Biochemistry in Assessing Tobacco Exposure -Smokers *versus* Non-smokers
Correlations with clinical practice

ANTIGONA TROFOR[1], OVIDIU PETRIS[2]*, LETITIA TROFOR[3], MILENA ADINA MAN[4], DUMITRU FILIPEANU[5], RAMONA MIRON[3]

[1]Grigore T.Popa University of Medicine and Pharmacy Iasi, Clinic of Pulmonary Diseases, 16 Universitatii Str., 700115, Iasi, Romania

[2]Grigore T.Popa University of Medicine and Pharmacy Iasi, Department of Cardiology, 16 Universitatii Str., 700115, Iasi, Romania

[3] Grigore T.Popa University of Medicine and Pharmacy Iasi, 16 Universitatii Str., 700115, Iasi, Romania

[4] University of Medicine and Pharmacy Iuliu Hatieganu Cluj, 8 Victor Babes Str., 400012, Cluj, Romania

[5]Technical University Gheorghe Asachi of Iasi, 67 Dimitrie Mangeron Blvd., 700050, Iasi, Romania

The present study evaluate medical history, smoking profile, serum cholesterol, triglycerides, creatinine in addition to uric acid and exhaled carbon monoxide levels (two biomarkers in use) for assessing tobacco exposure in 57 smokers versus 54 nonsmokers. Association of expired air carbon monoxide and plasmatic uric acid proved to be useful and necessary tool in evaluating smokers versus non-smokers, compared to medical history and serum cholesterol-triglycerides-creatinine profile.

Keywords: carbon monoxide, uric acid, biomarkers, smokers

All guidelines classify *tobacco use and dependence* as a chronic relapsing disease, due to *nicotine*, an addictive component present in any tobacco product, and recommend to mandatory identify and treat any smoker to quit [1]. Diagnosis of tobacco dependence considers both clinical and biological evaluation of the tobacco consumption disorder, together with a psycho-behavioral evaluation. Biological assessment refers to some specific biomarkers, allowing objective proof of tobacco exposure, like carbon monoxide in exhaled air, cotinine (a nicotine metabolite that can be measured in plasma, saliva, urine, hair and intranasal) but also anatabine, anabasine, thiocyanate, uric acid and nitric oxide, identified by more recent research [2].

Carbon monoxide (CO) can be most easily monitored and represents an indicator of tobacco consumption. CO levels can be easly evaluated by patient exhalations in a carbon monoxide analyzer. The CO unit is *ppm* (parts per milion), a parameter that can be expresed also as percentages of CO Hb (% carboxyhemoglobin) from total body hemoglobin, by the micro smokerlyzer device. Toxicity of CO is influenced by blood saturation, CO level in the air and breath air volume. Additional factors like environmental pollution (exhaust gas), passive smoking, professional exposure or smoke from biomass/coal burning may induce confusion in interpretating CO values, yet active smoking remains the major cause to increase CO levels. In normal conditions, in non-smokers, exhaled CO is < 4 ppm. Careful interpretation of CO is required in some special situations, when CO levels may register higher than estimated values, such as in COPD smokers, for example. This is due to either greater CO production resulting from more intensive inflammatory process of the chronic obstructive pulmonary disease or from more intensive smoking that usually is seen in this category of patients. Also, in asthmatic smokers, CO level may be confusing due to more intensive airway inflammation. Therefore, the *cut-off* optimal CO level, able to distinguish smokers from nonsmokers is of 10 ppm in asthmatic smokers and of 11 ppm in COPD smokers [3].

Serum uric acid

Uric acid is a degradation product from nucleic acids and the final result of the purine oxidation process. Its normal value is influenced by food, gender, age, genetic factors, physical effort and physiology [4]. It acts like a valuable antioxidant, including against oxidative stress caused by chronic tobacco smoking.

Besides this, smoking leads to higher triglyceride levels and to reduction in HDL cholesterol by increasing sympathetic activity. Smoking causes higher fasting plasma cortisol concentrations, resulting in an increase in visceral adipose tissue [5]. Studies assessing the impact on lipid profile of smokeless tobacco use, have come up with contradictory findings. While higher blood cholesterol, higher triglyceride and lower high-density lipoprotein levels have been reported in some studies, other similar researches have failed to confirm such associations [6].

