# ONCOLOGY

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## The analysis of the secondary structure of the proteins in blood serum of patients with multiple myeloma*

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Multiple myeloma (MM) is one of the most common hematologic diseases in the world, which is characterized by the proliferation of clonal plasma cells in the bone marrow producing a monoclonal immunoglobulin. In this research, analysis of the secondary structure of proteins in blood serum of patients with MM and healthy donors was carried out by an infrared spectroscopy method. There were shown a decrease in the average content of α-helical sites and an increase in the number of β-layered structures in blood serum proteins of patients with MM compared to healthy donors.

*Keywords*: multiple myeloma, infrared spectroscopy, the secondary structure of proteins.

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## Introduction

Oncogematological diseases (hemoblastoses) belong to an extensive group of neoplasms of hematopoietic and lymphoid tissues that include a variety of pathomorphological and clinical manifestations of the disease. Some of the most common variants of oncohematological diseases in the world, including Russia, are chronic lymphatic leukemia (CLL) and multiple myeloma (MM). Multiple myeloma (von Rustitsky–Kahler disease) — is a heterogeneous disease characterized by the proliferation of clonal (neoplastic) plasma cells in the bone marrow. Chronic lymphatic leukemia is a heterogeneous clonal malignant lymphoproliferative disease primarily localized in bone marrow [1]. To date, CLL and MM remain incurable diseases. Moreover, the molecular mechanisms for the occurrence, development, and progression of these diseases remain not fully understood. In the structure of cancer, multiple myeloma accounts for about 1 % of all cancers and about 10 % of malignant tumors of the hematopoietic system [2; 3]. Normal plasma cells produce and secrete various types of immunoglobulins (IgA, IgD, IgE, IgG, and IgM) which circulate in the blood and are a component of the immune system. Tumor cells produce a monoclonal immunoglobulin, commonly referred to as a "paraprotein" or "M-protein". The identification and quantification of "M-protein" play a leading role in the diagnosis of MM and the therapeutic control of disease course. This type of assay is typically performed using serum protein electrophoresis in an agarose gel [4]. Techniques developed to date allow the identification of monoclonal protein in blood serum in more than 90 % of patients with MM. This type of patient is dominated by monoclonal IgG and IgA comprising about 50 % and 20 % of all cases of MM, respectively [2; 3]. In recent years, several authors have shown that clonal plasma cells may exhibit abnormalities in the structural organization of certain proteins, as well as disorders in the folding processes of newly synthesized protein molecules [2; 5; 6]. These two circumstances led to the study of structural aspects of this type of pathogenesis, as well as the search for new and potentially promising approaches to the treatment of MM and CLL. Of particular interest, in our view, are approaches aimed at preventing "pathological" changes in the structure of protein molecules. However, at the present time, both the processes of occurrence and variation of structural transformations of blood proteins in MM patients and the effects on their structure of drugs remain hardly studied. Our research aims to study and compare the secondary structure of serum proteins in patients with MM and healthy donors using IR spectroscopy.

## Methods

***Blood sera samples***. S-Monovette tubes (Sarstedt, Germany) with coagulation activator were used to obtain blood serum samples. The collected samples were left in test tubes for 20–30 minutes at room temperature (18–24 °C) and centrifuged at 3000 rpm for 15 min using a Heraeus Labofuge 200 centrifuge (Thermo Scientific, United States). Before physicochemical studies, the samples were frozen and stored at –30 °C.

***IR absorption spectra*** were recorded in $D_2O$ solutions using Tenor 27 FTIR spectrometer (Bruker, Germany). Isotopic substitution was performed according to the previously described procedure [7]. We used demountable $CaF_2$ cells with an optical path length of 50 μm. The spectra were recorded with resolution of 2 $cm^{-1}$ in the range of 4000–800 $cm^{-1}$. The initial processing and analysis of the spectra were carried out using the software supplied by the instrument manufacturer.

***The secondary structure*** was analyzed by decomposing of the amide I band into components based on the analysis of the second derivative of the spectrum, as described earlier [8; 9]. Each obtained contour in decomposition was attributed with a certain type of secondary structure [10–13]. The fraction of each type of the secondary structure was determined by the fraction of the area of the corresponding contour in the Amide I band.

## Results and discussion

Bands arising from peptide bonds vibrations dominate in the mid-IR absorption spectra of proteins. Vibrations of C=O and N–H groups of the peptide bond are extremely sensitive to the geometry of the polypeptide backbone and observed in the spectra as well distinguished bands known as amide I and amide II. Earlier it was shown [8; 14; 15] that the analysis of the amide I vibrations allows one to obtain the most reproducible results in determining the secondary

In this study we performed a comparative analysis of the IR spectra of blood serum samples from healthy donors, patients with multiple myeloma (MM) and chronic lymphatic leukemia (CLL).

