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Immunologic Diagnosis of Neurotuberculosis

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1. Introduction

Tuberculosis (TB) is one of the most prevalent infectious diseases in the world, particularly in developing countries. The high incidence and mortality of TB in these countries mainly resides in the limited financial resources, the emergence of multiresistant strains and the spread of HIV infections. The key element for controlling TB is a rapid and early diagnosis which ensures the correct treatment and eradication of the infection source in the community. Current strategies for the development of a rapid and accurate diagnosis address 3 major objectives: improving the present diagnostic tools through better knowledge, developing new tests and increasing the accessibility to such diagnostic possibilities (Who Strategic Directions, 2006). The gold standard of diagnosis in TB is the identification of mycobacteria in the pathologic product using acid-fast smear microscopy and mycobacterial culture. Nevertheless the bacteriologic diagnosis is presently inefficient due to increasing number of smear-negative TB forms. This has led to the development of various complementary diagnostic methods such immunologic assays. The aim of the present chapter is to outline the literature data on the importance of the immunologic diagnosis in neurotuberculosis, a lethal localization of TB. It presents the current immunological assays and their practical value in the diagnosis of neurotuberculosis as complementary methods.

2. General data

Neurotuberculosis is represented by various central nervous system (CNS) tuberculous manifestations including : tuberculoma, tuberculous abscess, meningoencephalitis , spinal arachnoiditis and cerebral miliary tuberculosis. This diverse neurological frame mostly emerges as a result of the invasive potential as well as of the immune pathogeny of neurotuberculosis. The difficulty in the diagnosis of neurotuberculosis lies in the atypical clinical presentation and non-specific changes recorded by various diagnostic assays. The bacteriological exams of the cerebrospinal fluid (CSF) are still regarded as the sole diagnostic tools in the confirmation of neurotuberculosis. Nevertheless the bacteriological assay in neurological forms of TB has a low sensitivity and a considerable delay. As a rule, owing to the rapid evolution and increased mortality, the suspected neurotuberculosis

patients frequently receive antituberculous treatment before bacteriological confirmation (Donald & Schoeman, 2004). A complete antituberculous treatment requires 6-12 months, during which serious adverse reactions could occur. On the other hand nontuberculous CNS infections with different treatment and high mortality could be overlooked. Therefore multiple research studies based on new diagnostic methods have been developed over the last 30 years aiming to improve the diagnostic efficiency in neurotuberculosis.

3. Diagnostic methods in neurotuberculosis

CSF analysis represents the basic element in the diagnosis of neurotuberculosis. The isolation and identification of mycobacteria in CSF are crucial for the diagnosis of neurotuberculosis. However as the CSF is a paucibacillar product, these methods often fail. All the same the CSF could present characteristic cytological and chemical changes which could be suggestive for the neurotuberculosis diagnosis in a specific clinical context. In several cases, especially in children and immunosupressed patients, the cytochemical changes of the CSF lack even these particular findings and the neurotuberculosis diagnosis is then ommited. During the past decades various complementary methods have been implemented to assist these difficulties. These currently include : nucleic acid amplification tests (NAATs), CSF adenosindeaminase (ADA) detection and immunologic assays.

3.1 Advantages and disadvantages of current diagnostic techniques in neurotuberculosis

Below are the main findings, advantages and disadvantages of each current diagnostic method in neurotuberculosis (Desai et al, 2006; Sonmeza et al, 2008; Nyendak et al, 2009; Davies & Pai 2008)

a. **CSF cytology.** *Main findings*: at the onset mixed pleocytosis with mainly lymphocytes(80-90%) and the presence of neutrophils (10-20%); in the later stages, only lymphocytes.

Advantages: rapid, inexpensive, sensitive method. *Disadvantages*: 1) Variable CSF cytology in the evolution of the disease, 2) Many atypical CSF aspects at the onset or throughout the evolution in immunodepressed hosts and children 3)Low specificity; 4)Normal CSF cytology cannot rule out localized forms of neurotuberculosis

b. **Biochemical CSF exam.** *Main findings*: high CSF protein level and low glucose.

Advantages: rapid, inexpensive. *Disadvantages*: 1) Very low specificity: many infections of the CNS share similar changes ; 2) CSF biochemical analysis is normal in localized forms of neurotuberculosis.

c. Bacteriologic CSF exam.

CSF smear. *Main findings*: the CSF smear detects mycobacteria using acid-fast bacilli stains (Ziehl-Neelsen or the Kinyoun method) as well as fluorescence staining.

Advantages: rapid, inexpensive, simple, specific, low technical demand. *Disadvantages*: 1) Very low sensitivity of 13-53%; nevertheless, fluorescence microscopy and certain processing methods have been developed to improve the sensitivity; 2) The CSF smear

cannot distinguish between different species of mycobacteria ; moreover, it is difficult to detect nontuberculous mycobacteria; 3) The procesessing is prone to contamination with environmental or water-borne saprophitic mycobacteria; 4) Results depend on the CSF volume (a positive result requires >6ml of CSF) and on the number of samples (repeated analysis of lumbar punctures increases diagnostic yield); 5) False negative smear results in localized forms of cerebral TB.

CSF culture. *Main findings*: isolation and identification of mycobacteria on selective media (solid or liquid media).

Advantages: 1) Represents the definitive proof of active TB (gold standard). 2) Enables the performance of an antibiogram; 3) better sensitivity, than the CSF smear. *Disadvantages*: 1) Sensitivity depends on the number of samples, CSF volume and mycobacteria loading (CSF enrichment techniques could be used); 2) Large differences between media on efficiency and price; 3) Adequate laboratory infrastructure and trained personnel are necessary; 4) Detection requires minimum 14 days even on selective media; 5) Negative CSF culture in localized neurotuberculosis.

d. **Nucleic acid amplification tests (NAATs).** *Main findings*: detect mycobacteria nucleic acid in serum and CSF using the *PCR* assay.

Advantages: *NAAT*s exhibits a high specificity (88%-100%), good positive predictive value and rapid processing. Due to its specificity it could be used for treatment monitoring. *Disadvantages*: 1) *NAAT*s have a highly variable sensitivity and low negative predictive value, especially in smear-negative and extrapulmonary TB; the sensitivity of the *PCR* method (33% to 90%) is highly dependent on the mycobacteria loading and variable for each *PCR* technique; 2) *PCR* becomes rapidly negative after treatment; 3) In-house *NAAT*s produces highly variable results compared to commercial, standardized *NAAT*s. Nevertheless, commercial *NAAT*s show a potential role in confirming the diagnosis of TB meningitis, although their overall low sensitivity precludes the use of these tests to rule out the diagnosis with certainty; 4) The high price makes them prohibitive in poor countries with the highest prevalence of TB.

e. **The adenosine deaminase (ADA) detection**

Advantages: rapid; inexpensive; high diagnostic accuracy. *Disadvantages:* 1) Low specificity: it cannot rule out bacterial meningitis. 2) Not standardized.

f. **Histologic exam.** *Main findings*: reveals tissue mycobacteria and characteristic granulomatous aspects.

