1. **Abstract**

Ozone demonstrates strong oxidizing properties, has toxic effect on living organisms, causes damage to cell membranes. Ozone therapy improves microcirculation of blood with regard to tissues affected by hypoxia as well increases the level of cellular antioxidants, stimulates the immune system and has a beneficial effect on inflammatory reactions. Microparticles (MP) are spherical fragments of cell membranes with diameters ranging from 0,1 to 1.0 μm released from eukaryotic cells to the extracellular space. Formation of MP occurs during maturation, activation and apoptosis of a cell. There is no cellular core in microparticles and they don’t synthesize their own proteins, however, they may contain biologically active molecules, i.e. enzymes, membrane proteins, genetic material, adhesive proteins, coagulation factors and membrane lipids. All of them take part in regulation and coordination of many processes occurring within the organism, i.e.: inflammatory processes, neoangiogenesis, coagulation and fibrinolysis. Microparticles transmit signals between cells, they constitute a connection between the coagulation process and local inflammation. They also play an important role in the progression of cancer.

Ozone therapy has demonstrated a positive impact on the process of wound healing in patients with diabetes and peripheral artery diseases, increase of renal perfusion, healing of the dry AMD, improvement of motor functions of upper limbs after a stroke, beneficial changes in lipid metabolism and stimulation of the antioxidant system after a heart attack, alleviation of symptoms of asthma. Research on the outcomes of ozone effects on blood is inconclusive. There aren’t any reports on the impact of ozone on the creation of microparticles.

The aim of the research has been to assess the impact of whole blood ozonation on:

* + 1. The release of erythrocyte-derived microparticles (EMP), leukocyte-derived microparticles (LMP), endothelial-derived microparticles (ŚMP) and platelet-derived microparticles (PMP),
    2. The selected parameters of the hemostasis system.

Venous blood has been selected as the research material and subsequently collected from 20 healthy 25-35 year-old men, who weren’t on any medication at that time. 80 ml of blood has been collected to a container containing the solution of sodium citrate, phosphate and dextrose. After the blood has been collected, it’s been divided into three batches: the first one has been treated with air (P group), the second with 30 µg/ml of ozone (O1 group) and the third one with 60 µg/ml of ozone (O2 group). Blood that hasn’t been subject to aeration and ozonation constituted the control group (K group). A mixture of ozone and oxygen has been produced from medical oxygen in ATO-3 MINI ozone generator (CryoFlex, Poland) directly before blood ozonation. Ozone concentration in the gas mixture came to 30 µg/ml. Blood has been mixed with a single 10 or 20 ml dose of the mixture of ozone and oxygen or with 20 ml of air. Blood samples for flow cytometry and for the examination of the haemostasis system have been collected from each container before gas treatment and directly after ozonation/aeration. Samples for the examination of haemostasis have been centrifuged at the speed of 2000 × g for 10 min to acquire blood plasma. In order to mark the amount of MPs, blood samples have been centrifuged for 15 minutes at the speed of 1500 x to separate the morphotic elements. The supernatant has been centrifuged for 2 minutes at the speed of 13000 x g. Subsequently, the number and type of supernatant’s microparticles has been determined. Derivation of microparticles has been established on the basis of surface antigen expressions which are typical for cells that create those microparticles: erythrocyte-derived microparticles - CD235+, platelet-derived microparticles - CD 42+, leukocyte-derived microparticles - CD45+, MP endothelial-derived microparticles – CD144+. Samples have been analysed using the Facscalibur flow cytometer (Becton Dickinson, USA). APTT has been measured using the Hemostat APTT-EL reagent. Measurement of the PT time has been carried out with the use of Hemostat Tromboplastin SI reagent. Concentration measurement of fibrinogen has been determined with the use of the Hemostat Fibrinogen kit based on the Clauss method. Concentration of D-dimers has been determined using the qualitative test of the VIDAS system for immunoenzymatic marking of fibrin degradation products with the use of immunofluorescence technique (BioMerieux). Data analysis has been carried out with the use of STATISTICA 6.0.

Groups treated with ozonation have indicated statistically significant increase of D-dimers concentration in comparison with the control group and the P group. The prolonged APTT of both groups treated with ozone in comparison with K and P groups has been statistically significant but small and hasn’t exceeded the scope of the standard. The prolonged PT has reached statistical significance only in the group treated with 60 µg/ml of ozone, in comparison to the K group, the group subject to aeration, as well as the group treated with 30 µg/ml of ozone. A statistically significant decrease of fibrinogen has been noted in the groups treated with ozone, O1 and O2, in comparison to the K and P groups. Decreased concentration of fibrinogen in the O2 group when compared to the O1 group has also been statistically significant. Both groups treated with ozone, in comparison to the K and P groups, showed statistically significant increase in the number of PMPs and EMPs. The increased number of PMPs and EMPs in the O2 group when compared to the O1 group has also been statistically significant. Both groups treated with ozone and the group treated with air showed statistically significant increase in the number of EMPs and LMPs in comparison to the K group. Moreover, the increased number of EMPs in the O2 group in comparison with the P group has also been statistically significant just like the increase in the number of LMPs in the O1 and O2 groups when compared to the P group. The difference in the number of LMPs in the O1 and O2 groups has also been statistically significant.

The carried out own research provided evidence that blood ozonation results in the proportional to the ozone dosage release of microparticles from erythrocytes, leukocytes, platelets and even from endothelial cells. Moreover, blood ozonation results in the extended APTT and PTT as well as decrease of fibrinogen concentration and increase of d-dimers concentration.

Observations and conclusions:

1. Blood oxidation inhibits coagulation and activates fibrinolysis.
2. Blood oxidation increases the number of microparticles generated from blood and endothelial cells.
3. Increase in the amount of microparticles generated from blood and endothelial cells is in direct proportion to the ozone dosage.
4. Amounts of microparticles generated from individual types of blood cells and endothelial cells as a result of ozone treatment correlate with each other
5. Due to the impact of blood oxidation on the release of microparticles and the haemostasis system it seems that in the case of using blood saturated with ozone for transfusions it is necessary to consider the condition of the patient’s haemostasis system, and in particular to assess the thromboembolic risk.