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 CID, Faculty of Health Sciences, Tygerberg, Stellenbosch University

On 26 November 2007

the CID had the launch of the lectures series "Expert Opinion on Infectious Diseases and the lectures were accredited for 5CEU's level 1. Presentations were of exceptional quality. The prize for the best student presentation was awarded to Kim Hoek. Abstracts of this successful day are published in this issue of ~Bug's Life~

Special Interest:

- Best Student Presentation: FAST-RIF
- XDR-TB: Cause for Concern or Overreaction?

Highlights on page 2:

- Nuclear Medicine in Infections
- HIV exposed uninfected infants
- Host determining factors in susceptibility of TB

Rapid, Reliable and Easy Fluorometric Assay for Susceptibility Testing of Rifampicin in Mycobacterium tuberculosis (FAST-RIF)

Warren R.M., Hoek K.G.P., Moolman-Smook H., Carelse-Tofa K., Jordaan A., van der Spuy G., Streicher E., Victor T.C., Gey van Pittius N.C., van Helden P.D. *DST/NRF Centre of Excellence for Biomedical Tuberculosis Research / MRC Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Health Sciences, Stellenbosch University, South Africa.*

Rationale: The evolution of rifampicin-resistance is strongly associated with multi-drug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) which threaten TB control world-wide.

Objective: To develop a genotypic drug-susceptibility testing method that will enable the rapid diagnosis of rifampicin-resistance in *Mycobacterium tuberculosis* complex.

Methods: The rifampicin-resistance determining region (RRDR) of the *rpoB* gene from *M. tuberculosis* complex was amplified by polymerase chain reaction (PCR) from DNA templates extracted from either clinical isolates or the laboratory strain (H37Rv) in the presence of a fluorescent DNA binding dye. Subsequent mixing of the amplification products and thermocycling allowed for the formation of DNA duplexes. The thermal denaturation properties of these DNA duplexes were determined by measuring the derivative of the intensity of fluorescence at different temperatures (dF/dT). Genotypically drug-sensitive isolates were identified by homoduplexes (single peak), while drug-resistant isolates were identified by the presence homo- and heteroduplexes (two peaks).

Results: Analysis of DNA extracted 153 clinical isolates using the fluorometric assay for susceptibility testing of rifampicin (FAST-RIF) method showed a sensitivity of 0.96 and a specificity of 1.00 for the detection rifampicin-resistance when compared to the "gold standard" routine culture based phenotyping method. The positive predictive value was 1.00 and the negative predictive value was 0.96. No statistical difference was detected in the performance of the method when applied to crude DNA from 134 boiled cultures.

Conclusions: The FAST-RIF method allowed for the rapid, reliable and easy detection of genotypic rifampicin-resistance as a marker for MDR-TB and XDR-TB.

XDR-TB: Cause for concern or over-reaction?

R.M. Warren, Streicher E., Galver A., Mlambo C., Marais, E., Falmer A., Stolk M., van Helden P.D., Victor T.C. ¹DST/NRF Centre of Excellence for Biomedical TB Research/MRC Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Faculty of Health Science, Stellenbosch University. ²West Vaal Hospital. ³NHLS, Wits, Gauteng.

The evolution of drug resistance in *Mycobacterium tuberculosis* has largely been blamed on poor adherence to treatment. However, molecular epidemiological investigations have suggested that once drug resistance has evolved it spreads by transmission, a finding which has contradicted the dogma that suggested that drug resistant strains were less fit and were therefore less likely to be transmitted. Recently, the description of an outbreak of XDR-TB in Kwazulu Natal raised alarm bells suggesting that this highly resistant form of disease was rapidly spreading and was a threat to TB control. In this study, we have investigated the disease dynamics of XDR-TB in different regions of South Africa. Using molecular investigations we showed that the XDR-TB epidemic was context dependent as very little transmission of the XDR-TB strains occurred in immune competent individuals. We demonstrated that despite an excellent TB control program in certain settings, the emergence of resistance occurred as a consequence of the TB control guidelines used to fight the epidemic.

Nuclear Medicine in infection and inflammation – Current role and Research Possibilities

Rubow SM, Kaarse L, Ellmann A. Nuclear Medicine, Tygerberg Hospital and University of Stellenbosch

Imaging of infection using Nuclear Medicine techniques has two main indications: (1) The localisation of a focus of infection where other modalities have failed, e.g. in patients with fever of unknown origin; (2) To confirm or exclude the presence of infection as the cause of a localised abnormality found on clinical or radiological examination. A wide range of radiopharmaceuticals is available for imaging of inflammatory or infectious processes. In our situation, only Ga-67 and Tc-99m labelled white blood cells (WBC) are readily available, affordable and useful in daily practice.

