Western Blotting studies of IgG antibodies, IgG avidity and IgG subclasses during the follow up of patients with pulmonary tuberculosis, under treatment

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Background: WHO in 2007, estimated that the incidence of tuberculosis in worldwide was about 8.8 million new cases per year. In attention to WHO recommendations serological tests are currently being studied and standardized for the diagnosis of the active and latent disease.

Methods: From clinical strain of *Mycobacterium tuberculosis*, maintained in our laboratory, the antigen was prepared and after SDS PAGE, the proteins were transferred to nitrocellulose membrane for Western Blotting analysis. For IgG antibodies detections we used monoclonal antibodies anti IgG labeled with peroxidase diluted 1/500, and for IgG subclasses we used anti-IgG1 and anti-IgG3 labeled with biotin diluted 1/100 and streptavidin-peroxidase diluted 1/100. The reactions were revealed with a solution of 4 chloro-alpha-naphtol. For IgG avidity, after the first incubation with serum samples, the strips were washed with 8 M urea for 5 minutes.

Results: We tested serum samples from 5 patients, taken at the beginning and the end of treatment, after 6 months. The region of 30 kDa, related with metabolic activity of mycobacteriae, has been recognized in all samples for IgG antibodies, with intensity stronger in the last sample. There was the appearance of band in the region of 16 kDa in the first and in the last samples of two patients. In the others three patients this band appears only in the last sample, but after washing with urea less intensity of this band was observed. The region of 16 KDa is expressed by *M. tuberculosis* when it is maintained with low oxygen tension and it is associated with latent infection. The region of 6 kDa is better recognized in the last samples, and this region is not sensitive to urea treatment. With regard to IgG1, antibodies were most frequent in the region of 38 kDa, considered as specific protein of the *M. tuberculosis;* IgG3 antibody appears in serum samples from four patients reacting with bands in the regions of 30 kDa, 20 kDa and 6 kDa, at random.

Conclusion: The physiological pathological roles of antibodies reacting with the bands studied are being evaluated with a larger number of patients.

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A multiplex real-time PCR method for presumptive identification of NAP1 clone of *Clostridium difficile* from stools

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Background: Clostridium difficile infection (CDI) is the leading cause of nosocomial diarrhea in adults and paediatric patients. There has been significant morbidiity and mortality related to CDI due to the presence of hypervirulent strain (NAP1) associated with unregulated production of toxin. NAP1 has been responsible for a number of outbreaks in many countries. Rapid detection for the presence of NAP1 is essential for appropriate patient management and to minimize nosocomial transmission. Currently, pulsed-field gel electrophoresis (PFGE) and repetitive sequence-based (REP-PCR) methods which require bacterial culture have been used to identify NAP1. A 18 bp deletion in the *tcd*C gene and the presence of *cdt*A gene (binary toxin) have been shown to be associated with NAP1. We describe a rapid multiplex real-time PCR method for presumptive identification of NAP1.

Methods: Ninety blinded frozen stool samples previously tested for toxin A/B by CD TOX A/B® II EIA (TechLab, Inverness Medical) were used. A fragment of *tcd*C flanking the 18 bp deletion (surrogate marker for *tcd*A/*tcd*B), *cdt*A, and 16S rDNA (internal control) genes were amplified using Multiplex QuantiTechTM (Qiagen, Inc.) and detected by TaqMan probes. ProGastroTM (Prodesse, Inc.) kit that detects *tcd*B was used for comparison. DNA from stools were extracted using easyMAG (BioMerieux Inc) and tested accodring to manufacturers' specifications. Five !l of the same DNA was used for the in-house method. Both real-time PCR amplifications were done in a Rotor-Gene 6000 (Corbette, Inc.). PCR products from both *tcd*C and *cdt*A positive samples were separated on agarose gels to detect *tcd*C deletion.

Results: DNA from 85 specimens were positive for *tcd*C (toxigenic) and 53 of those showed the presence of *cdt*A gene (presumptive NAP1). DNA from none of the specimens were positive for *cdt*A only.The *tcd*B detection by ProGastroCDTM was concordant with the detection of *tcd*C. Gel electrophoresis of all *tcd*C and *cdt*A positive PCR products detected the 18 bp deletion in the *tcd*C amplicon and were confirmed as NAP1 by PFGE. All *cdt*A negative DNA samples were negative for 18 bp deletion in the *tcd*C.

Conclusion: Detection of toxigenic strains by the in-house method is comparable to the commercial assay and it presumptively identified the presence of NAP1.

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