Advantages: 1) Very high specificity. 2) The main diagnosis method for localised cerebral TB. *Disadvantages:* 1) Invasive method; 2) Histology does not distinguish between mycobacteria or different granulomatous diseases; 4)Belated result (requires one or two days)

g. **Imaging methods.** *Main findings:* indicates complications of neurotuberculosis (hydrocephalus, vasculitis) and localized forms of neurotuberculosis. *Advantages*: improves the differential diagnosis and treatment evaluation. *Disadvantages*: 1) Images are not pathognomonic for TB; 2) High exposure to radiation; 3) Very expensive.

Conclusion: None of the current methods meets the criteria required by an efficient diagnostic test: rapid, accurate and readily applicable. Treatment in neurotuberculosis is therefore implemented according to the association of epidemiological, clinical criteria, bacteriologic CSF examination and different complementary laboratory methods dependent on the technical level of each laboratory. The decision on which tests to use should consider country-level technical facilities and other relevant factors, such as cost and availability. The lack of standardisation in the use of these methods renders their comparison more difficult.

3.2 Reason for the development of immunologic assays in neurotuberculosis

Unlike latent TB, neurotuberculosis is characterized by active CNS lesions accompanied by an intense cellular and humoral immune response. Both T cells and B cells are active during mycobacteria replication suggesting the potential use of immune markers in cerebral TB. Immune diagnostic tools also assessed antigens released by *M.tbc* in the CSF or specific antibodies. Beginning with 1990, numerous antigens and antibodies were screened for the diagnosis of neurotuberculosis in endemic areas. Studies on these methods proved a good specificity and simple, inexpensive and rapid results. Nevertheless the low sensitivity and the development of molecular techniques decreased the importance of these methods in neurotuberculosis diagnosis. The immunological diagnosis continues to be considered in TB endemic areas due to the increasing number of immunological biomarkers detection (Walzl et al, 2011). Over the past years, T cell-based IFNg release assays (IGRAs) performed on T cell isolated from the CSF, were found senzitive and specific for TB meningitis. IGRAs restored the interest in the immunological methods for TB diagnosis even in countries with high financial support. (Thomas et al, 2008).

3.3 The advantages and disadvantages of immunologic diagnostic methods in the diagnosis of neurotuberculosis

a. Determination of mycobacterial antigens or antimycobacterial antibodies in serum and CSF fluid. (Thomas et al, 2008; Patel et al, 2010*)*

Advantages: 1) Rapid , inexpensive, simple technique, minimal training requirements compared to molecular methods. 2) Specific detection of the intrathecal synthesis of antimycobacterial antibodies and of mycobacterial antigens in the CSF.

Disadvantages: 1) Highly inconsistent estimates of sensitivity and specificity correlated with the method used, detected type of antigen or antibody and mycobacteria load; however combinations of select antigens provide higher sensitivity compared to single antigens. 2) False negative results particularly in immunodepressed hosts; 3) Specificity can be affected by cross reactions between different mycobacteria or other bacterial species (*Nocardia, Leishmania*); 4) Some methods (*ELISA*) require specific equipment, skilled technicians and refrigerated reagents.

b. **IFN-γ release assays (IGRAs)** The two methods presently used are *QuantiFERON-Gold (QFT-G)* method and *T-SPOT.TB* method. Both can substitute the tuberculin skin test (*TST*) in routine clinical practice, especially where *BCG* vaccination is prevalent**.**

Avantages: rapid and specific. 2) Applied in few studies of neurotuberculosis with a sensitivity of 90% and specificity of 100% in cases of negative bacteriological TB meningitis.

Disadvantages: 1) Requires appropiate lab-facility; 2) Expensive; 3) T cell lymphopenia and anergy resulting from disseminated TB or advanced HIV infection, lead to the lack of *ELISPOT* responses. 4) False negative results in patients with atypical CSF (few lymphocytes). 5) False positive results in the case of blood contamination during puncture.

Conclusion. The immunological diagnosis in neurotuberculosis reflects the present knowledge related to the immune pathogenesis of TB. Addition of new immunologic data and a more complex interpretation of the results could improve the diagnostic value of these methods.

4. Imunologic response in NTB

TB is primarily a pulmonary disease. The protective immunity in infections with *M.tbc* is ensured by the macrophageal activity, CD4T cell response and Th1 cytokines. Consequently, the immune response frequently leads to the formation of granuloma, an organized and efficient form of defence. The granulomas include mycobacteria with a modified metabolism and potential of active replication which increases in case of immunodepression. The granulomas adjacent to the meninges or cerebral vessels increase the risk of CSF mycobacteria invasion. Mycobacteria reaching the subarachnoidial space release antigens and induce an intense inflammatory response. Since 1990, these antigens have been acknowledged as potential markers for the diagnosis of TB meningitis.

4.1 Mycobacterial antigens (general data, classification, role)

M.tbc encodes about 4000 proteins. As mycobacteria are extremely dynamic they release a variable number of antigens according to the clinical form of TB, the duration of the infection and the host immune response. The recognition of relevant mycobacterial antigens by T cell subsets is an essential step in triggering the protective immunity in TB. A functional classification of these antigens is presently unavailable, nor is the selection of antigens for the development of efficient serological diagnostic tools. Sequencing of the M.tbc genome in 1998 and the knowledge in proteogenomics led to the identification of numerous immunogenic antigens specific for *M. tbc* and *M .bovis*. These specific antigens differ from environmental mycobacterias or BCG vaccine strains and could be used in the immunologic TB diagnosis. However no specific antigen or set of antigens has been yet recognized in TB and no set of antigens has been established for diagnosis with confidence. The release of a certain antigen is induced by a large number of factors such as the type of infection (active or inactive), the host immune status (imunocompetent or immunodeppressed), local immune metabolism or ph conditions, mycobacteria viability (viable mycobacteria release other antigens than dormant bacilli), mycobacteria virulence and the infection site. The immunogenicity of these antigens also differs: only a small number of the antigens released in the culture media (25%) induced the synthesis of specific antibodies. (Samanich et al, 2000). The variability of released antigens under different conditions and their immunogenicity hinders an accurate selection and represents a serious obstacle in the immunodiagnostic development of TB. A cocktail combining a large number of antigens (10 to 12 recombinant antigens or poly-proteins) appears to be a reasonable choice for increasing both the sensitivity and the specificity of immunologic diagnostic methods (Raja et al, 2008). The classification and nomenclature of mycobacteria antigens is

not unitary. There are more names and classifications for one antigen depending on localization (cellular wall, cellular membrane and cytoplasm antigens) and structure (lipids, proteins, polysaccharides and their complexes). Proteic antigens are secreted in active lesions and have proved a great potential in the serological diagnosis, either alone or in poly-protein complexes (Houghton et al, 2002), while glycolipidic antigens are released in immunodepressed hosts especially. A detailed presentation of mycobacteria antigens belongs to Young (Yang et al, 1992). Several of these proteic antigens have already been included in diagnostic tests for neurotuberculosis: antigens of 38 kDa, 16 kDa, 88 kDa, MPT51, CFP-10, antigen 85B (associated to the protein 30-31 kDa), lipoarabinomannan (LAM), antigen A60, antigen 5, cord factor. The roles assigned to mycobacteria antigens are often contradictory and not fully understood. The documented activity of mycobacteria antigens is diverse: enhancing the immune response (antigen 85, P320, A60), triggering the delayed-type hypersensitivity (proteic antigens), interfering with the adhesion of mycobacteria on host cells, promoting phagosome-lysososme fusion. Certain antigens (LAM, 30kDa antigen, antigen 6) also induce cellular immunodepression by inhibiting various functions of the macrophages, or T Lymphocytes and assisting in the formation of granulomas.

