that pleural effusions, ascites, and gallbladder wall oedema are commonly present during the critical phase of dengue and correlate with disease severity.[52–54] Thus, the spectrum of

plasma leakage associated with dengue seems to be broad, can be present in mild, as well as severe, disease and in primary, as well as secondary, infections, and start earlier than previously understood. The possibility exists that all dengue-infected individuals experience some degree of vascular leak, albeit at clinically undetectable levels in the majority of patients.[55]

Capillary leak in DENV infection is slow and persistent, contrary to the leak associated with bacterial septic shock, which is sudden and rapid and leads to cardiovascular collapse within hours. Slow leakage over several days, such that upregulation of homeostatic compensatory mechanisms can take place,[56,57] could explain one of the unusual clinical features that is often observed in patients with DSS. Narrowing of pulse pressure, in which systolic blood pressure is maintained at a normal level while diastolic pressure rises, is a characteristic feature of dengue that is not seen in other conditions in which hypovolaemic shock develops rapidly.[2,58] Rising diastolic pressure is thought to indicate maximal activation of the intrinsic protective mechanisms (renal, adrenal, and neurochemical) leading to increased systemic vascular resistance, designed to preserve critical organ perfusion in the face of gradually worsening hypovolaemia.[59] Although low degrees of pulse pressure narrowing clearly indicate some measure of circulatory compromise, when pulse pressure narrows to ≤20 mmHg accompanied by signs of impaired peripheral perfusion, the patient is defined as having established DSS and urgent fluid resuscitation is recommended.[2]

Close observation for signs of plasma leakage is critical from the end of the febrile phase. The most-common method of monitoring leakage relies on identification of relative haemoconcentration, determined by tracking changes in serial haematocrit measurements. Unfortunately, the method is rather insensitive, particularly if the patient is receiving parenteral fluid therapy and if the baseline haematocrit value for an individual is not known. Our group in Vietnam is investigating other potentially more-sensitive measures to assess intravascular volume depletion, including portable echocardiography, pulse waveform analysis,[60] and videomicroscopy of the microcirculation.[61]

Splanchnic circulatory changes

Some data exist on changes in the venous side of the vascular system in patients with dengue, particularly the splanchnic circulation. An ultrasound study of 45 patients with dengue, showed a dilated portal vein with lower flow velocity, a higher congestion index, and a smaller inferior vena cava in patients with DSS than in those with DF or DHF without shock, suggesting hepatosplanchnic circulatory dysfunction.[62] The mechanisms underlying these vascular changes are not clear, but might reflect the widespread endothelial and mircrovascular dysfunction that occurs in severe dengue.

Bleeding

Bleeding manifestations are often reported in dengue, typically in the form of minor mucosal bleeding, skin petechiae, and bruising (Figure 6). Major haemorrhage

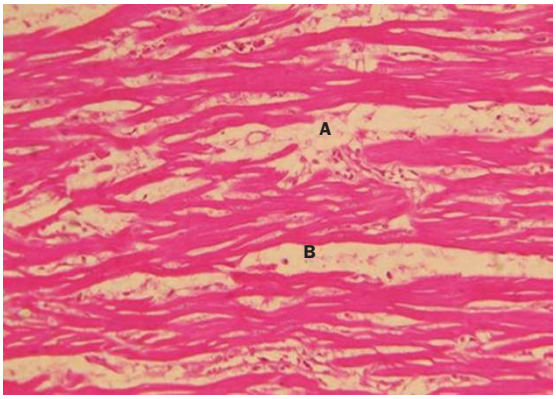

Figure 5 | Haematoxylin and eosin stained section of myocardium from a patient who died of dengue. Both infiltration of inflammatory cells with necrosis of myocardial fibres (A) and interstitial oedema (B) can be seen.

can occur in children, but is rare and usually associated with prolonged shock.[63] However, mucosal bleeding tends to be both more common and more severe in adults than in children.[4,64] Substantial increases in activated partial thromboplastin times with a reduction in fibrinogen levels have been noted in many studies of patients with dengue, in most cases with normal or only slightly prolonged prothrombin times and little evidence for the presence of fibrin degradation products.[63,65–67] Characteristically, coagulation abnormalities evolve during the various phases of the infection and correlate with vascular leakage severity rather than bleeding.[66,67] Several potential mechanisms underlying dengue-related coagulopathy have been suggested, including loss of anticoagulant proteins through leaks in capillaries; endothelial activation causing increased levels of soluble thrombomodulin and other procoagulant factors; viral induction of plasminogen; and release of a heparin-like circulating anticoagulant from the microvascular surface.[63,68,69]

