

Assessment of effectiveness and tolerance of polysaccharide extracts from medicinal mushrooms Podkovičník MIX (Black hoof mushroom, *Ganoderma lucidum*, *Grifola frondosa* and *Agaricus blazei* Murill) in treatment of oncology patients.



Clinical study



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Tested nutrition supplement:

Podkovičník MIX

Date of clinical study:

March 2009 – June 2009 (13 weeks)

Clinical study performed under guidance of:

Žitnooravská onkologická iniciatíva, o.z.
Oncology Clinic of the hospital NsP Dunajská streda,
a.s.
Management of the hospital NsP Dunajská Streda, a.s.
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Assessment of effectiveness and tolerance of polysaccharide extracts from medicinal mushrooms Podkovičník MIX (Black hoof mushroom, Ganoderma lucidum, Grifola frondosa and Agaricus blazei Murill) in treatment of oncology patients.

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Pre-clinical studies of medicinal mushrooms extracts clearly prove their direct antineoplastic, immunomodulation, anti-inflammatory effect and therefore our civic associations decided in close cooperation with the oncology clinic of the hospital in Dunajská Streda to test their assumed supportive effect on oncology patients during anti-tumour treatment. 40 patients with histologically verified oncology disease in III. and IV. clinical stage according to UICC 2002 were included in the clinical study. 38 patients were treated with specific oncology treatment (chemotherapy, molecular target treatment, radiotherapy), 2 patients were set to the best supportive care. The study took place in two randomization branches (Podkovičník MIX – mixture of mushrooms Black hoof mushroom, Ganoderma lucidum, Grifola frondosa and Agaricus blazei Murill versus placebo/maize starch, hereinafter as PODKOVIČNÍK MIX). The clinical monitoring was doubly blinded and took three months, during which we monitored in 4-week intervals changes of the life quality and total health condition of these patients, the effect of mushrooms extracts on bone marrow regeneration during the oncology treatment, changes of nutritional conditions of patients, changes of cardiovascular risk and internal environment.

After assessment of results we proved the reduction of risk of occurrence hematologic undesirable effects of anti-tumour treatment (especially chemotherapy, molecular target treatment and radiotherapy), we also determined in the PODKOVIČNÍK MIX branch the improvement of objective parameters of nutritional condition of patients. A global improvement of life quality and social functions of oncology patients occurred in the PODKOVIČNÍK MIX branch. The category of emotional functions was also improved. After assessment of the specific oncology treatment effect (restaging of health conditions), we determined that the PODKOVIČNÍK MIX branch provided a significantly larger number of the so-called large treatment responses (CR, PR, SD).

Introduction.

Mushrooms have been used in the traditional medicine of Japan, China and Korea for several millennia. Their joint feature is with regard to the content substances a significant representation of proteins, vitamins of B group and vitamin D, trace elements and unsaturated fatty acids. It is mentioned that mushrooms have positive impact on the increased blood sugar and cholesterol level; they improve the blood circulation, optimise functioning of immunity system and generally decrease the risk of cancer. They are rather rarely found in nature, they can be found in Asia and South America. These mushrooms are currently grown under controlled conditions and used for treatment purposes. Authors of this report stated in the introduction part scientific reasons for the performance of this clinical study.

Direct anti-tumour effect and their successful application as a supportive means in chemotherapy and radiotherapy of tumours have been published in numerous, mainly Asian, scientific studies. Chinese and Korean scientists proved the anti-tumorous effect of extracts from medicinal mushrooms, preferably from the hoof mushroom, on carcinoma of skin, lungs, colorectum and prostate. According to recent information from United States, this effect was proven also in case of the breast carcinoma, where it was determined that polysaccharides from this mushroom block the AKT enzyme, which stimulates the growth of tumour cells. Japanese scientists also proved the direct anti-tumour activity on tumour cultures (mouse S-180 cells of solid carcinoma). Water solutions of extracts from the Agaricus family reduced the population of tumour cells by 47.7%, extracts from the hoof mushroom by as much as 67.6%. It has been determined that this massive anti-tumour effect functions on the basis of **anti-angiogenesis** (reduction of formation of new tumour blood vessels) and subsequent stasis of growth of the primary tumour centre, further on the basis of **anti-metastatic** potential of these mushrooms, what prevents the creation of secondary tumour centres. The anti-tumour effect is dominantly ascribed to substances of **polysaccharide structure** (1-3- β -D glucans, neutral D-glucans, lentinan), to polypeptides, sesquiterpenes, polyenes and purine nucleosides.

The **immunomodulation effect** of extracts from medicinal mushrooms is based on the activation of phagocytosis and pinocytosis, increase of creation of TNF, IL-1, oxygen and nitrogenous radicals (H₂O₂, NO₂, NO) in macrophages, increase of creation of lymphotoxin (LT), which eliminates tumour cells directly and – last but not least – on the basis of increase of MIF creation in macrophages. This factor is known for its pivotal role in the so-called later hypersensitive defence reaction against tumour cells.

The **supportive effect** of these extracts during the oncology treatment has been originally assumed to be on the basis of works proving stimulation of stem progenitor cells in bone marrow (support of marrow regeneration during the treatment), on the basis of support of detoxication activity of liver and kidneys (support of biodegradation of system treatment). Extracts from these mushrooms have massive **anti-oxidation effect** dominantly at the level of support of the system of superoxide-dismutase (SOD). Their **global roborating effect** with positive change of the life quality of patients (nutritional conditions, weakness, loss of appetite, moodiness) is also indispensable.

Tumour diseases are chronic degeneration diseases, same as diabetes mellitus, arterial hypertension, ischemic heart disease, conditions after sudden brain events and therefore often occur synchronously in patients. Extracts from medicinal mushrooms have positive effect also on glycolipid metabolism by stimulating residual secretion of insulin from pancreas, increase of peripheral utilization of glucose, reduction of the value of total cholesterol, LDL fraction and triglycerides in blood. Their anti-sclerotic effect and support of regeneration of brain structures in cases of brain haemorrhage and brain ischemic ictus is also well mapped.



Clinical study



Methods and patients.

We used the product of the investigator/committer (PROVITEX s.r.o., Komárno) named Podkovičník MIX (hereinafter as PODKOVIČNÍK MIX) for the clinical monitoring. The product is officially registered in the territory of the Slovak Republic as a nutritional supplement and contains extracts from mushrooms Black hoof mushroom, *Ganoderma lucidum*, *Grifola frondosa* and *Agaricus blazei* Murill. The project executor was the civic association Žitnoostrovná onkologická iniciatíva. After the previous approval of the Ethical Committee of the hospital and after written information of patients and their informed consent, the project was performed at the Oncology Clinic of the hospital NsP Dunajská Streda, a.s. Recruitment of 40 oncology patients took place from 2.2.2009 until 2.3.2009. Selection and inclusion of patients into the clinical study was systematic, according to previously set algorithm. The inclusion criteria included age over 18, histologically confirmed advanced and/or metastatic tumour (clinical stage III., IV. according to UICC 2002), patient with or without specific oncology treatment, patient with overall health condition ECOG 0-2 event., Karnofsky index 70% and above, with suitable hepatic and renal functions. We chose 20 women and 20 men with age structure 37 – 79 years (median 62 years), 21 of which was in the III. clinical stage of disease and 19 in IV. clinical stage. 38 patients underwent specific oncology treatment (chemotherapy, molecular target treatment, radiotherapy). 2 patients were treated with the best supportive care and did not undergo any specific oncology treatment for the last 6 months before inclusion into the study. After fulfilment of these criteria, we received from the investigator (committer) on 2 March 2009 80 packages of the product (1 package contained 500 pieces of red capsules, total number of capsules was 40.000 for the whole study and 1.000 pieces for an individual during the study). 40 packages contained an intervention mixture of PODKOVIČNÍK MIX and 40 packages placebo with maize starch. All packages and pills were of homogenous identical design and individual packages were marked with random serial numbers (490 520-559). The table containing a code of double blinding was handed over to the management of the hospital NsP Dunajská Streda in a sealed envelope. The envelope was opened only after the completion of the period of clinical monitoring. We launched the study on 9.3.2009 after performance of input examinations. The active period of taking the tested substance ended on 9 June 2009. We continued to monitor patients until 2.7.2009. The dosing scheme was on the pyramid basis (gradually increasing) and that 3 days 3x1 cps á 500 mg, 3 days 3x2 cps á 500 mg, 3 days 3x3 cps á 500 mg, from the tenth day until 9.6.2009 4x3 cps á 500 mg in daily defined dose, always 20 minutes before meal. The distribution of tested packages took place by a simple random selection in form of concealed



image No. 1 method of electro interstitial somatography

randomization (the analysis of randomized branches of study see below).

