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Diabetes, cardiac disorders and asthma as risk factors for severe organ involvement among adult dengue patients: A matched case-control study

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Progression to severe organ involvement due to dengue infection has been associated with severe dengue disease, intensive care treatment, and mortality. However, there is a lack of understanding of the impact of pre-existing comorbidities and other risk factors of severe organ involvement among dengue adults. The aim of this retrospective case-control study is to characterize and identify risk factors that predispose dengue adults at risk of progression with severe organ involvement. This study involved 174 dengue patients who had progressed with severe organ involvement and 865 dengue patients without severe organ involvement, matched by the year of presentation of the cases, who were admitted to Tan Tock Seng Hospital between year 2005 and 2008. Age group of 60 years or older, diabetes, cardiac disorders, asthma, and having two or more pre-existing comorbidities were independent risk factors of severe organ involvement. Abdominal pain, clinical fluid accumulation, and hematocrit rise and rapid platelet count drop at presentation were significantly associated with severe organ involvement. These risk factors, when validated in a larger study, will be useful for triage by clinicians for prompt monitoring and clinical management at first presentation, to minimize the risk of severe organ involvement and hence, disease severity.

Dengue is currently the most important mosquito-borne viral pathogen affecting humans, and is emerging as a major threat to global health. It results in a significant public health and economic burden in the endemic regions^{1,2}, particularly in the World Health Organization (WHO) South-East Asia and Western Pacific Regions, accounting for nearly 75% of the current global dengue disease burden³. Best estimates indicate that some 3 billion people live in these tropical and subtropical regions where they are at risk of infection and that around 96 million symptomatic episodes and approximately 20,000 deaths occur each year⁴. The high burden was largely due to a lack of licensed vaccines then and specific therapies although there have been enormous research efforts focusing on these two areas. The ongoing implementation of a recently licensed Denvaxia[®] tetravalent dengue vaccine across various dengue-endemic countries⁵ may help to reduce these dengue burden in the near future.

Dengue is caused by infection with any one of four related dengue virus (DENV) serotypes that belongs to the genus *Flavivirus*. Although most dengue infections are asymptomatic, patients can present with a wide spectrum of clinical symptoms ranging from mild febrile illness through to severe manifestations of bleeding, organ involvement, and hypovolemic shock due to a systemic vascular leak syndrome. However, with good supportive care (primarily judicious use of parenteral fluid therapy to offset plasma volume losses due to leakage), mortality rates of less than 1% are possible even among severe cases^{6,7}.

Although the vast majority of symptomatic infections do not progress to severe disease, areas of high dengue transmission can have seasonal epidemics, which can quickly overwhelm health service capacity, particularly in the tertiary care settings. Thus, the ability to identify patients at high risk of severe disease progression, who are likely

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to benefit from close observation and early intervention with supportive therapy, has become the focus of intense research efforts in recent years. In recognizing the changing needs and increasing trends of difficulty in clinical application on the ground, the World Health Organization revised the previous dengue classifications⁸ (Dengue fever [DF], Dengue hemorrhagic fever [DHF] and dengue shock syndrome [DSS]) into two major entities, DF and severe dengue (SD), where DF comprises two subgroups of those with or without warning signs⁹ to help clinicians identify patients who are likely to develop complications during the critical phase of the illness¹⁰. However, the utility of warning signs were limited among adult patients^{11,12}, probably due to the small number of adult patients progressing to severe disease in the study that derived these warning signs¹³.

One of the major dengue complications of concern is the progression to severe organ involvement, which has been associated with SD, intensive care treatment, and fatality^{13–17}. Dengue associated severe organ involvement were cardiovascular¹⁸, hepatic^{14,19,20}, renal^{21,22}, as well as central nervous systems^{23,24}, and to some extent, respiratory^{25–27} and muscular systems^{28,29}. Different dengue serotypes may have different severe organ involvements³⁰. Moreover, severe organ involvement has been associated with adult dengue, and are likely to occur late in the disease course, but occurs rapidly when it happens, with limited time for optimal clinical management^{3,10}. Increasingly, pre-existing comorbidities such as diabetes have been shown to be a significant public health burden in the WHO Western Pacific and South-East Asia regions³¹, which are also dengue endemic regions. However, there is still limited understanding of the impact of pre-existing comorbidities³² and other early risk factors among dengue adults with severe organ involvement. Therefore, the aim of this retrospective case-control study is to characterize and identify risk factors that are useful to stratify adult dengue patients at a tertiary hospital at risk of progression with severe organ involvement.

Results

From year 2005 to 2008, there were about 6,300 dengue patients admitted into Tan Tock Seng Hospital. Among which, there were 174 (2.76%) dengue patients who had progressed with severe organ involvement. Among the cases, there were 106 (61%) who had hepatic involvement, 60 (34.5%) had renal involvement, 23 (13.2%) had neurological involvement, and 15 (8.6%) had both renal and liver involvement. The median age of these cases was 37.5 years old (Inter-quartile range: 27–52). Among these cases, 84 (48.3%) were female, 114 (65.5%) of Chinese ethnicity, and median duration of fever at presentation was 5 days (IQR: 4–6 days). In addition, 55 (31.6%) of these cases presented with pre-existing illnesses (Table 1).

