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CHANGES IN GLUCOSE LEVELS – A PREDICTIVE MARKER FOR AN ADEQUATE ENVIRONMENT AIMED AT *Mycobacterium tuberculosis* GROWTH

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Abstract

In the context of large interest of World Health Organization to progress in prevention of tuberculosis (TB) by avoidance of transferring pathogens from the Mycobacterium tuberculosis (Mtb) complex, and ensuring diagnosis and treatment of this disease at global, regional and country levels, it was found that most people who develop TB disease can be cured if well-timed diagnosis and correct treatment are performed.
This paper presents and discusses a case study, when a patient was rapidly diagnosed with Mycobacterium tuberculosis infection based on biochemical analysis of the cerebral-spinal fluid, especially high protein rhinorrhasia and low glycogenic glucose, in the absence of other pathological conditions. In these condition the therapy was immediately started. At discharge of the patient, lumbar puncture showed normal cellularity, with glucose levels below the normal range, but increasing relative to previous values, MRI examination was within normal limits, and motor deficit was minimal.

Key words: biomarkers, cellularity, cerebral-spinal fluid, environment, glycosylation, tuberculosis,

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1. Introduction

Tuberculosis (TB) is a transmissible infectious disease, which can be spread nearly fully by coughed aerosols transferring pathogens from the *Mycobacterium tuberculosis* (Mtb) complex (Goletti et al., 2016). In spite of prominent progress in the last years, TB continues to be one of the major infectious which causes mortality and morbidity globally, being still a public health problem in various countries. World Health Organization (WHO) reported that TB generated infections amid around 10 million people each year and 1.5 million deaths annually, and it is ranked together with the human immunodeficiency virus (HIV) as a foremost origin of death worldwide (Álvaro-Meca et al., 2016; Petruciolli et al., 2016; Raviglione and Sulis, 2016; WHO, 2015). Strategies to reduce TB morbidity and mortality, as well as Mtb transmission, count on effective treatment, accurate diagnosis, and preventive undertakings against contamination and disease (Dowdy et al., 2017).

New tools for diagnosis and new biomarkers become more and more essential to appraise both pathogen and host key elements of the answer to infection. Biomarkers that point out the start of effective treatment could enable progresses for alternative treatment approaches. The WHO End TB Strategy discussed in the Global Tuberculosis Report (WHO, 2017) is to "End the global TB epidemic". Taking 2015 year as baseline, the milestone and

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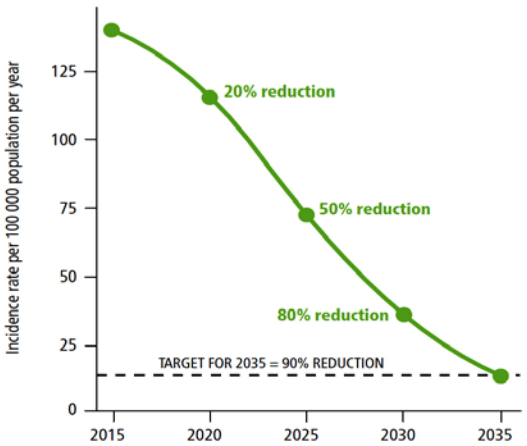
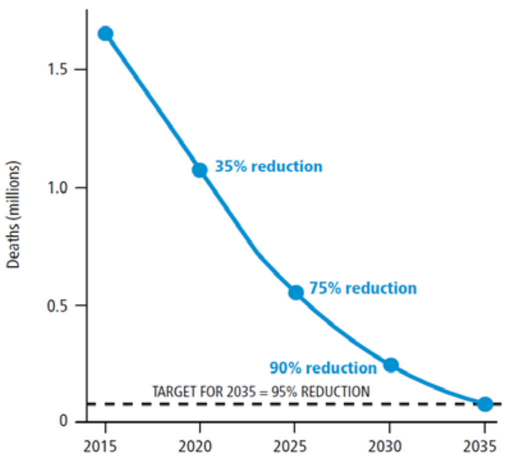
targets for ending the global TB epidemic (zero deaths, disease and suffering due to TB), are quantified in some indicators, as illustrated in Table 1. The graph on the incidence of tuberculosis and deaths required to achieve the milestones and targets are illustrated in Fig. 1.

Table 1. The WHO End TB strategy and indicators (WHO, 2017)

Indicators	***Milestones (%)***		***Targets (%)***	
	2020	***2025***	***2030***	***2035***
Percentage reduction in the absolute number of TB deaths (compared with 2015 baseline)	35	75	90	95
Percentage reduction in the TB incidence rate (compared with 2015 baseline)	20	50	80	90



a)



b)

Fig. 1. End TB Strategy incidence (a) and mortality (b) curves projected to reach during 2015–2035 (upon WHO, 2017)

The main pillars proposed to be established so as to fulfill these goals involve: integrated, patient-centred care and prevention, bold policies and supportive systems, intensified research and innovation. The integration of these three pillars would lead to "rapid and robust point-of-care diagnostics, technologies to reliably detect LTBI (latent TB infection) and accurate biomarker tests to monitor treatment response" (WHO, 2017). This is unequivocally necessary for ensuring a good environmental quality, so as to avoid the spread of disease generated by *Mycobacterium tuberculosis* occurrence and transmission powered by exposure to environmental risk factors.