Only few studies explicitly approached the relationship between smoking and creatinine levels. Dülger et.al. compared renal function in 24 active smokers vs. 20 passive smokers and vs. 20 controls. Authors found creatinine levels significantly higher in active smokers (p < 0.01) and concluded that kidneys and particularly glomerular function may be affected even by passive smoking. Also, the urine microalbumin/creatinine ratio was significantly increased in both active and passive smokers compared with controls, as a possible sign of increased atherosclerosis risk in these persons [7]. Other researchers, on the contrary, showed a significant lower creatinine level in smokers versus non smokers [8].

The objective was to assess clinical and smoking history, together with cholesterol, triglycerides and creatinine in the blood, plasmatic uric acid and CO in exhaled air, as useful biomarkers of tobacco smoking exposure in current clinical practice.

Experimental part
Material and method
The study was conducted between January- September 2013 in the Clinic of Pulmonary Diseases Iasi, Romania, according to the Helsinki declaration and Local Ethics

* email: ovidiupetris@yahoo.com Phone: 0040722550440

Committee provisions. A study group of 57 smokers and a control group of 54 non smokers were selected. All subjects were Caucasian, aged > 18, cooperating, able to perform study procedures, including biological sampling (fasting after 00.00 h) and first of all, provided a signed informed consent for study participation. Subjects in both groups were investigated their medical history, demographic data, alcohol and coffee use, dietary habits, smoking status, smoking profile according to a standard questionnaire (cigarette packs-years consumption, Fagerstrom nicotine dependence score, passive smoking) [9], and were determined serum cholesterol, triglycerides and creatinine, but also serum uric acid and carbon monoxide in exhaled air.

Lab normal range: serum cholesterol (normal: 100-200 mg/dL), triglycerides (normal: 0-200 mg/dL), creatinine (normal: 0.6-1.3 mg/dL), plasmatic uric acid (normal: 2-7.1 mg/dL), carbon monoxide in exhaled air (7 ppm certified active smoking).

Exclusion criteria: gout, chronic renal failure, renal lithiasis, alcohol/drug consumption, diabetus mellitus, any historical or current evidence of clinically significant or unstable disease, history of hyperuricemia, low protein diet, malnutrition, pregnancy, breastfeeding.

Statistical analysis: Data were loaded and processed by means of Microsoft Excel (Microsoft Corp.) and of the statistical analysis pack SPSS 17 (SPSS Inc.). Results were expressed as the average plus or minus standard deviation (SD). A statistically significant threshold was considered a p (Sig.) < 0.05. For a more detailed statistical analysis we used the Mann-Whitney U test, the independent t test and the correlation analysis.

Results and discussions

In total, 111 Caucasian patients (57 smokers and 54 non smokers) were enrolled in the study. Mean age according to group and gender was 48.38 ± 16.15 SD in males vs. 47.68 ± 17.49 SD in females for nonsmokers and 49.12 ± 10.0 SD in males vs. 38.67 ± 12.49 SD in females, for smokers. For the smokers group, analysis of the smoking profile showed a mean packs-years (PY) of 30.33 ± 15.07 SD in men and of 13.26 ± 9.56 SD in women and an average nicotine dependence (ND) Fagerstrom score of 5.58 ± 2.07 SD, with a mean dependence score by gender of 6.12 ± 2.05 SD in male and of 4.83 ± 2.05 SD in female subjects. By applying the Mann-Whitney U test, we found significantly higher ND scores in men than in women ($U=252$, $z=-2.3$ $p=0.018$) (table 1).

Passive smoking according to group has brought relevant data ($U=690$, $z=-5.7$, $p \leq 0.001$), as the smokers group reported it more frequently. Also, regarding this variable, there were significant differences depending on patient gender in the smokers group ($U=301$ $z=-2.0$, $p=0.045$), women being more often passively exposed to smoking than men.

In our study, subjects in the smokers group were found much more predisposed to respiratory co-morbidities (COPD, asthma, bronchiectasis, pulmonary fibrosis), compared to non smokers ($U=1056$, $z=-3.2$, $p=0.001$). Among other than respiratory burden, we identified cardiovascular (arterial hypertension, chronic heart disease, atrial fibrillation), digestive (Irritable bowel syndrome, duodenal ulcer, biliary lithiasis, hepatic steatosis/fatty liver) and metabolic (dislipidemia, type II diabetes, obesity)

	Person Correlation number of PY	p
Patient gender	-0.549	0.000
Patient age	0.708	0.000
Residence area	0.414	0.001
Fagerstrom test	0.419	0.001