Figures 1–3 demonstrate the difference in the form of the amide I bands in the spectra of MM samples in health and various oncohematological diseases, reflecting a change in the relative content of α-helical and β-layer conformations due to overproduction of immunoglobulins. To determine the specific content of various types of secondary structure in the sample, we have performed decomposition of the amide I bands [8]. The contribution of the bands corresponding to the α-helical conformations (1649–1656 $cm^{-1}$), β-layer regions (1630 ± 2 $cm^{-1}$), different β-turns (1660–1690 $cm^{-1}$), and disordered fragments (1645 ± 4 $cm^{-1}$) was analyzed. Based on the obtained data the average content of each type of secondary structures in all samples was determined.

MM samples reveal the opposite pattern: the β-structures demonstrate elevated levels (54 ± 5 %), while the fraction of the helical regions decreases down to 38 ± 2 %. In

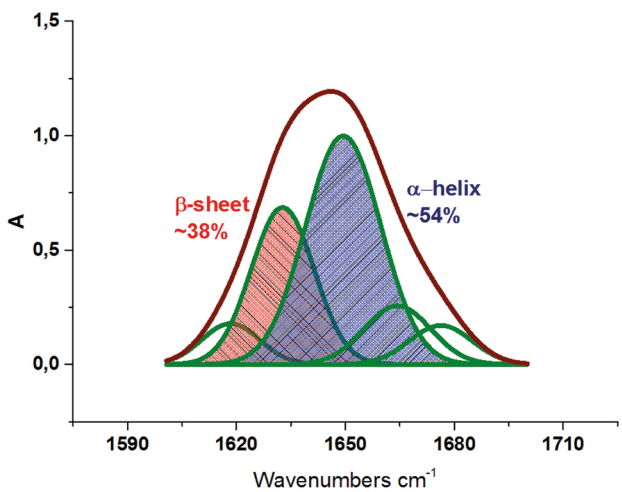


*Fig. 1.* Secondary structure composition of proteins in serum of healthy donors

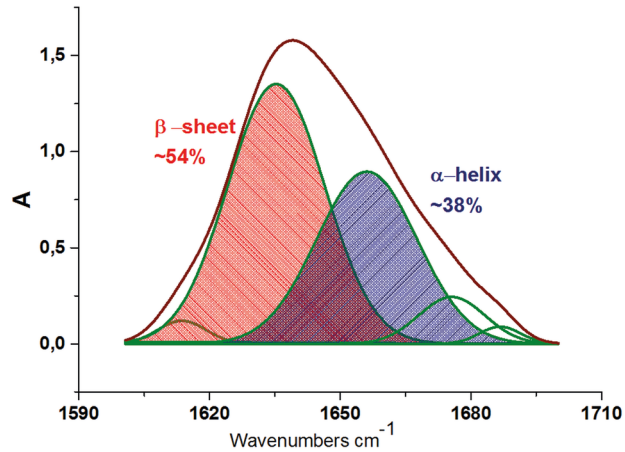


*Fig. 2.* Secondary structure composition of proteins in serum of MM patients

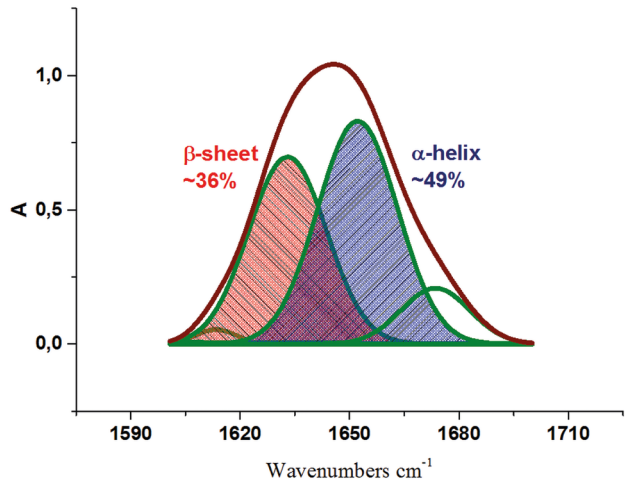


*Fig. 3.* Secondary structure composition of proteins in serum of CLL patients

general such a behavior of the protein secondary structure components can be explained considering the protein composition of the serum. The most abundant serum proteins, albumins and immunoglobulins, have remarkably different secondary structure. In the secondary structure of human serum albumin α-helical content is close to 65 %, while immunoglobulins have about 70 % of various β-structures. Hence increasing concentration of immunoglobulins readily results in increase of the β-structural regions. The observed decrease in α-helical content in CLL serum compared to the healthy donors can also indicate slight decrease in albumin concentration.

A comparison of the results for healthy donors, patients with MM and CLL allowed us to conclude that the most significant differences in the spectra were observed for the bands corresponding to the α-helical and β-layer regions. In the serum of healthy do-

nors and CLL patients, the polypeptide chains in α-helical conformations (54 ± 2 % and 49 ± 1 %, respectively) dominate, while the regions with different β-structures make a slightly smaller contribution (48 ± 3 % and 46 ± 2 %, respectively).

A decrease of fraction of albumin in the blood can be observed at various diseases and it is not a unique feature of CLL. Thus, we believe, that the described approach can be applied at investigation and diagnostics of some other diseases.

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