Labelled leukocytes are often regarded as the gold standard in infection imaging. At Tygerberg Hospital, Tc-99m WBC studies are routinely used, especially in the more acute setting. The most important indications are orthopaedic infections, abdominal infection and vascular prosthetic infection. However, labelled white cells have several disadvantages, such as the time consuming and labour intensive labelling process, the specialised facilities required, and the handling of large blood samples. The latter is especially a problem where there is a high incidence of hepatitis B and AIDS. There are also several clinical situations in which white cell studies are not indicated. Typical problems solved by Ga-67 imaging rather than WBC studies include tuberculosis, vertebral osteitis and fever of unknown origin. In immunosuppressed patients, Nuclear Medicine can be used for disease detection at an early stage and determination of the extent of disease. It is especially useful to assist clinicians in optimising therapy and assessing its efficacy.

World-wide, researchers are currently seeking more specific imaging agents for infection and inflammation. Promising results have been reported with labelled antibiotics and peptide radiopharmaceuticals, but these agents are not generally available. Labelled immunoglobulin (IgG) has been used for infection imaging, but is also not commercially available. It does not require complicated labelling procedures or the handling of blood samples. A project to investigate the role of Tc-99m labelled IgG in the African context and in HIV positive patients is currently underway at Tygerberg Hospital. This presentation summarises the current status and future prospects of scintigraphic infection imaging.

HIV Exposed, uninfected infants with immunodeficiency Diseases - A case study cohort

ESSER MM, Immunology Section, Div Medical Microbiology, TBH & NHL

Immunodeficiency and infection spectrum of the HIV infected infant and child are documented and treatment and follow up guidelines are in place. For the HIV exposed and PCR negative infant however there are no clear follow up guidelines. From observations of referrals of HIV exposed, PCR negative infants and children to the Immunology service at Tygerberg Hospital, 10 patients have been documented with life threatening infections, which are usually associated with Immunodeficiency.

All the children were born to HIV infected mothers and all had negative PCR's documented on two separate occasions. Viral loads where done were undetectable. The full lymphocyte subsets were available for several children and showed varying forms of lymphopaenia - in one child severe CD₁₉ -B-cell depletion. Infections included PCP pneumonias, CMV colitis, Group A Strep meningitis with endocarditis, unusual congenital syphilis. Other risk factors such as prematurity were absent. Most of the children required intensive care nursing as part of their hospitalization which in some cases exceeded 2 months of hospital stay. Where follow-up was documented the children appeared to be clinically well at 24 months of age.

On search of the literature there is one article documenting excess morbidity observed in this patient group and no literature on the full immunologic profile or suspected deficiency is documented. We hypothesize that exposure to HIV in utero, even with negative PCR and viral load may predispose the uninfected infant to serious infections and guidelines for follow up of these children may need to be adjusted.

Analysis of host determining factors in susceptibility to Tuberculosis in the South African Coloured Population

Erika Truter¹, Paul van Helden¹, Eileen Hoal¹ *Molecular Biology and Human Genetics, University of Stellenbosch, Stellenbosch, South Africa*

Background: The investigation of host genetic susceptibility to tuberculosis (TB) may result in new insights into the disease and suggest ways to combat it. However, it is necessary to perform this type of study in a number of different populations, so that one can ascertain which genes are universally important, and which are population specific, and may not be relevant in our population. Since TB is a complex disease, genetic studies in TB can be challenging, but several susceptibility genes such as *NRAMP* and *IFN-γ* have been identified. In complex diseases with many genes involved, gene-gene interactions may hold the key to elucidating pathways of resistance. We intend to investigate gene-gene interaction in a group of genes showed to be important in TB. The interaction of these human genotypes and mycobacterial strains will also be investigated which will lead to a unique study combining strain and genotype information.

Methods: This research tests the association of 8 potentially important genes including *SP-D*, *MBP*, *NRAMP*, *RANTES*, *IFN-γ*, *IFNGR1*, *IL-1Ra*, *IL-8* with TB in case-control association studies in the South African Coloured population. This includes 400 controls and 425 TB cases of whom 50 are TBM cases. Interaction will be measured by regression analysis. As the population under study has a complex make-up, 209 unlinked randomly selected microsatellites were used to test for population stratification using STRUCTURE.

Results: Polymorphisms studied in these 8 different genes were all in HWE and some demonstrated significant associations with TB. All genes, however, will be useful for gene-gene interaction studies. Analysis of the 209 microsatellites showed that our sample population was not stratified.

Conclusion: This study will give more insight into population structure of the South African Coloured population in the Western Cape as well as gene-gene combinations which play an important role in the development of TB.