Table 1
CORRELATIONS BETWEEN NUMBER OF PY, GENDER, AGE, RESIDENCE AREA AND FAGERSTROM TEST IN SMOKERS

Table 2
THE MANN-WHITNEY U TEST FOR THE CO-MORBIDITY VARIABLE, ACCORDING TO GEND

Patient group	Applied statistic test	Respiratory disease	Cardiovascular disease	Digestive disease	Metabolic disease
smokers group	Mann-Whitney U	315.5	333.0	356.5	354.0
	p	.330	.463	.848	.769
Smokers group	Mann-Whitney U	379.500	366.000	348.000	394.500
	p	.741	.394	.080	.973

disorders. Analyzing respiratory disease occurrence in the two groups, according to subjects gender, it was observed that there were no significant differences between men and women (U=315.5, z =-0.9, p=0.33, respectively U=379.5, z=-0.33, p=0.741) (table 2).

Correlations done in smokers for respiratory and cardiovascular co-morbidities and for passive smoking are described in table 3. Good correlations were found between respiratory and cardiovascular associated disease (r=0.32 p=0.015). This is consistent with our previously published data showing that cardiovascular co-morbidities associated to respiratory disorders had the highest rate and the greatest compliance to tobacco dependence treatment (48.8%) in our center. [10] By studying correlations of gender with passive smoking, it was seen that women declared more frequently they were passively exposed (r=0.26, p=0.004). As well, passive smoking was more often recorded in subjects provenient from urban areas (r=-0.4 p=0.001).

Table 3
CORRELATIONS WITH RESPIRATORY AND CARDIOVASCULAR DISEASE FOR THE SMOKERS GROUP

Pearson Correlation			p
Patient gender	Patient age	-0.42	0.001
	Residence area	-0.37	0.004
	Passive smoking	0.26	0.044
Patient age	Patient gender	-0.42	0.001
	Residence area	0.48	0.000
Residence area	Patient gender	-0.37	0.004
	Passive smoking	-0.43	0.001
Passive smoking	Patient gender	0.26	0.044
	Residence area	-0.43	0.001
	Cardiovascular disease	0.32	0.015

Laboratory findings

Carbon monoxide (CO) registered a mean value of 10.88 ppm ± 4.28 SD in smokers and of 0.24 ± 1.37 SD in nonsmokers. Smokers had higher exhaled CO concentrations and there were statistic significant differences between groups (U=26.5 z=-9.3, p ≤ 0.001). These results are concordant to literature, as in smokers, one should expect an expired air CO value of 10-20 ppm (this equals 2-5 % carboxyhemoglobin). Since it has been proved that in 24 h after last cigarette smoked, CO will come back to its normal values [9], it is accepted the fact that a carbon monoxide level > 8 ppm defines the status of current smoker [11, 12], while according to more actual data, the limit was set to 7 ppm.

Groups' gender analysis of CO range revealed a mean value of 12.36 ppm ± 3.97 SD in males, vs. 8.83 ppm ± 3.89 in females, for the smokers' group, respectively of 0.45 ± 1.86 SD vs. 0.00 ± 0.00 SD for nonsmokers. By applying the Mann-Whitney U test to check significant CO gender differences between the two groups, we noticed that women had lower CO than men, in nonsmokers

(U=321.5,z=-1.9 p ≤ 0.001,) and respectively in smokers (U=171.5, z=-3.6, p ≤ 0.001).

Concerning correlations obtained in the smokers group for carbon monoxide in exhaled air and gender, it was obviously that male smokers had higher values for exhaled CO (r=0.41 p=0.002); a possible explanation for this finding is the fact that this category of subjects presented also a more intense tobacco consumption and a more sever addiction to nicotine, by comparison to females.

Plasmatic uric acid results showed a mean 5.74 mg/dL ± 1.31 SD in males, towards 4.98 mg/dL i± 1.49 SD in females, if non-smokers and respectively 5.04 mg/dL ± 1.44 SD in males vs. 4.88 mg/dL ± 1.61 SD in females, for smokers. After careful exclusion of other factors that might affect uric acid determination, according to study exclusion criteria, overall, we found lower levels of plasmatic uric acid in smokers (mean value: 4.97 mg/dL), compared to nonsmokers (mean value: 5.39 mg/dL), but without significant differences, from statistic point of view.

