Abstract

The Relationship between Endothelial Dysfunction-Oxidative Stress-Smoking in Patients Exposed to Respiratory Insult from a Mining Basin

Lately, the medical scientific world is confronted with new concepts like "endothelial dysfunction", "oxidative stress" and "atherosclerosis", situations in which the cells of the body do not control anymore the excessive presence of toxic free radicals, which cause cardiovascular, pulmonary, kidney diseases, as well as diabetes, cancer.

The purpose of the study was to establish the relation between:

- Smoking and endothelial dysfunction in patients exposed to professional insult from the mining basin of Baia Mare
- Smoking and oxidative stress in patients exposed to professional insult from the mining basin of Baia Mare
- Smoking, endothelial dysfunction and echocardiographic parameters in patients exposed to professional insult from the mining basin of Baia Mare
- Smoking, pulmonary emphysema and CT parameters in patients exposed to professional insult from the mining basin of Baia Mare

To reach the above-mentioned purpose, we proposed the study of the following biochemical parameters:

- Blood: endothelin1 (ET1), total cholesterol, HDL cholesterol (HDLc), LDL cholesterol (LDLc), triglycerides, apolipoprotein B100 (apoB100), uric acid, fibrinogen, C-reactive protein, thrombocytes, albumin, glycemia, urea, creatinine;
- Saliva: uric acid, albumin, total antioxidant capacity (TAC), gamma-glutamyl transferase (GGT);
- Urine: microalbuminuria.

Meanwhile, we followed to establish the mechanisms which determine endothelial dysfunction, oxidative stress in pulmonary arterial hypertension and pulmonary emphysema, and the cardiovascular risk in patients exposed to professional insult from the mining basin of Baia Mare. The important cardiovascular risk factors are smoking, hyperlipidemia, arterial hypertension, obesity, diabetes and they have the same effect on the arterial wall, determining an increase in the production of free radicals, with a diminution of endothelium-dependent vasodilation, an increase of endothelial permeability with LDLc oxidation and atherosclerosis. Although the relation between smoking and atherosclerosis is well determined, the mechanisms triggering this causal relation are not completely elucidated. It is known that smoking is associated with the alteration of endothelial cells, hypercholesterolemia, the increase of plasma
levels of apoB100, LDLc, triglycerides, the increased formation of proatherogen oxidized lipids and of uric acid.

**Key words:** endothelial dysfunction, oxidative stress, pulmonary hypertension, emphysema

The four studies conducted were transversal studies, the equivalent of a thorough survey with quantitative information on risk factors in this population. The scientific research program was conducted in accordance with the norms of ethics in effect: protection of personal data, the use of biological samples for the intended purpose and the consent of the subjects. The study was approved by the Maramureș Department of Public Health and the Administration of the Maramureș Mining Direction.

In all the studies, the **statistical analysis** of the data was made with the Statistical Package for Social Sciences (SPSS-PC) software edition 7.5 and Stata 10. The data were presented as averages, standard deviations, series, depending on the situation. The *t* and Mann-Whitney tests were used for comparisons inside a group (smokers and non-smokers) and between groups. The Pearson and Spearman tests were used to evaluate the possible interrelations between different parameters. The graphic representation was made with Stata 10. The value *p* < 0.05 was considered as statistical significant.

In **study 1**, the purpose of the study was to establish the relationship between **smoking and endothelial dysfunction in patients exposed to professional insult from the mining basin of Baia Mare.**

The study group consisted of 51 volunteers exposed to insult from the non-ferrous metal mines from the Baia Mare mining basin (silicon and other non-ferrous minerals, Fe from FeS combination, Cu, Mg, S, Ca, Pb, Zn, Mn, Au, Ag). The average age was of 44.2 +/- 4.8 years. Out of the total, there were 29 smokers and 22 non-smokers. In the control group there were 17 volunteers from Baia Mare who did not work in the mining environment, had no pulmonary or cardiovascular disease antecedents, and were examined in the Ambulatory Facility of the Baia Mare Pneumology and Phthysiology Hospital. The average age was of 50.23 +/- 5.9 years. Out of the total, there were 10 smokers and 7 non-smokers.

The exclusion criteria were the presence of symptoms or signs of acute or chronic inflammatory respiratory and cardiovascular disease and the use of antibiotics or corticosteroids three weeks before being recruited for the study. All the participants in the study completed a questionnaire about the habit of smoking, respiratory symptoms, antecedents of pulmonary and cardiovascular disease. Blood samples were taken to determin the above-mentioned biochemical parameters.

