Vol 2:32-39

JEMI-PEARLS

Novel Omics Strategies To Combat Dengue Infection and Disease Progression

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BACKGROUND INFORMATION

Dengue fever is a debilitating disease caused by systemic infection with one of the four dengue virus (DENV) serotypes. DENV is transmitted between humans through the *Aedes* mosquito and is problematic in tropical and subtropical regions, where roughly 390 million DENV infections occur annually [1]. Unfortunately, due to global warming and increasing globalization, the virus is rapidly spreading to new areas around the world. It is currently predicted that half of the world's population, or roughly 3.9 billion people, are at risk of becoming infected with DENV [2].

Though the initial fever caused by DENV only rarely results in death, it has the potential to progress to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), both of which are severe and life-threatening diseases [3]. These diseases are characterized by severe hemorrhaging, plasma leakage that may lead to shock, and ultimately, organ impairment [4]. With prompt diagnosis and intervention, however, fatality rates of DHF and DSS may be reduced from over 20% to below 1% [5]. Unfortunately, rapid diagnosis and treatment of infection with DENV is currently limited by our knowledge of how the virus manifests the disease. Furthermore, there is no scientific consensus over the classification of DENV-associated diseases [6]. This lack of knowledge makes it difficult to predict which individuals have the highest risk of developing severe disease. At this time, there is also an absence of antivirals and specific treatments for treating dengue fever [3]. More research needs to be done focusing on the factors that mediate progression from dengue fever to more severe forms before we can rapidly diagnose patients and design novel treatments.

In recent years, advances in '-Omics' approaches have paved the way for the discovery of new diagnostic and prognostic markers which may be applied to viral infections. These '-Omics' approaches involve platforms that detect and quantify molecules such as DNA (genomics), RNA (transcriptomics), proteins (proteomics), or metabolites (metabolomics) in biological fluids [7]. In the context of DENV infection, studies have already found differences that may assist the diagnosis and prognosis of dengue fever to its more

Received: Feb./17 **Accepted: Feb**./17 **Published:** July/17

Citation Jazdarehee, A. 2017. Novel Omics Strategies To Combat Dengue Infection and Disease Progression. JEMI-PEARLS. 2:32-39.

severe forms. A recent study found 121 potential protein biomarkers which were differentially expressed in the plasma of patients with severe dengue compared to dengue fever [8]. Further, a proof-of-concept study recently explored metabolomic differences associated with individual outcomes of DENV infection in patients with and without the virus, specifically in Nicaraguan and Mexican populations [9].

> "It is currently predicted that half of the world's population, or roughly 3.9 billion people, are at risk of becoming infected with DENV"

When multiple '-Omics' platforms are used together, this allows for a holistic investigation of the mechanisms involved in viral disease progression and may also elucidate potential host pathways for novel treatment targets. In this review, we explore the potential of '-Omics' approaches to reduce mortality against viral infections. We focus on DENV and the mechanisms that are involved in progression from dengue fever to more severe forms of the disease, which will hopefully prove useful in future treatment of infected patients.

RESEARCH QUESTIONS

Orchestrating systems to efficiently and promptly diagnose and treat DENV-infected patients remains an issue as the virus continues to spread around the globe. Utilizing '-Omics' based platforms may uncover underlying physiological changes which could simultaneously result from DENV infection and predict disease progression. Fully elucidating the steps involved in the progression from dengue fever to its more severe forms will result in meaningful changes with regards to how DENV is diagnosed and treated to prevent disease progression. The preliminary studies which found differences in molecular profiles among different populations during DENV infection present an opportunity to personalize our treatment approaches in that we can target specific host pathways associated with disease progression in certain populations. Further investigation of '-Omics' based approaches could revolutionize how all infectious diseases, not simply DENV-associated diseases, are diagnosed and treated, which is an important target considering that millions of lives continue to be claimed worldwide due to infectious diseases [10].

Though improvements have certainly been made in the functionality and reliability of '-Omics' based platforms, careful planning is required before they can be applied in widespread clinical settings. First, we investigate how these '-Omics' based approaches can be used to facilitate diagnosis, prognosis, and treatment of viral diseases in specific populations. Specifically, we focus our discussion on genomic, transcriptomic, proteomic, and metabolomic approaches in the context of DENV infection. We then ask which steps are required in the progression of dengue fever to more severe outcomes of the disease. Due to the recent increase in observed Zika-DENV co-infections, it is important to explore the implications Zika infection has on DENV disease progression. Then we examine how infection with the Zika virus influences the progression of dengue fever to DHF and DSS.

