Environmental Engineering and Management Journal December 2018, Vol. 17, No. 12, 3007-3011
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

"Gheorghe Asachi" Technical University of Iasi, Romania

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

Stefania Alice Cislariu[1], Georgiana Alexandra Lacatusu[1], Alexandra Largu[1,2], Ioana Florina Iordan[1], Andrei Vata[1,2], Carmen Manciuc[1,2*]

[1]*„ Sf. Parascheva" Infectious Diseases Clinical Hospital, Iasi, 2 Octav Botez Street, Iași 700116, Romania*
[2]*„Grigore T. Popa" University of Medicine and Pharmacy, 16 Universității Street, Iași 700115, Romania*

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,

Received: August, 2018; Revised final: October, 2018; Accepted: November, 2018; Published in final edited form: December 2018

1. Introduction

Tuberculosis (TB) is a contagious airborne infectious disease, which is transmissible by cough aerosols contaminated with 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 accounting approximately for 10 million cases 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, depend on effective treatment, accurate diagnosis, and preventive undertakings against contamination and disease (Dowdy et al., 2017).

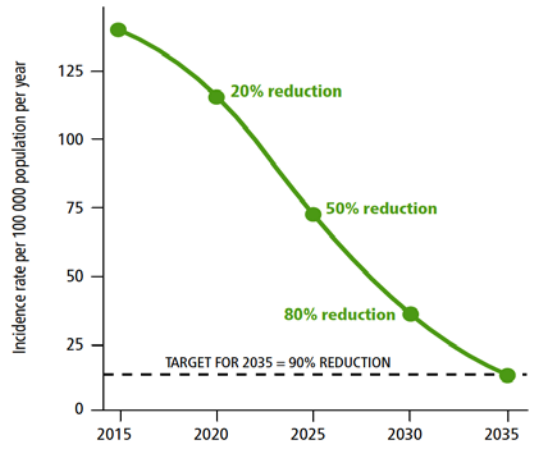
New tools for diagnosis and new biomarkers become more and more essential to appraise both pathogen and host key elements of the answer to infection. Biomarkers that point out the start of effective treatment could enable progresses for alternative treatment approaches. The WHO End TB Strategy discussed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

* Author to whom all correspondence should be addressed: dmanciuc@yahoo.com

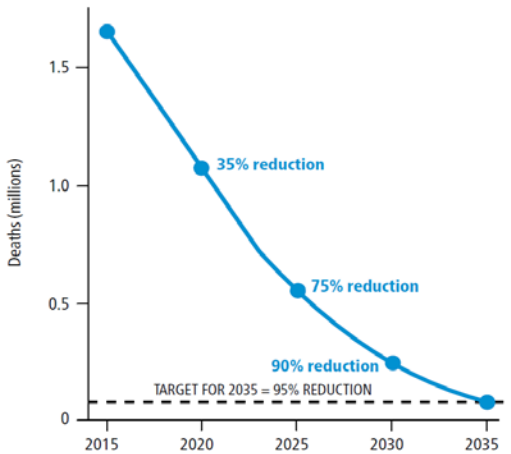
deaths, disease and suffering due to TB), are quantified in some indicators, as illustrated in Table 1. Graphon the incidence of tuberculosis and deaths required to achieve the milestones and targets are illustrated in Fig. 1 (WHO, 2017).

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 for future care involve: integrated, patient-centred care and prevention, efficient 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 resulting from exposure to environmental risk factors.

Previous research on new biomarkers has not been confirmed or validated enough. This situation requires ongoing efforts to fill this research gap on 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 recommended for avoiding 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 are absent. Lifetime latency in infected individuals is a normal occurrence. Estimates report that approximately one third of the global 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

Past research reveals a correlation 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 the 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 a severe influence over the psychological state occurs (Martínez Ortiz de Zárate et al., 2013; Morales-Casado et al., 2017; Waghdhare et al., 2011).

We present the case of a 42-year-old male patient from urban area with pathological history, athletic, risk-free, and with no contact with tuberculosis cases. He was 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 no acute cranial-cerebral lesions were revealed, and the appearance of CT scan was within normal limits. At admission,blood analysis showed neutrophilia (71.4%) and mild thrombocytosis, chest X-Rays were normal. The treatment was initiated with Clarithromycin *per os* and Ceftriaxone *intravenous*.