4.2 Antimycobacterial antibodies (general data, relevance, dynamics)

M. tbc is resistant to antimycobacterial antibodies. Stimulation of the humoral immunity using polysaccharide conjugated vaccines has not been efficient and antimycobacterial antibodies are not considered protective (Glatman-Freedman et al, 2000). Nevertheless their presence could influence the immune response. Thus the presence of B lymphocytes surrounding granulomas suggests the involvement of humoral immunity in latent forms of TB .The appearance of plasmocytes in the CSF also suggests a potential role of specific antibodies in TB meningitis. Numerous studies have attempted to identify specific antibodies in active TB but the present data is incomplete. The antigenic variability of mycobacteria is mirrored by the heterogenity of the released antibiodies. The repertoir of released antibodies appears to be diverse and correlated to many factors (the lesion's evolution and localization, the immune status etc) (Davidow et al, 2005). As in the case of antigens, the type of the released antibodies in different hosts and different forms of TB is unpredictable. Thus anti 38kDa are elevated in pulmonary TB while LAM antibodies and anti 16 kda increase in the CSF of patients with TB meningitis; anti 38 kDa have been associated with a poor outcome, while anti 19kda suggested a good prognosis. Anti38kDa are released in the presence of cavitary lesions and have not been found in patients who do not present such lesions such as immunodeppresed hosts. Anti 85 complex are present in large quantities in disease forms confirmed by positive smear as well as in severe forms. By comparison, lower amounts were found in forms with negative smears or minimal lesions. (Wiker & Harboe, 1992). Antibody responses are correlated with the bacillary load. Under these circumstances the level of IgG was regarded by some authors as an index for the antigenic load with possible implications in treatment follow-up (Fadda et al, 1992). The present knowledge on the role of antimycobacterial antibodies is the result of numerous immunologic diagnostic studies but their implication in the TB pathogenesis is still little known. IgM antibodies were found in different groups of patients with TB including vaccinated patients and are frequently found in HIV patients. Their presence marks the colonization with mycobacteria or the risk of TB relapse. IgA antibodies are rarely found.

They are detected in the CSF, pericardial and pleural liquid. They could appear in a state of anergy or in the absence of IgG antibodies, sometimes indicating an aberrant immune response Th2 like. Immune complexes are frequently correlated with IgA antibodies and severe disease. A protective effect of IgA of short duration has been observed in the early stages of TB. IgG antibodies appear during chronic disease; high titres are maintained even during treatment. These are the main type of antibodies observed in neurotuberculosis (Maes, 1991).

The dynamic synthesis of antimycobacterial antibodies

Different stimuli were documented to trigger the antimycobacterial antibodies in the primary versus post primary infection or active versus inactive TB. The hummoral immune response following BCG vaccination indicates that IgM anti PPD increase progressively until the third month, followed by an increase of IgG. The antibody dynamics in TB infection could be similar (Maes et al, 1989), but it also depends on the clinical form and treatment starting. IgA serum antibodies appear immediately after IgM and precede IgG, but the CSF dynamics could vary. IgG antibodies are prevalent in both active and inactive cases (Kaplan & Chase, 1980). During treatment for primary tuberculosis antimycobacteria antibodies are present in low titres, exhibit a slow increase and are directed against a low number of antigens. (Kaplan & Chase, 1980). Only 11-46% of patients display an initial titre of antibodies, but the number of cases subsequently increases to 60%. In relapse forms of TB the serum reacts with a larger number of antigens, the IgG titre is higher and rapidly increases under treatment. A detectable IgG titre is initially recorded in 66% of patients but reaches 75-100% of patients in the later stages. Similar conclusions were published in other studies in which TB relapse forms produced a positive serology in 100% of patients compared to 11% in primary TB (Julian et al, 1997). Seroconversion under treatment in patients with TB exceeds 3-8 weeks in patients with a negative titre at the onset, while in those with positive onset titres the antibody increase is rapid and immediate. The titre of antimycobacterial antibodies in the serum and CSF is higher in treated compared to untreated patients (Kaplan & Chase 1980). Some authors consider that in primary TB the maximum titre could emerge after 3 months of treatment. The presence of antimycobacterial antibodies in large quantities in relapse as well as in the treated forms is a significant advantage in the serodiagnosis of neurotuberculosis (as the latter frequently occurs as a relapse). The main disadvantage is related to the slow antibody dynamics in primary infections.

4.3 The intrathecal synthesis of antimycobacterial antibodies in NTB

CNS dissemination of mycobacteria is assisted by alveolar macrophages and their interaction with epithelial cells. Although the hematomeningeal barrier ensures the CNS protection from systemic immune reactions, the nevrax is still the site of intense inflammatory reactions. The intracerebral immune reactions are supported by the phagocytic cells of CNS, such as astrocytes and endothelial cells. (de Micco & Toga, 1988) The complex of astrocytes and endothelial cells is also connected to microglial cells, oligodendrocytes and hematopoetic stem cells. All these cells could activate lymphocytes and release inflammatory cytokines. Moreover, the lymphocytes crossing the hematomeningeal barrier synthesize intrathecal immunoglobulins (Ig). The intrathecal synthesis of specific antibodies was first observed in neurotuberculosis by Kinman. He showed that the stimulation of CSF lymphocytes by PPD leads to their intense proliferation

(Kinnman et al, 1981). Subsequent studies revealed a local production of antimycobacterial antibodies anti PPD in the CSF (Kalish et al, 1983). In 1990, Sindic proved the presence of IgG in the CSF against the antigen of *M. tbc* H37Ra and A60 (Sindic et al, 1990) In this study antimycobacterial antibodies appeared in the subarachnoid space as early as 15 days after clinical onset and persisted up to 69 months. The delayed immune response after the disappearance of the antigenic stimulus could persist as a result of immune disorders of active B cells clones. A similar response was also observed in other nontuberculous meningoencephalitis even 8 years later. IgG generally dominate the CNS humoral immune response, but in some cases IgA are also present (Felgenhauer & Schädlich, 1987). The antimycobacterial antibodies synthesis could be detected and quantified in the CSF, resulting in titres that could be higher in the CSF compared to the serum. Furthermore the increase of the Ig index in the CSF is a proof of the local synthesis of antimycobacterial antibodies (Kinman et al, 1981). The intrathecal synthesis of antimycobacterial antibodies released in the CSF is specific for cerebral TB and thus represents a solid argument in favor of the neurotuberculosis diagnosis.