PROJECT NARRATIVE

How Can '-Omics' Based Approaches Be Used to Facilitate the Diagnosis, Prognosis, and Treatment of Viral Infections?

In order to determine how viral infections impact complex host biological processes, our approaches must be holistic, yet integrated. Based on the close biological relationship between DNA, RNA, and proteins, studying genetic, transcriptomic, and proteomic profiles serves as a suitable starting point for investigation. Furthermore, it has been well established that viral infection perturbs the metabolism of carbohydrates, lipids, nucleotides, and amino-acids [11], so it would follow that metabolomic approaches will provide important insights as to how host metabolic pathways are altered. Together, these approaches allow us to peer into the bodies of infected patients and determine the molecular consequences of viral infection.

A holistic '-Omics' approach has not yet been utilized to investigate viral infections, but was used to explain the molecular mechanisms involved in breast tumorigenesis by deciphering the pathways perturbed in breast cancer [12]. Taking the analysis of '-Omics' data one step further, differences in molecular profiles among various populations may be exploited for the development of targeted therapeutics. As mentioned earlier, a study recently demonstrated that there are

distinct differences in metabolomic profiles after DENV infection between Mexican and Nicaraguan patients [9]. With data collection on a larger scale, these differences may be used to understand why certain populations are more susceptible to infection, which may in turn be used to design personalized treatments for those populations.

Such a widespread initiative requires collaboration with clinicians to administer tests and collect data while closely monitoring patients (Fig. 1). When a patient enters the clinic with signs associated with DENV infection, the first step would be to confirm infection with DENV and identify which serotype the patient is infected with. This may be accomplished using the Dengue Day1 Test, a rapid immuno-chromatographic test which detects DENV NS1 antigen and IgM/IgG antibodies specific to DENV in human blood [13]. These tests are highly sensitive and specific, and their low cost allows for widespread use even in remote clinics [13]. Following this initial diagnostic step, the patient's DNA would be sequenced and repeated measurements would be taken of their transcriptomic, proteomic, and metabolomic profiles over time. While the majority of patients will overcome the initial infection, the population which progresses to more severe forms of the disease will continue to be monitored and data collection will continue.

Up to now we have mentioned data collection but have not yet discussed what this data would actually comprise. The main goal is the collection of 'molecular profiles' in terms of which transcripts, proteins, and metabolites are present in what amounts over the course of a viral infection. Messenger RNA (mRNA) microarrays or RNA-Seq may be employed to study gene expression and collect transcriptomic profiles [14]. Multiple reaction monitoring (MRM) mass spectrometry may be used to probe for proteins present within specific biological samples collected from patients [15]. Profiles of metabolites present from biological samples may similarly be quantified and characterized using a combination of liquid chromatography and mass spectrometry [16]. Of course, integrated approaches remain a possibility to reduce the complexity of this data collection [17]. There are financial and bioethical limitations which we will consider later in the review.

Once this data is collected, it must be stored and analyzed. This approach may benefit from the advances made in cloud computing to create databases to share data among researchers around the globe. Various mathematical models can be applied to estimate which

