

Title	2. <i>Evaluation of the immune response against <i>Pseudomonas aeruginosa</i> in patients with cystic fibrosis: a comparison of methods</i>
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Study design	Prospective study, which compare different methods for the determination of antibodies anti- <i>Pseudomonas aeruginosa</i>
Grant by	Lega Italiana fibrosi cistica - Tuscany Association (2 years; Euro 42.000,00).
Background and aims	Monitoring infection by <i>Pseudomonas aeruginosa</i> (Pa) in patients with cystic fibrosis (CF) is crucial for the implementation of appropriate therapy to prevent chronic colonization of this pathogen; however, it is difficult to distinguish between chronic infection and intermittent colonization using only cultural methods in those patients who do not expectorate. Chronic Pa infection can be discriminated by the intermittent colonization by measuring the anti-Pa IgG antibody titer in the serum. So far to monitor the course of infection by Pa different serological tests with different antigens were used, but only few of them are commercially available or were compared to each other. The objective of this study is to evaluate the immune response with three anti-PA ELISA kits to identify the infection state in patients with intermittent colonization by Pa at risk of developing chronic infection.
Inclusion criteria	CF patients without infection or with intermittent Pa colonization defined according to the Leeds criteria as the presence of Pa positive cultures in less than 50% of samples tested in one year (with microbiological checks at least every three months). Healthy subjects were considered as negative controls.
Exclusion criteria	CF patients with chronic Pa, according to the Leeds criteria, assessed as the presence of Pa positive cultures in more than 50% of samples tested in a year (with microbiological checks at least every three months).
Methods	IgA and /or IgG antibodies titer will be analyzed by quantifying anti-Pa antibody in about 50 patients by ELISA method (enzyme-linked immunosorbent assay). The first test uses the antigen obtained by the partial purification of lipopolysaccharide Pa. The second test uses as antigen the total sonicated obtained from the 17 most common serotypes of Pa (O-1-O-17), in which are detectable 64 different antigens. Finally, the third test uses three different antigens obtained from the alkaline protease (AP), elastase A (ELA) and exotoxin (ExoA) of Pa.
Expected results and anticipated output	We want to find a reference method for monitoring the immune response to infection by Pa and a new cut-off level that could discriminate chronic infection from intermittent colonization.
Start of recruitment	January 2009
End of experimental plan	October 2010
Publication on medical Journal	Preliminary data will be presented at the 33rd European Congress on Cystic Fibrosis (ECFC 2010). Title of abstract: "Evaluation of the immune response against <i>Pseudomonas aeruginosa</i> before eradication therapy: results of two ELISA tests." Further publications are planned at the end of the experimental phase in 2010.