5. Immunologic methods used in the diagnosis of neurotuberculosis

Immunological assays are rapid and easy to process but their use in TB is disputed due to contradictory results on sensitivity. Nevertheless, immunologic methods could be considered as complementary diagnostic tools especially in poor areas and smear-negative CSF neurotuberculosis. The most commonly used serological assay in neurotuberculosis is enzyme-linked immunosorbent assay (ELISA). Rapid methods (such as immunodot) are only seldom used. ELISA is automated and able to simultaneously analyse a high number of samples and different antibodies (Ig G, IgM or IgA). The evaluation of serological assays requires the accuracy of the following parameters: the sensitivity (probability of a positive test in people with neurotuberculosis), the specificity (probability of a negative test in uninfected people), the positive predictive value (probability that the person is infected when the test is positive) and the negative predictive value (probability that the person is uninfected when the test is negative).

5.1 The enzyme-immuno-assay (ELISA) technique

ELISA technique was first introduced in the serological diagnosis of TB in 1976. It detects the antigen/antibody-enzyme linked complexes, with the antigen affixed to a solid adsorbent surface. Despite its highly variable sensitivity and specificity, studies on pulmonary TB proved that it is rapid and simple to process, suggesting its use in the extrapulmonary TB diagnosis. There are several comercial ELISA kits (Anda Biological using antigen A60, Omerga Pathozyme TB using antigen 38 kDa, Pathozyme TB complex with antigen 38 kDa and antigen 16 kDa, Pathozyme Myco with antigen 38 kDa and lypopolissaharidic antigen), and various in-house antibody-ELISA detection tests. Numerous ELISA studies were performed for the detection of different antigens and antibodies released in the pulmonary and extrapulmonary TB. Daniel and Debanne published a reference study in 1987 (Daniel & Debanne, 1987) analysing ELISA results in different forms of TB. They concluded that ELISA sensitivity of 25-100% and specificity of 76-100% were highly variable depending on the used antigen. A subsequent systematic revue on ELISA studies also revealed an extremely variable sensitivity of 0-100% and

specificity of 59-100% in pulmonary TB and modest results in TB meningitis (sensitivity of 48% and specificity of 82%) (Steingart et al, 2007).

5.2 Interferon-γ-release assays (IGRAs)

There are currently 2 methods measuring the IFN-γ released by sensitized T cells: QuantiFERON-Gold *In Tube* (Cellestis Ltd) which measures IFN-γ-released in whole blood following *ex vivo* stimulation with ESAT-6, CFP-10 and TB 7.7 antigens and the T-SPOT.TB method (Oxford Immunotec Ltd) which measures IFN-γ-released by peripheral blood mononuclear cells following *ex vivo* stimulation with ESAT-6 and CFP-10. These 2 methods were initially approved for the diagnosis of latent TB, displaying a higher efficiency compared to tuberculin skin tests (TST). Their use was later extended for the diagnosis of active TB including HIV-infected patients. Recent studies on TB meningitis using both methods revealed only modest results. (Thomas et al, 2008, Patel et al, 2010). Thus the specificity in the diagnosis of active TB was generally low (59-79%) while the sensitivity was between 79% (non HIV patients) and 64% (HIV patients) (Sester et al, 2011). Nevertheless more optimistic results in TB meningitis were also reported (Murakami et al, 2008).

5.3 Other techniques used in the immune diagnosis of neurotuberculosis

The immunoblot technique is based on the electrophoretic separation of proteins (antigens or antibodies from serum or CSF), blotting for separate fractions on nitrocelulosis and identification of each proteic fraction with a specific anti-serum conjugated with a complex of streptavidin-biotin preoxidase (calitative reaction). The immunoblot technique was previously used by several authors in the diagnosis of mycobacterial infections. Most antigens recorded in these studies weighted between 30-45 kDa. However, only a part of the serum antigens identified in pulmonary TB were also recorded in the CSF in TB meningitis. In a study by Mathai the patients with TB meningitis presented 27 kDa, 30kDa, 45 kDa and 5kDa *M.tbc* antigens (Mathai et al, 1994). The latter was found in another study in 70% of patients with TB meningitis (Mathai et al, 1991). Patil also identified 30-40kDa antigens in the CSF but lower amounts of 14kDa and 18-25kDa antigens (Patil et al, 1996). Katti identified mostly 30-32kDa and 71kDa antigens in the CSF of patients with TB meningitis (Katti, 2001). Literature data also reveals the predominance of 30kDa antigen in the CSF. Research proved that 30 kDa protein is also a member of the Ag 85 complex (Ag 85 A and Ag 85 B), an antigen frequently detected in TB meningitis. This emphasizes the importance of 30 kDa antigen as a CSF marker in various forms of TB meningitis. **ImmunoDOT** is an rapid-test format, dipstick ELISA that allows patients to be tested for multiple parameters simultaneously. Up to five or six different tests may be completed simultaneously on a single sample, making it ideal for users who require fast, and reliable diagnostic tests in a single patient format. ImmunoDot or other rapid techniques are suitable for low CSF volumes and limited resources laboratories. **Haemagglutination assays** such as reverse passive haemagglutination (RPHA) using polyclonal or monoclonal antibodies, could be used in the serum and CSF, with a variable sensitivity in the diagnosis of TB meningitis. (Venkatesh et al, 2007). The use of a polyclonal serum generally proved more efficient. The main limitation of this method was connected to the variable sensitivity (50-94%) and to the short shelf-life of red-cell labelled antibodies; both listed drawbacks could be amended (Katti, 2001). The method is interesting from the perspective of using a single sample for the

detection of both antibodies and antigens in biologic products and for measuring antigens and antibody dilutions needed in treatment monitoring. **Radioimmunoassay (RIA)** has been used for the TB diagnosis since 1987, with variable results. The method could follow the antigens level in the CSF during treatment which enables its use in treatment monitoring (Kadival et al, 1987). In conclusion, neurotuberculosis presently employs a wide range ofimmunologic diagnostic techniques. Their results however are not influenced by the method as much as by the detected antigens or antibodies. Presented below is the diagnostic value of the main antigens and antibodies detected in the serum and CSF in patients with neurotuberculosis.