We made examinations of the health condition of included patients (assessment of subjective condition, body weight, electro interstitial somatography, display diagnostics) in 4-week intervals during the 13-week monitoring.

The method of electro interstitial somatography (**EIS, image No. 1**) is a non-invasive examination that was – after successful registration studies – categorized by the European Union in 2006 as a medical tool of IIA category. It operates on the basis of a unidirectional current with voltage of 1.28V and 500-800 microA on the grounds of the so-called chronoamperometry, that provides image of activity of various body organs. The target space of EIS measurement is the inter-cell (interstitial) compartment of tissues of individual organs. Ions situated within the inter-cell space are decisive for the acid-alkali balance, but a sample cannot be taken from this space and thus the concentration measurement shall be done by a procedure that enables in vitro measurement. That is nothing else than chronoamperometry based on results of research of F. G. Cottrell (1877-1948) (**image No. 2**).

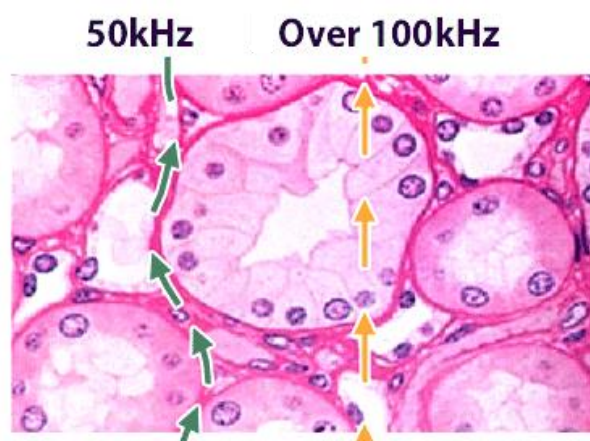


image No. 2 direction of the unidirectional current (green arrow) during EIS measurement, direction of alternating current (orange arrow) goes through the whole tissue structure

EIS is based on new principles of electronic measurement – computer adaptation of chronoamperometry – which was developed by Dr. Maarek, professor of neurology from the Paris Medical University, on the grounds of his more than 20-year long research. This measurement procedure is very quick (app. 3 minutes) and is non-invasive. Computer analyses during this period approximately 3 million of parameters, after assessment of which it makes a textual message and 3D graphically (**image No. 3**) provides very accurate image of the functional condition of internal organs of the patient. The main contribution of this examination with regard to the study was the dynamic monitoring of the actual condition of individual patients. We were able to detect the status of tissues of organ system with this device with each measurement at the level of acid-alkali balance, oxidative stress and free radicals, presence and level of acute and/or chronic inflammation of tissues during the oncology treatment, degree of organ damage risk, nutritional status according to the bioelectric impedance analysis (**BIA, image No. 4**) etc.



image No. 3 3D depiction of the EIS tissue scan

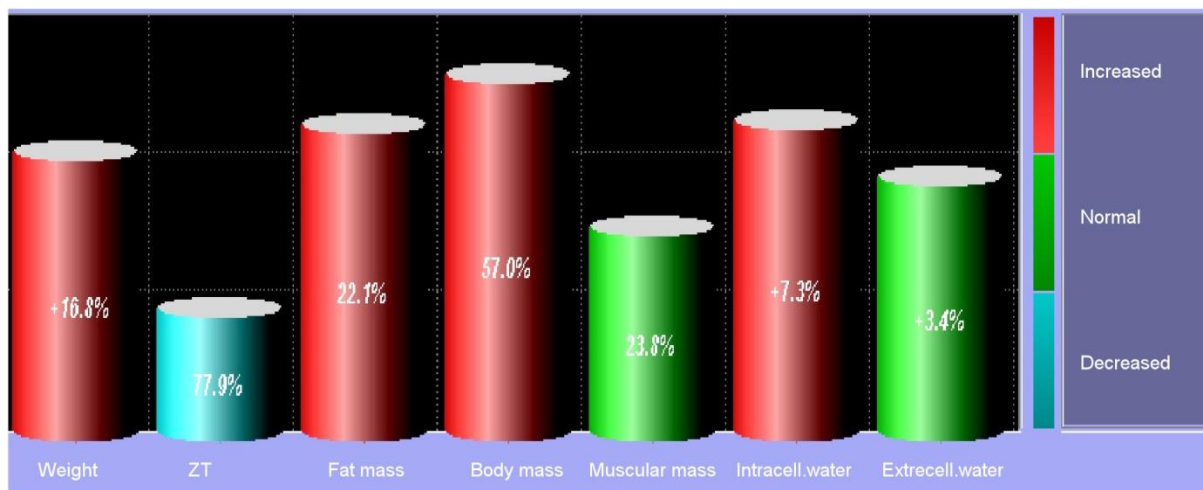


image No. 4 BIA (bioelectric impedance analysis) of body fluids

The assessment system of clinical monitoring were: 1. overall health condition of patients before, during and after the completion of the study (Karnofsky index), 2. assessment of subjective symptoms of patients dynamism (EORTC QLQ-C30



Clinical study



questionnaire system), 3. evaluation of objective disease indicators (RECIST NCI criteria from the year 2000 for the RR assessment: staging and restaging of disease, depiction diagnostic, dynamism of oncomarkers, weight, nutritional condition), 4. assessment of

undesirable effects of the official oncology system treatment (WHO criteria from the year 2000), 5. regular monitoring of internal environment of patients' organisms by means of electro interstitial scanning.

Objectives of clinical monitoring.

Primary aims of the study were the immunomodulation of patients during the oncology treatment and/or during the best supportive care, increase of effect of chemotherapy and/or molecular target treatment, reduction of undesirable effects of chemotherapy and/or molecular target treatment, improvement of daily life quality of an oncology patient. **Secondary aims of the study:** utilization of anti-metastatic and anti-angiogenic potential of polysaccharide structures from medicinal mushrooms in the treatment of oncology patients, monitoring of effect of the tested substance on the level of the cardiovascular risk, glycolipid profile of patients and parameters of oxidative stress.

Analysis of randomized branches

DESCRIPTION OF RANDOM. BRANCHES	PODKOVIČNÍK MIX branch (n = 19)		PLACEBO branch (n = 19)	
GENDER	No.	%	No.	%
men	8	42	11	58
women	11	58	8	42
GENDER				
age structure	39-79		37-74	
median	53.3		52.7	
UICC				
III. stage	8	42	12	63
IV. stage	11	58	7	37
ONCOLOGY TREATMENT				
chemotherapy	17	89	19	100
target treatment	7	37	5	26
radiotherapy	1	5	2	10
no treatment	2	10	0	0

Table No. 1

date of disclosure of randomized branches: 9.7.2009 (management of the hospital NsP Dunajská Streda, a.s.)

chemotherapy (paclitaxel, docataxel, gemcitabin, irinotecan, topotecan, epirubicin, cisplatin, carboplatin, oxaliplatin, 5-FU, capecitabin)

molecular target treatment (bevacizumab, trastuzumab, erlotinib, sunitinib)

radiotherapy (EXRT of chest, axial skeleton)

Results.

Changes of blood count values.

Leucocytic line.

We recorded an average decrease of white blood elements (total number of leucocytes by 8%, absolute number of neutrofiles by 18% and number of lymphocytes by 20%) in the placebo branch. We were forced to postpone the system treatment of 4 patients in this group (21% of the placebo group) and to treat the infection of upper and lower respiratory passages (3 patients, 16%) and we were forced to hospitalize one patient at the medical department due to a febrile neutropenia episode (5%).

