Indian Academy of Pediatrics (IAP)

nRICH

Newer **R**esearch and recommendations **I**n **C**hild **H**ealth

Lead Author
Snehal Mallakmir

UNDER THE AUSPICES OF THE IAP ACTION PLAN 2023

Upendra Kinjawadekar
IAP President 2023

GV Basavaraja
IAP President 2024

Remesh Kumar R
IAP President 2022

Vineet Saxena
IAP HSG 2022-23

Metagenomic next generation sequencing in infectious disease diagnosis

Snehal Mallakmir

MBBS,DCH, Fellowship in Clinical Genetics
Clinical Geneticist, Apollo Genomics Institutes, Navi Mumbai and Clinical Advisor, Medgenome Laboratories, Mumbai, India

BASED ON ARTICLE

Priya Edward, Andrew S. Handel. Metagenomic Next-Generation Sequencing for Infectious Disease Diagnosis: A Review of the Literature with a Focus on Pediatrics. Journal of the Pediatric Infectious Diseases Society 2021;10(S4): S71–7.

SUMMARY

The authors aim to study strengths of metagenomic next generation sequencing (mNGS) as well as weakness of this technique from current perspectives with focus on pediatric infectious diseases although they have also included some literature on adult infections. Conventional techniques to detect pathogenic organisms are usually sufficient for common infections. mNGS offers unbiased sequencing and identification of microbial genetic material. Unlike polymerase chain reaction assays, mNGS samples massive quantities of DNA or RNA fragments from a given specimen without the use of species-specific primers, allowing for the hypothesis-free identification of potential pathogens. Authors discuss few studies about the real-world impact of plasma microbial cell-free DNA mNGS on clinical care. Clinically, mNGS results may be interpreted and classified in many ways, as a single test may yield both relevant and irrelevant organisms. It is often obtained once conventional tests have returned negative, limiting clinical applicability, also studies vary significantly in defining basic outcome measures and interpreting the result significance.

In the studies mentioned by the authors, the majority of mNGS tests were ordered to evaluate a large range of clinical concerns, often among immunocompromised patients. It was common for mNGS results to identify multiple microbes, some of which were felt to be true pathogens while others were clinically irrelevant. Although the rate of relevant organism detection was high in some studies, clinical impact compared to conventional testing was low. Hence authors specified that it is crucial to note that "clinical impact" has no single definition. Each study assigned its own criteria, leading to significant variation in assessments of real-world utility. Proportion of pediatric patients in cohort in three studies considered by Lee, Rosoff and Niles was 100 % while it was 52.4 % in Hogan et al. Proportion of immunodeficient patients in cohort ranged from56 % to 76%. Common reasons for ordering mNGS were not stated by some while others noted concern for fungal infection, unexplained lesion on imaging, fever of unknown origin. mNGS positivity rate (regardless of result relevance) in study by Lee was 72% of tests/55% of organisms. Clinical impact of mNGS results differed widely for example in Lee et al study, it was 14% (8/59, all leading to antibiotic changes), 50% of mNGS-negatives were true-negatives while in study by Hogan et al beneficial impact was 7.3%, negative impact was 3.7%, no impact: 86.6% and unclear impact was 2.4%. Lee found 87.5% (7/8) of mNGS with impact occurred in immunocompromised patients (including 5 organisms identified by mNGS only). For Rosoff, sensitivity/specificity for identifying a clinically defined infection was 92%/64% for mNGS vs

77%/89% for CT. Of 39 invasive diagnostic procedures performed, plasma mNGS identified pathogen in 87% vs 67% by procedure. In study by Niles et al, no unusual organisms identified and on average, organisms were detected earlier by CT.

Authors have also discussed various other applications of mNGS from complex pulmonary illnesses with high potential for unusual or difficult-to-identify organisms to endocarditis, hepatitis, osteoarticular infections, immunocompromised hosts. In multiple retrospective pediatric reviews, infectious workup of pulmonary findings was the most common indication for requesting mNGS. Depending on testing availability, this may be performed using blood and bronchoalveolar lavage fluid. If the causative organism is detected by blood mNGS, a bronchoscopy or lung biopsy may potentially be avoided. In some studies, clinical management was changed based on mNGS results, antibiotics were narrowed based on mNGS results and antibiotics were changed to targeted therapy for specific organism. Identifying the source of fever among neutropenic patients, infectious or otherwise, is a common diagnostic challenge and by reducing the rate of false-positive results, mNGS has the potential to preemptively identify bacteremia among immunocompromised patients. The process of unbiased sequencing provides mNGS with the unique potential to identify novel or variant species. Diagnosis through sequencing also allows for the identification of specific genes of clinical relevance, including those encoding antimicrobial resistance.

