

Molecular Biomarkers at the Interface of Basic and Clinical Dengue Research

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The recent spike in dengue cases in Singapore brought to the foreground the need to triage dengue patients and stratify them for management by risk of complications. As of October 2013, Singapore has seen its highest number of dengue cases since 2005.¹ However, this epidemic had not resulted in a hospital bed shortage crisis that was experienced in 2005, where non-urgent medical/surgical procedures were postponed due to over-hospitalisation of dengue patients. The 2005 episode had served as a catalyst to re-examine and develop new clinical decision algorithms^{2,3} for hospitalisation of dengue patients. This has resulted in better use of hospital beds although the few fatal cases still argue for better tools to differentiate dengue patients that are more likely to develop severe disease from those with uncomplicated acute illness.

Dengue is caused by dengue virus of the *Flaviviridae* family. Without either a licensed vaccine or antiviral drug, dengue continues to inflict a substantial proportion of the population.⁴ Severity of dengue infections is correlated with the level of viremia⁵ and viruses are typically cleared by the onset of defervescence. However, it is at the point of defervescence that pathology underlying severe dengue, such as increased vascular permeability develops. This suggests that host responses are intricately tied to pathogenesis of severe dengue. Consequently, identifying the host responses that strongly correlate with onset of severe dengue could both improve our understanding of dengue pathogenesis and serve as biomarkers to screen for patients at risk of severe dengue.

Advances in technology, notably progress in omics sciences offer opportunities to study how diseases are brought about from a global molecular perspective. In a much shorter time, hundreds to thousands of genes, proteins and metabolites can be simultaneously studied and mined for information. Because of the global, systematic structure in omics technologies, all molecules are treated equally

without prescription to bias. This means that any prominent molecules that stand out are discovered objectively and more likely to be *bona fide* findings critical to the disease. Using mass spectrometry- and immunologically-based omics, we have begun to comprehensively profile cytokines, chemokines, RNA modifications, proteomes, lipidomes and metabolomes in dengue infected patients to uncover predictive soluble biomarkers.⁶⁻⁸ Using a prospective study,^{9,10} a set of serum biomarkers that potentially correlated with early host responses to dengue infections were revealed. However, we have only begun to scratch the surface. While these candidate biomarkers show promise, their translation into prognostic markers may not be as straightforward as originally thought. This is partly because dengue is caused by 4 different albeit related viruses. Although they are often thought of as antigenically distinct strains of one species, their *in vitro* phenotype and on several occasions, the disease outcome associated with infection appear to be significantly different. Phylogenetically, the genetic distance between the 4 “serotypes” is similar to the distance between Japanese encephalitis and West Nile viruses, which are distinct species within the *Flavivirus* genus.¹¹ Consequently, the pathogenic processes and hence the biomarker profiles identified through the study of infection of 1 or 2 dengue viruses may not be applicable across all 4 dengue viruses. Rather, we suspect the biomarker profile may be distinct for each of the 4 dengue viruses.

Besides viral differences, severe dengue also manifests as different clinical syndromes. The most common of which is vascular leakage of plasma that leads to hypovolemic shock. Other forms of complications arising from dengue include internal haemorrhage as well as severe organ dysfunction or even failure, both of which may arise without concurrent vascular leakage. The biomarker profile related to each of these outcomes could also be different, given that the disease manifestation is sometimes mutually

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exclusive. Studies on dengue must thus rely on carefully annotated clinical presentation, course and outcome of illness. Given that an average dengue patient is seen by the general practitioner, hospital-based physician in emergency, internal medicine or infectious disease departments and the confirmatory diagnosis relies on positive findings on appropriate laboratory tests, systematic annotation of dengue cases is practically challenging. Solving this at the national level by integrating data collection from different branches of the healthcare ecosystem could accelerate the discovery and translation of biomarkers for patient triaging and management.

Besides triaging patients for management, the availability of prognostic biomarkers could also be advantageous for clinical trials of anti-dengue drug candidates, several of which are currently under development.^{12,13} Biomarkers could aid the translation of a much needed therapeutic in several ways:

- While drugs are often developed to benefit all patients with a particular disease, in reality, the benefit is often more spectacular in a subset of patients.¹⁴ Invariably, patient selection (or non-selection) is one of the biggest issues in clinical trials and selecting the 'right' patient will accelerate drug development.
- An example of successful personalised medicine uncovered through biomarker-driven drug trials is Gefitinib (Iressa), an epidermal growth factor receptor (EGFR) inhibitor. In initial broad eligibility trials Gefitinib failed to show efficacy, but when patients were enrolled based on EGFR mutations, they were highly sensitive to Gefitinib.^{15,16} Another example is the development of Trastuzumab (Herceptin) to target 30% of women with breast cancer tumors that overexpresses Her-2.¹⁷
- The use of predictive, pre-screening biomarkers should be incorporated as early as possible to enrich for a subset of patients most sensitive to the effects of a drug. Using a biomarker-driven paradigm, in patients with severe dengue without vascular leakage^{18,19} molecular biomarkers may uncover patient subsets obscured by broad symptomatic grouping. While the use of biomarkers in personalised drug development in infectious diseases is not widely reported, we anticipate such patient targeting trial to ensue in the near future.

