Spatiotemporal Virus Surveillance for Severe Acute Respiratory Infections in Resource-limited Settings: How Deep Need We Go?

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(See the Major Article by Cummings et al. on pages 1118–25.)

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Following the discovery of molecular cloning, nucleic acid sequencing, and polymerase chain reaction, the wonderland of molecular biology has opened its doors again to virtually unlimited possibilities, as next-generation/deep sequencing or, better, massive parallel sequencing allows for the comprehensive analysis of all genomic nucleic acids of any origin in any given sample in real time. Initially applied to the bacterial microbiome, deep sequencing has similarly detected a multitude of viral genome sequences, and changed hitherto common views on persisting viral infections [1], putting forward the concept of the “virome” in general, and of the “human viromes” in particular [2]. While the numeric summary of viral genome sequences is mind-blowing, even after careful annotation and curation through refined bioinformatic tools, the utility of massive parallel sequencing has been prominently demonstrated in epidemiologic cluster analysis, for example, leading to the identification of a novel arenavirus as the cause of donor-derived death of 3 solid organ transplant recipients [3], or of a novel human polyomavirus causing lethal multiorgan failure in a pancreas transplant recipient [4]. Given the technical and bioinformatic advances as well as the declining laboratory costs, the application of deep sequencing to identify etiologic agents in clinical samples has been approached in different pathologies, including those caused by community-acquired respiratory viruses (CARVs).

Despite the general enthusiasm for metagenomics, it remains important to realize that there are caveats. In fact, multiple technical and conceptual aspects influence the results of deep sequencing, may cause significant differences in the read-outs, and hence bias their interpretation. Conceptually, it is clear that the human virome is different in different organ sites and body location (eg, the gastrointestinal, urogenital, or respiratory tract) and is subject to dynamic changes of the viral constituents, not only as a result of physiological changes (eg, dietary, hormonal, aging), but also due to complex pathological alterations such as trauma, inflammation, and immune responses [5], exemplified in inflammatory bowel disease or transplantation [6, 7].

Technically, nucleic acid extraction is presumed to be representative across all different agents, whereas in fact different sample preparations, buffers, purification, single fragment partitioning, and sequence analytic procedures may introduce an as-yet little-understood bias, which can lead to underrepresentation of certain viral genomes—for example, human coronavirus compared with paramyxovirus genomes (H. H. Hirsch, unpublished observation). Thus, every step of the wet and dry laboratory procedure is dependent on the methodology, and may yield potentially different results [8]. Independent confirmatory testing has become an essential part of the presumably hypothesis-free deep sequencing [9]. These aspects are important when discussing the results of massive parallel sequencing to either rule in or rule out the involvement of a pathogen in a given pathology.

With these caveats in mind, and given the significant global burden of viral respiratory tract disease in the very young and the very old [10–12], well-established researchers from the United States and Uganda have joined forces: Cummings et al. in this issue of Clinical Infectious Diseases characterize the potential role of noninfluenza viruses in spatiotemporal clusters of severe acute respiratory infection (SARI) in a resource-limited setting. The prospectively collected epidemiological data and nasopharyngeal/
opharyngeal (NP/OP) samples of SARI cases were identified through a national surveillance study conducted by the Uganda Virus Research Institute from 2010 through 2015. The virome present in influenza virus–negative NP/OP samples was analyzed by deep sequencing enriching for viral sequences. The epidemiologic data were collected by sentinel hospitals in 9 different geographic areas, and hence focused on SARI (eg, pediatric and adult patients admitted to hospital for severe influenza-like illness), thereby combining feasibility and clinical significance. The numbers are impressive, as 3921 SARI cases were identified, 335 (8.5%) of which were influenza-positive NP/OP samples. Of the remaining 3586 cases of influenza-negative SARI, 2901 had spatial and temporal data available to pinpoint 9 clusters involving 301 (10.4%) influenza-negative SARI cases. Notably, the demographic characteristics of the Ugandan SARI cases indicated that mostly young children were enrolled, 80% of whom were <1 year of age, both within and outside of clusters. Although coughing and shortness of breath were leading features of SARI cases of both clustered and nonclustered cases, fever with temperatures >38°C and stridor were significantly more frequent in cluster cases than in noncluster cases.