In the group of miners, significant correlations were recorded with the Pearson test, as follows: uric acid-total cholesterol (*r* = 0.39, *p* = 0.03), uric acid-trombocytes (*r* = 0.39, *p* = 0.04), total cholesterol-LDLc (*r* = -0.55, *p* = 0.002), total cholesterol-triglycerides (*r* = 0.43, *p* = 0.02), fibrinogen-apoB100 (*r* = 0.41, *p* = 0.03). With the **Spearman test**, the statistical significant correlations were: ET1-cholesterol (*r* = -0.43, *p* = 0.02) and ET1-LDLc (*r* = -0.48, *p* = 0.008). Taking into consideration the smoking criterion, the possible disparities between the different
parameters form the lot of miners were examined. Thus, for *ET1* there was a statistical significant difference of the values between smokers and non-smokers, in favour of the non-smokers (the Mann-Whitney test, *p* = 0.001). With the *t* test the average of uric acid values was statistically higher for non-smokers than for smokers (*p* = 0.005). The same *t* test recorded significantly higher values for smokers compared to non-smokers for: apoB100 (*p* < 0.05), total cholesterol (*p* < 0.05), triglycerides (*p* < 0.005), fibrinogen (*p* < 0.001) and trombocytes (*p* < 0.009). In the intergroup comparisons (group of miners and control group), the *t* test showed the following statistical significant differences: HDLc, in favour of the control group (*p* < 0.05); triglycerides, in favour of the group of miners (*p* = 0.06); uric acid, in favour of the group of miners (*p* < 0.001) and trombocytes in favour of the group of miners (*p* = 0.009). For ET1, the Mann-Whitney test shows a statistical significant difference in favour of the group of miners compared to the control group (*p* = 0.002). CRP was between normal limits (<6) for the control group, while for the group of miners pathological values (>6) were registered for 29.4% of cases.

The results show an increase of the level of ET1, triglycerides and total cholesterol in the plasma for the group of miners which explains the existence of *endothelial dysfunction* with the alteration of endothelial permeability, the disturbance of lipid transport and the risk of developing an atheromatous plaque.

**Smoking** is a traditional cardiovascular risk factor, while apoB100, LDLc, HDLc, total cholesterol, uric acid, fibrinogen and CRP are non-traditional risk factors, which however add plus value to the cardiovascular risk predicted by the Framingham table. The mechanism by which smoking concurs to the atherosclerosis process is by endothelial dysfunction which determines the increase of trombocyte adherence and of fibrinogen in plasma. Smoking is associated with • the alteration of lipid profile through the increase of total cholesterol, of LDLc and triglycerides and the decrease of HDLc and the formation of proatherogen oxidized lipids (LDLox) under the effect of oxygen free metabolites from the cigarette smoke • the increase of prothrombotic activity through the inhibition of the tissue plasminogen activator (t-PA) release in the endothelial cells • the increase of serum fibrinogen • of the tissular factor (TF) • of the thrombocyte activity with the increase of blood thickness and • of uric acid. **Smoking and insult from non-ferrous metal mines may determine through different mechanisms the increase of ET1 level, the disturbance of lipid metabolism and the alteration of the level of uric acid, fibrinogen and trombocytes in plasma.**

In conclusion, the level of ET1 in plasma can be considered an element of population screening in order to determine the risk of cardiovascular disease.

