ABSTRACT BOOK

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A rapid sputum PCR-SSCP test for guiding pyrazinamide use in tuberculosis therapy

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Rationale: Pyrazinamide susceptibility testing is usually too slow to guide initial therapy, so that many patients with pyrazinamide-resistant tuberculosis receive ineffective pyrazinamide therapy.

Objectives: To optimize and evaluate a rapid molecular test for tuberculosis drug susceptibility to pyrazinamide.

Methods: Tuberculosis polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) was optimized to test for mutations causing pyrazinamide resistance directly from sputum samples and Mycobacterium tuberculosis isolates. The reliability, sensitivity and specificity of PCR-SSCP in sputum (n = 65) and isolates (n = 185 from 147 patients), was compared with the Bactec-460 microbiological test (n = 139) that was considered to be the gold standard. Pyrazinamide susceptibility testing for all 185 isolates was also compared with the Wayne biochemical test, with DNA sequencing for pncA mutations that cause pyrazinamide resistance and with traditional microbiological susceptibility testing in duplicate broth cultures containing pyrazinamide.

Results: PCR-SSCP provided interpretable results for 96% (46/48) of smear positive, 76% (13/17) of smear negative sputum samples and 100% of M. tuberculosis isolates. PCR-SSCP had pyrazinamide susceptibility testing sensitivity and specificity of 88% and 93% for sputum and 89% and 95% for isolates. For isolates that yielded concordant results in conventional broth culture, sensitivity and specificity were 95% and 92%, compared with 88% and 100% for Wayne biochemistry. All 34 negative-controls were PCR-SSCP negative. Material costs were $1.36 per test and PCR-SSCP took one day, compared with 3-7 weeks for the other techniques.

Conclusions: PCR-SSCP is a rapid test that indicates which patients should receive pyrazinamide from the start of therapy, potentially preventing months of inappropriate treatment.