Age and existing co-morbidities as risk factors of severe organ involvement. Dengue patients within age group of 60 years of age and older had 2.75 times higher (AOR: 2.75; 95% CI: 1.3–5.8) risk than age group between 12–29 years of age. Patients who presented with any pre-existing co-morbidity had 1.63 times higher (AOR: 1.63; 95% CI: 1.07–2.49) risk than dengue patients who did not presented with any existing co-morbidity. In addition, dengue patients with two or more existing co-morbidities had 2.90 times higher (95% CI: 1.66–5.07) risk than dengue patients with no existing co-morbidities. Dengue patients with pre-existing diabetes had 2.21 times higher (AOR: 2.21; 95% CI: 1.10–5.02) risk than dengue patients without diabetes. Dengue patients with pre-existing cardiac disorder had 4.3 times higher (AOR: 4.30; 95% CI: 1.45–12.78) risk than dengue patients without cardiac disorders. Dengue patients with pre-existing asthma had 2.14 times higher (AOR: 2.14; 95% CI: 1.04–4.42) risk than dengue patients without asthma (Table 1).

Using these significantly associated co-morbidities as risk factors, the impact of dual pre-existing comorbidities was assessed. Dengue patients with both pre-existing diabetes and cardiac disorders had eight times higher risk of severe organ involvement (AOR: 8.02; 95% CI: 1.40–45.92) than dengue patients with none of these (Table 2). In addition, we observed that dengue patients with both diabetes and hypertension (AOR: 2.68; 95% CI: 1.07–6.68) or both diabetes and hyperlipidemia (AOR: 4.25; 95% CI: 1.34–13.52) or both cardiac disorders and hyperlipidemia (AOR: 5.79; 95% CI: 1.03–32.64) had significant increase in risk compared with dengue patients with only one of the comorbidities or none at all. There was a lack of controls for dual comorbidities involving asthma, and hence, it was excluded (Table 2).

Clinical severity & management characteristics as risk factors of severe organ involvement.

Based on WHO 1997 dengue classifications, dengue patients with initial diagnosis of DHF/DSS had 3.11 times higher (AOR: 3.11; 95% CI: 2–4.85) risk of subsequent severe organ involvement. However, any dengue warning signs category at presentation based on the WHO 2009 dengue classifications was not a risk factor for subsequent severe organ involvement. At discharge, both DHF/DSS and any dengue warning signs category were significantly associated with severe organ involvement. The median length of hospitalization was five days (IQR: 4–7 days) longer than dengue patients without severe organ involvement. Overall, 6.9% of those with severe organ involvement required intensive care admission, compared to none of the dengue patients without severe organ involvement. There were also significantly more dengue patients with severe organ involvement who required intravenous fluid (96%) with larger fluid volume (median: 6liters; IQR: 3.9–8.7liters), blood transfusion (5.8%) and platelet transfusion (33.3%) than dengue patients without severe organ involvement. There was no death in both groups (Table 3).

Signs and symptoms at presentation as risk factors of severe organ involvement. At presentation, abdominal pain (AOR: 2.02; 95% CI: 1.40–2.93), clinical fluid accumulation (AOR: 26.2; 95% CI: 2.51–274.3) and hematocrit rise and rapid platelet count drop (AOR: 6.67; 95% CI: 3.98–11.17) were specific warning signs significantly associated with severe organ progression. Dengue patients with severe organ involvement were significantly associated with nausea or vomiting (AOR: 1.65; 95% CI: 1.08–1.87), but not persistent vomiting at presentation. In addition, plasma leakage at presentation (AOR: 6.19; 95% CI: 4.09–9.40) was observed to be