In study 2, the purpose of the study was to establish the relationship between smoking and oxidative stress in patients exposed to professional insult from the mining basin of Baia Mare. The study group consisted of 30 volunteers exposed to insult from the non-ferrous metal mines from the Baia Mare mining basin (silicon and other non-ferrous minerals, Fe, Cu, Mn, Mg, Zn, S, Ca, Pb, Cr, Ni, Au, Ag). The average age was of 44.3 (DS 4.45; variation from 36 to 52). Out of the total, there were 16 smokers and 14 non-smokers. In the control group there were included
19 volunteers from Baia Mare who did not work in the mining environment, had no pulmonary or cardiovascular disease antecedents, and were examined in the Ambulatory Facility of the Baia Mare Pneumology and Phthysiology Hospital. The average age was 51.3 years (DS 5.57; variation from 36 to 59). Out of the total, there were 9 smokers and 10 non-smokers. Thus, for ET1 there was a statistical significant difference in favour of control group, (the Mann-Whitney test, $p < 0.001$) that demonstrat the presence of the endothelial dysfunction. In the intergroup analysis, the $t$ test for comparison between miners and control reveals that triglycerides, uric acid, thrombocytes were statistically higher for the miners ($p = 0.008$, $p < 0.001$, respective $p = 0.005$), which can be explained by the functioning mechanism of cigarette smoke. In the Mann-Whitney test there appears a statistical significant difference for ET-1 in favour of the group of miners ($p = 0.001$), which proves the presence of endothelial dysfunction. It is know that the redox status of uric acid can be modified by: the reactive oxygen species, the increase of endothelin-1 level, dyslipidemia-hyperlipidemia, hyperhomocysteinemia, angiotensin II. In our study, we can state that the increased plasma level of ET-1 is due to vascular permeability disorders, lipid metabolism, and the increase of platelet aggregation. The uric acid has a “redox shuttle” antioxidant/prooxidant condition and in the early stages of atherosclerosis it has an antioxidant role, being one of the determinants of plasma antioxidant capacity. In the intergroup analysis with the ANOVA test, taking into account the smoking criterion, for the uric acid a statistical significant difference can be noticed, thus: the average of smoker miners versus the average of non-smoker controls ($p = 0.009$); the average of non-smoker miners versus the average of non-smoker controls ($p = 0.001$); the average of non-smoker miners versus the average of smoker controls ($p = 0.01$). In all three comparisons, the values are in favour of the first mentioned averages. It is known that cigarette smoke generally produces 0.042 mg iron, and the iron deposit increases after the age of 20. The increase of iron concentration from cigarette smoke and the insult from the mine dust is a factor which enhances the formation of reactive oxygen species and oxidative stress. The cigarette smoke contains two types of free radicals: one in the (solid) tar phase which is a metal chelator that can tie up iron to produce tar-semiquinone + tar-Fe$^{2+}$, leading to H$_2$O$_2$ and another in the gas phase which contains small oxygen radicals, more reactive than the radicals of the tar phase. Saliva is the first biological liquid which encounters the inhaled cigarette smoke. Uric acid from saliva significantly decreases in the presence of cigarette smoke and much more during the solid phase, namely by 86.37% in the gas phase and by 98% in the solid phase. Both the gas phase and the solid phase of the cigarette smoke contain agents with a high capacity of reducing the ferrous ion to ferric ion, inducing the oxidative stress through the formation of hydroxyl radicals from the reaction with hydrogen peroxide. Thus, it can be stated that cigarette smoke induces oxidative stress by producing excessive free radicals and diminishing the antioxidants from saliva. In the analysis inside the group, in the group of miners there was a marginal significant negative correlation between the total antioxidant capacity (TAC) and uric acid ($r = -0.36$, $p = 0.05$), while in the control group the correlation was highly positive ($r = 0.76$, $p < 0.001$), which reveals that uric acid and TAC are antioxidants for the control group. In the intergroup analysis, the $t$ test revealed statistical
significant differences between the two groups for the average of all the tested parameters, thus: **albumin, GGT, TAC, uric acid** were statistically higher in the control group ($p = 0.004$, $p = 0.004$, $p < 0.001$, respective $p = 0.02$), demonstrating that these are highly antioxidant for this group. The ANOVA test, post-hoc correction performed on the two groups taking into consideration the smoking criterion, showed statistical significant differences between the TAC averages of the control group and the group of miners, in favour of the control group, thus: non-smoker control versus non-smoker miners ($p < 0.001$); non-smoker control versus smoker miners ($p < 0.001$); smoker control versus non-smoker miners ($p = 0.003$), smokers control versus smoker miners ($p = 0.005$). The decrease of TAC for the group of miners can be linked to the presence of mine insult and the increase of oxidative stress. It is known that the transition metals from the mine insult favour lipid peroxidation, affecting the total antioxidant capacity. The review of oxidative stress can be made through determining the antioxidants, and also the markers of oxidative stress which reflect the lipid oxidation, especially of LDLc. In the study, in the comparison between the two groups (miners and control), there are statistical significant differences for the average of all the tested parameters, thus: **albumin, GGT, TAC, acid uric** were significantly higher in the control group. Recent studies show that **GGT** has a direct role in the formation of reactive oxygen species, being a marker for oxidative stress, not only in serum, but also in plasma. The increased GGT activity may appear as a response to oxidative stress, leading to the accelerated transport of glutathione precursors in the cell. In our study, the low level of GGT in saliva for the group of miners can be explained by the fact that GGT in the presence of iron and other transition metals is partially absorbed by LDLc and included in the atheroma plaque. The catalyst activity of GGT induces oxidative stress in the atheroma plaque, encouraging plaque instability, its erosion and rupture, as well as the aggregation of trombocytes and the formation of the thrombus. Studies on non-selected populations and patients with known coronary affections show that GGT is an independent prognosis marker for the risk of cardiac death and reinfarction. Saliva has a very important antioxidant potential given by **albumin, TAC, GGT and uric acid**, which can be used as indicators for oxidative stress. The correlation between the biochemical parameters from saliva and blood suggests that antioxidants can be used to establish the risk of cardiovascular and cerebrovascular diseases. However, the results of studies regarding the antioxidant capacity of albumin, TAC, GGT, uric acid from plasma and saliva should be compared and completed with arguments based on experimental studies. These results are difficult to interpret because it is known that an increase of an antioxidant concentration in plasma or saliva may actually disguise a shortage of another antioxidant. An important issue is the inexistence of a **reference value** for saliva antioxidants, consequently the laboratories use various identification methods, without having established values for the healthy population.

In **study 3**, the purpose of the study was to establish the relationship between smoking, endothelial dysfunction and echocardiographic parameters in patients exposed to professional insult from the mining basin of Baia Mare.

The study group consisted of 35 volunteers exposed to insult from the non-ferrous metal mines.
from the Baia Mare mining basin (silicon and other non-ferrous minerals, Fe Cu, Mg, S, Ca, Pb, Zn, Mn, Au, Ag). The average age was of 44.17 +/- 0.83 years. Out of the total, there were 23 smokers and 12 non-smokers.