3.2. Diagnosis, evolution and therapy

Three days after admission, fever persisted, headache increased and photophobia occurred, that justified the lumbar puncture. The microscopic examination of the cerebrospinal fluid revealed increased cellularity (124 cm/mm^3) with 15% polymorphonuclear and 85% lymphocytes. Also, relevant are some of the biochemical results: low glucose level (30 mg/dL) and albuminorheia (0.63g/L) (Table 2). Since cerebrospinal fluid penetrating antibiotic therapy was already initiated and glycorrhachia was well below the lower limit, pre-treated bacterial meningitis was suspected. We initiated 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 glycorrhachia, we raised the suspicion of tuberculosis meningitis, despite the absence 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 glycorrhachia 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 glycorrhachia compared with the previous examination, but also the previously formulated suspicion, indicates PCR (Polymerase Chain Reaction) for rapid detection of *Mycobacterium tuberculosis* DNA. The result 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 (Hurmuzache et al., 2017). The fever syndrome was manifested up to 30 days associated with unsystematic fever.

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 persisted. A second HIV serology was negative (Costan et al., 2016; Teodor et al., 2013; Manciuc et al., 2010; Manciuc and Largu, 2014). The patient was

re-examined neurologically, by nuclear magnetic resonance (NMR), 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 triventricular dilation. Continuing tuberculostatic treatment in quadruple combination and steroidal anti-inflammatory therapy, showed a real improvement of neurological symptoms over the next few days and also 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).

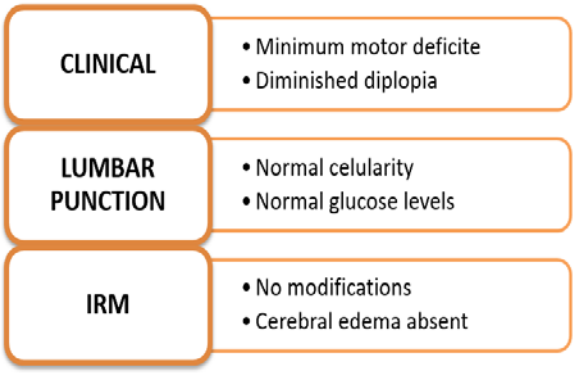


Fig. 2. Clinical and paraclinical data at discharge

3. Conclusions

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

For a clear 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 lumbar punctures. The results are obtained in 2-6 weeks, thus that PCR is the most promising method for rapid identification of Koch bacillus.

Tuberculosis meningitis often occurs in the absence of a pulmonary or extrapulmonary 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.

References

Alvaro-Meca A., Díaz A., Díez J.M., Resino R., Resino S., (2016), Environmental factors related to pulmonary tuberculosis in HIV-infected patients in the combined antiretroviral therapy (cART) Era, *PLoS One*, **11**, e0165944, DOI:10.1371/journal.pone.0165944.

Codina M.G., De Cueto M., Vicente D., Echevarría J.E., Prats G., (2011), Microbiological diagnosis of central nervous system infections (in Spanish), *Enfermedades Infecciosas y Microbiología Clínica*, **29**,127-134.

Costache D.V., Isac I., Mihăescu T., Manciuc C., (2016), TB Or Not TB In HIV Infection, *Pneumologia - Journal of the Romanian Society of Pneumology*, **65**, 210-211.

Goletti D., Petruccioli E., Joosten S.A., Ottenhoff T.H.M., (2016), Tuberculosis biomarkers: from diagnosis to protection, *Infectious Disease Reports*, **24**, 8(2): 6568, DOI: 10.4081/idr.2016.6568

Dowdy D.W., Grant A.D., Dheda K., Nardell E., Fielding K., Moore D.A.J., (2017), Designing and evaluating interventions to halt the transmission of tuberculosis, *The Journal of Infectious Diseases*, **216**, S654–S661.

Dye C., Scheele S., Dolin P., Pathania V., Raviglione M.C., (1999), Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO global surveillance and monitoring project, *The Journal of the American Medical Association*, **8**, 677-686.

ECDPC, (2016), *Handbook on TB Laboratory Diagnostic Methods in the European Union*, European Centre for Disease Prevention and Control, Stockholm, Sweden.

Hurmuzache M.E., Luca C., Lovin I., Dorobat C., (2017), Tuberculosis meningo-encephalitis with positive CSF for KB and slowly favourable evolution, *The Medical-Surgical Journal*, **121**, 804-807.

Incesu L., Khosla A., Levy L.M., (2015), Imaging in bacterial meningitis, Medscape, Nov. 28, On line at: https://emedicine.medscape.com/article/341971-overview.

Jeon C.Y., Murray M.B., (2008), Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies, *PLoS Med*, *15*, 5(7):e152. doi: 10.1371/journal.pmed.0050152.

Kompala T., Shenoi S.V., Friedland G., (2013), Transmission of tuberculosis in resource-limited settings, *Current HIV/AIDS Reports*, **13**, 3, DOI: 10.1007/s11904-013-0164-x.

Lachmandas E., Vrieling F., Wilson L.G., Joosten S.A., Netea M.G., Ottenhoff T.H., van Crevel R., (2015), The effect of hyperglycaemia on in vitro cytokine production and macrophage infection with Mycobacterium tuberculosis, *PLoS one*, https://doi.org/10.1371/journal.pone.0117941.