On the contrary, the total number of leucocytes increased by 15%, number of neutrofiles by 14% and number of lymphocytes by 22% in the PODKOVIČNÍK MIX branch. We did not detect an intercurrent infection in this branch, we were not forced to postpone the system treatment cycle of any patient and we did not record any case of febrile neutropenia. We show the comparison of values of absolute number of white blood elements (WBC leucocytes, NEUT neutrofiles, LYMF lymphocytes) in both randomized branches in **the table No. 2.**

Clinical study

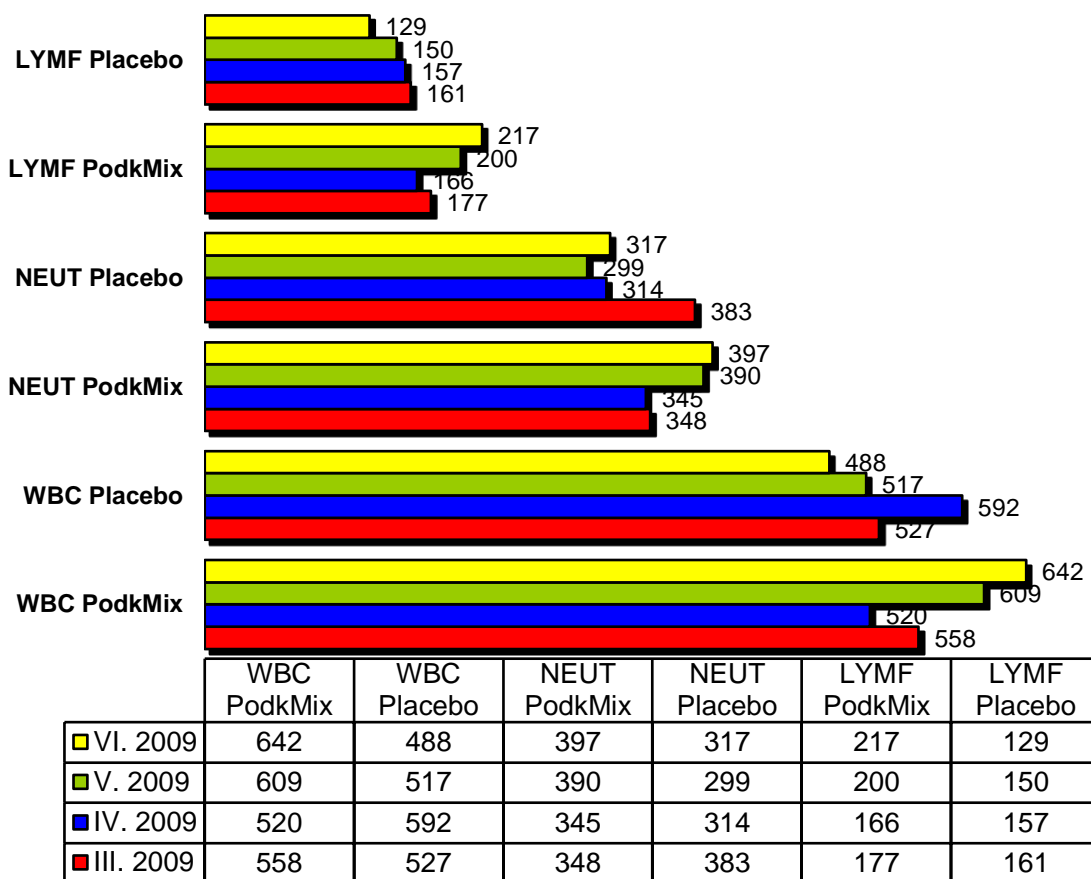
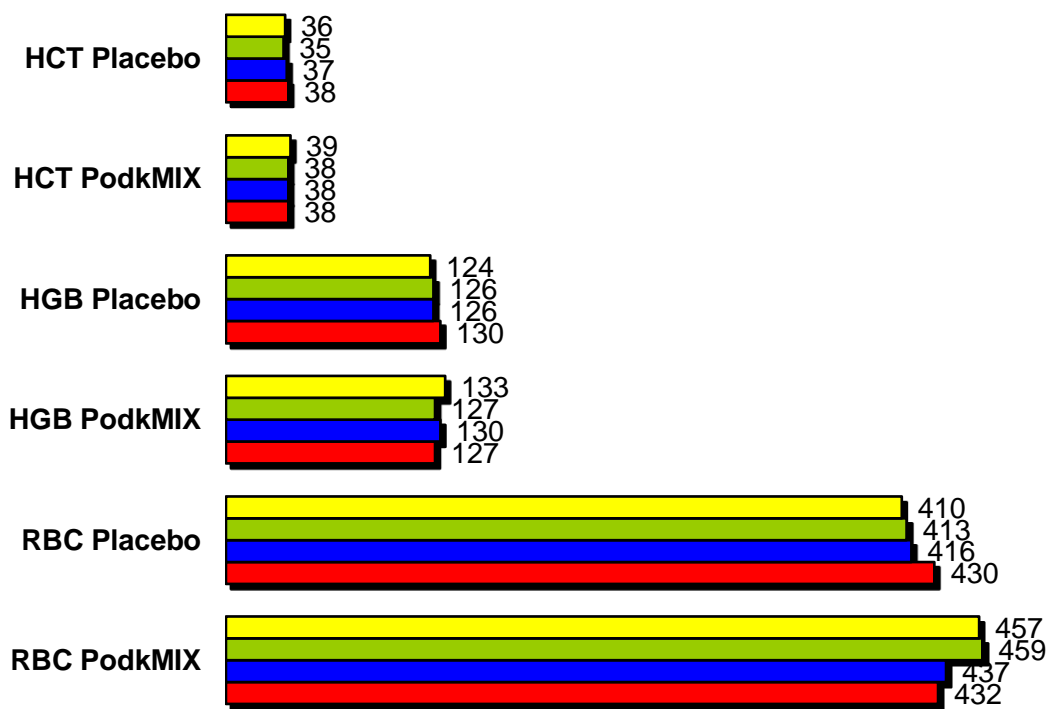


Table No. 2 analysis of values of leucocytic line in randomized branches.

Erythrocytic line.

We recorded in the placebo branch an average decrease of red blood elements, and that the total number of erythrocytes by 5%, value of haemoglobin by 5% and relative value of the haematocrit fell by 5%, whereas – during the clinical monitoring – we recorded a decrease of these elements of more serious grade (gr. 3-4) of 3 patients, requiring institutional care and repeated transfusions of erythrocytic masses, subsequent compensation with growth factors. The PODKOVIČNÍK MIX branch showed also a discrete positive change of these parameters, rather with a stabilization of all components (increase of the number of erythrocytes by 5%, haemoglobin by 4%, haematocrit by 4%), but we did not record any acute haematological insult in this group of patients. We were not forced to postpone the system treatment cycle, these patients did not need transfusions or erythrocytic growth factors. We show the comparison of values of absolute number of red blood elements (RBC number of erythrocytes, HGB value of haemoglobin, HTC haematocrit) in both randomized branches in **the table No. 3.**

Clinical study



	RBC PodkMIX	RBC Placebo	HGB PodkMIX	HGB Placebo	HCT PodkMIX	HCT Placebo
VI. 2009	457	410	133	124	39	36
V. 2009	459	413	127	126	38	35
IV. 2009	437	416	130	126	38	37
III. 2009	432	430	127	130	38	38

Table No. 3 analysis of values of erythrocytic line in randomized branches.

Thrombocytes.

Differences in this category were more significant. As compared to the control group, where we recorded an average decrease of the number of thrombocytes by as much as 19%, the PODKOVIČNÍK MIX branch showed on the contrary an increase by 7%. The course of the clinic monitoring showed 4 patients (21%) in the control group to have a decrease of the number of thrombocytes, 3 of which had a more serious one (gr. 3-4), in 1 case also with clinical correlate (epistaxis gr. 3), whereas in the PODKOVIČNÍK MIX branch we did not postpone any system treatment cycle, there were no haemorrhagic expressions and no decrease of thrombocytes of lower grade (gr. 1) was recorded. We state the comparison of values of the absolute number of thrombocytes (PLT) in both randomized branches in **the table No. 4**.

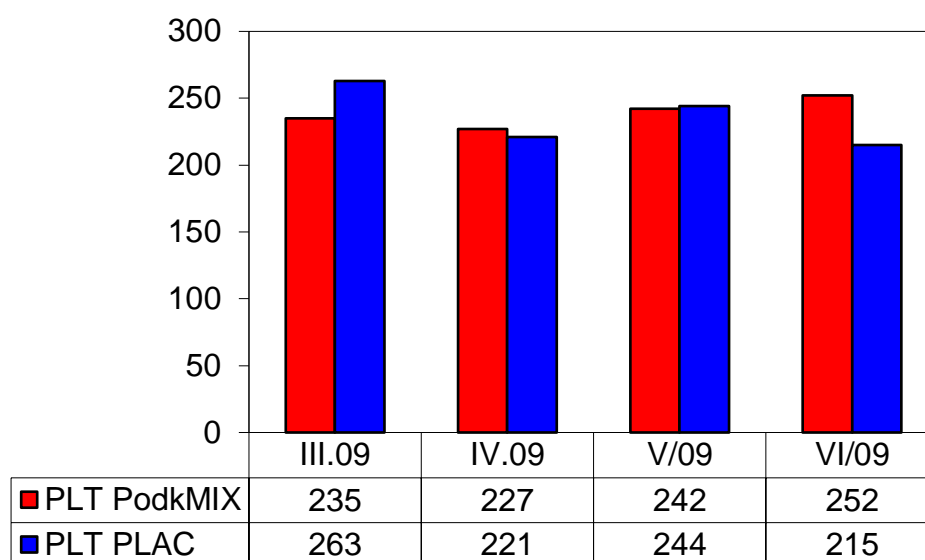


Table No. 4 analysis of values of thrombocytes in randomized branches.

