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TEACHING FILES (GRAND ROUNDS)

DISCORDANCE BETWEEN FLUOROQUINOLONE RESISTANCE RESULTS ON XPERT MTB/XDR PANEL, SECOND-LINE LINE PROBE ASSAY AND PHENOTYPIC DRUG SENSITIVITY TESTING- HOW TO INTERPRET?

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Clinical Problem:
A 10-year-old boy presented in February 2024 with fever for 4 months. His older sister had a past history of pulmonary multidrug-resistant tuberculosis (MDR-TB), microbiologically diagnosed 5 years back and who completed treatment after having received second-line antitubercular therapy (ATT) for 2 years. On presentation, his weight was 24 kg (10^{th}-25^{th} percentile according to Indian Academy of Pediatrics (IAP) charts) and height was 133.5 cm (25^{th}-50^{th} percentile according to IAP charts). General and systemic examinations were normal. Chest X-ray performed at another centre was suggestive of mediastinal lymphadenopathy. High-resolution computerized tomography thorax showed multiple enlarged pre- and paratracheal, pre- and subcarinal and hilar lymph nodes, of which the largest measured 2.6 x 1.5 cm. Few mediastinal lymph nodes showed calcification and multiple discrete nodules were also visualized in the left lower lobe. Bronchoscopy showed no endobronchial involvement. Urine examination found 6-8 pus cells/high power field (HPF), 50-60 erythrocytes/HPF. Abdominal ultrasound was normal. Mediastinal lymph node biopsy Xpert MTB/Rif assay detected medium load rifampicin-resistant *Mycobacterium tuberculosis* (MTB) complex. Xpert MTB/XDR performed on the same sample revealed resistance to isoniazid and kanamycin. Other investigations of the patient are shown in Table 1. He was initially diagnosed with pulmonary and mediastinal lymph node MDR-TB and was started on steroids and a shorter oral second-line ATT regimen consisting of high-dose isoniazid, pyrazinamide, ethambutol, ethionamide, bedaquiline, moxifloxacin and clofazimine. Histopathological examination of the mediastinal lymph node showed granulomatous inflammation with Langhans giant cells, tissue first-line line probe assay (LPA) showed resistance to isoniazid, rifampicin and second-line LPA showed resistance to fluoroquinolones. The mycobacterium growth indicator

Table 1. Investigations of the patient.

Parameters	Patient's Values	Reference Range
Hemoglobin (gm/dL)	9.5	11.5-15.5
TLC (cells/cumm)	6740	5000-13,000
ANC (cells/cumm)	3046	2000-8000
ALC (cells/cumm)	2501	1000-5000
Platelets (106 cells/cumm)	3.70	1.50-4.50
ESR (mm/hr)	50	<15
ALT (IU/L)	35	<41
ALP (IU/L)	144	51-332
BUN (mg/dL)	9	5-18
Serum creatinine (mg/dL)	0.35	0.3-0.59
Serum total protein (gm/dL)	7.4	6.00-8.30
Serum albumin (gm/dL)	3.6	3.80-5.40
PT (sec)	13.7	10-14
INR	1.19	0.8-1.2
Serum C3 (mg/dL)	158	88-201
Serum TSH (IU/mL)	2.1	0.5-5.0
Serum free T3 (pg/mL)	1.46	2.7-5.2
Serum free T4 (pg/mL)	6.55	0.8-2.1
QTcF at baseline (ms)	380	<450
HIV ELISA	Non-reactive	-
ASO titres	Non-reactive	-
Sputum Xpert MTB/Rif	MTBC not detected	-
Gastric lavage Xpert MTB/Rif	MTBC not detected	-
BAL Xpert MTB/Rif	MTBC not detected	-

Note: TLC- Total leukocyte count, ANC- Absolute neutrophil count, ALC- Absolute lymphocyte count, ESR- Erythrocyte sedimentation rate, ALT- Alanine aminotransferase, ALP- Alkaline phosphatase, BUN- Blood urea nitrogen, PT-

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Prothrombin time, INR- International Normalized Ratio, TSH- Thyroid stimulating hormone, T3- Triiodothyronine, T4- Tetraiodothyronine, HIV- Human immunodeficiency virus, ELISA- Enzyme linked immunosorbent assay, ASO- Antistreptolysin O, BAL- Bronchoalveolar lavage, QTcF- QT interval.

