The investigations of $^{31}$P spectra of sera and cells of patients with hematological malignancies (acute leukemia, malignant lymphoma, multiple myeloma) and other cancers: renal, thyroid, esophageal are a clinical trials over the introduction of MRS to monitoring of the therapy. The changes of concentrations of phospholipids in sera and cancer cells may explain their transport through cell membranes and significance of this mechanism in apoptosis.

Phospholipids (phosphoglycerides and sphingomyelins) are essential elements of cellular membranes. Phosphatidylcholine (PC) and sphingomyelin (SM) appear mainly in outer (exterior) leaflet of membrane. Phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) – are in inner leaflet. In plasma of healthy person PC, SM, LPC and PE prevail. PI and plasmalogens appear in plasma only in inconspicuous quantity. Cardiolipin (CL) is essential element of mitochondrial membranes. Sphingomyelin is the most known phospholipid in cancer disease as an important participant of signal transduction pathway.

In our investigation $^{31}$P NMR spectra were obtained from: sera, sera with added the sodium salt of cholic acid, methanol-chloroform extracts of phospholipids from: plasma, peripheral blood mononuclear cells (PBMC), bone marrow mononuclear cells (BMMC). Isopropanol-hexan extracts of phospholipids we used to extracted: peripheral blood erythrocytes (PBE), bone marrow erythrocytes (BME). Blood samples were collected by venous puncture after an overnight fast, for sera – 10 ml, for methanol chloroform extracts of phospholipid from plasma, PBMC, BMMC, PBE, BME 9 -15 ml. Cellular lipids were isolated from mononuclear cells Ficoll buffy coat centrifugation, and next underwent methanol-chloroform extraction. All spectra came from phospholipid extracts prepared from $60 \times 10^6$ cells for PBMC, BMMC and $5 \times 10^9$ for PBE, BME. Studies were carried out on AMX 300 Bruker and Avance III Bruker spectrometer 7.05 T.

In our preliminary studies we observed that $^{31}$P NMR spectra of normal serum consist of three peaks including a downfield peak due to Pi and two additional upfield field peaks from phospholipids PE+SM and PC. Spectra were performed in healthy volunteers, patients with acute leukemia, malignant lymphomas and multiple myeloma, at the time of diagnosis and repeated up to 13 times during chemotherapy. The sodium salt of cholic acid added to serum caused separation of three phospholipid peaks located upfield from inorganic phosphate. Contrary to the earlier studies, peaks from PE+SM and PC, and also a peak from LPC were observed. The above mentioned separation method had also been applied in investigations of patients with digestive tract tumors and with renal cell carcinoma. Changes in phospholipids in the $^{31}$P NMR spectra observed in these patients were primarily dependent on the advance of the disease. Long-term follow-up studies showed a good correlation between this $^{31}$P MRS evaluation of sera and the response of the disease to the therapy. At the time of diagnosis spectra showed strongly reduced peak areas and intensities from phospholipids (PC, LPC and PE + SM). During chemotherapy important changes in spectra
were observed: (1) in responding patients the spectral profile changed to resemble that of normal serum with increased peak intensities, (2) in non-responding individuals peak intensities were reduced. Spectra of patients suffering from acute leukemia or HD, who have achieved complete remission for 4-12 years did not differ from spectra of healthy volunteers. \( ^{31}\text{P} \) NMR spectra can prove the presence of the residual leukemia in patients when the number of leukemic cells equals to \( 10^9 \) and is not detectable in laboratory tests of the blood and bone marrow. \( ^{31}\text{P} \) NMR spectra can depict the function of the transplanted. It is possible to estimate the efficiency of cytostatics: e.g. DHAD & ARA-C as a first course treatment in patients with acute leukemia.

In our study \( ^{31}\text{P} \) MRS spectra of normal extract of serum consist of six peaks due to phospholipids. Beside previously identified, i.e. PC, LPC, PE and SM, some new ones were observed: PI, CPLAS. \( ^{31}\text{P} \) NMR spectra of phospholipid extracts from plasma. In patients with hematological cancers the values of peaks areas of PC, CPLAS, LPC, SM decreased. In some patients peaks from PI, PE were not observed. In responding patients the spectral profile changed to resemble that of normal extract. \( ^{31}\text{P} \) MRS spectra of extract of mononuclear cells consist of 9 peaks due to phospholipids: PC, CPLAS, LPC, SM, PE+PI, PS, CL. The peak of LPC that has the most prognostic value in sera, in extract of mononuclear cells was observed only in some healthy volunteers. Important meaning for prognosis of disease course has got a presence of CPLAS (PAF). In the case of both the PBMC and BMMC, the PAF concentration was significantly diminished in patients with ALL relative to the concentration for those with AML and for the healthy volunteers. No differences were observed in the PAF concentrations for the AML patients and the healthy volunteers. We focused on the significant difference in the integral intensities and phospholipid concentrations of SM and PS as well as CPLAS and PI+PE between the ALL and AML groups.

The experiences already achieved pointed out that \( ^{31}\text{P} \) spectra at present didn't allow for diagnosis of hematological disorders, but were of great importance in monitoring of therapy of the diseases under consideration. The changes of concentrations of phospholipids in sera and cells in hematological cancers are probably due to the increased uptake of phospholipid metabolites in proliferating blast cells, and their disturbed transport through cell membranes. It seems that SM and CPLAS (PAF) are of great importance in this process. It is very likely that observed reduction of the level of SM in blast cells’ extraction is due to activation of sphingomyelinase (SMnase) by for example tumor necrosis factor (TNF) or PAF. SMnase cleaves SM, generating choline phosphate and ceramide. The latter – among other things – mediates programmed cell death (apoptosis), induces differentiation and inhibits growth of leukemia cells. \( ^{31}\text{P} \) MRS it may be useful method for understanding the meaning of phospholipids in mechanism of cancer’s proliferation. In this review of our study, it was shown how \( ^{31}\text{P} \) MR spectroscopy can be applied for observations of changes of phospholipid concentrations in sera, plasma, PBMC BMMC, PBE, BME in patients with cancer diseases. This investigation points out that \( ^{31}\text{P} \) MR spectra allow monitoring of phospholipid concentration in body fluid as well as in cells. Moreover, among acceptable various applications of \( ^{31}\text{P} \) MRS, the possibility evaluating an advancement of disease, monitoring the treatment and forecasting resistance to the therapy seems to be the most promising. Simplicity of presented methods makes \( ^{31}\text{P} \) MR spectroscopy a very useful tool for investigations of phospholipid metabolism.