Literature in the field is relevant for a significant low level of uric acid in smokers, in a dose-relationship with cigarette consumption; also, a higher uric acid level was demonstrated in former smokers (618 mg/dL) vs. 5.98 mg/dL in current smokers [13, 14]. In 2008, Hanna et.al published effects of smoking on uric acid in a small sample of 60 smokers vs non smokers volunteers and calculated fraction excretion of uric acid (FE uric acid), by the following formula: FE uric acid = (Urine (U) uric acid x Serum (S) creatinine/S uric acid x U creatinine) x 100. No statistical significant differences were noted in the age, serum creatinine, urine uric acid/urine creatinine ratio and FE uric acid between the two groups (P>0.05), whereas serum uric acid was significantly lower in the smokers group (P<0.001). Authors concluded the significant low serum uric acid level in smokers was attributed to reduced endogenous production as a result of chronic exposure to cigarette smoke that is a significant source of oxidative stress and also, they draw attention on cardiovascular risk of smoking [15] Other data showed increased values of uric acid and reactive protein C in active smokers towards passive smokers and non smokers [16]. Such conflicting evidence suggests more research is needed to define the role of uric acid as a useful biomarker for tobacco use and cessation.

Mean *serum creatinine* was 0.66 mg/dL ± 0.153 SD in female vs. 0.73 mg/dL ± 0.135 in male for the non smokers group and 0.61 mg/dL ± 0 .133 SD in female vs. 0.71 mg/dL ± 0.117 SD in male for the smokers group. In our study, serum creatinine level was lower in smokers, but in general, it was shown that men in both groups had higher values of serum creatinine (r=-0.38 p=0.003). This finding confirms older data about a significant higher creatinine clearance and a significant lower serum creatinine in current smokers as compared to non-smokers [17]. Anyway, actual knowledge in the field of the renal risks of smoking has expanded rapidly in the past two decades. There is some evidence that smoking increases the risk of albuminuria / proteinuria and the risk of mild hyper filtration in the general population, as well as the risk of renal functional impairment particularly in men and in the elderly. Tobacco use has a negative impact on renal function even in subjects without apparent renal disease, but the adverse renal effects of smoking are particularly marked in patients with different types of kidney disease and also in hypertensive patients [17].

Cholesterol mean was 201.24 mg/dL ± 37.75 SD in female vs. 185.47 mg/dL ± 52.9 SD in male non smokers, compared to 209.20 mg/dL ± 58.56 SD in female vs. 194.08 mg/dL ± 49.22 in male smokers. Cholesterol level was

higher in the smokers group. There were higher cholesterol levels in women from both groups, without significant differences.

Triglycerides mean value was 124.3 mg/dL ± 98.44 SD in non smokers, compared to 115.56 mg/dL ± 60.355 SD in smokers. So, we found lower triglycerides levels in smokers and gender analysis revealed 140.2mg/dL ±121.48 SD in males vs. 105.9 mg/dL ±59.34 SD in females, for non smokers, respectively 117.74mg/dL ±53.49 SD in males vs. 112.58 mg/dL ±69.79 SD in females, for smokers.

The *independent t* test found no statistical significant differences between the two groups, from the point of view of total cholesterol and triglycerides.

However, statistic significant correlations were obtained between cholesterol and trigliceride (r=0.52 p≤ 0.001), such as high cholesterol levels associated to high trigliceride levels.

By comparing our findings to literature on this topic, we can see more or less similar results. Since 1989, Tucker has reported higher cholesterol levels in relation to tobacco consumption. Thus, in 2840 subjects (cigarettes and smokeless tobacco users), this author found 2 1/2 times the prevalence of hypercholesterolemia in smokeless tobacco users compared to non smokeless tobacco users, respectively twice higher cholesterol levels in heavy cigarette smokers and 1 1/2 times more elevated cholesterol in light/moderate smokers, compared to non users of cigarettes [18]. In a newly published study, there was a significant increase in total cholesterol and LDL-C in tobacco users, as compared to non tobacco users. These authors showed a mean serum triglycerides level with an increase of about 25.40% (p< 0.001) in tobacco smokers and of 33.35% (p< 0.001) in tobacco chewers versus non-smokers and respectively versus non-chewers [19]

On the other hand, it was observed in our study that high triglycerides levels were associated to high values for serum creatinine (r=0.37 p=0.004) In the smokers group, female gender was associated to lower serum creatinine levels (r=-0.389, p=0.003). Also in this group, high serum trigliceride were seen in the same time with increased cholesterol (r=0.52, p≤ 0.001) and creatinine (r=0.37, p=0.004), as medium powerful and highly significant statistic correlations.

Presence of respiratory co-morbidities among subjects in the smokers group was found in relation with significant lower values for serum cholesterol and for serum triglycerides (r=-0.43, p=0.001 respectively r=-0.28, p=0.033).

