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Identification of markers of acute inflammatory process in the pulmonary tuberculosis

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***Abstract*—Tuberculosis remains, according to the WHO report, one of the global problems in the world, especially in the form caused by drug-resistant mycobacteria. The identification of special biochemical markers allowing to assess the necessity of surgery or therapy prolongation remains a challenge. We consider promising markers – metalloproteinases – analyzing statistics of data obtained from patients with pulmonary tuberculosis forced by different strains of *Mycobacterium tuberculosis*. The discussed results could be used for further development of a precise biochemical data-based diagnostic and prognostic tool.**

***Index Terms*—tuberculosis data, heavy tail distributions, non-parametric statistics, data-based prognosis**

I. Introduction

The main factors of the pulmonary tuberculosis pathogenesis are not only the bacterial virulence and sensitivity of the host immune system to the pathogen but also the degree of destruction of lung tissue [1]. Such destruction processes lead to the development of the caverns that in most cases requires surgical intervention in addition to the drug therapy. Therefore, the identification of special biochemical markers allowing to assess the necessity of surgery or therapy prolongation remains a challenge. The promising candidate for possible markers is a group of special enzymes – matrix metalloproteinases (MMP), which are involved in the destruction of lung tissue. Under normal conditions, most of MMPs are not expressed, however, their overexpression is observed in inflammation, the intensity of which is regulated by anti-inflammatory cytokines and bacterial lipopolysaccharides [2]. The role of MMPs in the destruction of the connective tissue of the lungs mostly consisting of collagen – the main structural protein of the lung – caused by *Mycobacterium tuberculosis* (MBT) has not been yet fully investigated [3]. At the same time, there types of metalloproteinases, levels of which vary with the development of pulmonary tuberculosis, were identified [4]. The main role in the initiation of the destruction process is assigned to MMP–1 [5]. MMP-8 is a component of neutrophilic fractions, which modulates the activity of chemokines and increases in pulmonary tuberculosis along with MMP-9, reflecting manifestations of the severity of the process [6]. The recent clinical study [7] also implies that it would be possible to determine markers (MMPs) of severity and activity of the process: changes in MMP-1 concentration can be related to the presence of lesions and the type of anti-TB drugs sensitivity of isolates of the *Mycobacterium*. Increase in MMP-9 and MMP-8 concentrations can mark the destruction volume and the activity of the process, respectively.

Thus, our goal is to explore by statistical methods, which markers are most preferable for identifying the severity of the inflammatory process in tuberculosis and dependence of their concentration on on strains with different drug resistance.

II. Materials and methods

A. Clinical study

239 patients with pulmonary tuberculosis (TB) were examined. All of them were treated (six months) at the hospital of the Saint-Petersburg State Research Institute of Phthisiopulmonology and gave written consent to participate in the examination. The average age of the patients was 34 ± 0.9 years. The detection of mycobacteria from diagnostic material was performed using the following methods: 1) fluorescent microscopy; 2)strains isolation by the by Lowenstein–Jensen or Middlebrook 7H9 (BACTEC MGIT 960 automated system) medium; 3)real-time polymerase chain reaction (PCR-RV) (Sintol, Russia). To determine the drug sensitivity, cultures have been harvested in the presence of anti-TB drugs. Serum samples were collected for 3–4 months and stored at the temperature of $-70\ ^{o}$C. Blood samples (15 ml) were allowed to clot for 30 minutes. Then centrifuged for 10 minutes at 4000 g for 15 min. The concentrations of serum levels MMPs and TIMP-1 were measured using an ELISA technique. The procedures were performed according to the manufacturer's

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protocols. The final results were recorded at 450 nm on an ELISA plate reader (Bio Rad Laboratories, Inc.).

B. Data processing

The self-written MATLAB code was used for the general processing of the database collected during the clinical study. For solving particular statistical problems, the warrant MATLAB's functions were used. The database included clinical common characteristics (diagnosis, age and gender, drug-sensitivity of isolated strains) and clinical measurements of enzymes/inhibitors concentration in blood. Quantitative parameters were expressed as the median and 25th and 75th percentiles. To assess the significance of differences between categorical and metric values, Mann–Whitney U test was used (for small samples). At the same time, we also take a special attention to the features, which are not capturable by the common statistical approaches, namely, to the probability distributions with heavy tails. Their presence is related to rare event occurrence and draw a special modern interest in the field reliability of biomedical research [8].

III. RESULTS

We have processed data for 20 healthy persons and 239 patients with the pulmonary tuberculosis. As shown in the Fig. 1, the concentration of TIMP (inhibitor of metalloproteinases) slightly changes but the concentration of MMP-9 increased more then one hundred times. The level of MMP-8 concentration also grow up, but the concentration of MMP-1 practically does not change as it seem from the significantly overlapping interquartile distances for healthy persons and patients with tuberculosis. Therefore, only two biochemical markers, MMP-8 and MMP-9, can be considered as candidates for identifiers of the inflammation process degree.

However, exploring the sensitivity of changes in metabolites concentrations in the case of disease caused by drug-sensitive and drug-resistant results in more non-trivial conclusions, which may be missed by common statistical inferences, see Fig. 2. Drug-resistance of TB strains leads to an emergence of heavy tails in the probability density functions for the concentration of metabolites except for TIMP. For example, it means that even while median-based criterion for ensemble-based processing of data on MMP-1 concentrations does not distinguish between healthy and tuberculosis persons, the extreme events of extra large MMP-1 concentrations should caution about the possible presence of the drug-resistant strain in a patient's organism. Similarly, MMP-8 show different distributions for sensitive and resistant strains.

IV. CONCLUSION

Thus, we suggest a set of biochemical markers to identify the severity of the inflammation process at tuberculosis basing on the statistical analysis of clinical data. We have shown that it would be useful to consider the possibility of different distributions in addition to well-known statistical criteria such as Mann–Whitney U test. Also, the presence of outliers in the small size sample requires other methods that make it possible to define the various groups of markers more precisely.

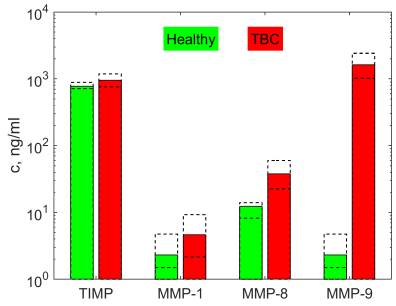

Fig. 1. Histogramm of MMPs/TIMP concentration difference for healthy persons and tuberculosis patients.The histogram amplitude denotes the median, while dotted lines mark 25 % and 75 % quartiles.

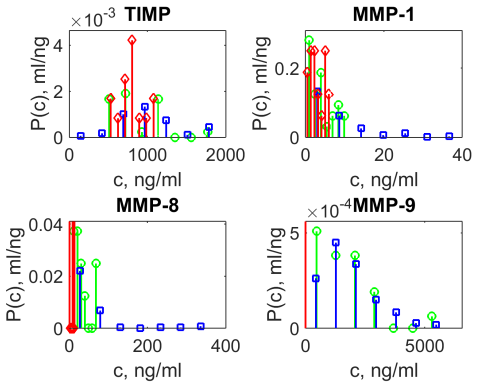

Fig. 2. Probability density function for the concentration of metabolites for healthy persons (red color) and patients with tuberculosis caused by drug sensitive (green) and drug resistant (blue) strains.

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