In the group of miners, significant correlations were recorded with the Pearson test, as follows: For echocardiographic parameters - biochemical parameters from blood: **ET1-RV wall thickness** ($r= 0.54, p= 0.0008$), RV short axis-RV wall thickness ($r= 0.54, p= 0.0007$), total cholesterol-RV wall thickness ($r= 0.34, p= 0.04$), apoB100-RV wall thickness ($r= 0.60, p= 0.0001$), apoB100-RV short axis ($r= 0.42, p= 0.01$), apoB100-total cholesterol ($r= 0.50, p= 0.002$), apoB100-LDLc ($r= 0.34, p= 0.04$), triglycerides-RV wall thickness ($r= 0.37, p= 0.02$), body mass index (BMI)-average of pulmonary artery pressure (sPAP) ($r= -0.33, p= 0.04$); for echocardiographic parameters-biochemical parameters from saliva: RV wall thickness-albumin ($r= 0.32, p= 0.05$). With the t test there appears a statistical significant correlation between the RV wall thickness-CRP ($p= 0.01$). With the t test for the smoking criterion there appears a marginal significant correlation between ET-1 ($p= 0.06$) and trombocytes ($p= 0.06$) in favour of the smokers and a statistical significant correlation between between BMI and smoking ($p= 0.002$). For the biochemical parameters from saliva the following correlations were established: GGT-uric acid ($r= -0.57, p= 0.01$), TAC-uric acid ($r= -0.33, p= 0.04$), TAC-GGT ($r= 0.51, p= 0.03$), showing that they are highly antioxidant. Pulmonary hypertension is an increase of the arterial pressure from the small circulation >25 mmHg in resting position and>30 mmHg in effort. The alterations at the level of pulmonary arteries are due to the **endothelial dysfunction** with the excessive production of vasoconstrictive substances (endothelin, thromboxane) and the decrease of the protective vasodilative substances. Echocardiography is considered the “**gold standard**” for the diagnosis of pulmonary hypertension, as it is a direct and non-invasive method. The echocardiographic signs of the right ventricular function are the dilation of the right ventricle, the paradoxal movement of the interventricular septum during systole and the tricuspid regurgitation flow. Recently, there were described two new echocardiographic parameters for the performance of the right heart for clinical evaluation and prognosis: **TAPSE index** (tricuspid annular plane systolic excursion) and the flow speed derived from the Doppler into the superior vena cava. In the study a strong statistical significant correlation appears between the level of ET1 in the plasma and the RV wall thickness ($r= 0.54, p= 0.0008$), RV wall thickness and the small axis at the base of RV ($r= 0.54, p= 0.0007$), apoB100-RV wall thickness ($r= 0.60, p= 0.0001$), triglycerides-RV wall thickness ($r= 0.37, p= 0.02$) which demonstrates the presence of endothelial dysfunction with the alteration of endothelial permeability and of the lipid transport with fat deposits in the RV wall, an early stage of pulmonary hypertension and the chronic pulmonary cord. In our study there is another correlation between RV wall thickness and the small axis at the base of RV ($r= 0.54, p= 0.0007$), which suggests the presence of concentric hypertrophy of RV, an early stage of pulmonary chronic cord and of pulmonary hypertension. In the study, for the smoking criterion there is a statistical significant correlation between the RV wall thickness and C-reactive protein ($p= 0.01$). Various studies show that the serum level of
CRP is correlated with the lipid profile and the RV wall thickness. CRP has a proinflammatory and prothrombotic role, inducing platelet hyperaggregability and lipid infiltration into the RV wall. CRP level can be an independent predictor for the systolic pulmonary artery pressure. In our study, there is a statistical significant correlation between TAPSE index (tricuspid annular plane systolic excursion) and smoking, which demonstrates a tendency for platelet hyperaggregability under the influence of insult from cigarette smoke and the professional environment which alter the characteristics of the tricuspid regurgitation jet. For the smoking criterion there is also a marginal significant correlation for ET1 ($p=0.06$) and trombocytes ($p=0.06$), in favour of the smokers. This can be explained by the fact that trombocytes are directly involved in the atherosclerosis process because they contain “trigger” substances for the platelet increase factor and prostaglandins which stimulate the smooth muscle cells from the artery wall.

With the Pearson chi$^2$ test, for the smoking criterion there was a difference between the statistical significant values for smokers and non-smokers for the tricuspid regurgitation (TAPSE) in favour of the smokers ($p=0.008$), explained by the increase of blood thickness due to an increase in fibrinogen level, platelet adherence, hematocrit and the decrease in the red blood cell deformability. It is difficult to establish an early diagnosis for pulmonary hypertension. In pulmonary hypertension, the presented studies have established the presence of inflammation through the high level of ET1. Smoking and insult from the non-ferrous mines can lead to endothelial dysfunction and oxidative stress with the alteration of echocardiographic parameters. The mechanisms that trigger endothelial dysfunction and oxidative stress form the pulmonary hypertension are not entirely elucidated.

In study 4, the purpose of the study was to establish the relationship between smoking, pulmonary emphysema and CT parameters in patients exposed to professional insult from the mining basin of Baia Mare.

The study group consisted of 26 volunteers exposed to insult from the non-ferrous metal mines from the Baia Mare mining basin (silicon and other non-ferrous minerals, Fe from FeS combination, Cu, Mg, Ca, Pb, Zn, Mn, Au, Ag). The average age was of $43.57\pm4.78$ years. Out of the total, there were 17 smokers and 9 non-smokers. The established value for pulmonary density, discriminatory in the CT exam, was of 910 HU. The value <80% from the prediction for FEV1 and <70% for the relation FEV1/FVC were considered as indicators of the early obstruction of the airways.

