

## Pneumologia

# Limited proteolysis and oxidative modification of proteins in the hepatocytes of patients with resistant forms of tuberculosis

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## Abstract

## English:

*The aim of this paper was to evaluate the oxidative modification of proteins and limited proteolysis in the hepatocytes of patients with resistant forms of pulmonary tuberculosis (TB).*

**Material and method:** This is a prospective pathomorphological study of 60 patients with confirmed pulmonary TB (clinically and anatomopathologically) deaths from various causes. To evaluate the intensity of free radical processes in hepatocytes, we used a methodology developed by Davydenko IS, 2003. Histological sections were stained with bromphenol blue by Michel-Calvo.

**Results:** When comparing the digital data indicated in our study, it is evident that changes in proteins properties in average tendencies run virtually identically in the hepatocytes of different zones of the comparison group. Thus, the difference is unlikely ( $p > 0.05$ ) when comparing red/blue ratio (specific staining of acid and basic proteins by Michel-Calvo) in the hepatocytes of zones I, II and III of the comparison group. A similar trend is observed in the analysis of average values of the optical density of a specific colouration on the free amino group of proteins by A. Yasuma and T. Ichikava.

The analysis of all parameters showed a linear increase in the intensity of oxidative protein modification and limited proteolysis from zone I to III (from the periportal to the centrilobular parts of the acinus) in all subgroups of the main group according to the acinus zone (by Rappoport).

**Conclusions:** Pulmonary TB leads to increased processes of free radical oxidation of proteins with characteristic effects – increased proteolysis and oxidation of amino groups of proteins – in the hepatocytes of the liver.

## Keywords

proteolysis • proteins metabolism • hepatocytes metabolism • pulmonary tuberculosis

## Proteoliza restrictivă și modificarea prin oxidare a proteinelor la nivelul hepatocitelor la pacienții cu forme rezistente de tuberculoză pulmonară

## Rezumat

## Romanian:

**Scopul cercetării:** Investigarea modificărilor generate de oxidarea proteinelor și a proteolizei restrictive la nivelul hepatocitelor la pacienții cu forme rezistente de tuberculoză.

**Material și metodă:** A fost efectuat un studiu anatomopatologic, prospectiv la 60 de pacienți cu tuberculoza pulmonară confirmată (clinic și anatomopatologic), decedați din diverse cauze. Pentru a evalua intensitatea proceselor cu radicali liberi în hepatocite, am folosit o metodologie implementată de Davydenko I.S., 2003. Secțiunile histologice au fost colorate cu albastru de bromfenol conform Michel-Calvo.

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**Rezultate:** Atunci când comparăm datele digitale indicate în studiul nostru, este evident că modificările proprietăților proteice din tendințele medii se execută practic identic în hepatocitele din diferite zone ale grupului de comparație. Astfel, diferența este puțin probabilă ( $p > 0.05$ ) atunci când se compară raportul R/B (colorarea specifică a acidului și proteinelor de bază de către Michel-Calvo) în hepatocitele zonelor I, II și III ale grupului de comparație. O tendință similară este observată în analiza valorilor medii ale densității optice a unei colorații specifice pe grupul amino liber de proteine de către A. Yasuma și T. Ichikava.

Analiza tuturor parametrilor a arătat o creștere liniară a intensității modificării proteinelor oxidative și proteolizei limitate din zona I la III (de la periportal la părțile centrolobulare ale acinusului) în toate subgrupurile grupului principal în funcție de zona acinusului (de Rappoport).

**Concluzii:** Tuberculoza pulmonară duce la creșterea proceselor de oxidare cu radicali liberi a proteinelor cu efecte caracteristice – creșterea proteolizei restrictive și oxidarea amino-grupărilor de proteine în hepatocitele ficatului.

#### Cuvinte-cheie

proteoliză • proteine – metabolism proteine • hepatocite – metabolism hepatocite • tuberculoza pulmonară

## Introduction

Tuberculosis (TB) continues to be a global problem for humanity (1–3). The prevalence of TB comorbidities has grown significantly over recent years. This creates some difficulties with the treatment prescription, requiring an individual approach to the choice of drugs, taking into account the possibility of adverse events and adverse drug interactions. An analysis of available literature sources showed that the digestive system pathology was detected in 62.5% of cases among patients with chemo-resistant TB (4,5). Immune homeostasis disorder, especially in patients with intense symptoms of exacerbation and prolonged relapse, takes an important part in the pathogenesis of the formation of both TB and chronic hepatitis (6,7). TB overlaps the course of chronic hepatitis in the case of comorbidity, which creates certain difficulties in the prescription of full-scale antimycobacterial therapy and reduces the efficacy of etiotropic treatment (8,9). The objective of this study was to investigate the oxidative modification of proteins and limited proteolysis in the hepatocytes of patients with resistant forms of pulmonary TB.