In two-thirds of the SARI cluster cases, a virus-capturing technique and massive parallel sequencing using the HiSeq Illumina system yielded informative genome sequences in 82%, identifying human rhinovirus in 34.5%, human parvoviruses/enteroviruses together accounted for >45% of the detected CARVs [13]. While this suggests that, by and large, a significant part of the pathogens could have been detected by multiplex NAT for CARVs, the deep sequencing approach of this study revealed a number of remarkable surprises, which may help to close the gap on at least 30%–50% of the CARV-negative multiplex NAT results [16].

Cytomegalovirus (CMV) was identified in 26.9% of the SARI clusters, and may either reflect reactivation as a bystander of significant inflammatory states including infections with other including viral pathogens [17–19], or may in fact reflect the manifestation of CMV primary infections as SARI, a hypothesis viable in view of the high seroprevalence in developing countries [20] and the very young age of 80% of the SARI cluster patients described here. Similarly, the discovery modus of deep sequencing identified more somewhat expected pathogens in that age group, such as human herpesvirus 6 and parvovirus B19, for which similar arguments could be made. By the same token, it is surprising that other viruses were not reported, some of which had been proposed to be associated with respiratory symptoms or febrile tonsillitis such as BK virus and other polyomaviruses [21]. Similarly, the complete absence of the apparently ubiquitous anelloviruses, which have received much attention in studies of respiratory samples from patients with inflammatory conditions or transplantation, is notable [7, 22–24]. However, no samples from the lower respiratory tract were available for this analysis.

The discovery potential of deep sequencing of this US–Ugandan study is, however, highlighted impressively by the identification of picobirnavirus in 2 cases, as well as measles, as the cause of SARI clusters in 18 cases, of which 13 belonged to genotype B3, which is endemic in Eastern Africa. Remarkably, 5 cases were caused by genotype B4, and could be traced back to an unvaccinated tourist from England and an outbreak in Manchester, United Kingdom, in 2011. Thus, global travel and outbreak have been linked. Even though undervaccination may play an important role in the Ugandan setting, it is clear that measles outbreaks have been a problem in resource-rich settings such as in Switzerland, where 105 cases have occurred in the year 2017 (incidence of 12.5 per million inhabitants), causing outbreaks among mostly unvaccinated individuals, despite the current 2-dose vaccination coverage of 85% and 93% of 2- and 16-year-old children, respectively [25]. Of note, measles virus also belongs to the human parvovirus family and is transmitted via respiratory fluids, but was detected alone as well as together with other viral pathogens in the present study by Cummings et al. Taken together, this report from resource-limiting settings is also of relevance for resource-rich countries and raises the question about how to best expand current first- or second-line testing for respiratory viral pathogens including CMV, parvovirus B19, and measles, and how to move to more deep sequencing virome analysis and comprehensive metagenomics in the near future.

The spatiotemporal cluster analysis, combined with viral metagenomics presented in this study, is of interest also for other settings, and raises the question of how and when this can be best delivered in real time. Such efforts are under way for influenza virus vaccine prediction [26], the pacemaker of respiratory viral research, and will be of utility for characterizing vaccine failure and anti-viral drug resistance.

The average Ugandan cluster consisted of 30 cases within a 13-km radius and 38 days, and viruses were detected in only some of the patients. Not unexpectedly, certain factors were associated with the Ugandan SARI clusters, such as human immunodeficiency virus (HIV) infection, urban location, higher population density (eg, 431.5 vs 250 persons/km²), or higher median annual rainfall of 1293 vs
1150 mm/year. These differences, though statistically significant, need to be put in perspective, as the study was anchored in sentinel hospitals, typically located in urban areas where HIV status is more likely to be known, whereas SARI cases in rural areas may not have been equally able to seek hospital admission. Given the >10-fold higher average population density of Boston (5000 persons/km²), or Basel (7500 persons/km²), one might suspect that the associations in the Ugandan setting may have been a surrogate for other factors such as general hygiene standards, other medical conditions, and socioeconomic factors in certain suburban quarters. Similarly, the differences in annual rainfall of <10% need to be balanced against larger seasonal changes in short-term higher rainfall, which may be more important for case clustering and should be addressed in future studies.

Despite a critical appreciation, this collaborative US–Ugandan study combining epidemiology with state-of-the-art modeling and virology not only sets standards for resource-rich industrial settings, but provides next to HIV, Ebola, and malaria another proof-of-concept of feasibility and impact of innovative joint projects and partnerships between advanced research groups and dedicated institutions in resource-limited countries.

Note

Potential conflicts of interest. Author certifies no potential conflicts of interest. The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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