Although there have been developed various studies on the discovery and application of new biomarkers, there is currently little information on their independent validation and confirmation. This situation requires ongoing efforts to fill this gap in research into new biomarkers and their validation alongside distinct clinical endpoints in different populations (Goletti et al., 2016). In this paper, the issue of robust diagnosis of TB is addressed by presenting and discussing a case study, where the correlation of Mtb presence and TB disease is performed via glycosylation level.

2. Diagnosis of tuberculosis

According to *Handbook on TB Laboratory Diagnostic Methods in the European Union* "*Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), is classified as a risk group 3 agent, which calls for a Biosafety Level 3 laboratory (BSL3) for culture, drug susceptibility testing and other laboratory examinations. Access to a safety laboratory should be restricted to staff members and accredited visitors" (ECDPC, 2016). Therefore, high levels of safety are imposed to avoid Mtb transmission.

A characteristic of *Mycobacterium tuberculosis* infections is that most infected individuals do not develop active tuberculosis. This condition is called *latent tuberculosis infection* (LTBI), when viable bacilli of Mtb are characterized by persistence and a low rate of multiplication (Tufariello et al., 2003).

Analyzes may indicate an immune response against the bacillus, but usually do not appear to be clinical manifestations and specific radiological evidence of active disease. This state of latency can be maintained for the lifetime of infected individuals, while estimates showed that approximately one third of the world's population can be suspected of this state of latency of Mtb (Dye et al., 1999; Styblo, 1985).

The definitive diagnosis of TB is achieved by isolating Mtb from a body's secretion: sputum culture, bronchoalveolar or pleural fluid lavage, or pleural biopsy or pulmonary biopsy (Pai et al., 2016a). In addition, diagnostic tools such as sputum acid-fast bacilli (AFB) smear and nucleic acid amplification (NAA) may also be used.

Rapid diagnosis of active TB favors therapeutic intervention in a timely manner and reduces the rate of transmission in the community (Lewinsohn et al., 2017; Pai et al., 2016b).

3. Case study

3.1. Case presentation

Some research revealed the correlations between glucose level in organism and TB. Type 2 diabetes mellitus (DM) has been increasingly recognized as an important risk factor for tuberculosis (TB). Epidemiological studies have demonstrated that adults with diabetes have a significantly increased risk of developing active TB and it is estimated that globally 15% of TB cases are attributable to DM (Jeon and Murray, 2008; Lachmandas et al., 2015; Ruslami et al., 2010).

It is well-known that glucose is the largest energy source for brain cells, extracted from circulation, from capillary blood, with normal values in cerebral-spinal fluid of 50-80 mg/dL, about 0.6-0.7 of the plasma concentration. An amount of glucose below the lower limit of normal in cerebral-spinal fluid can be found in many pathological conditions, of which more importantly are: bacterial infections of the nervous system, fungal infections, inflammation and central nervous system tumors, subarachnoid hemorrhage, chemical meningitis or even severe hypoglycemia. In these cases, the amount of glucose becomes insufficient for the proper functioning of the neurons, and psychic functions are severely influenced (Martínez Ortiz de Zárate et al., 2013; Morales-Casado et al., 2017; Waghdhare et al., 2011).

In this case study we report the case of a 42-year-old urban patient with no personal pathological history, athletic, risk-free, and with no contact with tuberculosis cases. He has been admitted in Infectious Diseases Clinical Hospital in Iasi, Romania, between August 22th, 2017 and September 30th, 2017. He presented the following symptoms at admission: intense headache; febrile syndrome; chills; hypertension. At clinical examination, the patient was conscious, cooperative and without pathological changes in other systems. Prior to admission to the Infectious Diseases Department, the patient was evaluated in the neurology service, where it was revealed that no acute cranial-cerebral lesions occurred, and the appearance of CT scan was within normal limits. From the paraclinical point of view, at admission, neutrophilia (71.4%) and mild thrombocytosis occurred, and chest radiographs were normal, thus starting with Clarithromycin *per os* and Ceftriaxone injectable therapy.

3.2. Diagnosis, evolution and therapy

Three days after admission, fever persisted, headache increased and photophobia occurred, which is why the lumbar puncture was decided. In the microscopic examination of the cerebrospinal fluid, increased cellularity (124 cm/mm^3) with 15% polymorphonuclear and 85% lymphocytes was observed. Also, relevant are some of the biochemical results – low glycosylation (30 mg/dL) and albuminorheia with values of 0.63g/L (Table 2). Since cerebrospinal fluid penetrating antibiotic therapy was already initiated and glycogens were well below the lower limit, pre-treated bacterial meningitis was suspected and it was decided to introduce Dexamethasone treatment regimen to diminish the inflammatory process.