Changes of nutritional condition of patients.

The cancer-induced weight loss (CIWL) is a non-remitting loss of weight due to metabolic abnormalities caused by the presence of tumour. It is not a simple caloric deprivation, but a consequence of tumour-synthesized induction paraneoplastic factors, as a result of which – apart from the decrease of caloric income – a degradation of proteins and muscle mass occur and therefore also of the total body weight without fat. And the loss of weight of an oncology patient adversely influences his/her living.

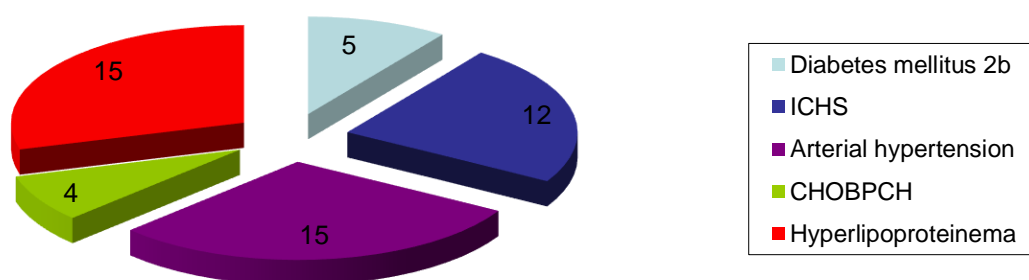
We monitored our patients – together with a regular measurement of the actual body weight – also with the bioelectric impedance analytical module EIS during the clinical study (changes of BMI parameters, % of body fat, % of muscle mass, % of body

mass above the ideal body weight, volume of body fluid in intracellular and extracellular compartments of patient's organism).

The analysis of results showed that patients in the PODKOVIČNÍK MIX branch, who had taken a total of 1000 pieces of extract from the above mentioned mushrooms, not only added on total body weight (average weight addition 4.25 kg with variance of 2-7 kg), but, more importantly, also the net muscle mass (increase of muscle mass by 4.07% with variance of 3-5.6%). The control branch showed weight loss by 3.9% (variance from -12% to + 1.8%). We did not record any such effect of muscle addition of patients in the control branch taking placebo (-1.5%).

Changes of lipid spectrum.

As the spectrum of clinically serious non-oncology co-morbidities of included patients (images No. 5 and 7) was known from the start and sufficiently presented from the quantitative point of view, we included among secondary aims of this clinical study also some selected parameters of cardiovascular risks (glucose, total cholesterol, LDL, HDL, triglycerides, homocysteine level, vitamin B₁₂ and blood folate levels; we also monitored with EIS – on the grounds of Davenport diagram – their changes in pH values, excess bases, HCO₃ level, pO₂ in interstitial compartment). **Only the following parameters were statistically assessed: 1. total cholesterol in blood, 2. triglyceride level in blood, 3. homocysteine level in blood.** A correlation of values between the intravascular and interstitial compartments, together with clinical indicators of individual patients, requires a deeper analysis.



image

No. 5: structure of co-morbidities signaling cardiovascular risk in the PODKOVIČNÍK MIX branch (from 19 patients)

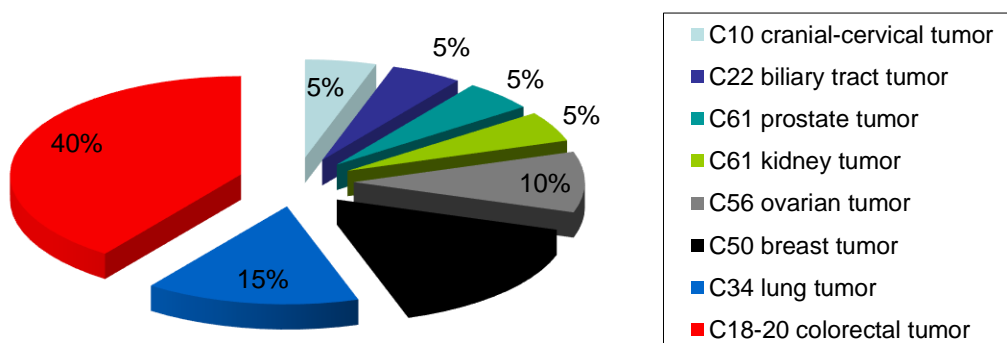


image No. 7: structure of oncology diagnoses in % in the PODKOVIČNÍK MIX branch

We determined in the **PODKOVIČNÍK MIX group** of patients, where 5 patients (26%) from the group suffered from 2nd type of diabetes and as much as 15 patients (79%) were treated for hyperlipoproteinemia and various grades of arterial hypertension, as much as 14% decrease of the total output cholesterol in blood as compared to the input level (average value of input level 5.8 mmol/l, average value of output level of 19 patients in this branch was 4.97 mmol). We noticed this tendency also in serum values of triglycerides, where we recorded the decrease by 20.1% (**table No. 5**). The **control group** with placebo showed only a stabilization of these markers (chol-c 1.12%, Tg 2.1%).

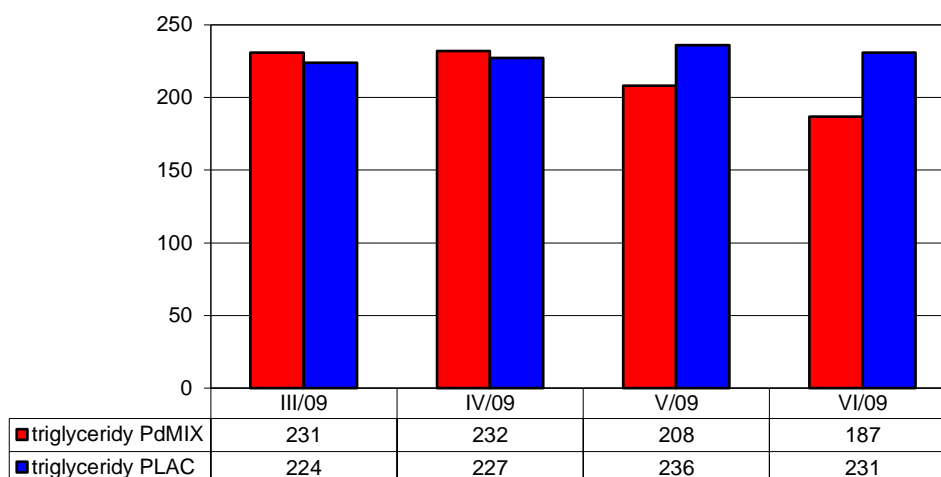


Table No. 5 analysis of values of serum triglycerides in branches.

The assessment of change in the level of **homocysteine** showed even more significant positive changes. We recorded an average reduction of this marker by as much as 34% in the PODKOVIČNÍK MIX branch, whereas an increase of the value of homocysteine in blood by 7% in the control branch (**table No. 6**).

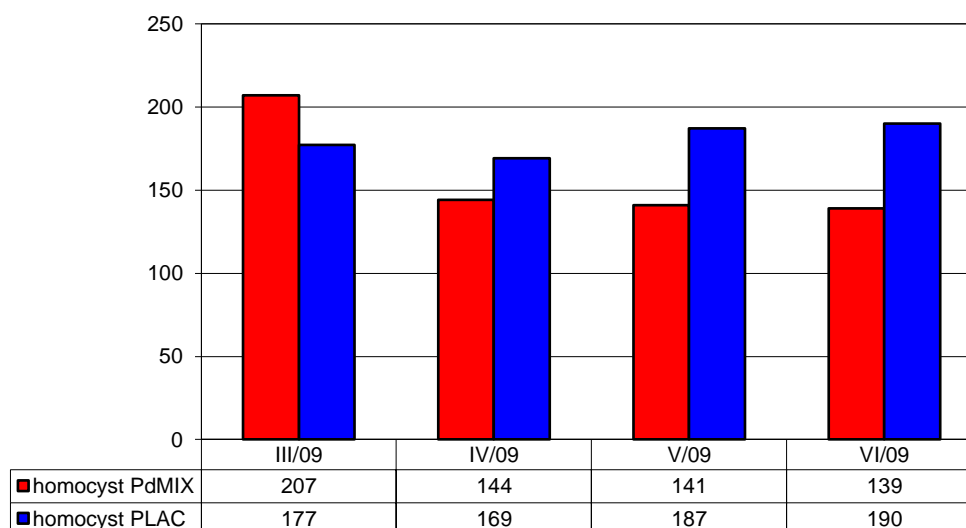


Table No. 6 analysis of values of serum levels of homocysteine in branches.

