Editorial

Dengue Research in Singapore Marking the Inaugural ASEAN Dengue Day

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Dengue is the most rapidly spreading mosquito-borne viral disease in the world. An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries. Some 1.8 billion (more than 70%) of the population at risk of dengue worldwide live in the World Health Organisation (WHO) South-East Asia Region and Western Pacific Region.[1] Case fatality rates of more than 1% have been reported in countries within the Southeast Asia region. Dengue has become a major public health issue in this region. In response, 15th June has been designated as the Association of Southeast Asian Nations (ASEAN) Dengue Day to raise awareness and commitment at all levels to reduce dengue incidence and burden.

Despite a widely hailed vector control programme, Singapore continues to see epidemics of dengue fever (DF). The largest dengue epidemics were seen in 2004 and 2005 with a total of 9459 and 14,209 cases notified to the Ministry of Health (MOH) respectively, including 168 and 393 cases of dengue haemorrhagic fever (DHF).[2,3] Aggressive vector control programmes over the years have reduced the "force of infection" that is the per capita rate at which susceptible individuals become infected. This, in turn, resulted in an epidemiological transition of dengue in Singapore from a childhood disease to an adult one.[4,5] The most recent sero-prevalence study by Yew *et al* reported that only 17.2% of young adults in Singapore were dengue IgG positive.[4] The age shift to older adults has become more apparent in recent years. In 2007 and 2009, incidence rates per 100,000 for 55 years and older age group were the highest among reported dengue cases.[6-9] In 2009, the incidence of DF/DHF was above 90 per 100,000 population in most age groups above 15 years of age compared to 43 per 100,000 and 23 per 100,000 population in the 5 to 14 years and under 5 years age groups respectively.[9]

Research Programme

Recognising the disease burden and a different epidemiological profile in Singapore compared to most other countries, a signature translational clinical research grant was awarded to study dengue in December 2008. This study was given the acronym STOP Dengue (Scientific exploration of disease pathogenesis, Translational research for improved clinical management, Operational evaluation for introduction of intelligent, vector control measures and Preventive measures through innovative treatment strategies for Dengue). The ultimate aim is to translate research into clinical management that results in zero dengue fatality in Singapore. STOP Dengue focuses on the main themes of finding innovative and cost-effective ways of early dengue diagnosis, early detection of severe illness, early intervention and prevention of infection and death.

Dengue Diagnosis and Classification

Prior to STOP Dengue, a dengue consortium was formed by a comprehensive group of researchers including clinicians and basic scientists immediately after severe acute respiratory syndrome (SARS) in 2003. This consortium launched the Early DENgue Infection Outcome study (EDEN) during the 2004 to 2005 dengue epidemics. EDEN has yielded many significant findings guiding the management of dengue infection. A simple decision algorithm on EDEN demonstrated an accuracy of 84.7% using clinical, haematological and virological data in differentiating dengue from non-dengue febrile illness within 72 hours of illness.[10] To enhance early detection, we studied the commercially available non-structural 1 protein (NS1) test kit (BioRad Laboratories) as a rapid test in the EDEN study and found an overall sensitivity and specificity of 80.5% and 100% respectively.[11] Another local study found it to be the most sensitive test (82%) in the primary health care setting.[12] Moving forward, STOP Dengue will validate these findings before promoting its widespread use in clinical settings. The Environmental Health Institute (EHI) has recently developed a saliva based test using an antigen capture anti-DENV IgA (ACA) enzyme-linked

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immunosorbent assay (ELISA) technique. This has been tested on saliva samples from an EDEN cohort presenting within 72 hours of illness. The sensitivity was 70% in the first 3 days of fever and 93% from days 3 to 5. The overall specificity of the ACA-ELISA test was 97%.[13] Saliva testing presents an attractive non-invasive testing modality particularly for children.

