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TEACHING FILES (GRAND ROUNDS)

DISCORDANCE BETWEEN MYCOBACTERIAL TUBERCULOSIS WHOLE GENOME SEQUENCING AND PHENOTYPIC DRUG SENSITIVITY - HOW TO INTERPRET

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Clinical Problem

A 14-year-old female presented in August with complaints of cough, easy fatiguability, shortness of breath for the past 2-3 months. She also had a 6.5 kg weight loss in the past 8 months and no menstrual cycles since the past 4 months. Eight months ago, she was diagnosed as pulmonary tuberculosis(PTB) and started on anti-tubercular treatment(ATT).

Her past reports in month of February were: CT chest showed Discrete and confluenting lymph nodes with necrosis in bilateral supraclavicular fossa, large subcarinal lymph node ruptured into the adjacent apical segment of right lower lobe of the lung with resultant consolidation with cavitation within, scattered centrilobular branching opacities suggestive of endobronchial spread. Sputum geneXpert MTB Rif assay positive with Rifampicin resistance indeterminate. Sputum TB MGIT culture was negative. TB whole genome sequencing showed resistance to Rifampicin, Isoniazid, Ethambutol, Stretomycin, Capreomycin(Cm), Kanamycin(Km), Amikacin (Am), Moxifloxacin(Mfx), Ofloxacin(Ofx), Gatifloxacin as in Table 1. Her TB drug regimen consisted of Clofazimine(Cfz), cycloserine(Cs), pyrazinamide(PZA), linezolid (Lzd), bedaquiline(Bdq).

Currently, on examination she was malnourished weighing 27.6 kg, heart rate of 110 beats/min, respiratory rate of 34 breaths/minute, oxygen saturation of 88% on room air, respiratory examination had bilateral crepts. Child was admitted in Airborne isolation war, started on oxygen at 4 L/min.

ATT treatment comprised of Cfz, lzd, sodium paraaminosalicylic acid(PAS), PZA which was continued and Bdq, delamanid (Dlm) were added. Hemogram showed hemoglobin 9.2 g/dl, white blood cell count of 26,890 cells/cubic mm with neutrophils of 87.7% and lymphocytes of 3.3% indicating severe lymphopenia with an absolute lymphocyte count of 880 cells/ cumm, platelet count of 2.74 lakhs/µl. 25 hydroxy Vit D3 levels were 3.89 ng/mL indicating severe vitamin D3 deficiency. Sputum TB MGIT culture was sent. Child showed improvement hence discharged and followed up on OPD basis. Sputum TB MGIT was positive with drug susceptibility testing showing pansensitivity - as in Table 2. ATT was continued. On follow up child improved symptomatically, had weight gain of 6.5 kg in 6 months of starting Bdq containing regimen.

CT chest, abdomen, pelvis 6 months after initiation of bedaquiline containing regimen reported complete resolution of previously seen cavitatory consolidation in superior/medial basal segment of right lower lobe, with multiple fibronodular, fibrocalcific and fibroatelectatic changes with marked resolution of previously seen enlarged conglomerated necrotic lymph nodes, multiple mildly enlarged lymph nodes with peripheral calcification and central necrosis, multiple enlarged discrete lymph nodes with central necrosis in periportal, portocaval and peripancreatic region which has increased in size as compared to previous CT suggesting mixed response to ATT with marked resolution of pathologies in chest and increased retroperitoneal lymphadenopathy in abdomen. Bdq and Dlm was stopped and child was continued on Cfz, Lzd, PAS and PZA. 12 months after initiation of Bdq containing regimen, CT chest and abdomen reported cavitation in superior/medial basal segment of right lower lobe, with multiple fibronodular, fibrobronchiectasis changes in right upper lobe and multiple mild enlarged lymph nodes with peripheral calcification and central necrosis in prevascular, paratracheal, subcarinal, hilar regions consistent with previous scan, multiple enlarged discreted lymph nodes with central necrosis

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Table 1. TGS Genome analysis report : Mycobacterium Tuberculosis detected of lineage – Beijing with coverage of 285.18 and 0.99 % of reads.

Antibiotic	Presence of drug resistance mutation
Rifampicin	Resistant
Isoniazid	Resistant
Ethambutol	Resistant
Pyrazinamide	No resistance detected
Streptomycin	Resistant
Capreomycin	Resistant
Kanamycin	Resistant
Amikacin	Resistant
Moxifloxacin	Resistant
Ofloxacin	Resistant
Gatifloxacin	Resistant
Ethionamide	No resistance detected
Linezolid	No resistance detected
Para-aminosalicylic acid	No resistance detected
Bedaquiline	No resistance detected
Clofazimine	No resistance detected
Delamanid	No resistance detected
Pretomanid	No resistance detected

Table 2. TB MGIT with phenotypic DST.