Changes of oncology diseases during monitoring.

The last secondary aim of the study was the objective monitoring of the condition of oncology diseases during the clinical study. As 94% of patients (36 from 38 patients, 2 excluded) were treated with specific oncology modalities, it was not possible to assess the direct anti-tumour effect of extracts from medicinal mushrooms in these cases (**images No. 6 and 8**).

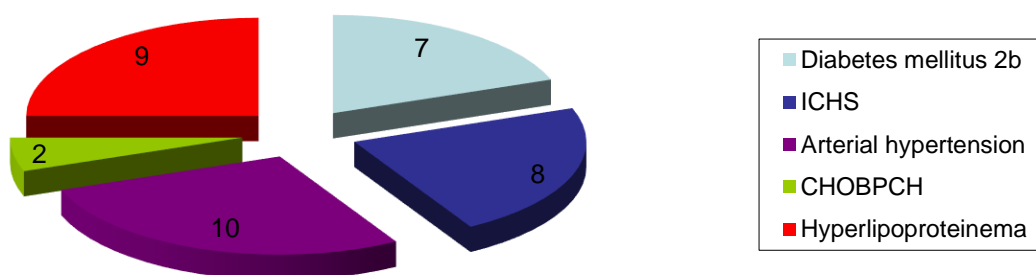


image No. 6: structure of co-morbidities in % in the PLACEBO branch

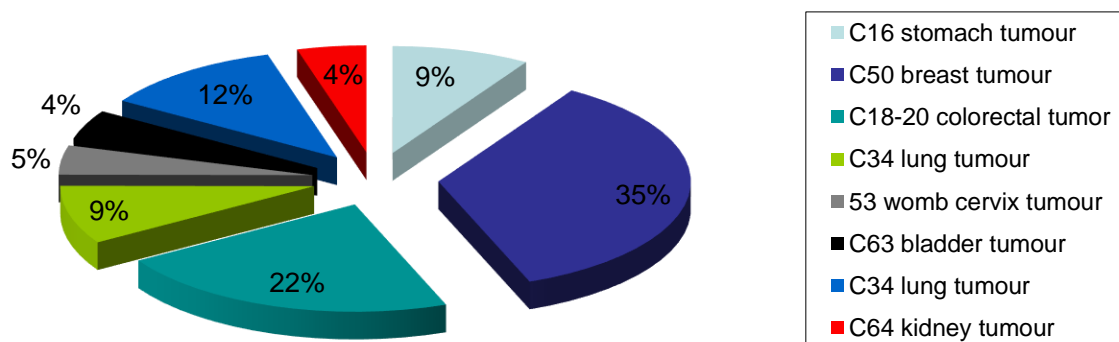


image No. 8: structure of oncology diagnoses in % in the PLACEBO branch

Despite that fact we found several remarkable matters of interest in the PODKOVIČNÍK MIX branch.

We had 11 patients in the IV. clinical stage in the PODKOVIČNÍK MIX branch, i.e. patients we treated for metastatic disease (58%). 6 patients from this group (5 patients with colorectum carcinoma C18-20 and 1 patient with breast carcinoma C50) were given – along with chemotherapy – also bevacizumab (Avastin), which is a monoclonal

antibody blocking angiogenesis of various solid malignities. We were curious whether the Podkovičník MIX gives potency to the effect of a synthetic angiogenic blockade. We recorded in this group of 11 patients 5 marker and size regressions of tumours (46%), significant decrease of the value of oncomarkers signalling a successful therapeutic effect), 4 marker and size stabilizations of tumours (36%) and the marker and size progression of only 2 patients (18%, increase of the value of oncomarkers signalling unsuccessful system treatment).

The **so-called large response to oncology treatment** is defined in the clinical oncology as a sum of all cases of complete and partial remissions, stabilizations of the disease in the respective set. In our case we recorded in the PODKOVIČNÍK MIX branch 9 large responses (81%, 5PR + 4 SD, without CR) and it is interesting that patients treated also with bevacizumab were among them (5 patients PR, 1 patient SD).

We had 7 patients in the control group in the IV. clinical stage (37%), 4 of which were treated with bevacizumab (3 patients with colorectum carcinoma and 1 patient with non-small-cell lung carcinoma). We recorded only one positive response among patients with the IV. stage (partial remission of disease) and 6 progressions of disease. End-points and comparison of both randomized branches is shown in the **table No. 7**.

DESCRIPTION OF RANDOM. BRANCHES	PODKOVIČNÍK MIX branch (n = 19)		PLACEBO branch (n = 19)	
	No	%	No	%
end-point analysis				
Patients with new recurrent disease	0	0	1	5
Patients with new metastatic lesions	2	10	4	21
Deaths	0	0	1	5
Overall patients with progression events, III.	0	0	0	0
Overall patients with progression events, IV.	2	18	6	85
Patients with the so-called large response in the IV. group (SD, PR, CR)	9	80	1	14

Table No. 7 end points of oncology patients analysis

We monitored 2 patients in the whole set of included patients, who were not treated with any specific oncology modalities during the last 6 months before inclusion into the study and which were after the disclosure of randomization branches shown to belong to the PODKOVIČNÍK MIX group of patients. As we also recorded remarkable moments with these patients (1st patient with head and neck tumour, IV. clinical stage: is in complete remission, 2nd patient with ovaries tumour, IV. clinical stage: we recorded a tendency to decrease of CA125 marker during the active taking), we decided to elaborate on these within casuistry. Casuistries are included in the attachment of this report.

Undesirable events.

Patients taking the Podkovičník MIX initially complained about borborygmus and increased flatulence, which were temporary, lasting for app. 6 – 8 days. We do not assess this effect as being an undesirable event, rather as a consolidation of intestinal dysmicrobia. However, due to this effect on the large intestine, we were forced to exclude one patient from the clinical study. It was a patient with the colorectum carcinoma with extensive carcinosis of visceral and parietal peritoneum, whose intestinal gasses distended curves of the large intestine and caused tenesmus similar to subileous conditions with absence of stool. One patient complained about intermittent pain in the epigastrium of mild level 1.5 – 2 hours after taking the Podkovičník MIX. Toxoallergic exanthema did not occur.

Conclusion and discussion.

Pre-clinical examinations with Black hoof mushroom, *Ganoderma lucidum*, *Grifola frondosa* and *Agaricus blazei* Murill (polysaccharide components of 1-3-β-D glucans) showed a direct anti-tumour effect on the grounds of anti-angiogenesis and anti-metastatic potential; further they had antioxidant, anti-inflammatory and immunomodulation effect. A positive effect on glycolipid metabolism was also proved, their anti-sclerotic effect on the arterial wall is also significant. These beneficial effects are ascribed to polysaccharide substances, especially beta-D-immunoglucans. Our civic

association in cooperation with the oncology clinic of the hospital NsP Dunajská Streda, a.s. decided to design a clinical study that would focus on the assessment of the effectiveness of these results under clinical conditions on a set of oncology patients. The main objective of this monitoring was not as much the proof of statistical significance of these hypotheses, as the conviction about the clinical benefit of oncology patients from taking extracts of medicinal mushrooms in practice.