| | Controls [^] (N = 865) | % | Cases (N = 174) | % | cOR | p-value | 95% CI | 95% CI | AcOR | p-value* | 95% CI | 95% CI |
|--------------------------------|------------------------------------|---------|--------------------|---------|-------------|-------------------|-------------|--------------|-------------|-------------------|-------------|--------------|
| Age (median) | 33 | (24–42) | 37.5 | (27–52) | 1.03 | <0.0001 | 1.02 | 1.04 | 1.01 | 0.112 | 0.99 | 1.03 |
| Age groups | | | | | | | | | | | | |
| 12–29 | 342 | 39.5 | 51 | 29.3 | 1 | | | | 1 | | | |
| 30–39 | 241 | 27.9 | 45 | 25.9 | 1.25 | 0.318 | 0.81 | 1.93 | 1.25 | 0.318 | 0.8 | 1.95 |
| 40–49 | 167 | 19.3 | 30 | 17.2 | 1.17 | 0.531 | 0.72 | 1.9 | 1.07 | 0.792 | 0.62 | 1.72 |
| 50–59 | 77 | 8.9 | 18 | 10.3 | 1.58 | 0.128 | 0.88 | 2.84 | 1.11 | 0.751 | 0.52 | 1.99 |
| ≥60 | 38 | 4.4 | 30 | 17.2 | 5.29 | <0.0001 | 3 | 9.35 | 2.75 | 0.008 | 1.3 | 5.8 |
| Gender | | | | | | | | | | | | |
| Female | 347 | 40.1 | 84 | 48.3 | 1.41 | 0.039 | 1.02 | 1.96 | 1.7 | 0.09 | 0.95 | 1.93 |
| Ethnic Groups | | | | | | | | | | | | |
| Chinese | 593 | 68.6 | 114 | 65.5 | 1 | | | | 1 | | | |
| Malay | 84 | 9.7 | 20 | 11.5 | 1.26 | 0.403 | 0.74 | 2.14 | 1.09 | 0.76 | 0.62 | 1.93 |
| Indians | 86 | 9.9 | 17 | 9.8 | 1.03 | 0.915 | 0.59 | 1.8 | 1.15 | 0.647 | 0.64 | 2.07 |
| Others | 102 | 11.8 | 23 | 13.2 | 1.19 | 0.501 | 0.72 | 1.96 | 1.51 | 0.129 | 0.89 | 2.57 |
| Epidemic Year [^] | | | | | | | | | | | | |
| 2005 | 585 | 67.6 | 117 | 67.2 | | | | | | | | |
| 2006 | 115 | 13.3 | 23 | 12.2 | | | | | | | | |
| 2007 | 75 | 8.7 | 16 | 9.2 | | | | | | | | |
| 2008 | 90 | 10.4 | 18 | 10.3 | | | | | | | | |
| DPF presentation | 5 | (4–6) | 5 | (4–6) | 0.89 | 0.038 | 0.8 | 0.99 | 0.93 | 0.186 | 0.83 | 1.04 |
| IgG positive | 241 | 93.8 | 70 | 98.6 | 1.85 | 0.579 | 0.21 | 16.23 | 0.88 | 0.917 | 0.08 | 10.2 |
| Detection Assay | | | | | | | | | | | | |
| Serology+ | 582 | 67.3 | 114 | 65.5 | 1 | | | | 1 | | | |
| PCR+ | 283 | 32.7 | 60 | 34.5 | 1.10 | 0.609 | 0.77 | 1.55 | 0.96 | 0.851 | 0.66 | 1.42 |
| Any Pre-existing illness | | | | | | | | | | | | |
| Yes | 147 | 17 | 55 | 31.6 | 2.22 | <0.0001 | 1.53 | 3.22 | 1.63 | 0.023 | 1.07 | 2.49 |
| Number of Pre-existing illness | | | | | | | | | | | | |
| 0 | 718 | 83.0 | 117 | 67.2 | 1 | | | | 1 | | | |
| 1 | 99 | 11.5 | 24 | 13.8 | 1.42 | 0.177 | 0.85 | 2.36 | 1.19 | 0.515 | 0.70 | 2.02 |
| ≥2 | 48 | 5.6 | 33 | 19.0 | 3.92 | <0.0001 | 2.45 | 6.29 | 2.90 | <0.0001 | 1.66 | 5.07 |
| Diabetes | 27 | 3.1 | 23 | 12.2 | 4.75 | <0.0001 | 2.63 | 8.56 | 2.21 | 0.027 | 1.1 | 5.02 |
| Hypertension | 70 | 8.1 | 34 | 19.5 | 2.77 | <0.0001 | 1.77 | 4.33 | 1.02 | 0.935 | 0.54 | 1.95 |
| Heart Failure | 2 | 0.2 | 0 | 0 | | | | | | | | |
| Hyperlipidemia | 29 | 3.4 | 21 | 12.1 | 3.65 | <0.0001 | 2.03 | 6.57 | 1.46 | 0.347 | 0.66 | 3.22 |
| Cardiac Disorder | 9 | 1 | 13 | 7.5 | 8.59 | <0.0001 | 3.41 | 21.66 | 4.30 | 0.009 | 1.45 | 12.78 |
| Lung Disorder | 14 | 1.6 | 4 | 2.3 | 1.43 | 0.529 | 0.47 | 4.34 | 0.46 | 0.278 | 0.12 | 1.86 |
| Liver Disorder | 11 | 1.3 | 1 | 0.6 | 0.45 | 0.443 | 0.06 | 3.52 | 0.39 | 0.383 | 0.05 | 3.23 |
| Renal Disorder | 3 | 0.4 | 1 | 0.6 | 1.67 | 0.658 | 0.17 | 16.02 | 1.25 | 0.85 | 0.12 | 13.2 |
| Asthma | 34 | 3.9 | 13 | 7.5 | 2.05 | 0.039 | 1.03 | 4.04 | 2.14 | 0.039 | 1.04 | 4.42 |

Table 1. Demographic and comorbidities risk factors at first presentation of dengue patients with severe organ involvement outcome (Cases) compared to matched[^] dengue patients with no severe organ involvement outcome (Controls). DPF - Days post fever. [^]Matched by year of presentation as the surrogate marker for predominant serotype to minimize the bias due to the effect of different predominant serotypes. *Adjusted by age group, gender, day post fever duration at presentation, diabetic, hypertension, hyperlipidemia, cardiac disorder and asthma.

significantly associated with dengue patients with severe organ involvement, but not hemorrhagic manifestations at presentation (Table 4).

Clinical laboratory characteristics at presentation as risk factors of severe organ involvement. White blood cell (median: 3.3; IQR: 2.1–4.7) and neutrophil counts (median: 61.7; IQR: 50–70) were significantly higher at presentation among dengue patients who progressed with severe organ involvement compared with dengue patients without severe organ involvement. However, lymphocyte (median: 20.2; IQR: 15–28.9) and eosinophil counts (median: 0.1; IQR: 0–0.5) were significantly lower at presentation among dengue patients who progressed with severe organ involvement compared with dengue patients without severe organ involvement. Alanine (median: 77.1; IQR: 174–1242) and aspartate aminotransferases (median: 91.5; IQR: 91.5–717)