Pathogenesis of vasculopathy

The pathogenesis of dengue-associated vasculopathy is likely to be multifactorial, including a variety of host and viral factors.[70] Complement activation mediated by soluble and membrane-associated NS1might have a role through generation of anaphylatoxins and the terminal SC5b-9 complement complex.[71] High levels of NS1 and SC5b-9 in the plasma of patients with dengue correlated with disease severity and were also found in large amounts, together with the anaphylatoxin C5a, in the pleural fluid of patients with DSS.[71] NS1 bound to endothelial cells can then be targeted by cross-reactive NS1 antibodies during secondary infections, leading to complement-mediated cytolysis and endothelial cell damage.[72,73] Immunopathogenic theories suggest a greater T cell response in severe DENV infection than in mild infection, with cells that produce high levels of cytokines predominating.[74] The resulting excessive proinflammatory response, particularly by tumour necrosis factor (TNF), has been implicated in increased vascular permeability. Sera from patients with dengue has been

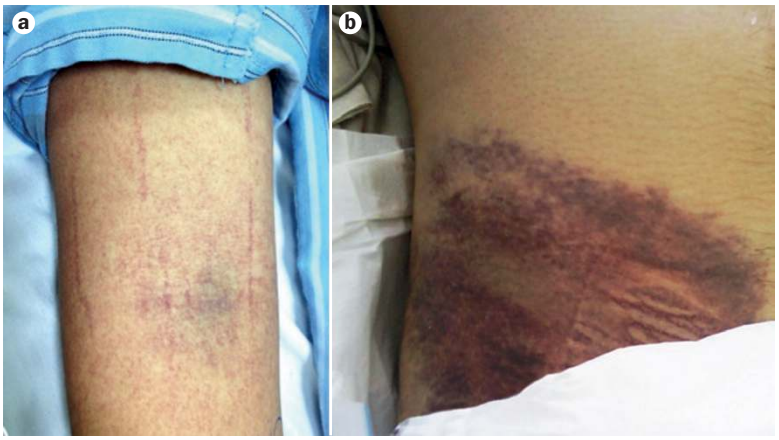

Figure 6 | Bleeding manifestations in patients with dengue. **a** | Antecubital fossa bruising following venepuncture and linear petechiae after blood pressure cuff inflation. **b** | Abdominal wall haematoma, complicating a femoral line attempt in a patient with dengue and marked coagulopathy and thrombocytopenia.

shown to induce endothelial cell activation *in vitro*, and this effect can be inhibited by anti-TNF monoclonal antibodies.[75] However, the timing of peak TNF levels does not coincide with the peak of the microvascular leakage.[76] Gene expression profiling demonstrated that increased expression of an interferon–mediated signalling pathway predominates early in the course of dengue,[77] and these proteins have been shown to suppress the effect of TNF on endothelial cells *in vitro*.[78] The vascular endothelium could be protected in the first few days of infection through DENV-mediated type I interferon-dependent CD73 (also known as 5'-nucleotidase) upregulation.[79] Later in the disease course, TNF and other vasoactive mediators can enhance vascular permeability.[80] The vasoactive cytokine vascular endothelial growth factor (VEGF) and its soluble receptors might also have a role in increasing vascular permeability, with high levels of VEGF correlating with disease severity in one study,[81] and elevated levels of free, but not total, VEGF-A correlating with plasma leakage in another study,[82] although these results have not been supported by findings from other studies.[83,84]

In addition to the potential effects of dengue on the endothelial cell layer, the importance of viral and NS1 interactions with the surface glycocalyx layer in the pathogenesis of the vascular leakage is becoming increasingly apparent. The endothelial glycocalyx layer consists of a negatively charged mesh of glycoproteins, proteoglycans, and glycosaminoglycans covering the luminal surface of the microvascular endothelium. The glycocalyx layer is now considered to be the primary barrier to the movement of water and other molecules out of the microcirculation, according to the size, charge, and shape of these molecules.[85] The layer also acts as a transducer of sheer stress and regulates leucocyte and platelet adhesion, as well as complement and clotting activation.[86]

Visualization of the surface glycocalyx layer is currently not possible using conventional microscopy techniques; therefore, the effects of DENV infection on this layer have not been evaluated. However, the DENV E protein and the NS1 antigen have been found to bind to heparan sulfate, the major glycosaminoglycan in the microvascular glycocalyx layer, with the resulting disruption implicated in increased vascular permeability.[87,88] Soluble NS1 has been found to preferentially bind to microvascular endothelial cells, and might provide an explanation for the tissue-specific vascular leakage that occurs in dengue.[88] Urinary excretion of heparan sulfate was also found to be raised in children with DSS,[89] and elevated plasma levels of heparan sulfate have been detected even in the early febrile phase of the disease.[90]

Cardiovascular therapeutic targets

No antiviral agents are licensed for dengue, and the current treatment for severe disease is supportive, primarily focused on cautious fluid resuscitation, aiming to give just sufficient intravenous fluid therapy to maintain adequate tissue perfusion during the critical period of capillary leakage. If too much fluid is given, or the infusion is continued into the recovery period, the risk of iatrogenic fluid overload and associated morbidity is substantial. Rarely, inotropic support is required. In a large series of patients with DSS, managed over 10 years in a single institution in Vietnam, only 4% of patients required extra haemodynamic support, with the majority recovering with standard crystalloid resuscitation or following a single colloid infusion.[48] Bedside echocardiography should be considered for patients with dengue and cardiac symptoms, as well as those with fluid refractory shock or haemodynamic compromise disproportional to their capillary leakage, to tailor specific supportive therapies.