40 oncology patients were included into the study, who were in III. or IV. clinical stage of the disease pursuant to the UICC. Their age variance was 37 – 97 years. We selected 20 men and 20 women for the group. We treated 38 patients (94%) with a specific oncology treatment (chemotherapy, so-called molecular target biological treatment and radiotherapy). 2 oncology patients had not underwent any such treatment during the last 6 months before inclusion into the study and were set for the supportive care. We chose a double blinded, placebo controlled randomization as the

clinical experiment. The division of testing packages took place with a simple random selection by form of concealed randomization. All packages and capsules were of homogenous identical design. Patients were taking the tested substance according to previously set dosing scheme with a gradual, so-called pyramid titration to a daily defined dose of 12 capsules á 500mg divided to 4 daily doses. The active period of study took 13 weeks (03-06/2009). We completed the clinical study at the beginning of July 2009 and subsequently on 9.7.2009 the randomized branches were disclosed. Results we found out during the clinical study and assessed after the analysis of randomized groups can be summarised as follows:

1. We confirmed the positive effect of Podkovičník MIX on the regeneration of bone marrow, dominantly at the level of the regeneration of leucocytic and thrombocytic line. The effect of Podkovičník MIX on the erythrocytic line was – as compared to the control group – stabilizing. We did not record in any case in the intervention branch an acute haematological insult, we were not forced to use leucocytic or erythrocytic growth factors, patients in this group did not need any blood transfusion and we did not need to postpone any system treatment cycle. On the contrary, in the control branch we had to manage all types of acute haematological episodes. We recorded clinically significant differences between randomized branches.
2. We confirmed the positive effect of Podkovičník MIX on the nutritional conditions of oncology patients during the specific oncology treatment. Patients taking Podkovičník MIX added on the total body weight by 4.5 kg (variance 2 – 7 kg), but also on net muscle mass (4.07%), whereas we recorded weight loss in the control branch by 3.9% and reduction of muscle mass by 1.5%. The reduction of the body weight, dominantly of muscle mass, is at the same time a neuralgic point of continuation of active oncology treatment and adversely influences also the living of an oncology patient.
3. We confirmed the positive effect of Podkovičník MIX on the lipid metabolism, parameters of oxidative stress and cardiovascular risk. As much as 79% of patients in the intervention PODKOVIČNÍK MIX branch had in their personal case history information about the failure of lipid spectrum and various forms of arterial hypertension, 26% of which were treated for the 2nd type of diabetes. We recorded in the PODKOVIČNÍK MIX group

a 14% decrease of the total cholesterol level and 20% decrease of the level of serum triglycerides as compared to input values. Even more significant changes were recorded in the level of homocysteine. We recorded an average reduction of the level of this marker in the PODKOVIČNÍK MIX branch by as much as 34%, whereas values of homocysteine in blood increased by 7% in the control branch.

4. We recorded remarkable changes in the PODKOVIČNÍK MIX branch also with regard to oncology diseases. We had 11 patients in IV. clinical stage of disease (58%) in this branch. We recorded the so-called large response in 9 patients (81%), whereas

5 patients had partial remission of the disease and we achieved stabilization of disease of 4 patients. An interesting thing is that among the above mentioned 9 patients were also the 6 patients we treated during the clinical study – along with chemotherapy – with bevacizumab, a monoclonal antibody blocking angiogenesis of various solid tumours. The control group contained 7 patients in the IV. clinical stage of the disease, whereof 1 patient achieved partial remission of the disease and we proved a progression of the disease of 6 patients. The progressing cases included also patients we treated with bevacizumab, therefore there is a reasonable suspicion of a synergy between the synthetic and natural form of angiogenesis, what could present a subject of another and more detailed clinical research.

5. The group Podkovičník MIX contained also 2 patients who were not treated with specific oncology modalities. The reason for this was also their disagreement with continued oncology treatment. Their story is described in detail in casuistries, because one of them got into a complete remission during the clinical monitoring and a significant decrease of cardiovascular risk was recorded for the second patient, where we determined a decreasing tendency in his marker values. We included these two patients into a strict observation and recommended them a change of dietary regime and continuation in therapy with this nutritional supplement.
6. We confirmed a total roborating effect of the Podkovičník MIX on oncology patients during the oncology treatment. We recorded in the intervention PODKOVIČNÍK MIX group an improvement of the total health condition (Karnofsky index) as compared to the control group, improved appetite during system treatment cycles, positive change in the daily frequency of stools, mitigation of algic manifestations (visual analogous scale of pain) and positive changes of patients' moods. We stated a global improvement of the life quality of patients taking Podkovičník MIX as compared to the control group at the end of the clinical monitoring.

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Reference values of blood parameters:

Blood count.

- total number of leucocytes 4-9 $10^9/l$
- erythrocytes 3.8-5.2 $10^{12}/l$
- haemoglobin 12-15 g/dl
- number of thrombocytes $10^9/l$

Biochemical parameters.

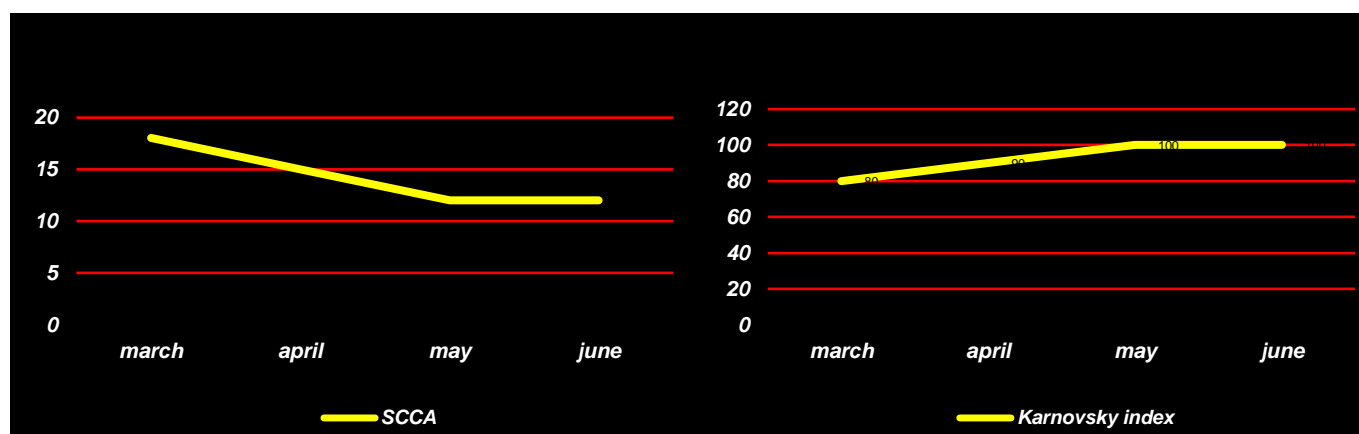
- total cholesterol in blood 2.8 – 5.52 mmol/l
- triglycerides in blood 0.45 – 1.82 mmol/l
- homocysteine in blood 5-17 $\mu\text{mol}/l$

CASUISTIC No. 1

This is a 58-year old patient – writer and journalist by vocation – strong smoker and regular consumer of alcoholic drinks, with positive family anamnesis of oncology disease (his mother died of stomach carcinoma). He has been treated for an ischemic heart disease with dysrhythmia since 1997, otherwise without any serious anamnesis.

The patient was taking antibiotics in June 2007 due to a painful tumescence of the left side of the neck. A circularly thickened oropharynx and a submandibularly solid formation with size 55x40mm on the neck were detected during the CT of his head and neck in the same month. A suspicion of MTS in lymphatic ganglia was expressed. A low differentiated epidermoid carcinoma of oropharynx with MTS into regional lymphatic ganglia, cTxN3M0, III. stage was histologically verified at the otorhinolaryngology clinic in September 2009. The patient took – within neoadjuvant treatment – 4 cycles of chemotherapy of TPF protocol (docetaxel + 5-FU + cisDDP) in the period between 11/2007 – 02/2008 at the oncology institute Onkologický ústav Sv. Alžbety, Bratislava (OUSA) and subsequently, at the same institute, in mediations 03-05/2008 a locoregionally concomitant radio chemotherapy to TD 56Gy with cisDDp 40mg/m² i.v. The patient was after these treatment modalities restaged (CT, MRI, SCCA) and consulted at the head and neck committee at the OUSA.

The committee assessed the condition as inoperable due to the direct contact of the residual tumour with large vessels (ACC I.sin.) and the patient was sent to the workplace of the regional oncology clinic in the hospital NsP Dunajská Streda, where we continued in the period 07-09/2008 with the system treatment (4 cycles docetaxel + cDDP + cetuximab biweekly). At the 2nd restaging – at MRI of the neck (4.12.2009) – a stabilization of a solidly cystic formation of the residual tumour with size 30x20mm was described as compared to MRI images from 06/2007 and 02/2008. As we achieved a marker (SCCA 1.8) as well as size stabilization of the disease, we were inclined to continue with the original treatment, but the patient refused this possibility and signed negative document. The patient was monitored at our clinic only in 1-month intervals from 12/2008 until 03/2009. He was included in the clinical study Podkovičník MIX on 1 March 2009.