| | Controls [^] (N = 865) | % | Cases (N = 174) | % | cOR | p-value | 95% CI | 95% CI | AcOR | p-value* | 95% CI | 95% CI |
|---|------------------------------------|-------|--------------------|-------|--------------|-------------------|-------------|---------------|--------------|--------------|-------------|--------------|
| Diabetes, Cardiac disorder | | | | | | | | | | | | |
| No DM, no CD | 831 | 96.07 | 144 | 82.76 | 1 | | | | 1 | | | |
| DM, no CD | 25 | 2.89 | 17 | 9.77 | 3.99 | <0.0001 | 2.09 | 7.61 | 2.36 | 0.028 | 1.10 | 5.08 |
| CD, no DM | 7 | 0.81 | 7 | 4.02 | 6.73 | 0.001 | 2.18 | 20.83 | 4.85 | 0.011 | 1.43 | 16.41 |
| DM and CD | 2 | 0.23 | 6 | 3.45 | 18.93 | <0.0001 | 3.74 | 95.74 | 8.02 | 0.019 | 1.40 | 45.92 |
| Diabetes, Hypertension | | | | | | | | | | | | |
| No DM, no HT | 783 | 90.52 | 133 | 76.44 | 1 | | | | 1 | | | |
| DM, no HT | 12 | 1.39 | 7 | 4.02 | 3.66 | 0.012 | 1.34 | 10.04 | 2.37 | 0.131 | 0.77 | 7.26 |
| HT, no DM | 55 | 6.36 | 18 | 10.34 | 1.96 | 0.024 | 1.09 | 3.51 | 1.21 | 0.572 | 0.62 | 2.37 |
| DM and HT | 15 | 1.73 | 16 | 9.20 | 5.95 | <0.0001 | 2.85 | 12.43 | 2.68 | 0.035 | 1.07 | 6.68 |
| Diabetes, Hyperlipidemia | | | | | | | | | | | | |
| No DM, no HL | 779 | 90.06 | 136 | 78.16 | 1 | | | | 1 | | | |
| DM, no HL | 10 | 1.16 | 3 | 1.72 | 1.86 | 0.362 | 0.49 | 7.08 | 1.50 | 0.562 | 0.38 | 5.94 |
| HL, no DM | 68 | 7.86 | 20 | 11.49 | 7.08 | 0.098 | 0.92 | 2.75 | 5.94 | 0.436 | 0.27 | 1.77 |
| DM and HL | 8 | 0.92 | 15 | 8.62 | 10.89 | <0.0001 | 4.41 | 26.85 | 4.25 | 0.014 | 1.34 | 13.52 |
| Cardiac disorder, Hypertension | | | | | | | | | | | | |
| No CD, no HT | 793 | 91.68 | 133 | 76.44 | 1 | | | | 1 | | | |
| CD, no HT | 2 | 0.23 | 7 | 4.02 | 21.83 | <0.0001 | 4.44 | 107.30 | 15.79 | 0.002 | 2.87 | 86.76 |
| HT, no CD | 63 | 7.28 | 28 | 16.09 | 2.62 | <0.0001 | 1.60 | 4.29 | 1.41 | 0.275 | 0.76 | 2.61 |
| CD and HT | 7 | 0.81 | 6 | 3.45 | 5.42 | 0.003 | 1.75 | 16.84 | 2.64 | 0.141 | 0.73 | 9.57 |
| Cardiac disorder, Hyperlipidemia | | | | | | | | | | | | |
| No CD, no HL | 829 | 95.84 | 146 | 83.91 | 1 | | | | 1 | | | |
| CD, no HL | 7 | 0.81 | 7 | 4.02 | 6.21 | 0.001 | 2.03 | 19.03 | 4.52 | 0.016 | 1.32 | 15.52 |
| HL, no CD | 27 | 3.12 | 15 | 8.62 | 2.80 | 0.002 | 1.44 | 5.44 | 1.46 | 0.357 | 0.66 | 3.23 |
| CD and HL | 2 | 0.23 | 6 | 3.45 | 17.28 | 0.001 | 3.44 | 86.91 | 5.79 | 0.046 | 1.03 | 32.64 |

Table 2. The risk effect of severe organ involvement with two pre-existing comorbidities. [^]Matched by year of presentation as the surrogate marker for predominant serotype to minimize the bias due to the effect of different predominant serotypes. *Adjusted by age group, gender, day post fever duration at presentation, diabetic, hypertension, hyperlipidemia, cardiac disorder and asthma.

were observed to be significantly higher at presentation among dengue patients who progressed with severe organ involvement compared with dengue patients without severe organ involvement (Table 5).

Discussion

Severe organ involvement has been associated with severe dengue, intensive care requirement, and fatality^{13–17}. Chronic medical disorders have also been implicated in the development of severe dengue diseases³². However, there is still a lack of understanding on the impact of comorbidities on adult dengue patients who progressed with severe organ involvement, which is one of the criteria for severe dengue classification. Identification of these risk factors at presentation may guide early triage and clinical management of adult dengue patients who are at high risk of severe organ involvement.

Age group of 60 years old and above was observed to be an independent risk factor of severe organ involvement. Even after adjusting for the potential confounding effect of pre-existing comorbidities, age group of 60 years old and above has about three times higher risk for severe organ involvement than those aged 12–29 years old. This may not be surprising as elderly were also reported to be an independent risk factor for severe dengue in Thailand³³, Malaysia³⁴, Taiwan³⁵ and Singapore³⁶, and organ impairment was significantly associated with increased age in Vietnam³⁷. This may be due to immunosenescence among elderly, which predisposes them to infectious diseases³⁸. Immunosenescence is characterized by reduced natural killer cell cytotoxicity; reduced number and function of dendritic cells in blood; decreased pools of naive T and B cells; and increases in the number of memory and effector T and B cells³⁹. In addition, elderly tend to have impaired functional reserve for various organ systems, and with the reported tropism of dengue virus for liver, spleen, lymph node, kidney, bone marrow, lung, thymus and brain⁴⁰, may increase the risk of severe organ impairment.