In the group of miners, for the $t$ test, statistical significant correlations were recorded between $FEV1-CT$ ($r=-0.49$, $p=0.009$), $FEV1FVC$-fibrosis/silicosis ($p=0.05$); $PLE/CLE$-time of exposure ($p=0.008$); $BMI$-fibrosis/silicosis ($p=0.03$) and for the smoking criterion between CT-smoking ($p=0.02$). Epidemiologic studies conducted until now have established that smoking and pollution at work remain the main risk factors for morbidity and mortality in COPD. However, these studies have not singled out emphysema from the other components of COPD. This epidemiologic study is performed for the first time on a population exposed to professional insult from the Baia Mare mining basin. Generally, studies suggest the use of high resolution CT
for an objective quantification of emphysema to replace the subjective method of visual
diagnosis. Some studies have established that in the case of smokers with normal pulmonary
function, the CT quantititative evaluation of the hyperinflation of lung parenchyma can be an
**independent predictor** of the down-grade of pulmonary function in two years. High resolution
CT is the most sensitive method of easily quantifying emphysema in smokers with no symptoms,
who can develop COPD. Early emphysema is associated much more with the reduction of air
flow obstruction. In our study we found no correlation between centrilobular emphysema and
smoking. Patients suffered from panlobular emphysema in a higher degree which can be
explained by association with interstitial pulmonary fibrosis injuries caused by insult from the
professional environment. It has been established that patients with emphysema present a
significant decrease of FEV1/FVC relation. It seems that the predictor factors for emphysema are
FEV1 and the emphysema score in CT. In the study, the average values of FEV1 and FEV1/FVC
were very close to normal, suggesting a low degree of airway obstruction. The average
pulmonary density in CT correlates significantly with the FEV1 obstruction index and with the
FEV1/FVC, but does not correlate significantly with VC. In the case of miners, the focal or
diffuse emphysema areas in CT are due to the combination between “air trapping”, the decrease
of pulmonary perfusion and the affection of the capillary bed, and not from the abnormal
enlargement of air spaces distal to the terminal bronchiole accompanied by destruction of the
alveolar wall, the anatomopathological characteristic of emphysema. Studies show that mild-
moderate emphysema may be present even without the reduction of MEV(S). Numerous studies
show that pulmonary density evaluated in CT for emphysema is more extended in smokers with
CLE. In our study there appears a statistical significant correlation between FEV1/FVC-
fibrosis/silicosis ($p=0.05$) and PLE/CLE-exposure period. In the group of miners, the panlobular
(PLE) or centrilobular (CLE) type modifications correlate statistically with the period of
exposure (the average exposure was of 15.5 +/- 2.73 years). The FEV1FVC relation correlates
statistically with the group of miners that had lesions of diffuse interstitial fibrosis and silicosis
type, as the pulmonary volume increases with the decrease of the decline rate of FEV1 and in
functional pulmonary tests the obstruction of airways is associated with hyperinflation and the
increase of pulmonary volumes. In the group of miners there appears a statistical significant
correlation between BMI-fibrosis/silicosis ($p= 0.03$). The study reveals that the aggravation of
pulmonary function in patients with fibrosis/silicosis correlates with the extension of the
emphysema. Thus, in smokers with normal FEV1, CT scan can point out pulmonary
hyperinflation, predicting the rapid annual decline of FEV1. High resolution CT is the most
sensitive method for quantifying mild emphysema in smokers with no symptoms who can
develop COPD, due to the relationship between the degree of obstruction limiting of the airflow
and emphysema which demonstrates the link between the extension of emphysema and the
pulmonary function. The average pulmonary density in CT is significantly correlated with the
obstruction index, FEV1 and FEV1/FVC. The early emphysema is associated more with the
reduction of obstruction of the airflow. The inflammatory modifications especially influence the
peripheral airways, being able to lead to the development of emphysema.
CT is more sensitive than functional pulmonary tests for the diagnosis of pulmonary emphysema, suggesting that it can evaluate the severity of the emphysema. This epidemiologic study is the first of its kind on a population exposed to professional insult from the Baia Mare mining basin.

Generally, the following risk factors are taken into consideration: gender (M), age between 35-64 years, family antecedents of cardiovascular disease (chronic ischaemic cardiomyopathy, arterial hypertension, myocardial infarction), smoking, type II diabetes, HDLc. The plasma level of LDLc is linked to the associated risk factors, thus, if there exists a cardiovascular risk factor, the LDL level should be <1.9 g/l, if there are two risk factors than the LDLc level should be <1.6 g/l and in case of a high cardiovascular risk, the LDLc level should be <1 g/l.