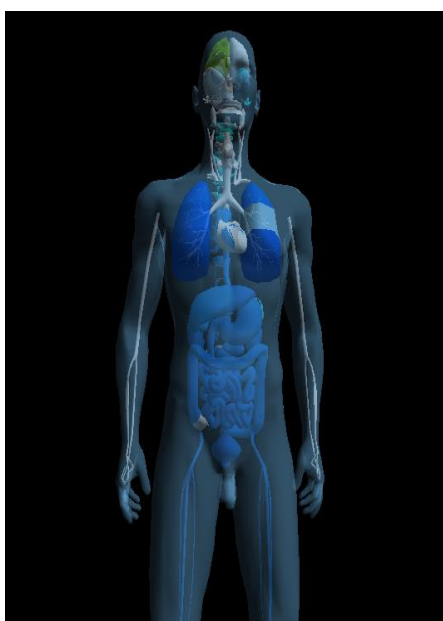


Clinical study

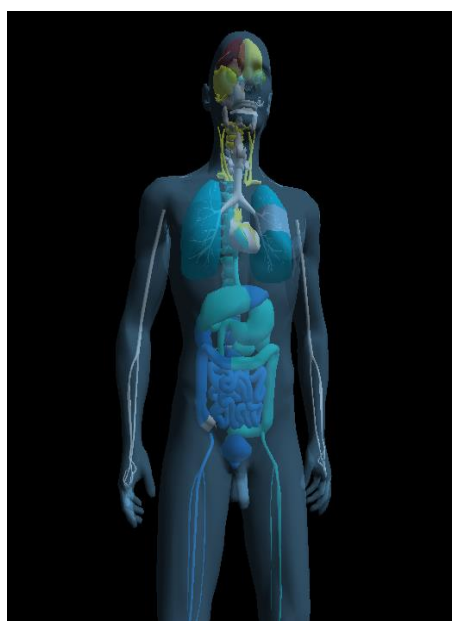


The daily quality of his life improved during the 13-week monitoring. Deglutination problems as well as appetite and subsequently his nutritional condition improved. The

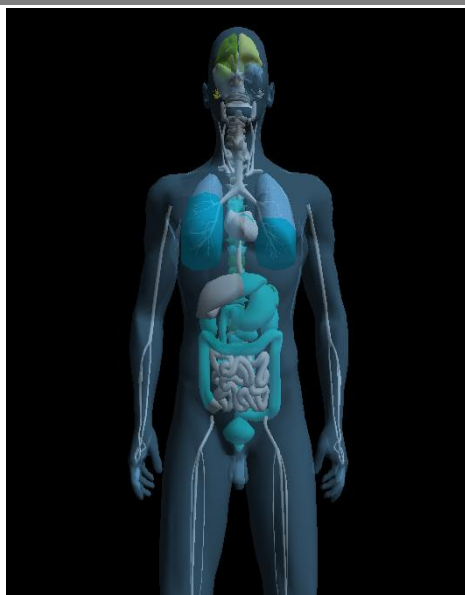
patient was initially extremely acidotic in interstitium (Davenport diagram: metabolic acidosis pH7.19, BE-11; the ABR correction was at the level pH7.27, BE-4 at the last EIS control). The extremely deep chronic inflammation in the interstitium of vital organs (up to the level of chronic degeneration with relative values – 85 to – 90) improved and – in some localities – completely normalized. We noted the normalization of the SCCA marker starting from the 8th week. We learned on 9.7.2009 – after disclosure of randomized branches – that he was taking Podkovičník MIX. On 10.7.2009 – at the control MRI of the neck – the radiologist did not describe any tumorous changes in the oro- and hypopharyngeal and in the area of large vessels (ACC I.sin.) only fibrotic changes were present. I assess the patient as a complete remission of the disease.



EIS 03/2009



EIS 04/2009



EIS 05/2009

CASUISTIC No. 2

This is a 65-year old diabetic on diet and PAD, hypertonic patient who has been treated for years also for an algic form of the ischemic heart disease with dysrhythmia (KES). Positive family anamnesis of both oncology and cardiovascular diseases (both sisters died of consequences of the breast carcinoma, mother of myocardial infarction). She walks with French crutches due to the lumboischialgic syndrome with right-side radiculopathy. Her active mobility is made more difficult also due to the extreme proportional obesity (weight 130kg, BMI 47.75 kg/m²).

A total hysterectomy with bilateral adnexectomy was performed at the Gynaecology clinic of the hospital NsP Dunajská Streda on 9.11.2005 due to the susp. tumour endometry (posit. hyst. curettage for metroragy for endometrial carcinoma). According to the post-chirurgic definitive histology, it was a duplex gynaecology malignity, namely an endometrial adenocarcinoma G1 without invasion into myometrium, without presence of lymphovascular invasion, pT1aNxMx, FIGO IA. The second malignity (random finding) was a serious papillary carcinoma G2 of the left ovary with invasion into vessels, with damaged case of the left ovary. The second operation for the purpose of making pelvic and paraaortal lymphadenectomy was not performed due to the extreme obesity and increased cardiovascular risk.

The patient was sent to our oncology clinic of the hospital NsP Dunajská Streda in January 2006. We made CT of chest, abdomen, MP with described axial LAP in retroperitoneum (paraaortally) and in mediastinum, marker CA125 1240 U/ml, within the fixation of the disease condition and completion of the proper staging. We gave within the first line palliative treatment (02-08/2007) 6 cycles of CHT (paclitaxel + carboplatinum) with achieving a complete remission that had lasted until 01/2008. Due to the elevation of CA125 elevation and reoccurrence of original tumorous LAP lesions

Clinical study

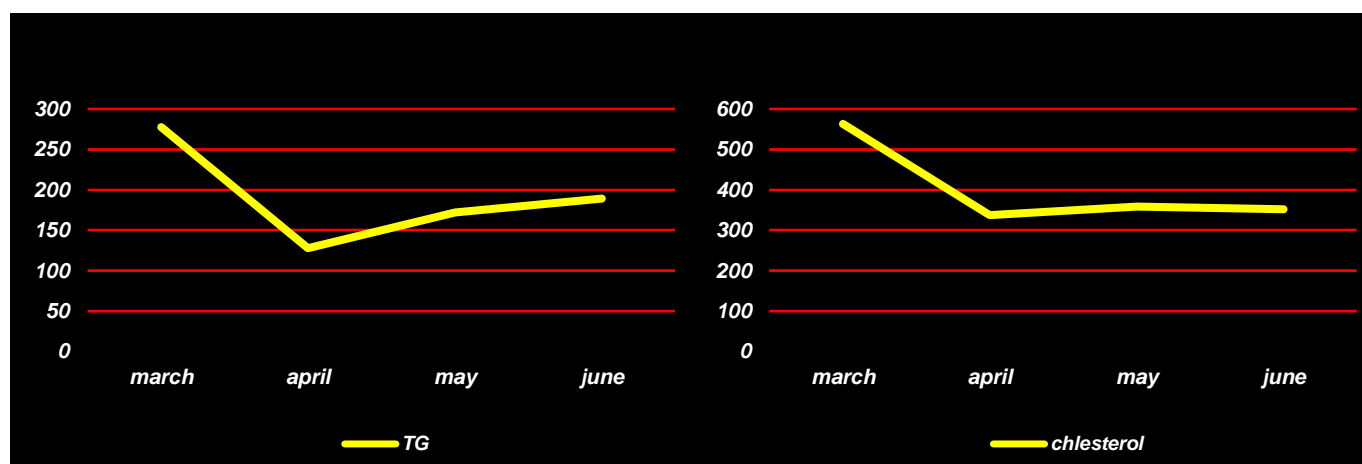


we gave another 3 cycles of the original first line mode without any effect. After the change of treatment (4 cycles gemcitabin + carboplatinum) we achieved another episode of complete remission (09/2008) that had lasted only until 10/2008 (CT, CA125). The patient did not agree with another chemotherapy. The disease was in time of signing the negative document localised in the area of mediastinum (LAP 30x35mm) and retroperitoneum paraaortally (LAP 38x33mm) and small pelvis (LAP in the area of iliac vessels 31x25mm). CA 125 between 800-1200 U/ml. She was included into the clinical study Podkovičník MIX on 23.2.2009.