## Materials and methods

A prospective anatomopathological study was conducted on 60 patients with confirmed pulmonary TB (clinically and anatomopathologically) deaths from various cases. The main group was divided into three subgroups depending on the clinical forms and variants of TB. The first subgroup consisted of 19 cases in which the clinical diagnosis of the newly diagnosed tuberculosis was established, the second subgroup included 21 cases of polydrug-resistant tuberculosis (PDR-TB) and the third subgroup included 20 cases of multidrug-resistant tuberculosis (MDR-TB). The comparison group included 20 corpses without any pathology of the hepatobiliary system and morphological signs of TB infection. The primary medical

records were reviewed: in-patient medical records (form No. 003/o) and postmortem examination reports (form No. 103/o). The methodology developed by I. S. Davydenko in 2003, which combines the long-established method of Michel-Calvo staining with bromophenol blue to visualize the ratio of “acid” (with predominance of carbonyl groups) and basic (with dominant amino groups) proteins and computed spectral analysis of the digital copy of the optical image, was used for the evaluation of the intensity of free radical processes in hepatocytes. Histological sections were stained with bromophenol blue using the Michel-Calvo method. The optical images were then digitalised using a digital camera (Target Image File Format, without a compression algorithm), and the latter were analyzed using the GNU Image Manipulation Program (GIMP) computer program (GPL license, 2012, version 2.82) by probe-computed microspectrometry in the RGB (red, green, blue) colour analysis system. The intensity of red and blue in colour staining was evaluated according to the given colour analysis system. The degree of oxidative protein modification (OPM) can be evaluated by detecting the relationship between the intensity of colouration in both colours (spectral regions), since the red colour by the Michel-Calvo method corresponds to the carbonyl groups and the blue colour corresponds to the amino groups of the proteins. Thus, the degree of OPM in histochemical specimens was judged by the magnitude of the red/blue (R/B) ratio, which was determined separately in the hepatocytes of the first, second and third zones (10).

The stoichiometric ninhydrin-Schiff reaction according to A. Yasuma and T. Ichikava, allows evaluating the degree of limited proteolysis, as a result of which the hidden amino groups of proteins are partially “opened”, was used to verify and quantify the free amino groups of proteins. The quantitative measure of limited proteolysis was the value of optical density in relative units of optical density (from 0 – no

colour, absolute transparency – up to 1 – maximum colour, absolute opacity), which was measured on digital monochrome copies of the image by computed micro densitometry using a computer GIMP program (GPL license, 2012, version 2.82). Determination of the optical density of a specific colour on the free amino groups of proteins was performed separately in the hepatocytes of the first, second and third zones of the acinus.

### Statistical analysis

StatSoft, Inc. version 10.0.228.8, was used for statistical analysis of data. Differences were considered as reliable at levels of significance  $p < 0.05$ . Descriptive statistics was used to represent statistic data with mean values, median, minimum and maximum values, standard deviation and 95% and 99% confidence intervals. The probability of a possible error for each parameter was calculated by the statistical parametric Student's *t*-test (with a normal sample distribution). The Pearson and Spearman correlation factors (in normal distribution of the sample and different from normal distribution) were used to characterize the degree of interconnection between variables.

## Results and discussion

Oxidative stress leads to an increase in the production of reactive oxygen species and, as a result, oxidative modification of proteins (11–13).

Taking into account the different functional activities of hepatocytes of different zones of the acinus (by Rappoport)

according to the literature (7,14) and the results of our own studies, R/B ratio at a specific colouration on the acid and basic proteins by Michel-Calvo and the mean values of the relative units of optical density of a specific colouration on free amino groups of proteins according to A. Yasuma and T. Ichikava were determined separately in the hepatocytes of the first, second and third zones.

When comparing the digital data indicated in our study, it is evident that changes in proteins properties in average tendencies run virtually identically in hepatocytes of different zones of the comparison group. Thus, the difference is unlikely ( $p > 0.05$ ) when comparing R/B ratio (specific staining of acid and basic proteins by Michel-Calvo) in the hepatocytes of zones I, II and III of the comparison group. A similar trend is observed in the analysis of average values of the optical density of a specific colouration on the free amino group of proteins by A. Yasuma and T. Ichikava (Table 1).