6. Diagnostic value of immunological assays in studies of adults with neurotuberculosis

6.1 Mycobacterial antigens detection in neurotuberculosis

The mycobacterial antigens detection in CSF samples evidence active meningeal infection and could hold a diagnostic value. Hence, several *M.tbc* antigens have been analyzed as potential markers for the TB menigitis diagnosis. Moreover, quantitative determination of antigens could be useful for treatment monitoring. Attempts to detect mycobacterial antigens in neurotuberculosis have been made since 1984 employing mostly agglutination tests, *ELISA* with different variants or other methods (Venkatesh et al, 2007; Sumi et al, 2002; Radhacrishnan et al, 1990; Katti, 2001; Kashyap et al, 2009). Numerous antigens were evaluated: 55-kDa antigen, 14 kDa, PPD, 62 kDa, ag 85 complex , 30-32 kDa protein, LAM, Ag Mtbc, *M. tbc* H37Rv**.** The main conclusions of these studies are listed below.

a. **Method efficiency.** The specificity of methods: 69-100%. The sensitivity variation depended on the diagnosis criteria: in bacteriologically confirmed TB meningitis 79-100%; 67-92% in unconfirmed cases and 50-100% in both confirmed and unconfirmed cases.
b. **Comments**. The evaluated antigens were extremely different for each study. The sensitivity of methods was highly variable too. No method or specific antigen rendered a higher efficiency, although more studies have confirmed the presence of 30Kda antigen (ag 85 complex) in the CSF and simultaneous use of more antigens improved the methods sensitivity. The highly variable results could derive from the reduced level of antigen in the CSF, as well as from the lack of complete information connected with the precise type of antigens released in the CSF in TB meningitis.

6.2 Antimycobacterial antibodies detection in neurotuberculosis

Irrespective of localisation, antigentic stimulation in mycobacteria infections induces the synthesis of specific antibodies in the serum. In neurological localizations however, antibodies also appear in the CSF as a result of intratechal synthesis (Sindic et al, 1990; Kinmann et al, 1981; Kalish et al, 1983) and active secretion by choroidal plexuses. False positive antibodies in the CSF are possible in areas endemic for TB, where healthy persons or patients with pulmonary TB present high serum levels of antimycobacterial antibodies able to difusse from the serum to the CSF. The assesment of the intratechal synthesis of antimycobacterial antibodies could help ascertain such false positive reactions. Most studies analyzing the presence of the antimycobacterial antibodies in CSF were performed in

imunocompetent adults with TB meningitis. The detected antibodies involved more antigens either isolated or simultaneous (LAM, PPD, A60, M.tbc, Ag 5, 14kDa, 19 kDa, 27 kDa, 30 kDa, 35 kDa, 40 kDa, Ag H37Ra, LAM, 30 kDa, 65 kDa heat shock protein, ESAT-6 antigen). In addition, most authors focused on the detection of IgG type antibodies using ELISA. Below are the main findings of these studies (Thakur & Mandal, 1996; Maheshwari et al, 2000; Patil1et al, 1996; Kashyap et al 2009; Mudaliar et al, 2006).

a. **Method's efficiency**. The specificity of antimycobacterial antibodies detection ranged between 92-100% but the positive predictive value was high. False positive results were recorded in patients who also presented pulmonary TB or as a result of cross-reactivity; 65 antigen kDa belonging to stress proteins was most frequently involved in cross reactions with other bacteria. The sensitivity of methods was highly variable: 30-100% in the studies with a single tested antigen and 61-100% when testing multiple antigens Chandramuki et al, 1989; Mathai et al, 1990a). The sensitivity was correlated with diagnosis criteria as follows: 80-90% sensitivity in bacteriologically confirmed tuberculous meningitis, 30-62% sensitivity in unconfirmed cases, 58% sensitivity in histologically confirmed tuberculomas and 70-87% sensitivity in clinically TB meningitis. Large differences between studies were also noticed depending on antibodies detection (for *LAM*, the sensitivity varied between 58-85% and for A60, the sensitivity ranged from 38 to 100%).

b. **Comments.** Almost all studies were perfomed in patients with TB meningitis. Nevertheless, a study on tuberculoma revealed A60 antibodies in these patients too. This indicates the potential use of the serodiagnosis in localized forms of neurotuberculosis, in which noninvasive diagnotic tools are presently unavailable. The variability of the recorded results was connected with more factors: 1) the use of different antigens (several studies indicate *LAM* antigen as immunodominant in the CSF; 2) the use of different dilutions: higher dilutions of the CSF decreased the sensitivity; 3) the immune status of different patients: low antibody detection in immunodepressed patients (only 10%) compared to immunocompetent hosts (50%); 4) almost all studies analyzed IgG antibodies, while only few also considered IgM or IgA. The antigens which induced IgM antimycobacterial antibodies were different from those responsible for IgG. IgG antibodies were mostly directed against *LAM* or 14 kDa antigen (74% of the total patients), suggesting that using these antigens could raise the efficiency. 5) some studies disclosed that simultaneous detection of serum and CSF antimycobacterial antibodies improved the CSF results compared to serum detection only. (Srivastava et al, 1994); however other studies reported inferior CSF sensitivity compared to the serum sensitivity.(Ghoshal et al, 2003). 6) the sensitivity of antibodies detection increased during treatment with 18% for both IgG and IgA detection.

Conclusion. There is a large number of serological studies dedicated to neurotuberculosis, with extremely different results. They are hard to compare as a result of using different study protocols and different antibodies detection. Despite the unconfindence which these studies arose, serologic diagnosis is widely used in poor countries. The standardisation of the methods in what regards the recommended antigens (type, concentration) and the immunological assay, could help improve the immunological diagnosis. One advantage of this method is the CSF specificity of antigens and intrathecal antimycobacterial antibodies

synthesis and also the increased antibody level during antituberculous treatment, which renders this method useful for a retrospective diagnosis.

6.3 Intrathecal synthesis of antimycobacterial antibodies in neurotuberculosis

TB meningitis prompts a vigorous humoral local response proved by CSF intrathecal synthesis of antimycobacterial antibodies. Sindic proved that most antibodies synthesized intrathecally are directed against antigen A60 (Sindic et al, 1990). He also revealed the presence of Ig in the subarahnoidian space after 14-27 days of disease and their persistence after several years. A higher Ig G index is presently regarded as a strong argument in favor of the humoral local response induced by mycobacteria. All the same not all Ig detected in the CSF belong to antimycobacterial antibodies. Unspecific IgG were also found in other diseases affecting the CNS as a results of an immunosuppresive abberation. This immune impairment allows B cell multiplication and antibody persistence accounting for false positive results. Fals positive CSF results could also be found after passive transfer of antimycobacterial antibodies from the serum in the subarachnoid space. The mathematical evaluation of the intrathecal synthesis takes into account the presence of antibodies and IgG level in serum and CSF could highlight these false positive results.

6.4 Personal contribution. The intrathecal synthesis of antimycobacterial antibodies in patients with TB meningitis

The sole presence of antimycobacterial antibodies in the CSF cannot define the local inflammatory response since any form of TB accompanied by high titres of antibodies could promote their passive transfer in the CSF. The intrathecal synthesis was instead acknowledged as a typical finding in neurotuberculosis and an alternative for improving the specificity and positive predictive value of diagnosis in TB meningitis.The aim of our study was to evaluate the presence of antimycobacterial antibodies released as a result of the intrathecal synthesis in TB meningitis and to differentiate them from unspecific Ig.

Subjects. The study was performed on 21 adult patients with TB meningitis. A total of 34 CSF samples were collected, all having proved positive for antimycobacterial antibodies. The samples were collected after starting the antituberculous treatment, between 3 and 140 days after the clinical onset of the disease. Diagnosis criteria of TB meningitis were based on clinical characteristic features, CSF characteristic features (CSF lymphocytosis, increased protein and decreased sugar), evidence of pulmonary or extrapulmonary TB and positivity of CSF Lowenstein culture (for 3 patients only). Antimycobacterial antibodies were detected through ELISA using glycolipidic (GL) antigens and A60antigen.