In the conducted studies, the following correlations were found between cardiovascular disease risk factors and family antecedents of cardiovascular disease (arterial hypertension, chronic ischaemic cardiomyopathy, diabetes):

• in the control group, fibrinogen has a statistical significant meaning of arterial hypertension risk \( (p < 0.03) \), uric acid in blood has a marginal statistical significance of arterial hypertension \( (p = 0.07) \), the high plasma level of ET1 has a statistical significant meaning of diabetes risk \( (p > 0.001) \) and the level of HDLc has a statistical significant meaning of myocardial infarction \( (p = 0.05) \)

• in the group of miners, uric acid has a statistical significant meaning of risk for CCI \( (p > 0.005) \) and diabetes \( (p = 0.05) \) and C-reactive protein has a statistical significant meaning of arterial hypertension risk \( (p = 0.0004) \) and of CCI \( (p = 0.01) \)

• according to the smoking criterion, in the group of miners the correlation between ET1-glicemy has a marginal statistical significance of diabetes \( (p = 0.07) \), while in the control group is has a statistically higher risk significance \( (p = 0.04) \)

• according to the smoking criterion, in the control group there appears a statistical significant risk correlation for diabetes \( (p = 0.04) \), while in the group of miners there is a marginal statistical significance for risk of diabetes \( (p = 0.07) \). In the group of miners, for the smokers there appears a marginal statistical significant correlation as risk factor for CCI \( (p = 0.06) \). Meanwhile, the average value of uric acid in those with no family diabetes risk factors is statistically higher than in those with family risk factors, \( p > 0.05 \). The plasma level of ET1 statistically significantly higher in the control group compared to the group of miners, \( p > 0.001 \). In the group of miners, HDLc was statistically significantly higher in those without risk factors for CCI, than those presenting risk factors, \( p > 0.05 \).

• in the studied groups, no correlations were found between the plasma level of ET1-urea and creatinin, respective glycemia-urea and creatinin, which demonstrates that although there is endothelial dysfunction, an early sign of atherosclerosis, there are no visceral or renal affections.

The patients from the two groups (miners and control) can be considered as being in risk group A (no risk factors, no vascular affection, no cardiovascular clinical affection) or risk group B (at least one risk factor excluding diabetes, no vascular affection, no cardiovascular clinical
It can be concluded that in the conducted studies, in the group of miners the plasma level of HDLc, uric acid and CRP are strongly correlated with the family risk factor of arterial hypertension, CCI, diabetes, while in the control group ET1, fibrinogen, uric acid, glycemia, smoking. Based on the data gathered from the study groups, along the family risk factors of cardiovascular disease and smoking we can include in the Framingham equation the following risk factors: plasma level of ET1, CRP, uric acid, HDLc, fibrinogen and glycemia. The study gives the possibility of layer classifying a population with no clinical signs of disease exposed to smoking and professional insult, depending on the risk factors of cardiovascular disease, with no clinical signs of disease, being a proper basis for a cohort study project to confirm the role of these factors in the studied population.

We can propose a layer classification of the cardiovascular risk, for the group of miners and for control group by determining the plasma level of ET1, CRP, fibrinogen and uric acid, glycemia, HDLc. We believe that a set of biochemical tests on a certain population can offer more data on cardiovascular disease risk factors than one single parameter. Smoking, as a traditional cardiovascular risk factor along with the non-traditional risk factors: apoB100, LDLc, HDLc, total cholesterol, uric acid, fibrinogen, CRP are parameters which can help in calculating the cardiovascular disease risk in a population exposed to respiratory insult. Thus, it can be stated that the association of several risk factors multiplies the cardiovascular risk. Consequently, studies have established that two risk factors determine a 2.5 x increase of cardiovascular risk, three factor 7 x, four factors 16 x. The metabolic syndrome increases cardiovascular risk by accumulating the risk of each if its constitutive factors. Due to the complex etiology, at present only a small proportion of cardiovascular diseases is acknowledged to be of professional origin. However, it is admitted that the exposure to preprofessional insult contributes to the development of cardiovascular and pulmonary diseases in the working environment.

The prevention of cardiovascular diseases at population level is based on the sanitary education with the help of doctors, sanitary educators, sick people’s associations, the media. The primary prevention measures which do not imply medicaments are the fight against smoking (banning smoking at work, increasing the price of cigarettes) by prevention campaigns at work with the involvement of family and specialist doctors, occupational medicine doctors, the fight against sedentariness by prevention campaigns for daily physical activity, the fight against obesity and eating disorders by national nutritional health programs. The Framingham study, begun in 1948, continues today with the SABRe (Systems Approach to Biomarker Research in Cardiovascular Disease) initiative to study the biomarkers of cardiovascular diseases and the DNA, in order to understand the genetic map and to identify people with cardiovascular risk, as well as to establish the means of intervention to prevent the development of the atherosclerotic disease. The patients from the studied group of miners, B, with an average age of 48-52 years, are exposed at work to respiratory insult, large temperature differences, noise, vibrations caused by boring machines. We can propose a prevention and health promoting program at work. In our country, until now no study has been conducted in a mining basin to understand the
cardiovascular disease risk factors. In Romania, it is necessary to have a national strategy for the
detection of endothelial dysfunction, an early, reversible and detectable stage of cardiovascular
disease, at work. The endothelial dysfunction can be quantified through simple, non-invasive
tests with validated equipment in numerous centers from the world, from Europe and the USA.
In Romania, since 2008, there is a National Smoking Cessation Program ("STOPFUMAT") of
counseling for giving up smoking. Since 2009 the National Program for the Prevention of
Cardiovascular Diseases has been initiated and also a National Program for Nutritional Health is
on the way of being started.
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General and medical studies