Molecular biomarkers could also act in reverse by elucidating the proof of mechanism of underlying pathological mechanisms which in turn generates novel drug targets. Using work by Cocchi et al²⁰ as an illustration, they showed that the production of specific chemokines, RANTES, MIP-1 α and MIP-1 β by T lymphocytes suppresses

the release of HIV suppressive factors.²⁰ Subsequent studies revealed mutations in CCR5 chemokine receptor gene, the gene that encodes for the receptor for RANTES, MIP-1 α and MIP-1 β , that provide HIV resistance in individuals²¹ and based on this proof of mechanism led to the development of Maraviroc (Selzentry).²² In a similar fashion, candidate predictive biomarkers^{6,7} can be validated in independent patient cohorts to test for consistency and also show proof of mechanism and implemented to monitor drug efficacy in trials such as Celgosivir, an antiviral, α -glucosidase I and II inhibitor currently in Phases I and II trials (NCT01619969) for treating dengue fever.

Predictive biomarkers may serve well to bridge translational and clinical sciences to bring success to triaging dengue patients and anti-viral dengue clinical trials. Subtyping patients and their responses may commit toward strengthening prognosis and better management of patients displaying varying degrees of disease severity and symptoms. Likewise, biomarkers may build toward stratified dose-regimes or treatment types and reduce the number of enrolled volunteers and patients in anti-viral trials. Biomarker and hypothesis-driven trials may be a rational strategy forward to accelerate dengue drug development to ultimately benefit patients.

REFERENCES

1. Koh BKW, Ng LC, Kita Y, Tang CS, Ang LW, Wong KY, et al. The 2005 dengue epidemic in Singapore: epidemiology, prevention and control. *Ann Acad Med Singapore* 2008;37:538-45.
2. Thein TL, Leo Y-S, Lee VJ, Sun Y, Lye DC. Validation of probability equation and decision tree in predicting subsequent dengue hemorrhagic fever in adult dengue inpatients in Singapore. *Am J Trop Med Hyg* 2011;85:942-5.
3. Lee VJ, Lye DC, Sun Y, Leo YS. Decision tree algorithm in deciding hospitalization for adult patients with dengue haemorrhagic fever in Singapore. *Trop Med Int Health* 2009;14:1154-9.
4. Lee LK, Earnest A, Carrasco LR, Thein TL, Gan VC, Lee VJ, et al. Safety and cost savings of reducing adult dengue hospitalization in a tertiary care hospital in Singapore. *Trans R Soc Trop Med Hyg* 2013;107:37-42.
5. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 2000;181:2-9.
6. Kumar Y, Liang C, Bo Z, Rajapakse JC, Ooi EE, Tannenbaum SR. Serum proteome and cytokine analysis in a longitudinal cohort of adults with primary dengue infection reveals predictive markers of DHF. *PLoS Negl Trop Dis* 2012;6:e1887.
7. Cui L, Lee YH, Kumar Y, Xu F, Lu K, Ooi EE, et al. Serum Metabolome and Lipidome Changes in Adult Patients with Primary Dengue Infection. *PLoS Negl Trop Dis* 2013;7:e2373.
8. Dong H, Chang DC, Hua MHC, Lim SP, Chionh YH, Hia F, et al. 2'-O Methylation of Internal Adenosine by Flavivirus NS5 Methyltransferase. *PLoS Pathog* 2012;8:e1002642.

9. Low JGH, Ooi EE, Tolfvenstam T, Leo YS, Hibberd ML, Ng LC, et al. Early Dengue infection and outcome study (EDEN) - study design and preliminary findings. *Ann Acad Med Singapore* 2006;35:783-9.
10. Low JGH, Ong A, Tan LK, Chatterji S, Chow A, Lim WY, et al. The early clinical features of dengue in adults: challenges for early clinical diagnosis. *PLoS Negl Trop Dis* 2011;5:e1191.
11. Gould EA, de Lamballerie X, Zanotto PM, Holmes EC. Origins, evolution, and vector/host coadaptations within the genus *Flavivirus*. *Adv Virus Res* 2003;59:277-314.
12. Watanabe S, Rathore APS, Sung C, Lu F, Khoo YM, Connolly J, et al. Dose- and schedule-dependent protective efficacy of celgosivir in a lethal mouse model for dengue virus infection informs dosing regimen for a proof of concept clinical trial. *Antiviral Res* 2012;96:32-5.
13. Nguyen NM, Tran CNB, Phung LK, Duong KTH, Huynh H le A, Farrar J, et al. A randomized, double-blind placebo controlled trial of balapiravir, a polymerase inhibitor, in adult dengue patients. *J Infect Dis* 2013;207:1442-50.
14. Freidlin B, Simon R. Adaptive signature design: an adaptive clinical trial design for generating and prospectively testing a gene expression signature for sensitive patients. *Clin Cancer Res* 2005;11:7872-8.
15. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA* 2004;101:13306-11.
16. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1497-500.
17. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;235:177-82.
18. Deen JL, Harris E, Wills B, Balmaseda A, Hammond SN, Rocha C, et al. The WHO dengue classification and case definitions : time for a reassessment. 2006;368:170-173.
19. Thein TL, Gan VC, Lye DC, Yung CF, Leo YS. Utilities and limitations of the world health organization 2009 warning signs for adult dengue severity. *PLoS Negl Trop Dis* 2013;7:e2023.
20. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science* 1995;270:1811-5.
21. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 1996;86:367-77.
22. Dorr P, Westby M, Dobbs S, Griffin P, Irvine B, Macartney M, et al. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. *Antimicrob Agents Chemother* 2005;49:4721-32.