Co-morbidities such as diabetes mellitus, cardiac disorders and asthma are among the few leading causes of mortality and morbidity in Asia³¹. However, there are still limited reported systematic studies so far to assess the association between these comorbidities and severe organ involvement due to dengue as a form of disease severity³². In this study, having any pre-existing comorbidity was observed to be an independent risk factor of severe organ involvement. Similarly, this was reported to be associated with dengue hemorrhagic fever (DHF) and severe dengue³². Furthermore, the risk of severe organ involvement was found to be three times higher among patients with at least two pre-existing comorbidities compared to patients with none. This observation suggests that there could be multiplicative effect modification. Diabetes, cardiac disorders and asthma were observed to be independent risk factors of severe organ involvement. Diabetes was well-reported to be a risk factor for

| | Controls [^] (N = 865) | % | Cases (N = 174) | % | AcOR | p-value | 95% CI | 95% CI |
|--|------------------------------------|-------|--------------------|-------|--------------|-------------------|--------------|---------------|
| Classification at Presentation | | | | | | | | |
| DHF/DSS (WHO 1997) | 96 | 11.1 | 49 | 28.2 | 3.11 | <0.0001 | 2 | 4.85 |
| WS (WHO 2009) | 567 | 65.6 | 125 | 71.8 | 1.38 | 0.95 | 0.95 | 2.02 |
| Classification as final outcome | | | | | | | | |
| DHF/DSS (WHO 1997) | 167 | 19.3 | 81 | 46.6 | 3.95 | <0.0001 | 2.68 | 5.82 |
| WS (WHO 2009) | 656 | 75.8 | 152 | 87.4 | 1.93 | 0.008 | 1.19 | 3.14 |
| Severe dengue (WHO 2009) | 104 | 12 | 174 | 100 | 62.81 | <0.0001 | 31.37 | 125.76 |
| Liver involvement | 0 | 0 | 106 | 61 | | | | |
| Renal involvement | 0 | 0 | 60 | 34.5 | | | | |
| CNS involvement | 0 | 0 | 23 | 13.2 | | | | |
| Renal & liver involvement | 0 | 0 | 15 | 8.6 | | | | |
| Median LOS (IQR) | 4 | (3–5) | 5 | (4–7) | 1.4 | <0.0001 | 1.27 | 1.54 |
| ICU admission | 0 | 0 | 12 | 6.9 | | | | |
| IV Fluid | 748 | 86.5 | 167 | 96 | 3.46 | 0.003 | 1.53 | 7.80 |
| Blood transfusion | 4 | 0.5 | 10 | 5.8 | 11.92 | <0.0001 | 3.55 | 40.6 |
| Platelets transfusion | 80 | 9.3 | 58 | 33.3 | 5.12 | <0.0001 | 3.21 | 8.16 |

Table 3. Differential severity and clinical management among dengue patients with severe organ involvement outcome compared against matched dengue patients with no severe organ involvement outcome. DHF - Dengue haemorrhagic fever. DSS - Dengue shock syndrome. WS - Dengue warning signs (WHO 2009 dengue classification). CNS - Central nervous system. LOS - Length of stay. IQR - Interquartile range. ICU - Intensive care unit. IV - intravenous. [^]Matched by year of presentation as the surrogate marker to minimize the impact of different predominant serotypes of that year. *Adjusted by age group, gender, day post fever duration at presentation, diabetic, hypertension, hyperlipidemia, cardiac disorder and asthma.

dengue severity^{41–43}, as well as acute kidney injury²². However, the pathophysiology behind diabetes leading to severe organ involvement outcome is not well understood yet, even though numerous studies had suggested that diabetes mellitus can result in immune and endothelial dysfunction^{44,45}. Pre-existing cardiac disorders have also been reported as a risk factor for dengue fatality⁴⁶. Cardiac output, is one of the major determinants of renal blood flow autoregulation. Renal blood flow is highly regulated to ensure oxygen delivery for normal renal function⁴⁷. If cardiac output is compromised, ischemic and toxic injury to the kidney can occur, resulting in severe impairment⁴⁸ especially when dengue virus has also been reported to affect kidney function²². Cardiovascular manifestations due to dengue has been reviewed¹⁸, however, there was no dengue-related myocarditis reported as disease outcome in this study. Asthma has been reported as a risk factor for DHF^{49–51}. It was observed that dengue virus enhancement within peripheral blood leukocytes was more significant among the asthmatic group compared to non-asthmatic group⁵², which may promote higher viral load leading to severe organ involvement. Asthma is a disorder of variable airflow obstruction in association with airway hyper-responsiveness (AHR). T Helper 2 (T_H2) cell-type cytokines orchestrate the allergic inflammatory cascade that occurs in asthma⁵³. Hence, with dengue virus infection, it may potentially activate the allergic inflammatory cascade, that may also result in selective organ damage. On the other hand, as an intrinsic abnormality, impaired TLR3-mediated interferon- β (IFN- β)⁵⁴ and IFN- λ ⁵⁵ production by asthmatic epithelial cells may also predispose to dengue virus indirectly^{56,57}. This allergic inflammatory cascade may also play a role in the development of acute respiratory failure, which was reported to be a risk factor of dengue fatal cases²⁷. Future studies will be required to validate this hypothesis.

Interestingly, having two or more comorbidities was observed to be an independent risk factor of severe organ involvement after adjusting for age as potential confounder. So far only diabetes with hypertension was reported as a risk factor of DHF⁴². Similarly, diabetes with hypertension remained an independent risk factor of severe organ involvement. Hypertension was reported to be a risk factor of rhabdomyolysis²⁸. In addition, diabetes with hyperlipidemia, diabetes with cardiac disorders and cardiac disorders with hyperlipidemia were observed to be independent risk factors of severe organ involvement. The underlying mechanism of diabetes, hypertension, hyperlipidemia, and cardiac disorders is inter-related and involves endothelial dysfunction⁵⁸ and increased nitric oxide⁵⁹, which has been shown to result in increased cytokine tsunami⁶⁰ and vascular permeability, leading to DHF⁶¹. This further suggests that these comorbidities have a major but indirect impact on the dengue disease progression with severe organ involvement⁴⁸, particularly on hepatic, renal and central nervous systems as observed in this study. Future studies focusing on the impact of medications for management of these pre-existing comorbidities and the underlying immunopathogenesis leading to severe organ involvement are needed to provide a better understanding on the impact of the interaction between dengue virus and non-communicable diseases.