It was established that the results of the study of R/B ratios and the mean values of the optical density of a specific colouration of the free amino group of proteins by A. Yasuma and T. Ichikava in the first subgroup of the main group significantly differ from those in the comparison group ( $p < 0.01$ ). The average R/B ratio and the optical density of a specific colouration of the free amino group of proteins are reliable in the hepatocytes of the second group of the main group – 1.3 and 1.2 times higher than those in subgroup I, respectively, and the parameters of subgroup I exceed those of subgroup III – 1.7 and 1.3 times, respectively ( $p < 0.05$  in all cases). This tendency indicates an increase in the processes of free radical oxidation of proteins with characteristic effects – the growth of

**Table 1.** R/B ratio with specific staining of acid and basic proteins by Michel-Calvo in the hepatocytes of patients with pulmonary tuberculosis and in the comparison group ( $X \pm Sx$ ). Computed microspectrometry.

Acinus zone (by Rappoport)	Study groups			
	Comparison group ( $n = 20$ )	Main group		
		Subgroup I ( $n = 19$ )	Subgroup II ( $n = 21$ )	Subgroup III ( $n = 20$ )
Zone I	$1.09 \pm 0.008$	$1.27 \pm 0.014^*$	$1.73 \pm 0.041^{**}$	$2.05 \pm 0.072^{\#}$
Zone II	$1.11 \pm 0.004$	$1.39 \pm 0.009^*$	$1.81 \pm 0.032^{**}$	$2.34 \pm 0.094^{\#}$
Zone III	$1.13 \pm 0.012$	$1.48 \pm 0.041^*$	$1.95 \pm 0.063^{**}$	$2.68 \pm 0.108^{\#}$

Notes: \* – reliable compared to the comparison group at  $p < 0.01$ ; \*\* – reliable compared to subgroup I at  $p < 0.01$ ; # – reliable compared to subgroup II at  $p < 0.01$ . R/B, red/blue.

**Table 2.** Quantitative parameters of optical density of a specific colouration on the free amino group of proteins by A. Yasuma and T. Ichikava ( $X \pm Sx$ ) in the hepatocytes of patients with pulmonary tuberculosis and in the comparison group, relative units of optical density. Computed microdensitometry.

Acinus zone (by Rappoport)	Study groups			
	Comparison group ( $n = 20$ )	Main group		
		Subgroup I ( $n = 19$ )	Subgroup II ( $n = 21$ )	Subgroup III ( $n = 20$ )
Zone I	$0.195 \pm 0.0012$	$0.221 \pm 0.0016^*$	$0.249 \pm 0.0012^{**}$	$0.302 \pm 0.021^{\#}$
Zone II	$0.196 \pm 0.0031$	$0.245 \pm 0.0009^*$	$0.262 \pm 0.0021^{**}$	$0.318 \pm 0.008^{\#}$
Zone III	$0.198 \pm 0.0012$	$0.251 \pm 0.0012^*$	$0.274 \pm 0.0013^{**}$	$0.327 \pm 0.014^{\#}$

Notes: \* – reliable compared to the comparison group at  $p < 0.01$ ; \*\* – reliable compared to subgroup I at  $p < 0.01$ ; # – reliable compared to subgroup II at  $p < 0.01$ .

limited proteolysis and oxidation of amino group proteins in the hepatocytes of patients with PDR-TB and MDR-TB (Table 2). The analysis of the parameters showed a linear increase in the intensity of OPM and limited proteolysis from zone I to zone III (from the periportal to the centrilobular parts of the acinus) in all subgroups of the main group according to the acinus zone (by Rappoport).

## Conclusions

Pulmonary TB leads to increased processes of free radical oxidation of proteins with characteristic effects – the growth of limited proteolysis and oxidation of amino groups of proteins in liver hepatocytes. The intensity of the limited proteolysis and oxidation of the amino group of proteins has a linear growth pattern from zone I to zone III of the acinus.

The average R/B ratio for a specific colouration of the acid and basic proteins by Michel-Calvo and the optical density of a specific colouration on the free amino group of proteins by A. Yasuma and T. Ichikawa were 1.32 and 1.15 times higher in patients with resistant forms of TB compared with those with susceptible TB, and 1.71 and 1.25 times higher in those with multidrug-resistant pulmonary TB, respectively.

Prospects for further research lie in a more detailed study of changes in oxidative processes in hepatocytes under the influence of long-term chemotherapy of multidrug-resistant and expanded resistant forms of TB.

## Ethics approval and consent to participate

Inform consent was obtained from the patients in order to participate to the study and write the article.

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