Authors caution that as the technique is being used more and more, it also gave insights into benefits as well as limitations. mNGS is an exciting, versatile tool giving the way in which infectious diseases are diagnosed. The literature reviewed demonstrates many hypothetical applications for mNGS, though most have yet to be fully realized. Potential benefits for patients include avoidance of invasive diagnostic procedures, simplification of fever of unknown origin evaluations, and identification of pathogens otherwise undetected by conventional testing. The current technology is expensive and is not easily accessible at most institutions, though these are improving rapidly. Result interpretation is complicated, multiple organisms may be reported in an infection due to only one pathogen. Antibiotic resistance gene detection by deep sequencing has tremendous potential, but interpretation remains a challenge. In conclusion, mNGS is a rapidly evolving technology with tremendous potential as a diagnostic tool, but with important limitations. Much research is needed to determine optimal mNGS use and implementation, highlighting the need for collaboration between laboratory, bioinformatics, and clinical experts.

COMMENTARY

Nearly all infectious agents contain DNA or RNA genomes and hence sequencing their genome is thought to improve preciseness and rapidity of pathogen detection. The cost of high-throughput NGS technique has been significantly reducing since its advent in 2004, and it has emerged as an enabling technological platform for the detection and taxonomic characterization of microorganisms in clinical samples from patients (1).

In view of growing complexities arising due to atypical presenting phenotypes, mutated organisms, novel pathogens, associated immunological complications in certain situations and deciding therapeutic measures like specific antibiotic to prevent resistance, precision and quick diagnosis is gaining importance. NGS, also termed as a high-throughput or massively parallel sequencing, is a technology that allows for thousands to billions of DNA fragments to be simultaneously and

independently sequenced. mNGS has been helping for diagnosis and therapeutic decisions in give answers to deal with complex infections and antibiotic applications in pediatrics. unexplained fever (2), various difficult to treat infections and situations with difficulties in obtaining samples like neurology cases (3). In pediatric practice, neonatal critical care mainly requires rapidity of detection as well as judicious use of antibiotics use in vulnerable babies as prevention of damage to vital organs is time bound and hence various mNGS studies are being reported (4)

Applications of NGS to infectious diseases are wide ranging and include lineage tracing, drug-resistance testing of viruses or culture isolates, and microbiome studies. NGS-based virus detection technique has also been shown to be useful in surveillance of vector-borne and zoonotic viruses (5). Due to different anthropological activities, extensive globalization of travel, rapid urbanization, deforestation and many factors, epidemiology of viral diseases have changed significantly as with recent experience of corona virus. This change has also led to the increased exposure of different human populations to newer pathogens, including viruses, mostly zoonotic in nature for example, the emergence of Ebola virus, Nipah virus, Sin Nombre Hantavirus, SARS (severe acute respiratory syndrome), Influenza viruses (H1N1, H7N9) etc.

Wide ranging utility of NGS in pathogen detection, high sensitivity, detection of novel mutations or novel pathogens also leads to chances of unintentional contaminated sequences results. There is then need of further cell culture or animal studies for confirmation, which limits the utility. The studies from various parts of the world and different populations are very essential due to widely variable susceptibility to endemic infections, vaccine coverage for known pathogens, antibiotic use and corelation with emerging studies of susceptibility genes in various immunological diseases.

Hence much focus is on collaboration between clinicians, laboratories, bioinformaticians as well as research studies. Nonetheless, mNGS is looked at with hopes to give answers to deal with complex infections and antibiotic applications in pediatrics.

REFERENCES

1. Wei Gu1, Steve Miller1, and Charles Y. Chiu. Clinical Metagenomic Next-Generation Sequencing for Pathogen Detection. Annu Rev Pathol. 2019 January 24; 14: 319–338. doi:10.1146/annurev-pathmechdis-012418-012751.
2. José Francisco Fernandes, Florian Laubscher, Jana Held et al. Unbiased metagenomic next-generation sequencing of blood from hospitalized febrile children in Gabon. Emerg Microbes Infect. 2020; 9(1): 1242–1244
3. M.R. Wilson, H.A. Sample, K.C. Zorn et al. Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis. N Engl J Med. 2019 June 13; 380(24): 2327–2340.
4. Yuhao Chen, Thomas C. Brook, Cho Zin Soeet al. Preterm infants harbour diverse Klebsiella populations, including atypical species that encode and produce an array of antimicrobial resistance- and virulence-associated factors. Microb Genom. 2020 Jun; 6(6): e000377
5. Sibnarayan Datta, Raghvendra Budhauliya, Bidisha Das et al. Next-generation sequencing in clinical virology: Discovery of new viruses. World J Virol. 2015 Aug 12; 4(3): 265–276.