Having any dengue warning signs at presentation was not a risk factor as a potential triage for severe organ involvement. However, it was observed in other published studies that any warning signs had very high sensitivity but only modest specificity for predicting DHF and severe dengue progression^{11,12}. Abdominal pain, clinical fluid accumulation, and hematocrit rise and rapid platelet count drop at presentation were significantly associated with severe organ involvement progression, which were also shown to be associated with severe dengue progression^{11,13}. Hematocrit rise and rapid platelet count drop and neutrophil count at presentation were associated with intensive care unit admission⁶². Dengue patients with severe organ involvement were significantly associated with

| | Controls [^] (N = 865) | % | Cases (N = 174) | % | AcOR ⁺ | p-value | 95% CI | 95% CI |
|--|------------------------------------|-------------|--------------------|-----------|-------------------|-------------------|-------------|--------------|
| Haemorrhagic manifestation | 394 | 45.6 | 77 | 44.3 | 0.96 | 0.833 | 0.68 | 1.36 |
| Rash | 413 | 47.8 | 75 | 43.1 | 0.92 | 0.66 | 0.65 | 1.31 |
| Leucopenia | 593 | 70.4 | 92 | 53.5 | 0.92 | 0.659 | 0.65 | 1.31 |
| Nausea/Vomiting | 604 | 69.8 | 135 | 77.6 | 1.65 | 0.019 | 1.08 | 2.51 |
| Aches pains | 655 | 75.7 | 135 | 77.6 | 1.23 | 0.327 | 0.81 | 1.87 |
| Abdominal pain | 226 | 26.1 | 70 | 40.2 | 2.02 | <0.0001 | 1.4 | 2.93 |
| Cough | 188 | 51.2 | 36 | 48.7 | 0.94 | 0.852 | 0.50 | 1.76 |
| Persistent vomiting | 0 | 0 | 0 | 0 | | | | |
| Clinical fluid accumulation | 1 | 0.1 | 3 | 1.7 | 26.2 | 0.006 | 2.51 | 274.3 |
| Mucosal bleeding | 199 | 23 | 37 | 21.3 | 1.02 | 0.936 | 0.67 | 1.56 |
| Lethargy | 289 | 33.4 | 55 | 31.6 | 0.85 | 0.39 | 0.58 | 1.23 |
| Hepatomegaly | 7 | 0.8 | 4 | 2.3 | 2.91 | 0.136 | 0.71 | 11.83 |
| Haemocrit \geq 20% & Platelet $<$ 50 | 45 | 5.4 | 49 | 28.7 | 6.67 | <0.0001 | 3.98 | 11.17 |
| Haemocrit \geq 20% | 79 | 9.4 | 72 | 41.9 | 5.91 | <0.0001 | 3.89 | 8.98 |
| Hypoproteinemia | 149 | 19.7 | 46 | 30.3 | 1.86 | 0.007 | 1.18 | 2.94 |
| Plasma leakage 2009 | 80 | 9.3 | 73 | 42 | 6.19 | <0.0001 | 4.09 | 9.4 |
| Thrombocytopenia | 731 | 86.9 | 163 | 95.3 | 2.93 | 0.005 | 1.38 | 6.22 |
| Tachycardia | 183 | 21.3 | 46 | 26.4 | 1.2 | 0.393 | 0.79 | 1.81 |
| Hypotension for age | 54 | 6.2 | 14 | 8.1 | 1.4 | 0.302 | 0.74 | 2.63 |
| Narrow pulse pressure | 3 | 0.4 | 2 | 1.2 | 2.2 | 0.422 | 0.32 | 15.08 |
| Shock | 221 | 25.6 | 55 | 31.6 | 1.24 | 0.271 | 0.84 | 1.83 |
| GIT bleed | 15 | 1.7 | 5 | 2.9 | 1.56 | 0.433 | 0.51 | 4.73 |
| Severe bleeding | 32 | 3.7 | 12 | 6.9 | 1.9 | 0.091 | 0.9 | 3.99 |
| Altered level of consciousness | 0 | 0 | 12 | 7.8 | | | | |
| Jaundice | 6 | 0.7 | 1 | 0.6 | 1.1 | 0.934 | 0.12 | 9.78 |
| Median temperature (IQR) | 37.6 | (36.9–38.4) | 37.7 | (37–38.4) | 1.15 | 0.104 | 0.97 | 1.37 |
| Median blood pressure (IQR) | 103 | (98–111) | 103 | (98–112) | 0.98 | 0.019 | 0.97 | 0.99 |
| Median pulse (IQR) | 76 | (67–87) | 75 | (65–84) | 0.99 | 0.035 | 0.97 | 0.99 |

Table 4. Differential clinical signs and symptoms at first presentation among dengue patients with severe organ involvement outcome compared against matched dengue patients with no severe organ involvement outcome. IQR - Interquartile range. [^]Matched by year of presentation as the surrogate marker to minimize the impact of different predominant serotypes of that year. ⁺Adjusted by age group, gender, day post fever duration at presentation, diabetic, hypertension, hyperlipidemia, cardiac disorder and asthma.

nausea or vomiting, but not persistent vomiting at presentation. This may not be a useful risk factor as most dengue patients with mild disease may present with nausea or vomiting⁶³. In addition, plasma leakage was observed to be significantly associated with dengue patients with severe organ involvement, but not hemorrhagic manifestations at presentation. This is consistent with the reported observation that plasma leakage has a role in the development of severe organ involvement⁶⁴. However, the underlying mechanism of the role of plasma leakage is not clear and requires further studies.