Methods. Two types of comparative antigens were used for ELISA: GL antigen (0,1 ml sol of 10micrograms/ml of purified glycolipid in hexan obtained from Cantacuzino laboratories, Romania) and A60 (Andaelisa mycobacteria IgG, IgM, IgA kits, from Anda Biologicals, Strasbourg, France).

a. Elisa anti GL antigen detection method steps: 0,1 ml of ½ diluted CSF and 0,1 ml of conjugate (protein A–peroxidase) were distributed in microplates at 37^0 C for 1 hour; 0,1 ml *OPD* (peroxide and ortophenylendiamine) substrate in citric acid-sodium citrate buffer, 0,1 M, pH =5, was used. The reaction was prolonged for 30 minutes, at 37^0C, at

dark. The optical densities (OD) were read with a Multiscan MCC Reader, at 450 nm. Serum Ig isolated from immunised rabbits with *Mtbc* suspension were used as positive controls. Cut off was established at two standard deviations above the level considered normal. The level of antibodies was expressed in international units (UI). The baseline serounit for the CSF fluid was 0 UI.

b. Elisa antiA60 detection steps: the CSF fluid was diluted 1/10 according to the kit instructions; anti-human IgG, IgA, IgM were conjugated to peroxidase (one hour at 37^0C). The baseline serounits were: 125 for IgG, 200 for IgA serounits and 0.8 for IgM antibodies. Supplementary analysis in all subjects included: serum and CSF albumin expressed in mg/ml) (Ortodiagnosis) and serum and CSF Ig (radial passive immunodifussion). Albumin index, IgG index and IgG intratechal synthesis were calculated using the following mathematical formula (Tibbling 1977):

1. **Albumin index** (mg/ml)= CSF albumin/ serum albumin ratio. The normal value of CSF albumin was considered < 35mg% and between 3500-5000 mg% for serum albumin. The normal value of the *albumin index* is < $7x10^{-3}$ (0.007). Higher values suggest the permeability of the hematomeningeal barrier and the possibility of a passive transfer of antimycobacterial antibodies from the blood to the CSF.

2. **IgG index** (mg/ml)= (CSF IgG/serum IgG) / (CSF albumin /serum albumin) ratio.The normal value of the IgG index is ≤0.7. Higher values indicate increased IgG synthesis.

3. **IgG intratechal synthesis** of antimycobacterial antibodies was acquired using the formula: (CSF antimycobacterial antibodies of IgG type/CSF IgG) / (serum antimycobacterial antibodies of IgG type/serum IgG) ratio. Antimycobacterial antibodies of IgG type were measured in *ELISA* units. Serum and CSF IgG were quantified in mg/ml. The normal value of the intratechal synthesis calculated using this formula is < 1.

Results. The intrathecal synthesis was present 3 days after clinical onset and persisted up to 140 days after onset. The intrathecal synthesis against GL antigen was assessed in 31 samples while anti A60 antibodies were assessed only in 19 samples, out of the total of 34 CSF samples. 19 CSF samples were studied comparatively for the intrathecal synthesis of anti GL antibodies and antiA60: anti GL antibodies were detected in 7 samples only (22,58%) and anti A60 in 15 patients (78,94%).Eighteen from the 19 CSF samples (94,73%) presented the intrathecal synthesis of either anti GL antibodies or anti A60.Results are displayed in table 1.

Antimycobacterial antibodies of IgG type	Total CSF samples	CSF samples positive for the intrathecal antibody synthesis	CSF samples positive for a high albumin index	CSF samples positive for **a high** IgG index
GL-IgG antibodies	31	7 from 31 samples (22,58%)	33 from 34 samples (97,05%)	24 from 34 samples (70,58%)
A60-IgG antibodies	19	15 from 19 (78,94%)		
GL and A60 IgG antibodies	19	18 from 19 (94,73%)		

Table 1. Results of the albumin index, IgG index and the intrathecal synthesis of antimycobacterial anti GL and A60 in patients with TB meningitis

Observations. High values of the IgG index were recorded in 70, 58% cases and high values of the albumin index suggesting an increased permeability of the hematomeningeal barrier were noticed in 97,05% of patients. Only a part of the patients positive for CSF anti GL or A60 antibodies also exhibited an intrathecal synthesis. This finding confirms the transfer of antibodies from the serum into the CSF, through the hematomenigeal barrier. We also observed a significantly lower anti GL intrathecal synthesis compared to anti A60. In our study the simultaneous detection of intrathecal synthesis increased the CSF detection of antimycobacterial antibodies.

Conclusions. This study recorded the intrathecal synthesis of at least one type of antimycobacterial antibodies (GL or A60) in 94,73% of the TB meningitis patients even after specific treatment starting. The current study is also one of the few confirming the intrathecal synthesis of antimycobacterial antibodies in TB meningitis. Our findings support the value of the immunologic diagnosis, be it retrospective, after specific treatment starting.

7. Diagnostic value of immunological assays in children with neurotuberculosis

TB meningitis in children develops after hematogenic dissemination from the pulmonary infection site. Thus it manifests as a progressive primary disease, while in adults it commonly arises as a relapsing form of TB. The neurologic signs and symptoms become obvious 2 weeks after the onset. CSF changes are frequently unspecific and cases with a normal CSF examination have also been described in TB encephalopathy. The first obstacle in the diagnosis lies in obtaining adequate CSF and sputum samples. Moreover the bacteriological diagnosis in TB meningitis is disappointing: acid fast bacilli stain is positive in only 15% of cases and culture in only 30% of cases. The prognosis is poor and a rapid diagnosis is imperious. Most treatment regimens in children TB meningitis are initiated based on clinical, epidemiological data and pulmonary radiological features, without a bacteriological confirmation. The most adequate methods in the neurotuberculosis diagnosis in children are the molecular techniques (Lawn & Zumla, 2011). These are rapid and specific, but the high cost prevents their use on a wider scale in poor countries. At the same time, some countries are reluctant to employ molecular techniques in the TB diagnosis in children (Consensus Statement on Childhood Tuberculosis, 2010). Therefore the serological methods could be an alternative, but there are few studies on children and their efficiency is variable. The lack of knowledge related to the type of antigen released in this age group causes highly unspecific results. The serologic diagnosis is based on antigens randomly chosen, considered as immunodominant in adult TB (Raja et al, 2001). However certain results yielded by serologic techniques deserve credit for having revealed additional information on the immune pathogenesis of TB in children. According to these the titre of antimycobacterial antibodies and TST in children under 2 years are influenced by BCG vaccination which prompts persistent titres of antimycobacterial antibodies. Children between 0-4 years display a decreased humoral immune response despite a strong cellular immune response. (Seth et al, 1993). Subsequently the titre of antimycobacterial antibodies increases with age (Delacourt et al, 1993). Children with primary lesions of TB or calcified lesions also present increased titres of serum antibodies. As a result there is a high risk of false positive reactions in TB endemic areas. Thus the serologic diagnosis of active TB is not regarded as a plausible alternative for diagnosis in children. In addition, the serologic

