Tuberculosis and its sequelae
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2015

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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In this thesis, we touched on molecular diagnostics and epidemiology, as well as treatment aspects of tuberculosis (TB) and a condition that may ensue, once TB – or any other devastating infectious condition of the lungs – is cured: non-CF bronchiectasis, or structural airways disease.

In chapter 2 we compared the major molecular tests for the diagnosis of TB and we studied an optimized method of fingerprinting – Variable Number of Tandem-Repeats (VNTR) typing generally used for molecular epidemiology of TB.

In chapter 2a we focused on the rapid molecular diagnostics of TB and the different in-house and commercially available PCR assays. This was the first study to include comparisons of a large number of diagnostic PCR assays currently available for TB. In general, the bacterial load of the specimen correlates with analytical performance of a test. We mimicked the bacterial load of specimens by dilution steps, with a high dilution mimicking a low bacterial load. Both analytical sensitivities and the detection limits appeared to differ between the PCR tests, with the AmpliSens MTC-FRT PCR kit, the in-house real-time PCR IS6110: 10 μl DNA input performing best in both analytical sensitivity and detection limit and with the GeneXpert MTB/RIF assay having the lowest analytical sensitivity.

In chapter 2b we evaluated a modified method for VNTR typing. VNTR typing is the current gold standard for performing molecular epidemiological studies with *M. tuberculosis* (MTB) strains. The modification of 7 primers of the original in-house method resulted in a high percentage of complete profiles in the first multiplex PCR run. This reduces workload and laboratory turnaround time and it is much cheaper than the commercially available VNTR method. We tested analytical sensitivity of this optimized VNTR method in standardized broncho-alveolar lavage fluid (BALF) spiked with MTB as we described in the study presented in chapter 2a. This resulted in an analytical sensitivity of 1:10 – or in an almost perfect analytical sensitivity of 1:100. The latter dilution showed a perfect score except for one of the three VNTR analyses with a strain with a single copy of IS6110 that yielded results for only 22 loci instead of 24. These results show that the assay may provide an asset for clinicians; they may take clinical decisions for empirical treatment based on fast molecular, and standard phenotypic drug sensitivity results if their patient appears infected with an isolate from a known cluster; or, in discriminating between a relapse or a new infection, comparing two different isolates over time in the same patient.

In chapter 3 we focused on results of epidemiological linking of individual patients. In chapter 3a we described an outbreak among non-human primates and a human using results
obtained by another molecular fingerprint method – spoligotyping. With the clinical and radiological data and with the use of this molecular epidemiological technique we described the transmission of TB from great apes to humans. Transmission from animals to humans has rarely been described; our clinical and radiological data appeared highly suggestive that the route of transmission was in fact from non-human primates to humans. Further evidence of the route of transmission was given by spoligotyping resulting in the general conclusion that the animals, and not the human, were the source of the infection.

In chapter 3b we described a case study of the results using a blood test, and not only a skin test for TB contact tracing, to detect any transmission from a patient with TB. For the first time, the Quantiferon (QFT) was used next to the tuberculin skin test (TST) for contact tracing around an index case with bovine TB. We showed the discrepant results between the TST and the QFT in three rings of contact tracing. The QFT targets the ESAT-6 and CFP10 gene products coded by the Region of Difference-1 and TB7.7; these genes are almost unique for the *M. tuberculosis* complex. Therefore the results in this study in bovine TB met our expectations, as in human TB the discordant results of both the TST and the QFT in contact tracing have been reported and discussed in the literature. The results of the molecular-epidemiological test showed a possible link to another case but thorough investigation by the public health TB department could not relate our patient to this source.

In chapter 4a we reported the drug concentrations of second-line anti TB drugs at the site of the infection, in this case, the lung of a patient that needed resection. The hypothesis to be tested by measurements of drug concentrations in the resected lung tissue was that the second-line TB drugs would not reach the severely injured, poorly perfused lung tissue. The hypothesis of insufficient blood supply to the destroyed lung was based on imaging studies of the lungs using technetium-99m-labeled macro-aggregates of albumin, showing that lung perfusion of the injured lung was poor. Surprisingly, even with the caseated tissue of the destroyed lung, tissue drug concentrations were sufficient for antimicrobial killing. The study model presented here is a step forward in gaining more knowledge of the concentrations of these second-line TB drugs at the site of the TB infection. In the letter in chapter 4b we commented on a study describing the pharmacokinetics of high dose of rifampicin and moxifloxacin in both serum and cerebrospinal fluid in TB meningitis. We argued that there is a need for studying this devastating type of extra-pulmonary TB that still carries a poor prognosis. Several of the first-line TB drugs have poor penetration into cerebro-spinal fluid; rifampicin has poor penetration, but isoniazid does not. The latter drug should therefore be analyzed for its contribution relative to the other treatment components. Drug exposure
of the different TB drugs is best studied as continuous variable to estimate their relative pharmacokinetic contribution. Studies of the pharmacotherapy and pharmacokinetics of patients with TB meningitis are typically small, and every effort should be made to analyze all available data to help design future studies in this daunting condition.

In chapter 4c we commented on a study that related plasma concentrations of isoniazid, rifampicin and pyrazinamide to sputum culture status after 4 and 8 weeks. Only a few studies have related low drug exposures to treatment response. We propose a limited sampling strategy for calculating the pharmacokinetics of the drugs instead of using 2-hour post ingestion as moment to measure levels. For the correct interpretation of the pharmacokinetics, especially the area under the curve, the actual MIC of the isolates is needed. In this way the best predictor of efficacy, the AUC/MIC ratio, can be measured.

In chapter 5a we built a model to predict systemic levels of drugs by measuring blood concentrations by absorption in the airways following inhalation. In this model, we describe the different stages of absorption, distribution and clearance of an antimicrobial agent following inhalation. The model includes knowledge about the device used for inhalation and the physico-chemical properties of the antimicrobial product used.

In chapter 5b we studied the local tolerability and pharmacokinetics of dry powder tobramycin using a novel device called the Cyclops. We tested four different doses of tobramycin in patients suffering from non-Cystic Fibrosis Bronchiectasis. In general, the tobramycin was well tolerated – in fact much better than currently available methods to deliver tobramycin to the airways of such patients that usually report adverse effects like cough and dyspnea side effects resulting from inadvertent drug resorption from the airways into the blood stream. There were only mild complaints of cough in two out of eight study participants, each at only one of the four visits. Bad taste reported after inhalation of the first dose initially reported was abolished after we introduced rinsing the mouth with water. Pharmacokinetics were calculated and compared to the Tobi Podhaler. The $C_{\text{max}}$ in our study was lower compared to the $C_{\text{max}}$ values of the Podhaler in studies with healthy volunteers and patients with CF-bronchiectasis.
References

