Abstract

Status of tuberculosis infection in an individual

In a first aspect, the present invention relates to a method for diagnosing or determining the status of tuberculosis infection in an individual afflicted with or suspected to be afflicted with tuberculosis infection. In a further aspect, a method for the stratification of the therapeutic regimen of an individual with tuberculosis infection is provided as well as a method for predicting a clinical outcome or determining treatment course in an individual afflicted with tuberculosis infection. Moreover, the present invention provides a method for monitoring the change from latent into active status of tuberculosis infection or vice versa in an individual. Furthermore, the present invention relates to a kit for use in diagnosing or detecting the status of tuberculosis infection as well as to Mycobacterium tuberculosis alanine dehydrogenase for use in specifically differentiating latent status from active diseases status of tuberculosis in an individual.

AMENDED CLAIMS

1. A method for diagnosing or determining the status of tuberculosis infection in an individual afflicted with or suspected to be afflicted with tuberculosis infection, comprising:

a) determining the level or amount of cytokines released or produced by mononuclear cells after stimulation, whereby said mononuclear cells are from said individual; and

b) determining or diagnosing the status of said individual based on the level or amount of cytokines released or produced from the mononuclear cells,

characterized in that the mononuclear cells are stimulated with mycobacterial Alanine Dehydrogenase (AlaDH), in particular, of *Mycobacterium tuberculosis*, and
wherein the cytokines to be determined are selected from IL-2 and interferon Gamma.

2. A method for the stratification of the therapeutic regimen of an individual with tuberculosis infection comprising:

a) determining the level or amount of cytokines released or produced from mononuclear cells after stimulation, whereby said mononuclear cells are from said individual; and

b) determining the status of tuberculosis infection based on the level or amount of cytokines released or produced from said mononuclear cells after stimulation allowing differentiation of a latent and active status of tuberculosis infection in said individual, characterized in that the mononuclear cells are stimulated in the presence of with mycobacterial Alanine Dehydrogenase (AlaDH), in particular, of *Mycobacterium tuberculosis*, and

wherein the cytokines to be determined are selected from IL-2 and interferon Gamma.

3. A method for predicting a clinical outcome or determining the treatment course in an individual afflicted with tuberculosis infection comprising:

a) determining the level or amount of cytokines released or produced from mononuclear cells after stimulation, whereby said mononuclear cells are obtained from said individual; and

b) predicting the clinical outcome or determining the treatment course based on the level or amount of cytokines released or produced from said mononuclear cells after stimulation, characterized in that the stimulation includes cultivation of said mononuclear cells with mycobacterial Alanine Dehydrogenase (AlaDH), in particular, of *Mycobacterium tuberculosis*, and
wherein the cytokines to be determined are selected from IL-2 and interferon Gamma.

4. The method according to claim 1 for monitoring the change from latent to active status of tuberculosis infection or vice versa in an individual comprising:

a) determining the level or amount of cytokines released or produced from mononuclear cells from said individual after stimulation at a first point in time;

b) determining the level or amount of cytokines released or produced from mononuclear cells after stimulation, whereby said mononuclear cells are obtained from said individual at a second point in time; and

c) comparing the level or amount of cytokines determined in step a) to the level or amount determined in step b) or to a reference value whereby

an increase in the level or the amount relative to a reference value or to the level or amount determined in step a) is indicative for a transition from latent to active status and a decrease in the level or the amount relative to a reference value or to the level or amount determined in step a) is indicative for a transition from active to latent status characterized in that the mononuclear cells are stimulated with mycobacterial Alanine Dehydrogenase (AlaDH), in particular, of *Mycobacterium tuberculosis*.

5. The method according to any one of the preceding claims wherein the mononuclear cells are stemming from a sample of the individual including a blood sample or other body fluids including Bronchoalveolar lavage and urine and tissues.

6. The method according to any one of the preceding claims wherein the level or amount of cytokines is determined at protein level, in particular, by an immunoassay, like, ELISpot or ELISA or at nucleic acid level, in particular, by PCR techniques.

7. The method according to any one of the preceding claims wherein the cytokine to be determined is IL-2.

8. The method according to any one of the preceding claims wherein the reference value in IL-2 Elispot is below 25.

9. The method according to any one of the preceding claims wherein the individuals are children.

10. The use of a kit for diagnosing or determining the status of tuberculosis infection, or for predicting a clinical outcome or determining the treatment course in an individual afflicted with or suspected to be afflicted with tuberculosis infection, or for the stratification of the therapeutic regiment of an individual with tuberculosis infection or for monitoring the

progression of tuberculosis infection, or for determining the transition from latent to active status of tuberculosis infection in an individual, said kit comprises means for determining the level or amount of cytokines released or produced from mononuclear cells after stimulation, whereby said mononuclear cells are obtained from said individual, a stimulating agent which is mycobacterial Alanine Dehydrogenase (AlaDH), in particular, of *Mycobacterium tuberculosis*, and instructions on how to use said test kit for a method according to anyone of claims 1 to 9, wherein the cytokines to be determined are selected from IL-2 and interferon Gamma.