results in children with pulmonary and extrapulmonary TB exhibit an extremely varied sensitivity (20.7%-85%) (Rosen, 1990, Alde et al, 1989). Results with a better sensitivity were retrieved for A60, antigen 85 complex, 30 kDa or combinations of multiple antigens (Delacourt et al, 1993, Dayal et al, 2008, Raja et al, 2001). The specificity depends on the antigen used or the type of detected antibodies (Raja, 2001, Delacourt et al, 1993). There are also studies suggesting a correlation between the titre of antibodies and the antituberculous treatment. This implies the monitoring of antibodies titre in the pediatric management of TB. (Sireci et al, 2007). However the above mentioned results are considered of little practical value and irrelevant for the current diagnostic methods in TB. Other immunologic assays such as IGRAs although unrelated to the BCG vaccination and with a high specificity appear not to be advantageous in all cases of active TB in children. (Kampmann et al, 2009). There are few serologic studies in children with TB meningitis which displayed a high variable sensitivity. A comparative analysis on these studies is hindered by the diverse antigens used and discordant results (Dole et al, 1989; Srivastava et al, 1998) (table 9). Serious errors of interpretation are important for the above mentioned reasons.

The efficiency of serologic techniques in children with neurotuberculosis is low: sensitivity of 30%-100% and specificity of 62-96%. The sensitivity was influenced by the CSF dilution, the type of detected antibody and the chosen antigen. The sensitivity was similar for the serum and CSF.

Comments. ELISA was the technique most commonly used. Best results involved antimycobacterial antibodies A60, 30 Kda and *M. bovis* BCG towards Ag 5 and LAM. Some studies considered the detection of IgM more relevant than IgG. The detection of antigens in the CSF proved more useful compared to the finding of antibodies in TB meningitis.

8. Diagnostic value of immunological assays in studies of HIV patients with neurotuberculosis

The neurotuberculosis diagnosis in HIV infected patients is probably overevaluated. The TB patients with HIV often present miliaria forms, extrapulmonary localisations of TB and extensive vasculisis, accompanied by specific HIV manifestations. The difficulty to recognize the neurologic forms of TB increases with the advancing immunodepression. The low inflammatory response in HIV patients generates an atypical CSF aspect and repeated confusions with meningitis of other aetiology. At the same time, the TB aetiology should always be included in the differential diagnosis due to its frequency in the evolution of the HIV infection. TB meningitis usually appears as a relapse and only rarely during primary infection. Bacteriological confirmation is delayed and its sensitivity is under dispute: some authors recorded a higher number of false negative smear sample results while others maintained that the sensitivity is similar in both HIV and non-HIV infected patients. Therefore in order to raise the cases diagnosed one should consider the addition of invasive investigations and high cost molecular techniques. The immunologic methods of diagnosis in immunodepressed hosts have been little evaluated. HIV patients display a decreasing Th1 count, sustaining the Th2 stimulation of B cells and the synthesis of antimycobacterial antibodies. Specific tests of cell immunity (IGRAs, TST) are disappointing (Pai & Lewinsohn, 2005) but the humoral immune response appears to persist for a long time. An advantage of the serologic diagnosis is that of the high titres of antimycobacterial antibodies in relapse forms which comprise the majority of TB forms in HIV patients. Certain authors observed

an increased IgG in the serum of HIV patients with pulmonary TB compared to controls (Van Vooren et al,1994). No difference has been observed between pulmonary TB and extrapulmonary TB in what regards humoral immunity (Daleine et al, 1995). The essential difference resides in the released antigens. A serologic study in HIV patients with TB infection concluded that only 14 antigens induced the antibody synthesis in these patients. Interestingly, most antigens between 32-45KDa (except for 38 Kda) failed to elicit a humoral reponse (Zhou et al, 1996). The best results involved glycolipidic antigens , especially LOS, LAM, DAT, PGL (Patel et al, 2009) and high molecular weight proteic antigens (ag 85Kda, 88Kda) (Laal et al, 1997). LAM positivity has been associated with HIV co-infection and low CD4 T cell count. Below are the conclusions of ten studies performed in patients with HIV and TB infection using Elisa and numerous antigens (LOS, LAM , 88kDa , PPD , 38 kDa, 88 kDa, A60 or antigen combinations such as PPD and DAT, PPD and SLIV or PPD and LAM.

Main findings. Various studies revealed an extremely variable sensitivity (0-95%), not only for different antigens included in the same study, but also for the same antigen in different studies (such as the high variable sensitivity for LAM in different studies: 35-95%). Only the selection of LOS antigen has led to concordant results between studies. Despite the dysfunctional cellular immune responses in HIV patients, several investigators have reported the presence of antibodies against *M. tbc,* TB16.3, TB9.7, MPT 51, MTB 81 and 88 kDa antigens (Laal et al, 1997). Comparative studies also revealed that the sensitivity for certain antigens (PPD, *M.tbc*) doubles in patients who are HIV negative compared to HIV positive. Moreover the sensitivity is higher in HIV patients compared to AIDS patients. However the level of anti A60 IgG remains elevated in patients with AIDS or with a negative TST result. Despite the low sensitivity the specificity of the Mycodot test was high.

Comments. Secondary TB elicits a stronger serologic response than primary TB and could account for the differences between the different groups of patients (Maes, 1991). However none of the mentioned studies classified the patients as primary or secondary TB. On the other hand an attempt to classify according to the bacteriological confirmation revealed a higher detection sensitivity for antimycobacterial antibodies in patients with confirmed TB (57%) than in patients with unconfirmed TB (30%) (Kameswaran et al, 2002). Antimycobacterial antibodies could predict tuberculosis in HIV patients in some studies. A study by Amicosante proved the appearance of antimycobacterial antibodies one year before the clinical onset of tuberculosis in 67% of HIV infected patients. (Amicosante et al, 1994). The early prediction and diagnosis of neurotuberculosis rendered by antibodies are extremely important for an early starting of antituberculous chemotherapy. Unfortunately only few HIV patients with neurotuberculosis are recorded in the evaluation of extrapulmonary TB and it is difficult to estimate the real value of serologic methods.

9. Diagnostic value of immunological assays in patients with neurologic nonmycobacterial infections

Nontuberculous mycobacteria infections with neurologic or systemic localizations exhibit a low titre of IgG (Oliver et al, 2001). In addition the presence of corresponding antibodies in HIV patients suggests colonization (usually involving the digestive tract) rather than infection (Maes 1991). Thus studies on patients with AIDS and disseminated infection with

M.avium failed to detect any IgG antimycobacterial antibodies in the serum, unlike patients with AIDS and pulmonary infection with *M.avium* (Daniel et al, 1990). A study on the IgG, IgM, IgA released against antigen LAM has triggered no immune response in mycobacteria infections other than TB (Demkow et al, 2006). There are also studies upholding the possibility of a serologic diagnosis in HIV patients with nontuberculous mycobacteria infection using antigens extracted from PPD-B /M. intracellulare, PPD-Y/M. kansasii, PPD-F/M. fortuitum) Nevertheless the serologic diagnosis is presently impractical in the case of nontuberculous mycobacteria infections and there are no available studies regarding HIV patients with neurotuberculosis.