Drug	Sensitivity	Concentration
Rifampicin	Susceptible	0.5 mcg/ml
Isoniazid	Susceptible	0.1 mcg/ml
Kanamycin	Susceptible	2.5 mcg/ml
Ofloxacin	Susceptible	2.0 mcg/ml
Amikacin	Susceptible	1.0 mcg/ml
Clofazimine	Susceptible	1.0 mcg/ml
Capreomycin	Susceptible	2.5 mcg/ml
Moxifloxacin (0.25)	Susceptible	0.25 mcg/ml
Moxifloxacin (1)	Susceptible	1.0 mcg/ml
Linezolid	Susceptible	1.0 mcg/ml
Ethambutol	Susceptible	5.0 mcg/ml
Ethionamide	Susceptible	5.0 mcg/ml
PAS	Susceptible	4.0 mcg/ml
PZA	Susceptible	100.0 mcg/ml

in periportal, portocaval and peripanreatic region which had decreased in size of lymph nodes as compared to previous CT suggestive of good response to AKT, hence regimen was continued. After 18 months of initiation of this regimen, child had weight gain of 12 kg with no symptoms, regular menstrual cycles. CT chest report suggestive of persistent calcific, fibronodular lesions with bronchiectatic changes and areas of cystic parenchymal destruction in the right lung is largely unchanged compared to the previous study with fibroatelectatic changes in the superior segment of right lower lobe at the site of previous cystic lesion with no new lung infiltrates or consolidation, persistent unchanged necrotic mediastinal and bilateral hilar nodes with peripheral calcification.

How to interpret the findings in whole genome sequencing compared to TB MGIT phenotypic DST ?

Discussion

Drug susceptibility testing (DST) of Mycobacterium tuberculosis (Mtb) is essential to formulate the most appropriate individualised TB treatment especially for MDR–TB cases. There are 2 types of DSTs – molecular and phenotypic. Phenotypic tests have been the preferred method for a significant period, but they are time-consuming as they involve the culture process. On the other hand, advanced molecular techniques such as Whole Genome Sequencing (WGS) analyze the complete DNA of the bacteria, theoretically providing comprehensive information about the bug's susceptibility or resistance to various drugs.[1,2] Coll et al., (2015) published data regarding the a of sensitivity and specificity of WGS in predicting drug resistance which showed a high sensitivity and specificity values for the first-line drugs: 96.2% and 98.1% for rifampicin; 92.8% and 100% for isoniazid; 88.7% and 81.7% for ethambutol; 87.1% and 89.7% for streptomycin.[3]

Yusoof et al stated that MGIT phenotypic DST is consistent for Isoniazid, Rifampicin, Kanamycin, Amikacin, ofloxacin and levofloxacin. However for other drugs, there is limited data.[4] This was also emphasized by a study from Latvia, which showed that for isoniazid and rifampicin phenotypic DST could potentially be substituted by genotypic DST given their tests exhibited a 100% concordance rate. However, this equivalency wasn›t observed for other tuberculosis drugs.[2]

Feliciano et al.'s research aimed to resolve the disparity between the outcomes of the two tests, but their publication only covered the findings related to rifampicin. Within their study, two isolates exhibited resistance according to phenotypic DST despite the absence of identified mutations in WGS. Conversely, one isolate displayed the opposite pattern. The researchers suggested that this discrepancy could be attributed to the potential presence of multiple strains within the identical clinical sample.[1]

Hence the discrepancy in our results can also be answered by some contamination in our samples or presence of bugs that have mutated. Future research on the concordance of these 2 testing modalities might be needed to provide strong evidence on the topic.

Compliance with Ethical Standards

Funding None

Conflict of Interest None

References

1. Feliciano CS, Namburete EI, Rodrigues Plaça J, Peronni K et al. Accuracy of whole genome sequencing versus phenotypic (MGIT) and commercial molecular tests for detection of drug-resistant Mycobacterium tuberculosis isolated from patients in Brazil and Mozambique. Tuberculosis (Edinb). 2018/04/05 ed. 2018 May;110:59-67.
2. Vīksna A, Sadovska D, Berge I, Bogdanova I et al. Genotypic and phenotypic comparison of drug resistance profiles of clinical multidrug-resistant Mycobacterium tuberculosis isolates using whole genome sequencing in Latvia. BMC Infectious Diseases. 2023 Sep 28;23(1):638.
3. Coll F, McNerney R, Preston MD, et al. Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. Genome Med 2015;7(1):51
4. Yusoof KA, García JI, Schami A, Garcia-Vilanova A, Kelley HV, Wang SH, et al. Tuberculosis Phenotypic and Genotypic Drug Susceptibility Testing and Immunodiagnostics: A Review. Front Immunol. 2022 Jul 7;13:870768-870768.