As this is a retrospective study, the quality of the study was dependent on the quality of the data available and collected. Information bias was minimized by the use of the standardized dengue care path for consistent clinical documentation. Reporting bias was minimized by the fact that patients with comorbidities tend to know their existing conditions and are on regular medications. However, undetected existing comorbidities among the controls could not be excluded in this retrospective study. In addition, there may be selection bias because the subjects were all hospitalized and hence were likely to be more severe and/ or have a more active health seeking behavior who may not represent the general dengue population in the community. We did not have patient-specific dengue serotype data and could only minimize the confounding effect of different serotypes by matching each case to five controls by year of presentation. Lastly, we understand the importance of accounting for prior infection as it is a main risk factor for severe dengue disease. The result of IgG test carried out within seven days of fever onset can be used to classify patients with or without prior infection. From our limited data of potential secondary infections, we showed that secondary infection was not significantly associated with severe organ involvement in adult patients (Table 1). Further studies involving larger number of patients with acute secondary infections are required to confirm this hypothesis.

Conclusion

Severe organ involvement results in severe dengue disease. Age group of 60 years or older, diabetes, cardiac disorders, asthma, and two or more pre-existing comorbidities were independent risk factors of severe organ involvement. Abdominal pain, clinical fluid accumulation, and hematocrit rise and rapid platelet count drop at

| | Controls [^] (N = 865) | Interquartile range | Cases (N = 174) | Interquartile range | AcOR* | p-value | 95% CI | 95% CI |
|--------------------|------------------------------------|------------------------|--------------------|------------------------|-------|---------|--------|--------|
| Median WBC | 2.7 | (2–3.8) | 3.3 | (2.1–4.7) | 1.18 | <0.0001 | 1.09 | 1.27 |
| Median neutrophil | 53.8 | (40.6–67.2) | 61.7 | (50–70) | 1.02 | <0.0001 | 1.01 | 1.03 |
| Median lymphocytes | 26 | (18.5–35.2) | 20.2 | (15–28.9) | 0.96 | <0.0001 | 0.95 | 0.98 |
| Median monocytes | 10 | (6.9–13.1) | 8.6 | (5.4–12.8) | 0.97 | 0.14 | 0.94 | 1.01 |
| Median basophils | 0.2 | (0–0.6) | 0.2 | (0–0.5) | 0.89 | 0.541 | 0.61 | 1.3 |
| Median eosinophil | 0.2 | (0–1) | 0.1 | (0–0.5) | 0.84 | 0.047 | 0.7 | 0.99 |
| Median hematocrit | 43.1 | (40–45.8) | 44 | (40–46.9) | 1.06 | 0.001 | 1.01 | 1.11 |
| Median hemoglobin | 14.6 | (13.5–15.6) | 15 | (13.6–16) | 1.2 | 0.006 | 1.06 | 1.37 |
| Median platelet | 70 | (47–87) | 51 | (25–73) | 0.98 | <0.0001 | 0.98 | 0.99 |
| Median sodium | 137 | (134–139) | 135 | (132–137) | 0.84 | <0.0001 | 0.8 | 0.88 |
| Median potassium | 3.6 | (3.3–3.9) | 3.6 | (3.3–4) | 1.41 | 0.092 | 0.95 | 2.09 |
| Median urea | 3.4 | (2.5–4.4) | 4.3 | (2.8–7.4) | 1.42 | <0.0001 | 1.29 | 1.57 |
| Median creatinine | 76 | (62–88.5) | 85 | (65.3–128.3) | 1.03 | <0.0001 | 1.02 | 1.04 |
| Median bilirubin | 11 | (8–15) | 13 | (9–18) | 1.06 | <0.0001 | 1.04 | 1.09 |
| Median AST | 112.5 | (65–207.3) | 771 | (174–1242) | 1 | <0.0001 | 1.004 | 1.006 |
| Median ALT | 71 | (39–148) | 389 | (91.5–717) | 1.006 | <0.0001 | 1.005 | 1.008 |
| Median protein | 68 | (64–72) | 66 | (61–71) | 0.96 | 0.016 | 0.93 | 0.99 |
| Median albumin | 39 | (36–41) | 37 | (33–40) | 0.89 | <0.0001 | 0.85 | 0.934 |

Table 5. Differential clinical laboratory factors at first presentation among dengue patients with severe organ involvement outcome compared against matched dengue patients with no severe organ involvement outcome. WBC - White blood cells. AST - Aspartate aminotransferases. ALT - Alanine aminotransferases. [^]Matched by year of presentation as the surrogate marker to minimize the impact of different predominant serotypes of that year. *Adjusted by age group, gender, day post fever duration at presentation, diabetic, hypertension, hyperlipidemia, cardiac disorder and asthma.

presentation were significantly associated with severe organ involvement. With larger prospective studies to validate these findings, they may be useful to guide triage at presentation of adult dengue patients who are at higher risk of severe organ involvement.