Lewinsohn D.M., Leonard M.K., LoBue P.A., Cohn D.L., Daley C.L., Desmond E., Keane J., Lewinsohn D.A., Loeffler A.M., Mazurek G.H., O'Brien R.J., Pai M., Richeldi L., Salfinger M., Shinnick T.M., Sterling T.R., Warshauer D.M., Woods G.L., (2017), Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of tuberculosis in adults and children, *Clinical Infectious Diseases*, **15**, 111-115.

Manciuc C., Nicu M., Largu A., Filip-Ciubotaru F., Dorobat, C., (2010), Sustainable development and key health trends: case study of dislipidemy in HIV patients, *Environmental Engineering and Management Journal*, **9**, 495-502.

Manciuc C., Largu A., (2014), Impact and risk of institutionalized environments on the psycho-emotional development of the HIV-positive youth, *Environmental Engineering and Management Journal*, **13**, 3123-3129.

Martínez Ortiz de Zárate M., González del Castillo J., Julián-Jiménez A., Pinera Salmerón P., Llopis Roca F.,

Guardiola Tey J.M., Chanovas Borrás M., Ruiz Grinspan M., García Lamberechts E.J., Ibero Esparza C., Moya Mir M.S., González Martínez F., Candel González F.J., (2013), INFURG-SEMES study: epidemiology of infections in hospital emergency departments and evolution during the last decade (in Spanish), *Emergencias*, **25**, 368–3378.

Morales-Casado M.I., Julián-Jiménez A., Lobato-Casado P., Cámara-Marín B., Pérez-Matos J.A., Martínez-Maroto T., (2017), Predictive factors of bacterial meningitis in the patients seen in emergency departments, *Enfermedades Infecciosas y Microbiología Clínica*, **35**, 220-228.

Nakao J.H., Jafri F.N., Shah K., Newman D.H., (2014), Jolt accentuation of headache and other clinical signs: Poor predictors of meningitis in adults, *The American Journal of Emergency Medicine*, **32**, 24-28.

Pai M., Nicol M.P., Boehme C.C., (2016a), Tuberculosis diagnostics: state of the art and future directions, *Microbiology Spectrum*, **4**, ID: UNSP TBTB2-0019-2016, DOI: 10.1128/microbiolspec.TBTB2-0019-2016.

Pai M., Behr M.A., Dowdy D., Dheda K., Divangahi M., Boehme C.C., Ginsberg A., Swaminathan S., Spigelman M., Getahun H., Menzies D., Raviglion M., (2016b) Tuberculosis, *Nature Reviews Disease Primers*, **2**, ID16076, https://www.nature.com/articles/nrdp201676.

Petruccioli E., Scriba T.J., Petrone L., Hatherill M., Cirillo D.M., Joosten S.A., Ottenhoff T.H., Denkinger C.M., Goletti D., (2016), Correlates of tuberculosis risk: predictive biomarkers for progression to active tuberculosis, *European Respiratory Journa*l, **48**, 1751-1763.

Raviglione M., Sulis G., (2016), Tuberculosis 2015: burden, challenges and strategy for control and elimination, *Infectious Disease Reports*, **24**, ID 6570, DOI: 10.4081/idr.2016.6570.

Ruslami R., Aarnoutse R.E., Alisjahbana B., van der Ven A.J., van Crevel R., (2010), Implications of the global increase of diabetes for tuberculosis control and patient care, *Tropical Medicine & International Health*, 15,1289-1299.

Styblo K., (1985), The relationship between the risk of tuberculosis infection and the risk of developing infectious tuberculosis, *Bulletin of the International Union against Tuberculosis and Lung Disea*se, **60**, 117-119.

Teodor A., Prisăcariu L.J., Manciuc C., Nicolau C., Jugănariu G., Teodor D., Dorobăț C., (2013), Tuberculous meningitis: presentation, diagnostic and outcome in HIV-infected individuals from regional center Iași, *BMC Infectious Diseases*, **13**, (Suppl 1): P2, https://doi.org/10.1186/1471-2334-13-S1-P2.

Tufariello J.M., Chan J., Flynn J.L., (2003), Latent tuberculosis: mechanisms of host and bacillus that contribute to persistent infection, *The Lancet Infectious Diseases*, **3**, 578-590.

Waghdhare S., Kalantri A., Joshi R., Kalantri S., (2010), Accuracy of physical signs for detecting meningitis: A hospital-based diagnostic accuracy study, *Clinical Neurology and Neurosurgery*, **112**, 752–757.

WHO, (2015), *Global Tuberculosis Report 2015*, World Health Organization, On line at: http://www.who.int/tb/publications/global_report/en/.

WHO, (2017), *Global Tuberculosis Report 2017*, World Health Organization, https://www.who.int/tb/publications/global_report/gtbr2017_main_text.pdf.