In table 4, correlations between associated respiratory diease, metabolic disease, CO in exhaled air, plasmatic uric acid, colesterol, triglycerides and serum creatinine are reproduced.

Our findings showed that high cholesterol levels were associated with presence of metabolic co-morbidities (r= -0.28, p=0.03) and with increased triglycerides (r = 0.52, p ≤ 0.001). From another point of view, high levels of exhaled air carbon monoxide in smokers was correlated to greater levels of serum creatinine (r=0.39, p=0.002). High value for serum creatinine was associated, in its turn, with an elevated level of triglycerides (r=0.37, p=0.004). As regards a higher than expected level of uric acid we found in the smokers group, this was observed in relation with increasing cholesterol (r=0.44, p ≤ 0,001) and triglycerides (r=0.39, p=0.002).

All in the smokers group, significant correlations were established between plasmatic uric acid levels and serum creatinine (r=0.29 p=0.02) respectively and triglycerides (r=0.39 p=0.002).

Tabel 4
CORRELATIONS BETWEEN ASSOCIATED RESPIRATORY, METABOLIC DISEASE, CO IN EXHALED AIR, PLASMATIC URIC ACID, COLESTEROL, TRIGLICERIDE AND SERUM CREATININE, FOR THE SMOKERS GROUP

Pearson Correlation			p
Respiratory disease	Uric acid	-0.43	0.001
	Cholesterol	-0.283	0.033
Metabolic disease	Cholesterol	-0.284	0.032
Exhaled air CO	Creatinine	0.399	0.022
Uric acid	Cholesterol	0.449	0.000
	Triglycerides	0.399	0.002
	Creatinine	0.29	0.027
Cholesterol	Triglycerides	0.52	0.000
Triglycerides	Creatinine	0.37	0.004

Carbon monoxide in exhaled air was found much more increased in smokers (confirming it represents a biomarker of sure tobacco exposure); mean CO value was obviously higher in male versus female smokers and this gender difference applied also to the non smokers group. Higher exhaled CO in male smokers was well correlated to an increased PY consumption and to greater Fagerstrom ND scores. We have documented lower levels of plasmatic uric acid in smokers compared to non smokers, but not statistically significant. The importance of serum creatinine, cholesterol and triglycerides to act as biomarker for tabagism was not supported in our study due to no statistic significant differences between the two groups. On the other hand, there were major correlations between those two tests, as higher cholesterol was associated to higher triglycerides (r=0.52 p ≤ 0.001). We have also noticed that female smokers had the lowest serum creatinine levels, and that higher CO concentrations in smokers were described in relation to higher serum creatinine levels. As well, independently of any existing co-morbidity, when smokers had higher serum triglycerides values, it was seen also a high serum cholesterol and a high serum creatinine. Furthermore, smokers with respiratory co-morbidities had lower cholesterol and lower triglycerides serum levels compared to smokers without respiratory disorders. Finally, the highest serum cholesterol values were found in smokers with both metabolic and respiratory co-morbidities.

Conclusions
It is highly recommended to fully evaluate the impact of tobacco on exposed subjects, due to not only new scientific challenges but also to actual public health and regulatory requirements . Only the clinical assessment of smokers is not enough, but adding a biological evaluation will give the great picture of the problem. Biomarkers of tobacco exposure are most useful tools for this purpose. Carbon monoxide concentration in exhaled air represents a sure proof of recent smoking and our data confirm higher than

7 ppm CO values in active smokers. Even if we found not so lower as expected levels of plasmatic uric acid in smokers, we strongly recommend introducing serum uric acid testing in current clinical practice for smokers, as a valuable marker of oxidative stress induced by chronic tobacco smoking. Our findings concerning proposed laboratory profile (cholesterol, triglycerides, creatinine) in relation to medical history and to smoking pattern suggest firstly that smokers develop most frequently respiratory and cardiovascular co-morbidities. Such data make us also speculate that respiratory co-morbidities might affect levels of cholesterol and triglycerides in smokers, since lower values of these parameters were observed compared to non respiratory co-morbidities smokers. Also, in this respect, in smokers, higher CO levels may associate with higher serum creatinine and when higher serum triglycerides values are found - serum cholesterol and creatinine may increase, in the same time. As our analysis is based on a small sample of subjects, we trust future larger and longer studies in the field could advance the idea of the association of carbon monoxide and uric acid for an useful evaluation of smokers versus non-smokers, in current clinical practice.

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