11. The use of a kit according to claim 10 whereby said kit is an ELISpot or ELISA or said kit is a PCR kit.

12. Mycobacterial Alanine Dehydrogenase, in particular, of *Mycobacterium tuberculosis*, for use as a stimulant for differentiating latent status and active status of tuberculosis infection in an individual afflicted with or suspected to be afflicted with tuberculosis infection.

13. The mycobacterial Alanine Dehydrogenase (AlaDH), in particular, of *Mycobacterium tuberculosis*, for use in differentiating between latent and active tuberculosis infection according to claim 12 wherein the individuals are children.

14. An assay for conducting the method according to any one of claims 1 to 9 comprising means for culturing mononuclear cells, means for determining the level or amount of cytokines and mycobacterial Alanine Dehydrogenase (AlaDH), in particular, of *Mycobacterium tuberculosis*, and wherein the cytokines to be determined are selected from IL-2 and interferon Gamma.

15. Composition comprising mycobacterial Alanine Dehydrogenase (AlaDH), in particular, of *Mycobacterium tuberculosis,* and ESAT-6 and CFP-10 for use

in determining tuberculosis infection in an individual, in particular, for use in determining tuberculosis infection in an individual, using the components of the composition as stimulant.

Dated this 19th day of January 2015

(Nisha Austine)
of Khaitan & Co
Agent for the Applicant
Reg No IN/PA-1390

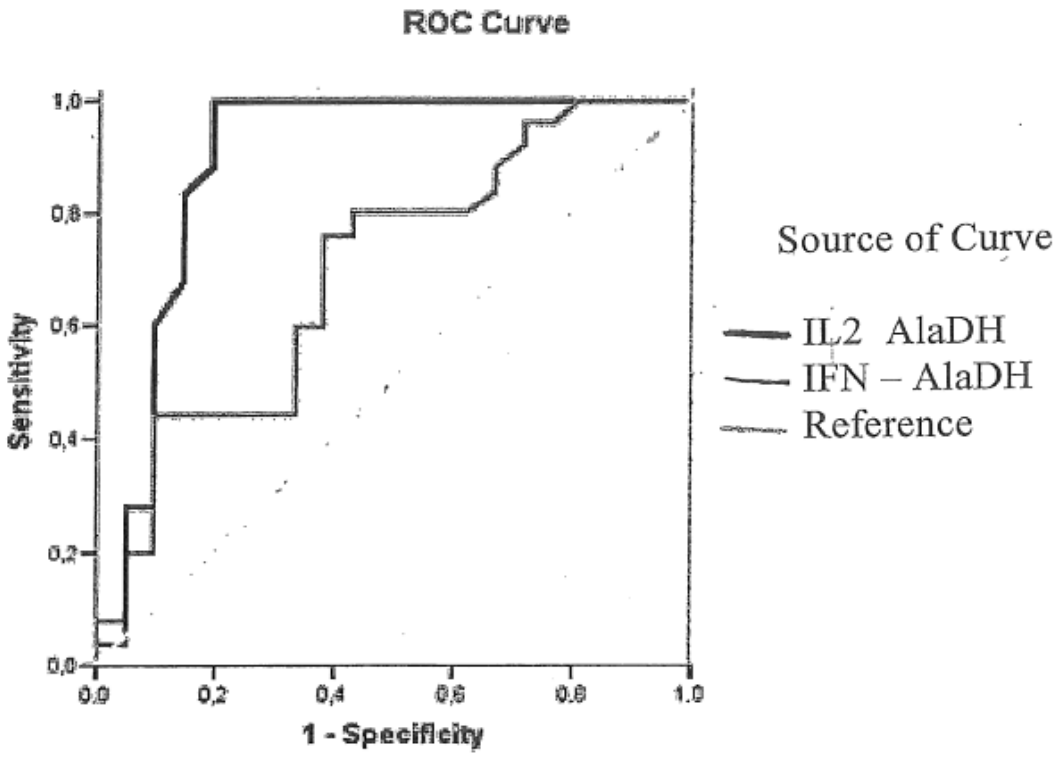

Figure 1

LIONEX GmBH
By their Agent

(Nisha Austine)
of Khaitan & Co
Reg No IN/PA 1390