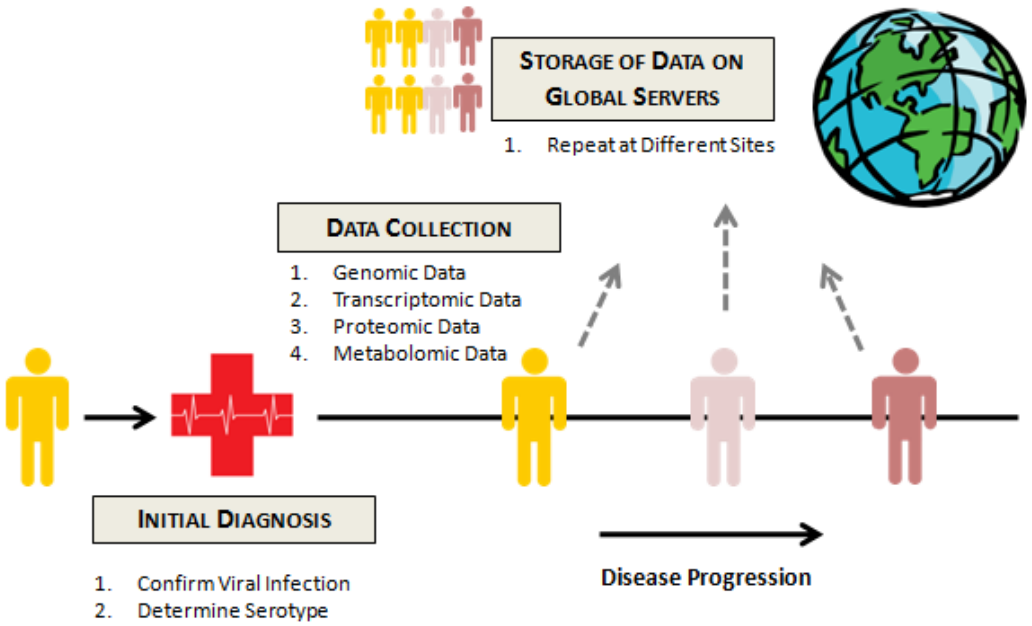

FIG. 1 Collection of data requires careful planning and collaboration with clinicians. Patients entering the clinic with symptoms associated with viral infection are first diagnosed with a rapid test. This confirms viral infection and identifies the specific viral cause and subtype. Molecular data is collected periodically by clinicians while the patient is being monitored. Though viral infections are often resolved, progressing disease manifestations will continue to be monitored with additional data collection. Data collected is stored on servers which may be accessed by researchers around the globe.

molecular signatures are associated with different disease states, such as the progression from dengue fever to severe dengue [18]. The associations revealed may be starting points for molecular investigations to elucidate mechanisms of viral infection and disease progression. Additionally, associated pathways may reveal host pathways contributing to viral infection and disease, which may be exploited as novel targeted treatments.

We have described this holistic system as a potential means to further study DENV infection and disease progression in vast clinical settings. Although we are quite far from adapting these emerging '-Omics' platforms for widespread clinical use, studies incorporating single '-Omics' platforms in the lab have found differences in the molecular profiles of DENV infected patients compared to healthy individuals [8, 9, 19]. In the following section, we will discuss relevant findings pertaining to DENV infection and disease progression, and explore how an integrated approach would allow us to build upon these preliminary findings.

What are the required steps in the progression of Dengue fever to severe Dengue?

As we've discussed, DENV remains a threat to global health due to its increasing geographic distribution and incidence rate [20]. Though the progression to severe dengue is relatively rare, occurring in roughly 5-10% of DENV-infected individuals, these diseases may be fatal unless promptly diagnosed and treated [21]. A greater understanding of the molecular mechanisms involved in DENV disease progression is required to facilitate disease diagnosis and prognosis as well as elucidate novel targets for treatments against the disease.

Progression to severe dengue typically occurs 4-7 days after the initial infection, at a time when viral levels are typically low and immune responses are well-established [22]. Though the mechanism of disease progression has not yet been determined empirically, there is increasing evidence to support the idea that our host immune responses facilitate the progression of the disease and result in the hemorrhaging observed in DHF and DSS.

A recent study found 551 overabundant transcripts in severe dengue patients compared to serotype-matched patients who did not develop severe complications [23]. Almost a third of these overexpressed genes encoded products that are involved in neutrophil activation and degranulation, including neutrophil elastase (ELA2), cathepsin G (CTSG), myeloperoxidase (MPO), and the defensins DEF1A and DEF4A [23]. Interestingly, ELA2 and CTSG are serine proteases which have been shown to cleave vascular endothelial cadherins, resulting in reduced integrity of the vascular endothelium [24]. Reduced integrity of the vascular endothelium may contribute to hemorrhaging.