Tube (MGIT) culture of the tissue sample grew MTB and subsequent phenotypic drug-sensitivity testing (DST) showed resistance to pyrazinamide and high-level moxifloxacin. Gastric lavage and sputum MGIT cultures were negative. He was diagnosed with pre-extensively drug resistant TB (preXDR-TB) and isoniazid, pyrazinamide, moxifloxacin and ethambutol were stopped. The patient was shifted onto a longer oral regimen consisting of bedaquiline, linezolid, clofazimine, cycloserine and ethionamide.

How to interpret the discrepancy between Xpert MTB/XDR panel, second-line LPA and phenotypic DST for fluoroquinolone resistance?

Discussion:

Xpert MTB/XDR is a nested real-time polymerase chain reaction-based test used to detect resistance to isoniazid, fluoroquinolones, amikacin, kanamycin, capreomycin and ethionamide. It detects fluoroquinolone resistance through the amplification of gyrA and gyrB genes.[1] A recent Cochrane review estimated the sensitivity and specificity for Xpert MTB/XDR to detect fluoroquinolone resistance in sputum samples comparison to phenotypic DST to be 93.2% (ranging from 88.1-96.2%) and 98.0% (ranging from 90.8-99.6%).[2] Despite the high sensitivity, our patient was falsely reported negative for fluoroquinolone resistance. One possible reason for such a discrepancy could be due to improper sample handling, storage and other technical errors which may lead to a reduction in the number of organisms present in the sample, thus affecting detection of MTB deoxyribonucleic acid.[1] This is unlikely to be the case in our patient as the MTB-load detected for the same sample on Xpert MTB/Rif was medium. Another possible explanation for this result lies in the type of sample tested. Unlike sputum, tissue samples have not been evaluated for testing by the Xpert MTB/XDR panel. In addition, blood and white blood cells are considered as "potentially-interfering substances" for the Xpert MTB/XDR test and their presence in the lymph node sample may result in aberrant results.[1] Interference by organisms can also affect the reporting of fluoroquinolone resistance by Xpert MTB/XDR. The ATCC 0927 strain of M.marinum at concentrations greater than 104 colony forming units (CFU)/mL can interfere with the gyrA signal and thus leads to the suppression of one or more melting temperature peaks. However, this would lead to the reporting of a fluoroquinolone indeterminate call.[1,3] The phenomenon of heteroresistance may also explain these discordant results. If the tissue sample contained a mixture of fluoroquinolone sensitive and resistant strains, Xpert MTB/XDR may not be able to pick up the resistant strain, if the resistant strain was present at levels below the limit of detection (LoD). Cao et al.[3] found that mixtures of populations with A90V and S91P gyrA mutations were not reliably reported to have low fluoroquinolone resistance by the Xpert MTB/XDR panel. Rather, they were either reported as fluoroquinolone resistant or sensitive by the assay. They also found that reporting of gyrB mutations in mixed samples as fluoroquinolone resistant, required a prevalence of 60% of the mutant strains.[3] However, heteroresistance is also an unlikely cause for the discrepancy in our patient as the estimated LoD of Xpert MTB/XDR (71.9 CFU/mL for MTB detection and 95.5 CFU/mL for fluoroquinolone susceptibility detection) is much lower than that of LPA (~10,000 CFU/mL).[3,4] Lastly, the presence of mutations or polymorphisms in probe bindings regions or the primer region may result in an inability to be detected by the Xpert MTB/XDR panel and result in a falsely drug-sensitive result. Cao et al.[3] reported that while the assay was vigorous in detecting gyrA mutants, it missed detecting one gyrB mutant (C5389A) and this could lead to the reporting of a false-sensitive result. Ultimately, since the World Health Organization considers phenotypic DST on liquid media such as the MGIT system as the reference standard and finds fluoroquinolone resistance detection by this method to be reliable, we treated our patient in concordance with the DST results as preXDR-TB.[5]

Compliance with Ethical Standards

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Conflict of Interest : None

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