General education high-school, special class of biology-chemistry Baia Mare, national olympiads of biology and chemistry

The Faculty of General Medicine, Cluj-Napoca, 1980

Internship at the Baia Mare County Hospital, 1980-1983

Specialisation in pneumology at "Marius Nasta" Pneumology Institute in Bucharest, 1984-1986

Consultant practice at "Marius Nasta" Pneumology Institute in Bucharest, 1993

Exam for Head of Department status in Bucharest, 1996
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<td>Specialisation in pneumology at &quot;Marius Nasta&quot; Pneumology Institute in Bucharest, 1984-1986</td>
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Training courses:

- "Emergency in Intensive Care", 2000, Bucharest
- "New Theories in the Diagnosis and Treatment of Bronchial Asthma", 2006, Bucharest
- "Diagnosis Difficulties in Non-specific Pleuro-pulmonary Infections", 2004, Cluj-Napoca
- "Assisted Ventilation", 2008, Constanța
- "Chronic Respiratory Failure", 2004, Sibiu
- "Diffuse Interstitial Pneumopathy", 1996, Bucharest
- "Systemic Symptoms of Respiratory Disorders during Sleep", 2002, Timișoara
- "Bronchial Asthma and Chronic Obstructive Pulmonary Disease – News in Diagnosis and Treatment", 2004, Bucharest
- "CT Semiology", 2006, SRP, Cluj-Napoca
- "Course on Sarcoidosis", 2006, Cluj-Napoca
- "Implementation of the National DOTS Strategy", 2004, Cluj-Napoca
- "Diagnosis and Treatment Strategies in Systemic Vasculitis", 2007, Baia-Mare
- "Pre-conference courses - The National Conference on Clinical Allergology and Immunology", 2006, Cluj-Napoca
Specialisation courses:

**Tabacology:**

“Main Elements in Nicotine Dependence Therapy”, 2007

“Smoking Induced Epidemiology and Pathology, Nicotine Dependence and Pharmacological and Non-Pharmacological Therapies”, 2007, Craiova

“Therapeutic Strategies in Nicotine Dependence”, 2008, Bucharest

“Therapeutic Strategies for Giving Up Smoking”, 2008

“Therapeutic Strategies in Quitting Smoking”, 2008, Brașov

“Basic Course in the Therapy of Nicotine Dependence”, 2008, Constanța

“Smoking Cessation Courses”, ERS, 2008

“The 3rd National Conference on Tabacology” 2009, Iași

**Sleep medicine:**

“Systemic Symptoms of Respiratory Disorders during Sleep”, 2002, Timișoara

“Diagnosis and Treatment Algorithm of the Sleep Apnea Syndrome”, 2004, Timisoara

“The First National Sleep Medicine Conference – Comorbidities in Sleep Pathology”,
2005, Bucharest

Course: “Diagnosis and Treatment Algorithm of the Sleep Apnea Syndrome”, 2005, Bucharest

“The Second National Sleep Medicine Conference”, 2007, Oradea

“Course on Polysomnography”, 2007, Bucharest

“Course on Sleep Medicine”, 2007, Iași

Workshop “Forensic Implications in Sleep Medicine”, 2007, Iași

“Screening in Sleep Apnea Syndrome”, 2007, Oradea

“The Third National Sleep Medicine Conference – Sleep Apnea Syndrome and Related Diseases”, 2009, Craiova

Course: “Sleeping Disorders – Diagnosis and Therapy in a Pluridisciplinary Framework – Presentation of Novelties in the Field”, March 2010, Bucharest

Course on Sleep Medicine: “Sleep Medicine – Program for Central Europe”, March 2010, Bucharest

Participation in National Symposia and Congresses:

“The Congress of the Romanian Pneumology Society”, 2002, Bucharest

“Difficulties in Pneumologic Diagnosis”, 2004, Suceava


“The Third National Bronchology Conference”, 2005, Constanța

“Treatment Strategies for Reaching and Maintaining the Control over Asthma”, 2006, Baia-Mare


“The 4th Edition of MaraMedica”, 2007, Baia Mare


“The 7th Edition of MaraMedica”, 2010, Baia Mare


“Home Care of Patients with Chronic Respiratory Affections. Perpectives for Implementing a National Program”, 2004, Călimănești

“The 44th Annual Scientific Session”, 2007, Bucharest

“We Can Help COPD Patients Live Longer and Better”, 2007, Baia Mare

“INSPIR Pneumology Conference with International Participation”, 2007, Iași

“The Annual Clinical Allergology and Immunology Conference”, 2008, Baia Mare

“The Congress of the Romanian Pneumology Society”, 2008, Constanța

“The Big Picture – A New Perspective in COPD”, 2008, Satu-Mare

Web-based course on “Smoking Cessation” organised by ERS School, November-December, 2008

Web-based course on "COPD" organised by ERS School, February-March, 2009

“The Second National Sleep Medicine Conference, topic: Sleep Apnea Syndrome and Related Diseases”, 2009, Craiova

Course on “Pulmonary Artery Hypertension”, 07-05.2009, Craiova

Course on “ Novelties in the Management of Internal Affections”, 20-21.03.2009, Baia Mare