Perhaps a more convincing finding that host factors are involved, however, is the observation that blood cells of severe dengue patients have increased levels of transcripts encoding matrix metallopeptidase (MMP) 8 and 9 compared to dengue fever patients and healthy controls [25]. While these multifunctional proteins primarily play a role in tissue remodeling, studies have shown that increased MMP levels result in permeability of capillaries and direct damage to endothelial tissues through induction of inflammatory mediators [26-28]. Notably, increasing serum levels of MMP 9 have been strongly associated with severity of plasma leakage during DENV infection in two separate studies [29, 30]. A recent study found 121 differentially expressed proteins in the plasma of DHF patients compared to dengue fever patients, 15 of which were statistically associated with disease severity [8]. This included the anti-protease alpha-2-macroglobulin (A2M), whose serum levels were higher in recovering dengue fever patients and less in DHF patients, compared to healthy controls [8]. Perhaps increased protease activity in DHF patients due to low levels of A2M may be contributing to the hemorrhaging observed in these patients. Upon further study, these differentially expressed proteins may serve as biomarkers to diagnose DENV infection as well as predict and monitor disease progression. Similarly, metabolites found in certain bodily fluids may serve as promising biomarkers. Studies have found differences in the metabolomic profiles of DENV-infected patients based upon geographic location, age, and gender [9, 19].

These preliminary findings of host pathways associated with DENV disease progression serve as starting points for the production of novel anti-DENV therapeutics which target host factors. As an illustration, synthetic inhibitors of MMPs have been in development for the past two decades due to the association of MMPs with several cancers and inflammatory diseases [31, 32]. Unfortunately, poor study design and low inhibitor stability have led to the failure of all clinical trials [31]. Continued development of these MMP inhibitors will remain an active area of research in the near future given the lack of currently approved antivirals against DENV. Furthermore, the finding that anti-protease A2M levels are decreased in

severe dengue patients may warrant investigation into analogue drugs to prevent vascular leak.

In order to design personalized treatments appropriate for specific populations, genetic backgrounds must also be considered. Genomic studies have revealed associations between gene polymorphisms and DENV disease severity. One study found that the rs3765524 polymorphism of the phospholipase C epsilon (PCLE1) gene is strongly associated with progression to DSS [33]. Similar PCLE1 mutations have been associated with nephrotic syndrome, a kidney disorder characterized by glomerular basement membrane dysfunction, which results in decreased vascular oncotic pressure and edema [34]. While these associations provide insight into the severe forms of DENV infection, they certainly do not imply causation, and a bigger picture is required in order to understand the steps involved in disease progression upon infection. The holistic approach described in the previous section would allow for a deeper understanding of the effects of DENV infection on different populations.

In line with a big-picture approach, it is necessary to examine the interplay between different viruses. Mono-infections with DENV are becoming increasingly rare as it has been shown that DENV is co-circulating with the Zika and Chikungunya viruses [35]. Given the continuing spread of the Zika virus where DENV is endemic, the virus will remain a focus of the field, and so we must next consider how infection with the Zika virus affects DENV disease progression.

How does co-infection with the Zika virus influence Dengue disease progression?

Investigating the effect Zika virus could potentially have on DENV disease progression may be accomplished using the clinical approach described earlier. A simple adjustment to the initial diagnostic test would be needed to test for co-infection with the Zika virus. This would require improvements to the current ELIZA performed as a test for Zika-specific IgM antibodies, as cross-reactivity remains an issue [36]. A possible alternative to achieve this is the multiplex reverse transcription PCR (RT-PCR) recently developed for the simultaneous detection of the Zika, Chikungunya, and Dengue viruses [37].

Co-infection scenarios involving both DENV and the Zika virus have only been documented over the past 2-3 years. Co-infection was described in two patients from New Caledonia in 2014 [38]. Neither patient developed hemorrhagic or neurologic conditions often associated with severe dengue and no synergistic effects of the two viruses were observed. Other DENV-Zika co-infections have been documented [39, 40], but again, no synergistic effects were reported. In-depth molecular studies are required in order to uncover possible influences one virus has on the other.

Molecular studies suggest that DENV antibodies may be facilitating Zika infection, resulting in increased viral replication [41]. It is plausible that Zika may conversely influence DENV infection and disease progression. It has been shown that the Zika NS5 protein targets STAT2, ultimately resulting in dampened type I interferon responses [42]. As DENV similarly has multiple mechanisms to dampen type I interferon signaling [43], it is interesting to investigate whether co-infection results in a synergistic dampening of these responses. Holistic '-Omics' approaches may perhaps reveal decreased type I interferon signaling in co-infected patients. It is a possibility that a reduction in this signaling may result in an increased viral load, potentially amplifying host responses that may be contributing to the progression to severe dengue. This would have severe implications in how future co-infected patients may be treated to combat progression to severe disease forms at an early stage.