No cardiac-specific treatments for dengue myocarditis exist, but standard treatment for cardiac failure (β-blockers, angiotensin-converting-enzyme inhibitors, and diuretics) has been used successfully in these patients.[15] Antiviral and immunomodulatory treatments, interferon beta, corticosteroids, and intravenous immunoglobulins, have been used in patients with myocarditis of other viral aetiologies.[91,92] However, the evidence comes from small case series and nonrandomized trials, with benefit only shown in specific subgroups, and cannot, therefore, be extrapolated to dengue myocarditis.

Therapeutics targeting vascular leakage have shown some promise in murine models of DSS, but have not been used in humans with dengue.[93] Modulating the inflammatory response with corticosteroids has been attempted in paediatric patients with established DSS and also in patients with early acute dengue, but no benefit has been shown.[90,94] Evaluation of immunological correlates in a trial of early prednisolone therapy in Vietnamese paediatric patients with dengue, showed no change in the 11 cytokines tested, but did demonstrate a small change in the whole-blood gene-expression profile in the high-dose prednisolone arm 2 days after commencing treatment compared with placebo.[95] Specifically, transcripts encoding T cell and natural killer cell activity were less abundant. However, this finding did not translate into impaired viral clearance, and viraemia levels were not altered in either the low-dose

or high-dose steroid arms of the study compared with the placebo group.[95]

A study of lovastatin in early dengue is ongoing in Vietnam with the hope that the antiviral properties of this drug, along with its known stabilizing and anti-inflammatory effects on the endothelium, will modulate disease severity.[96,97] Other beneficial effects of statins, identified in studies of sepsis, include upregulation of endothelial nitric oxide synthase and downregulation of inducible nitric oxide synthase, both of which might help to restore endothelial function and microvascular tone and integrity.[98]

To date, no adjunctive therapies have demonstrated benefit in preventing the development of complications in dengue,[90,99,100] which might be partly due to timing. Initiating treatment within the first 72 h of fever might be too late to impact on virally induced immunopathogenesis, because viral replication is likely to have peaked by this time point and subsequent viral clearance is typically rapid. The future development of effective therapies will depend on an improved understanding of the exact mechanisms underlying the cardiovascular manifestations of dengue.

Conclusions

The spectrum of cardiovascular manifestations in dengue is broad, ranging from myocardial impairment and arrhythmias to vascular barrier dysfunction causing plasma leakage and haemodynamic compromise. Myocardial impairment can contribute to haemodynamic instability during the critical phase of capillary leakage. These complications are often under-reported, highlighting large gaps in our knowledge. Pathogenesis studies are needed to address the underlying mechanisms of capillary leak and endothelial dysfunction so that effective therapeutic targets can be identified. Studies are also required to improve our ability to predict which patients are likely to develop clinically relevant capillary leak, and investigate new techniques to monitor intravascular volume in these patients.

Definitive evidence of direct viral invasion of myocytes, or of an immune-mediated injury that might explain the cardiac involvement in dengue, is lacking. Ongoing research to elucidate the relative contribution of these two aetiologies is crucial to further our understanding of the disease, and to allow rational choices for individual case-management and for future therapeutic intervention trials targeting the virus or immune modulation.

Where available, echocardiography should be performed in patients with dengue, particularly in those with severe disease and refractory shock, to tailor their management. Future studies need to define systematically the true incidence of cardiac involvement in dengue, particularly in high-risk groups such as elderly patients (age ≥65 years) and those with comorbidities. As portable echocardiographic equipment and expertise in dengue become more-readily available worldwide, this goal should now be achievable. The use of new echocardiographic techniques, including strain echocardiography, could increase the sensitivity and specificity of diagnosing myocarditis.[101] In addition, the use of CMR imaging as a research tool will provide specific information on the presence and type of myocardial tissue injury, but is likely to have a limited role in routine clinical practice in dengue-endemic areas.[102]

Review criteria

We searched the PubMed database for articles published between 1970 and 2014, using the search term "dengue myocarditis" alone, or the combination of "dengue" with "cardiac function", "electrocardiogram", "histopathology", "capillary leakage", "ultrasound", "endothelial dysfunction", "glycocalyx", and "therapeutics". In addition, bibliographies of the selected articles were reviewed for further relevant articles. Only articles published in the English language were included.

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Author contributions

S.Y. and B.W. wrote the manuscript. H.W., C.P.S., G.S., and B.W reviewed and/or edited the manuscript before submission.