Methods

A retrospective case-control study was conducted using data collected from all adult dengue patients admitted from 1 January 2005 to 31 December 2008 to the Department of Infectious Diseases at Tan Tock Seng Hospital (TTSH). It is the largest hospital in Singapore for the treatment of dengue patients where dengue patients were managed using a standardized dengue care path. Hospital electronic medical records were used for extraction of administrative, laboratory, microbiological and radiological data. Data extraction was performed by medically trained research assistants. Rule-based data validation was performed for the entire data set. In addition, 10% of the cases were randomly selected for repeat data entry by another research assistant; data discrepancy was resolved by independent medical case note review by one of the authors. The extracted data was de-identified in analysis.

Probable dengue patients had positive acute dengue serology, as measured by Dengue Duo IgM & IgG Rapid Strip Test (Panbio Diagnostic, Queensland, Australia), and fulfilled clinical diagnostic criteria of dengue fever by WHO 1997. Confirmed dengue patients had positive dengue polymerase chain reaction (PCR) assay. Patients were classified into the WHO 1997 and 2009 dengue severity categories with available clinical, laboratory and radiological data from the entire clinical course till hospital discharge for inpatients and end of acute follow up for outpatients with strict application of the two WHO classifications. As per previous published study⁴⁶, cardiac disorder is defined as having ischemic or other heart diseases excluding heart failure and peripheral vascular diseases. Renal disorder is defined as having renal failure, calculi or other kidney diseases.

WHO 1997 Classification. Based on WHO 1997 classification⁸, classification as dengue fever (DF) requires the presence of fever and two or more of the following: headache, retro-orbital pain, myalgia, arthralgia, rash, leukopenia, or hemorrhagic manifestations. The tourniquet test was not performed. Diagnosis of DHF required fever and all three of: hemorrhagic tendencies; thrombocytopenia (platelet <100 000/mm³); and evidence of plasma leakage (hematocrit change of $\geq 20\%$ or clinical fluid accumulation or hypoproteinemia [serum protein 63 g/dL]). For DSS, DHF cases required either (i) tachycardia (pulse > 100/minute) with narrow pulse pressure (<20 mmHg) or (ii) hypotension for age (systolic blood pressure <90 mmHg).

WHO 2009 Classification. Based on WHO 2009 classification⁹, classification as probable dengue required fever with two or more of: nausea/vomiting, rash, aches/pains, leukopenia and any warning sign. Warning signs used were: abdominal pain/tenderness, persistent vomiting (≥ 2 consecutive days), clinical fluid accumulation (pleural effusion or ascites on examination or radiography), mucosal bleeding, liver enlargement, and increase in hematocrit concurrent with rapid decrease in platelet count (interpreted as any hematocrit $\geq 20\%$ over baseline).

with platelet $<50000/\text{mm}^3$). Lethargy was not used as it was not routinely recorded in the dengue care path. For severe dengue, the criteria were: (i) plasma leakage (either clinical fluid accumulation or evidence of hematocrit change of $\geq 20\%$) and shock [with at least one of tachycardia (pulse $> 100/\text{minute}$), hypotension (systolic blood pressure $<90 \text{ mmHg}$), or narrow pulse pressure ($<20 \text{ mmHg}$)] or respiratory distress; severe bleeding was defined as WHO Grade 2 or above: hematemesis, melena, menorrhagia or clinical drop in hemoglobin requiring whole blood or packed red cell transfusion; severe organ involvement comprised hepatic injury (aspartate [AST] or alanine transaminase [ALT] levels $> 1000 \text{ unit/L}$), renal impairment (Stage 2 Acute Kidney Injury⁶⁵ defined as serum creatinine increase of 100% over baseline or calculated norm for age/gender/race), or encephalopathy. No dengue-related myocarditis was found in this cohort.

Statistical methods. Univariate and multivariate conditional logistic regression were used to calculate crude and adjusted odds ratios (cOR, AcOR), respectively, and their 95% confidence intervals (CI) were used to assess the association of the variables with DHF. Confounding effect was minimized by performing multivariate conditional logistic regression adjusting for potential confounders identified in the univariate analysis. These potential confounders are exposures at initial presentation that were found to be statistically different ($p < 0.05$) between cases and controls in Table 1. Severe organ involvement is the disease outcome, which is a part of the disease severity (particularly for WHO 2009 classification), that we are interested to understand more in this study. One of the principles for confounding factors states that a confounding factor should not be in the (known) causal pathway of the outcome of interest. In addition, there is high collinearity between severe organ involvement (as outcome of interest) and severe dengue classification (as potential confounding factor) as shown in Table 3. As such, we did not perform statistical adjustment using disease severity as one of the confounding factors to achieve a more realistic interpretations. In Singapore, dengue infections were predominantly due to dengue serotype 1 (detected in 75% to 100% of dengue samples collected each month) during the epidemic in the year 2005 and 2006, and dengue serotype 2 (detected in up to 91% of dengue samples) during the epidemic in the year 2007 and 2008. Given that different dengue serotypes may cause different disease severity, each case was matched to 4–5 controls by epidemic year to minimize confounding effect due to different predominant dengue serotypes. All statistical analyses were performed using Stata 10.0 (Stata Corp., College Station, TX, 2005). All tests were conducted at the 5% level of significance, with OR, P-value and corresponding 95% CI reported where applicable.

Ethics statement. The National Healthcare Group Domain Specific Review Board granted ethics approval of the study with a waiver of informed consent for collection of anonymized case note data and the data were analyzed anonymously (DSRB B/05/115, DSRB E/08/567).

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Author Contributions

J.P. and D.L. conceived the project and designed the study. J.P. and J.P.H. analyzed the data. T.W.Y. and Y.S.L. helped partly in study design. J.P. and J.P.H. collected the data, supported by T.W.Y., Y.S.L., and D.L. J.P. and D.L. wrote the manuscript. All authors read and approved the final manuscript.

Additional Information

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