Evolution at 24 hours after the first lumbar puncture was not favorable, the patient suddenly installing diplopia. In conjunction with low glycogen, this raised the suspicion of tuberculosis meningitis, despite the failure of a primary affection or radiological imaging for tuberculosis, and in the context lack of epidemiological data.

Table 2. Analysis results of the first lumbar puncture

Analysis	***Results***
Cellularity (ecn/mmc)	124
Polymorphonuclear neutrophils (% PMN)	15
Lymphocytes (% Ly)	58
Albumin (g/L)	0.63
Glucose (mg/dL)	**30**
Chlorine (g/L)	6.8
Others	Increased cellularity Pathological CFS glucose levels

It was thus decided to re-evaluate the cerebral-spinal fluid by a new lumbar puncture, where cellularity is increased, but decreasing compared to the previous examination (76 cc/mm^3), while the glycaemia decreased to 16 mg/dL (Table 3).

Table 3. Analysis results of the second lumbar puncture

Analysis	***Results***
DNA *Mycobacterium tuberculosis*	positive
Cellularity (ecn/mmc)	76
Polymorphonuclear neutrophils (% PMN)	32
Lymphocytes (% Ly)	64
Macrophages (%)	4
Glucose (mg/dL)	**16**
Others	Glucose at critical levels

Decreased glucose levels compared with the previous examination, but also the previously formulated suspicion, lead to PCR (Polymerase Chain Reaction) for rapid detection of *Mycobacterium tuberculosis* DNA, the result of which was positive, thus confirming the tuberculosis etiology. HIV serology was negative (Codina et al., 2011; Manciuc et al., 2010; Manciuc and Largu, 2014). Quadruple combination therapy (Etambutol, Pirazinamide, Isoniazid, and Rifampicin) was initiated, with waviness and feverish hooks up to 30 days (Hurmuzache et al., 2017).

The condition of the patient remained stationary 7 days after initiation of tuberculostatic therapy. A sudden hemiparesis installed on the left side of the mouth, diplopia gets worse and fever persists. A second HIV serology was negative (Costan et al., 2016; Teodor et al., 2013). The patient was re-examined neurologically, by nuclear magnetic resonance, and by a new lumbar puncture, targeting growing cellularity (370 ecn/mm^3), but with DNA for *Mycobacterium tuberculosis* - undetectable. The MRI (Magnetic Resonance Imaging) examination highlights the maintenance of cerebral edema and slight trientricular dilation. Continuing tuberculostatic treatment in quadruple combination and steroidal anti-inflammatory therapy, results in a net improvement of neurological symptoms over the next few days and the febrile syndrome remission (Nakao et al., 2016).

At discharge, lumbar puncture showed normal cellularity, with glucose levels below the normal range, but increasing relative to previous values, MRI examination - within normal limits, and motor deficit was minimal (Fig. 2) (Incesu et al., 2015).

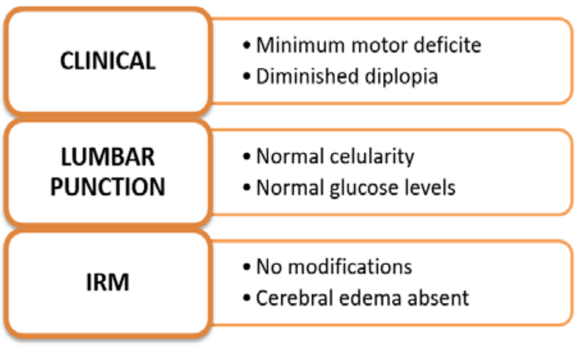


Fig. 2. Clinical and paraclinical data at discharge

3. Conclusions

The biochemical analysis of the cerebral-spinal fluid, especially high protein rhinorrhasia and low glycogenic glucose, in the absence of other pathological conditions, should be correlated with epidemiological, clinical and paraclinical data. In this case, low glycosylation in the CSF has indicated a favorable environment for the development of *Mycobacterium tuberculosis*.

For a certain diagnosis of *Mycobacterium tuberculosis* infection, positive cultures are ideal, but require a large amount of cerebral-spinal fluid, 30-50 mL and approximately 3-5 repeated lumbar punctures. The results are obtained in 2-6 weeks, thus that PCR is the most promising method of rapid identification of Koch bacillus.

Tuberculosis meningitis often occurs in the absence of infection in other extra pulmonary sites and, although suspected in immunosuppressed patients with diabetes, alcohol consumption, HIV-positive or endemic TB, the disease also occurs in healthy and risk-free individuals and even the slightest suspicion should lead to specific investigations.

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