10. The interpretation of serologic results in the diagnosis of neurotuberculosis. Reasons for false positive and negative results

Immunoenzymatic techniques are the most frequently used techniques for the detection of antimycobacterial antibodies in the CSF. The detection of CSF and serum antimycobacterial antibodies through such methods is specific, inexpensive, rapid, but displays a moderate sensitivity and requires a correct interpretation of the data.

Reasons for false negative results. Most false negative results are related to the low level of antimycobacterial antibodies in the serum or CSF, along with inadequate technical parameters. The low titre of antibodies could be the consequence of: a) decreased antigenic stimulation induced by reduced metabolism of mycobacteria; b) reduced antibodies synthesis as a result of a congenital or acquired immunodeficiency; c) antigen fixation in the immune complexes; d) early detection. The use of inadequate technical parameters correlates with: a) a cut off too highly set; b) too high dilutions (the current CSF dilutions of 1/10 appear to exceed the titre of antibodies); c) belated CSF processing; d) improper storing conditions.

Reasons for false positive results in the CSF: a) excessive amount of serum antibodies able to cross the blood barier (a finding usually related to pulmonary TB); b) cross reaction with other infections (Nocardia, Corynebacterium) , the rheumatoid factor, or other diseases (sarcoidosis, pulmonary neoplasm, autoimmune diseases); c) immune hiperreactivity unable to suppress the antibody synthesis after the disappearance of the stimulus; f) insufficient purification of the antigens; g) high prevalence of TB and a low cut-off.

11. Improvement possibilities of serologic techniques in neurotuberculosis

The improvement of immunologic results in neurotuberculosis diagnosis is first related to the knowledge advance of TB immunopathology. The current improvement strategies focus on the following: a) The discovery of highly specific antigens; b) Polyproteins or multiple antigens concurrent detection; c) Cut-off value adapted to the geographic area TB endemicity and to the CSF sample (the predicted cut off value for the CSF is of 40 serounits of IgG instead of 200 serounits required by pulmonary TB); d) The removal of immune complexes; e) Repeated serologic detection after onset and during treatment; f) Simultaneous screening for antibodies IgM, IgG, IgA; g) Simultaneous detection of antibodies and antigenes using rapid techniques; h)The use of low dilutions for the immunosuppressed patients); i) Intrathecal synthesis detection; j) Correlation with

immunologic markers (the CD4 level, immunogram) for a correct interpretation of the immune status.

12. Conclusions

a.   The value and limits of the immunologic diagnosis in NTB

The detection of mycobacteria antigens and antimycobacterial antibodies in the CSF, as well as in the serum of patients with neurotuberculosis could augment the diagnosis suspicion and ensure a rapid treatment. Increasing the efficiency of the immunologic diagnosis in neurotuberculosis requires antigens that are specific for neurological localizations as well as standardized and sensitive methods. Immunoserological studies on neurotuberculosis generally involve methods with a good specificity and acceptable senzitivity. The rapidity and increased specificity are the main advantages of these methods. Still, these diagnostic methods cannot replace the bacteriological exam and are presently unstandardised, which hinders the results comparison. In addition, the correct interpretation requires complex data (not always available) related to the onset, treatment, medical history, other localizations of tuberculosis and the immunologic status. In the absence of these data, the results obtained through serological methods cannot be relevant.

b.   The possibilities of performing the immunologic diagnosis in NTB

Despite the numerous diagnostic tools presently available, the neurologic forms of TB often remain undetected and lead to an increased mortality. Neurologic localizations are mostly smear negative and require a rapid diagnosis. The only rapid diagnosis method employs molecular techniques which are too expensive for developing countries. Moreover, starting antituberculous treatment before the collection of specific pathological products decreases the sensitivity of both bacteriological and molecular methods. The immunologic methods are inexpensive and could also be used after treatment starting. The rapid methods (Dot) do not even require trained personnel, the results are available without delay and the reagents are easy to store. As long as the interpretation of the results is correct, the serological methods deliver significant information and a rapid orientation in the diagnosis at a low cost.

c.   The practical value of the immunologic diagnosis as a complementary method in developing countries with a high prevalence of TB

The immunologic methods studied in neurotuberculosis hold a practical value once their results are included in an internationally accepted algorithm for diagnosis. However, the use of these diagnostic methods and reports on cost-efficiency are regarded with reluctance. Literature data published between 2004-2008 on TB case definitions includes only 1 study which considers ELISA IgM detection useful in TB meningitis (Kalita et al, 2007).Three other studies consider that a positive TST test could exhibit only a potential utility in the diagnosis of TB. (Marais et al, 2010) There is also insufficient experience in order to appreciate the efficiency of IGRAs assays in the diagnosis of neurotuberculosis. Therefore the immunologic diagnosis in neurotuberculosis is not currently accepted in the international guidelines as a complementary diagnosis for the confirmation of the diagnosis of neurotuberculosis. Nevertheless serologic diagnostic methods are in use in developing countries and provide

rapid orientative data in TB meningitis. However each assay should be validated using controls from that specific area and a part of the technical criteria are also to be adapted to that area in terms of cut off value and used antigens. Standardised diagnostic criteria for TB meningitis as well as standardised immunologic methods could render correct forthcoming comparisons between immunologic studies. It could also establish the real value of these methods especially in poor countries with a high prevalence for tuberculosis and reduced possibilities of diagnosis.

13. Acnowledgments

14. References

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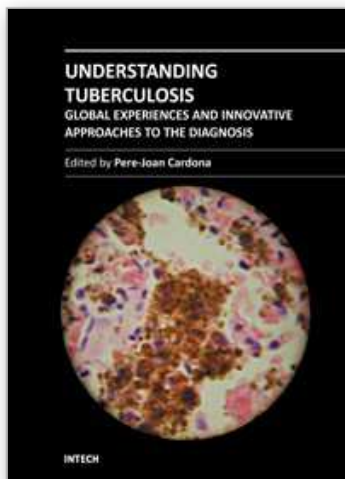
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Mycobacterium tuberculosis is a disease that is transmitted through aerosol. This is the reason why it is estimated that a third of humankind is already infected by Mycobacterium tuberculosis. The vast majority of the infected do not know about their status. Mycobacterium tuberculosis is a silent pathogen, causing no symptomatology at all during the infection. In addition, infected people cannot cause further infections. Unfortunately, an estimated 10 per cent of the infected population has the probability to develop the disease, making it very difficult to eradicate. Once in this stage, the bacilli can be transmitted to other persons and the development of clinical symptoms is very progressive. Therefore the diagnosis, especially the discrimination between infection and disease, is a real challenge. In this book, we present the experience of worldwide specialists on the diagnosis, along with its lights and shadows.

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