There occurred **3 remarkable moments** in her clinical and paraclinical parameters during the 13-week monitoring. The **first** one was a significant improvement of the daily life of the patient: the depressive and querulous woman became a person of good mood and a psychically compensated personality. Pains of weight-bearing joints and lower areas of the axial skeleton were significantly mitigated (according to a visual analogous pain scale from 8-9 to 2-3 with visual improvement of active mobility). The stool frequency was also improved. On 9.7.2009 – after disclosure of randomized branches – it was learned that she was taking Podkovičník MIX.

She did not add on weight during the monitoring (she completed the study with 130 kg), what was typical for the group of other patients. The **second** significant moment occurred at the assessment of the period of clinical monitoring, namely the significant decrease of the value of total cholesterol (from 5.63 mmol/l to 3.52 mmol/l),

LDL fraction (from 3.17 mmol/l to 1.54 mmol/l) and values of serum triglycerides (from 2.78 mmol/l to 1.89 mmol/l) without her taking any hypolipidemics. Values of FW and CRP (from 37.7 mg/l to 7.27 mg/l) also decreased.

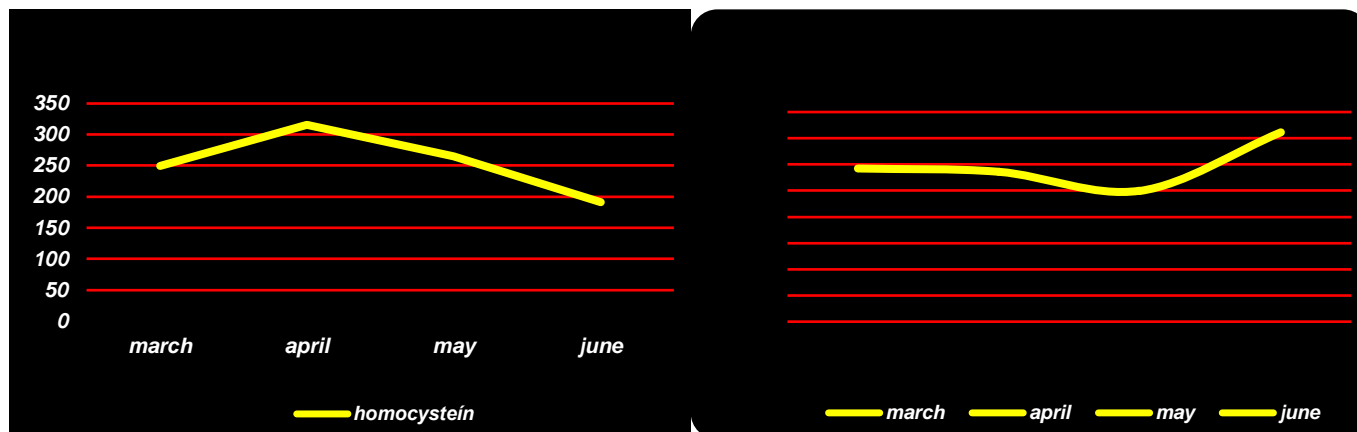


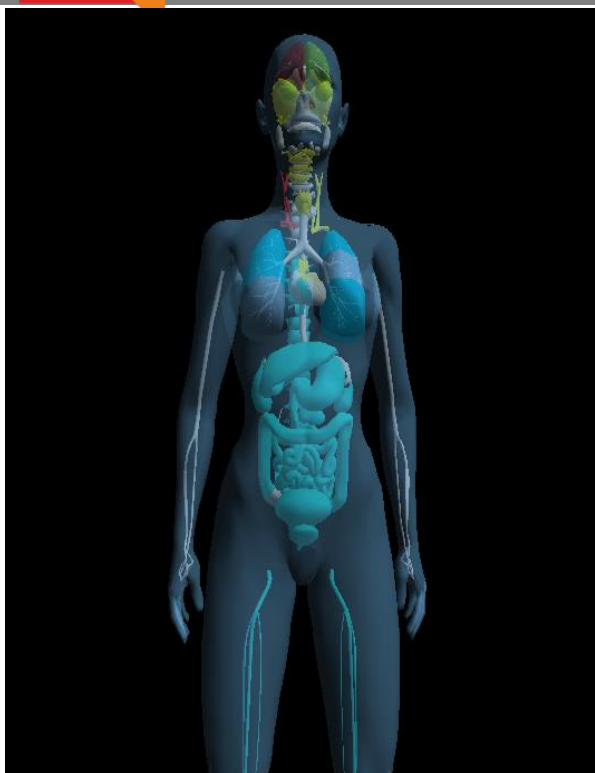
She had the starting concentration of homocysteine in blood 31.50 umol/l with output value 17.19 umol/l (standard is between 5-17- umol/l). She was extremely alkaline in interstitium (EIS, Davenport diagram: pH 7.43, BE +9.00 – metabolic alkalosis), but she has at her last EIS control the ipH 7.39 and BE +3.00 (the standard is pH 7.29-7.37 and BE -2/+2). The **third** moment was the dynamism of values of the CA125 oncomarker. The input value of CA125 was 1.168 U/ml, which during the active

Clinical study

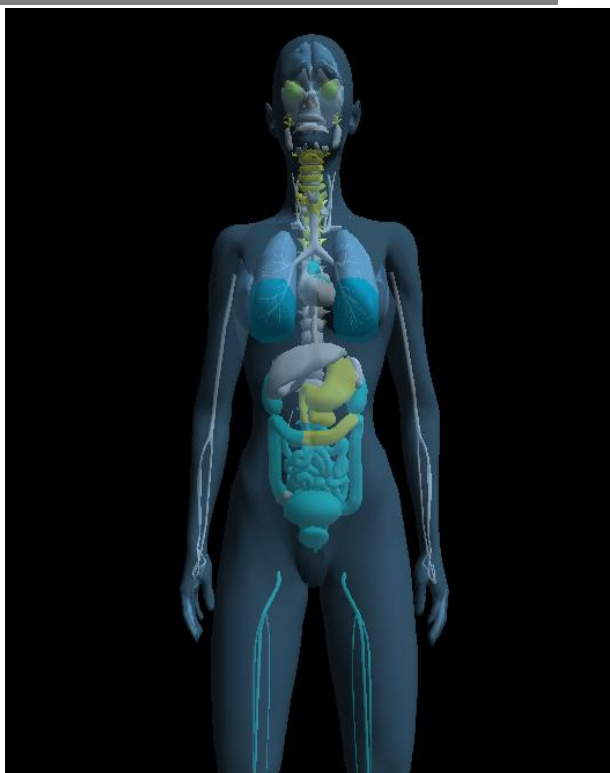


taking of Podkovičník MIX (09.03.2009 – 09.06.2009 12 capsules per day) showed **falling tendency (1.168...1.143...1.192...998 U/ml)**. The value of CA125 was on 02.07.2009, i.e. app. 4 weeks after the termination of taking extracts from medicinal mushrooms, at the level of 1.447 U/ml, which was **higher by 24%** than the input value upon inclusion into the clinical study and was **by 45%** higher than the lowest CA125 value recorded during the active taking of capsules!

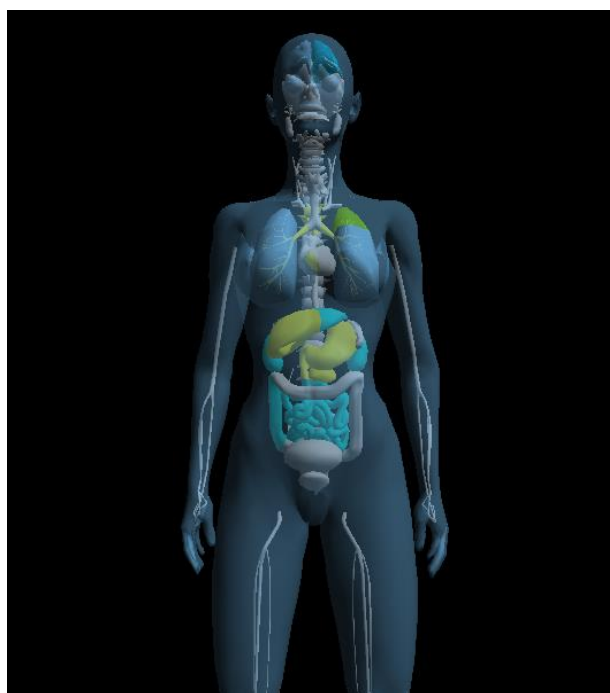




EIS 3/2009



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Clinical study