The disease manifestations and outcomes of dengue infection differ between children and adults. Two local studies demonstrated that fewer adult infections fulfilled the classification of DHF or dengue shock syndrome (DSS) as defined by the second edition of the WHO dengue guidelines,[6,14] which was published in 1997 using the data mostly from children.[15] The disease severity was classified as dengue fever, and a more severe form as DHF and the most severe form with evidence of shock as DSS. The differing clinical pictures in adults and increasing reports on cases with severe outcomes not fulfilling the 1997 WHO disease severity classification led to the review of the WHO guidelines.[16,17] The third edition of guidelines for dengue was published in 2009.[1] Several distinct differences from the previous guidelines include the introduction of gastro-intestinal symptoms and warning signs as additional criteria to probable dengue diagnosis, the use of warning signs for triage to determine the level of care and a complete change of disease severity classification. The latest guideline classifies disease severity into dengue infection, dengue with warning signs and severe dengue. The criteria for severe dengue include severe plasma leakage, severe bleeding and/or severe organ involvement. This has become an area of intense study comparing the applicability of the two sets of the WHO guidelines in each geographic area that might differ in demographic and disease patterns. Local studies comparing the 2 sets of WHO guidelines found discrepancies in assigning disease severity outcomes. A proportion of subjects fulfilling the 2009 guidelines of severe disease did not fall into the more severe form of dengue disease using the 1997 guidelines. These findings concur with an international multi-centre study.[18,19]

Hospital Admission and Treatment

High hospital admission rates that overwhelmed healthcare delivery were reported during the 2004 to 2005 dengue epidemics. STOP Dengue utilises a two-pronged approach based on retrospective and prospective cohorts to study who should be hospitalised for dengue treatment. The retrospective cohort based on cases managed in Tan Tock Seng Hospital (TTSH) cases in 2004 applied the WHO 1997 severity classification to yield 2 predictive models (a probability equation and a simple decision tree) for disease progression. The models were able to assist the primary care physicians in predicting whether the patients will subsequently develop DHF: parameters predicting disease progression were clinical bleeding, high serum urea, low serum protein with or without low lymphocyte proportion.[20] Applying the models on a 2007 TTSH cohort showed a comparable sensitivity and specificity.[21] Of note, the predominant serotype in the year 2004 was dengue serotype 1 compared to the predominant serotype 2 in the year 2007. Research using similar models to predict progression to severe disease using the WHO 2009 classification will be done next. The Prospective Adult Dengue Study (PADS) follows subjects on a daily basis during acute illness to examine the applicability of warning signs in triaging care and to identify predictive markers of disease severity for hospitalisation. This prospective cohort will be used to validate the predictive models developed from our retrospective cohorts. The findings of these studies will contribute greatly to evidence-based treatment approaches for dengue.

It is of concern that clinical data on dengue in the elderly is extremely lacking. A few recent reports suggested that older patients had fewer symptoms including fever that may contribute to missed or late diagnosis.[7,22] A local study showed similar findings in other reports indicating a higher mortality among older patients.[23] Such disconcerting findings emphasise the need for further research to improve clinical care in this vulnerable population.

The mechanisms underlying dengue-associated vascular leakage has yet to be fully elucidated. The lack of relevant animal models has greatly hampered the understanding of the pathogenesis of dengue infection. In this edition of the *Annals*, Tan *et al* describe a mouse model in which systemic manifestations of dengue resembling human disease can be elicited by subcutaneous administration of a non-mouse-adapted DEN2 virus strain (D2Y89P-PP1) into AG 129 mice. This offers an opportunity to identify the molecular players involved in the pathogenesis of dengue infection and provides a platform to test novel prophylactic and therapeutic agents. There are currently no licensed antiviral available for the treatment of dengue infection. Treatment of dengue is largely supportive, i.e. rest and appropriate fluid management. Thrombocytopenia, a hallmark of dengue infection and bleeding is also eloquently discussed by Kurukularatne *et al* in this edition. Platelet transfusion, a common practice that lacks supporting scientific evidence is up against STOP Dengue's prospective study which assesses the risks and benefits of platelet transfusion for dengue infection.

The long and winding journey of dengue vaccine development appears more positive in recent years. However, dengue vaccines need to be inexpensive as the majority of countries experiencing dengue epidemics are developing countries.[24] It will take considerable time for

dengue vaccines to be administered widely to populations at risk. There is thus an urgent need for dengue research to develop low cost, simple, accurate, and acceptable tools to enable early diagnosis, early prognosis and appropriate treatment so as to achieve the best possible outcomes.

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