“ The Third National Tabacology Conference”, 25-27.06.2009, Iași

“ The First Romanian-British Meeting”, October 2009, Vama

Course on “Novelties in Pulmonary Rehabilitation and Non-Invasive Mechanical Ventilation at Home”, May 2010, Iași

The Congress of the Romanian Pneumology Society: “The Lung - Part of a Whole”, 9-12 June 2010, Bucharest

**Participation in National Congresses and Conferences on Pneumology, Allergology, Tabacology, Sleep Medicine**

Professional activity (position, duration, …)

Senior consultant pneumologist since 1993

Head of Pneumology I Department from the Pneumophysiology Hospital in Baia Mare since 1996

Graduand in pneumology since 2006, title of the doctoral thesis:

"The Relationship between the Endothelial Dysfunction - Oxidative Stress - Smoking in Patients Exposed to Respiratory Insult in a Mining Basin"

Experience in university medium (position denomination, lectures, …)
Scientific Activity (published articles, books, …)

General Papers, subject: "Functional Aspects in Diffuse Interstitial Pneumopathy"

"Ethiopathogenetic aspects of bilateral pleurisis"

Study together with „Marius Nasta” Pneumology Institute and the Romanian Academy, subject:

"Epidemiological Survey on a Representative Pattern for an Adult Urban Population (Baia Mare), the Prevalence of Respiratory Symptoms Suggestive for Bronchial Asthma, of Bronchial Asthma and of Chronic Bronchitis”, 1994

Papers presented at national and international symposiums and congresses:

"The Erasmus Syndrome - Case Study” presented at the Suceava Symposium, 2003

Ligia Puiu

"Scleroderma -A Clinical Case” presented at the National Respiratory Physiopathology, Târgu-Mureș, 2004
Ligia Puiu

"The Caplan Syndrome - case study" presented at the Baia-Mare Symposium, 2005

Ligia Puiu


Ligia Puiu

"The Sleep Apnea Syndrome and Homocysteine" presented at the Third National Sleep Medicine Conference, Craiova 05-07.2009

Ligia Puiu1, Andreea Didilescu2, Călin Pop3, Maria Greabu, Alexandra Totan5, Cristian Didilescu6


Ligia Puiu1, Andreea Didilescu2, Ovidiu Fira-Mlădinescu3, Călin Pop4, Stela Carmen Hanganu5, Cristian Didilescu6

„SALIVARY OXIDATIVE STRESS MARKERS AT MINERS FROM BAIA MARE METAL MINES”

17th Meeting of Balkan Clinical Laboratory Federation, 2009
Miricescu D., Totan A., Didilescu A., Puiu L., Greabu M., Mohora M., Hanganu C., Radulescu R.

"The Role of Smoking in Altering Certain Essential Parameters in the
Homeostasis of the Organism”
Didilescu AC, Hanganu SC, Galie N, Greabu M, Totan A, Stratul SI, Puiu L.

„The Relationship between the Endothelial Dysfunction and Biochemical Parameters of the Blood in Patients Exposed to Respiratory Insult”
Romanian Review of Laboratory Medicine, September, 2010
Ligia Puiu1, Andreea Didilescu2, Ovidiu Fira-Mădăinescu3, Călin Pop4, Maria Greabu5, Alexandra Totan6, Stela Carmen Hanganu7, Cristian Didilescu8

„Evaluation of the Relationship between Oxydative Stress and Biochemical Parameters from Blood and Saliva and the Influence of Respiratory Insult”
Study for the doctoral thesis.

„The Relationship between Emphysema and Tomodensitometric Parameters in Patients Exposed to Respiratory Insult”
Study for the doctoral thesis.

„The Relationship between Endothelial Dysfunction and Echocardiographic Parameters in Patients Exposed to Respiratory Insult”
Study for the doctoral thesis.

„The Endothelial Dysfunction”. General Paper
“Clujul Medical” Magazine, the July issue, 2010, Ligia Puiu

„Evaluation of Salivary Antioxidant Biomarkers in Neferrous Metals Mine Workers”
IADR General Session (July 14-17, 2010), Barcelona
Miricescu D., Totan A., Didilescu A., Puiu L., Greabu M., Mohora M., Hanganu C., Radulescu R.
Possible Relationships between Sleep Apnea Syndrome, Blood Biochemical Parameters and Cardiovascular Risk

ERS Barcelona, September, 2010

Ligia Puiu, Andreea Didilescu, Ovidiu Fira-Mădălinescu, Călin Pop, Maria Greabu, Alexandra Totan, Vanda Roxana Nimigean, Cristian Didilescu

Membership in Medical Societies or International Bodies

Member of the Romanian Pneumology Society
Member of the Tabacology Division of the Romanian Pneumology Society
Member of the Sleep Medicine Division of the Romanian Pneumology Society
Member of the Bronchology Division of the Romanian Pneumology Society
Member of the Respiratory Physiopathology Division of the Romanian Pneumology Society
Member of the European Respiratory Society (ERS) since 1998, Gold Member since 1999
Member of the French Pneumology Society (SPLF) between 1998-2004
Member of WASOG (Word Association of Sarcoidosis and other Granulomatous Disorders) since 2006
Fellow of the American College of Chest Physicians (ACCP)