Elucidating any potential differences with respect to disease pathogenesis in co-infected patients may have important consequences during this time where DENV, Zika, and Chikungunya are rapidly spreading through subtropical regions. Accounting for potential influences of DENV disease pathogenesis lead us closer to explaining the scarcity of Zika infections in India, for example.

SUMMARY AND CONCLUSION

We have discussed the potential of an widespread, integrated '-Omics' approach to increase our understanding of viral diseases. In the context of DENV, a continuing threat to public health, this approach may be used to solve unanswered questions surrounding viral pathogenesis and disease progression. Early '-Omics' studies suggest that the progression from dengue fever to severe dengue is mediated, in part, by host responses. It remains unclear how co-infection with Zika may influence this disease progression, but further research will uncover associations that may reveal interactions between the two infections.

While '-Omics' approaches have been widely used to study human cancers and facilitate drug development, these approaches have received less attention when it

comes to infectious diseases, specifically viral infections. These approaches have proved useful when used in the field, however. Proteomic approaches have greatly increased our understanding of how herpes simplex virus type 1 (HSV-1) interacts with host cells [44]. Building off of these preliminary studies, a holistic approach involving the study of multiple molecular profiles is required to truly understand the implications of viral infection.

Future studies using the strategies explained in this review could link specific molecular profiles to host microbiota. It is well established that the host microbiota vastly influences human health and it has in fact been shown that our microbiota inhibits infection with some viruses and promotes infection with others [45]. It is plausible, then, that our microbiota may influence host pathways involved in the progression of viral diseases, such as that of dengue fever to severe dengue. A recent study has found a correlation between lung microbiota to specific metabolic profiles in human immunodeficiency virus (HIV)-infected patients [46]. Investigating the role commensal microbes have on viral infection and disease progression will continue to be an area of interest.

There are numerous limitations which must be considered before such widespread studies may take place. First and foremost is the issue of cost. The majority of the tests and assays described in this review are currently too expensive for widespread clinical use. Improvements have been made, however, in sequencing and molecular profiling technologies which are making these platforms more accessible [47, 48]. It must also be considered that the transition from research grade '-Omics' assays to those done in the clinic requires extensive development and validation [49]. The data collected is sensitive to differences in how specimens are collected, processed, and stored [50], which will vary in different geographical settings unless standardized procedures are set and enforced.

Our described approach would have to comply with existing bioethical guidelines and also adapt to emerging guidelines associated with collection of large, personalized databases. Widespread training and education of clinical workers is required to ensure informed consent from subjects before data is collected. The molecular profile data is personal and potentially identifying, so appropriate measures must be taken to encrypt the data and ensure it is secure. Stringent guidelines must be followed regardless of geographic locations. Recent publications have outlined several steps which may be taken to ensure these study comply with ethical, legal, and regulatory frameworks [49].

Improvements in '-Omics' technologies and platforms present opportunities to address many unanswered questions pertaining to viral infection and infectious diseases beyond DENV infection. Given the continuing worldwide mortality attributed to infectious diseases, these approaches will continue to provide novel insights for years to come.

ACKNOWLEDGEMENTS

I would like to sincerely thank Dr. François Jean for his keen guidance and endless support throughout the preparation of this document. His meaningful feedback, along with the contributions from my peers, is much appreciated.

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ACRONYMS

Alzheimer's disease (AD), herpes simplex virus type 1 (HSV1), central nervous system (CNS), apolipoprotein E type 4 (*APOE ε*4), amyloid-beta (Aβ), neurofibrillary tangles (NFTs), hyperphosphorylated Tau (p-Tau), peripheral nervous system (PNS), herpes simplex encephalitis (HSE), amyloid precursor protein (APP), trigeminal ganglion (TG), heparin sulphate proteoglycan (HSPG), latency associated transcript (LAT), infected cell polypeptide (ICP) interleukin (IL), transforming growth factor beta (TGF-β), complement factor H (CFH), nerve growth factor (NGF), signal transducers and activators of transcription (STAT), reactive oxygen species (ROS), paired helical filaments (PHFs), glycogen synthase kinase 3 beta (GSK3β), protein kinase A (PKA), Caspase 3 (Cas3), protein kinase R (PKR), valacyclovir (VCV), Immunoglobulin M (IgM), phosphorylated APP (p-APP)