Is metagenetic next-generation sequencing (mNGS) the future of infectious disease diagnosis?

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BACKGROUND

While traditional diagnostic methods often rely on culture techniques, PCR amplification or serological assays which can be time-consuming and may fail to identify the causative pathogens, metagenomic next-generation sequencing (mNGS) allows unbiased comprehensive analysis of genetic materials from all microorganisms present in a specimen.

The clinical application of mNGS has gained momentum, especially in cases of undiagnosed infections. The use of mNGS facilitates rapid diagnosis, enabling timely treatment decisions that can significantly enhance patient outcomes. Here we explore the clinical utility of mNGS in infectious diseases management.

METHODOLOGY

Total DNA was extracted using commercial kits and DNA libraries were prepared according to the manufacturer's instructions using universal adaptor. The prepared libraries were quantified using Qubit 1X dsDNA HS Assay Kit and were qualified using Agilent D1000 ScreenTape system. The DNA libraries were sequenced in one lane per panel on the Illumina NovaSeq PE150 platform.

The resulting fastq data was subsequently uploaded to Chan Zuckerberg ID (CZID), a cloud-based metagenomics platform. Data analysis with CZID was conducted using the Illumina mNGS pipeline v8.3 with default parameters that include human genome filtering. Positive mNGS results were reported based on criteria described in the study by Miao *et al.* 2018. The results of mNGS were compared with regular medical care.

RESULTS

In this case series, mNGS displayed excellent performance on detecting pathogens ranging from bacteria, fungi to parasites in clincal specimens. Appropriate treatment could be prescribed after identifying the causative pathogens by mNGS analysis.

In case 2, mNGS successfully identified the parasite as *Dibothriocephalus nihinkaiense.* After receiving a single oral dose of praziquantel, no ova or proglottids were found when the patient visited again after 1 week for stool examination. There was no evidence of relapse after the treatment.

In case 4, no bacterial culture was performed on the biopsy sample. Therefore, mNGS can only be done on formalin-fixed paraffin-embedded (FFPE) sample. Cervical actinomycosis was confirmed based on the detection of *Actinomyces israelii* DNA by mNGS and the clinical presentation of the patient. The patient recovered completely after 6 months of oral amoxicillin treatment.

Case no.	Case presentation	Specimen type	Culture results	PCR results	mNGS results
1.	A 48-year-old man presented with generalized lymphadenopathy, long term chronic malaise, pelvic pain and change of body odour.	Left groin lymph node biopsy	Bacterial and fungal culture negative	Bacterial and fungal PCR negative	*Toxoplasma gondii*
2.	A 40-year-old woman presented with a complaint of epigastric pain and diarrhea. A curvilinear opacity was seen at the upper quadrant of the abdomen.	Incomplete strobila of parasite	Not performed	Not performed	*Dibothriocephalus nihinkaiense*
3.	A 55-year-old woman presented with fever and bone pain.	Right iliac bone biopsy	Negative	*Mycobacterium tuberculosis* PCR negative	*Mycobacterium canettii*
4.	A 39-year-old woman presented with an enlarging right neck mass and dysphagia after steroid exposure for treatment of De Quervain thyroiditis.	Right neck Tru-cut biopsy (FFPE)	*Mycobacterium tuberculosis* culture negative	*Mycobacterium tuberculosis* PCR negative	*Actinomyces israelii*
5.	A 59-year-old woman presented with an enlarging right neck mass. Histological findings suggested granulomatous inflammation with necrosis.	Right neck core biopsy (FFPE)	Not performed	*Mycobacterium tuberculosis* PCR negative	*Burkholderia* spp.
6.	A 66-year-old man with fungal infection and organizing pneumonia. Fungal hyphae were found in the lung biopsy using Grocott stain.	Left lung biopsy (FFPE)	Not performed	Not performed	*Malassezia* spp.
7.	A 21-year-old man with infective endocarditis. Antibiotic treatment was initiated.	Peripheral blood	Blood culture negative	Not performed	*Streptococcus oralis*

Table 1. Selected cases using mNGS to aid in infectious diseases diagnosis.

Figure 1. Incomplete strobila of parasite received in case 2.

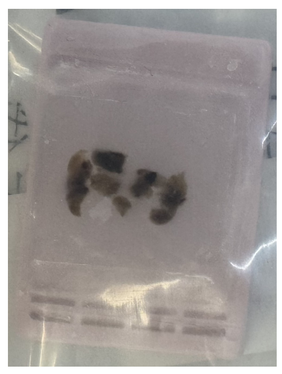

Figure 2. Right neck Tru-cut biopsy (FFPE) received in case 4.

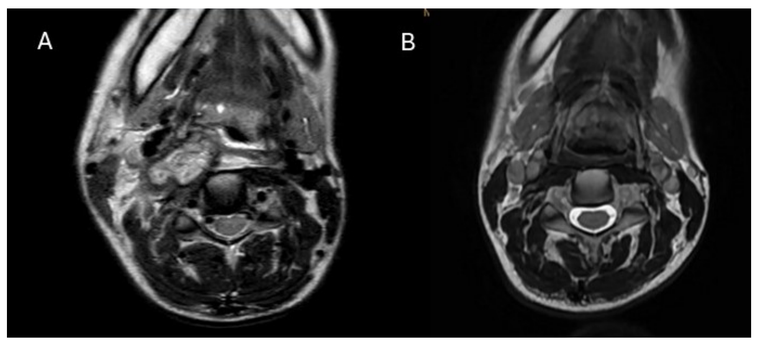

Figure 3. The axial T2-weighted MRI of case 4 shows the presence of irregular infiltration mass over the right side with extension to retropharyngeal space and carotid space before treatment started (A). The mass resolved after starting appropriate treatment (B).

CONCLUSION

These cases showcased the high value and excellent performance of mNGS in the diagnosis of infectious diseases caused by pathogens ranging broadly from bacteria, fungi to parasites. The integration of mNGS holds promise for more effective diagnostic and therapeutic strategies of infectious diseases.