BIOMARKERS OF TOBACCO EXPOSURE- RELEVANT DIAGNOSTIC IMPLICATIONS IN DAILY PRACTICE

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ABSTRACT

Aim of the study was to present available data on biomarkers of tobacco exposure and to inform about their utility in daily practice, focusing on expertise in the field in the Clinic of Pulmonary Diseases of Iasi, Romania. Data overview Smoking status declared by patients must be objectively validated by biochemical tests to determine biomarkers of tobacco exposure. The main biomarkers utilized by practitioners are: carbon monoxide in expired air and plasma, saliva, urine, hair and intranasal cotinine. More recently, researchers have studied also the role of some new biomarkers: anatabine, anabasine, thyocianate, serum uric acid and nitric oxide in expired air. We present up to date data about advantages and disadvantages of each such biomarker and practical details on clinical use, as some of them are also markers of chronic airway inflammation. Conclusions Laboratory tests for tobacco biomarkers offer the opportunity to measure constituents of tobacco smoke and to show exposing to toxicants in tobacco, regardless smoking behavior. In daily clinical practice of the Clinic of Pulmonary Diseases of Iasi have been successfully introduced carbon monoxide and nitric oxide in exhaled air, serum uric acid and urinary cotinine determinations.

Keywords: biomarkers, tobacco exposure, cotinine, nitric oxide, carbon monoxide

INTRODUCTION

Tobacco smoking is the single greatest cause of preventable premature death worldwide. Well known as the main risk factor for lung cancer, tobacco use also promotes the development of vascular disease and, thus, is at the origin of ischemic and peripheral heart disease and stroke. Epidemiological studies have confirmed the contribution of active smoking to cardiovascular risk, by finding an odds ratio (OR) for acute myocardial infarction of 2.87 for current versus never smokers[1]. Moreover, exposure to passive smoking has been found to increase the risk of cardiovascular disease by approximately 30%, more than would be expected based on comparable amounts of active exposure to tobacco smoke [2]. Nowadays, despite scientific evidence about harmful effects of tobacco, almost one-third of the global adult population currently smokes. Even if strong expert decision exist to eliminate smoking for reducing
cardiovascular disease, the World Health Organization estimates that worldwide, the number of smokers is expected to increase from 1.3 billion to 1.7 billion by the year 2025 [3].

Whether so called usually as smoking or its synonym - *tobacco use and dependence* -, this is considered by all current manuals and guidelines as a chronic relapsing disease, due to *nicotine*, an addictive component present in any tobacco product. Thus, it is unanimously recommended to mandatory identify and treat any smoker to quit [4]. Diagnosis of tobacco dependence takes into account both clinical and biological evaluation of smoking/tobacco use, together with a psycho-behavioral evaluation. Biological (laboratory diagnosis) refers to some specific biomarkers, allowing objective proof of active or passive tobacco exposure, such as: carbon monoxide in exhaled air, cotinine (a nicotine metabolite that can be measured in plasma, saliva, urine, hair and intranasally), but also the more recently identified anatabine, anabasine, thiocyanate, uric acid and nitric oxide [5].

**MATERIAL AND METHODS**

**Clinical diagnosis of tobacco use and dependence**

Before more in depth approach of the biomarkers importance for daily practice of assisting smoking patients, we shall briefly describe below the criteria for clinical diagnosis of tobacco (nicotine) dependence, as defined by the most recently agreed European smoking cessation guideline, published in 2012[4]. So, basic clinical evaluation should consider:

- *Smoking status* (smoker/ex-smoker/non smoker); smokers are defined as at least 6 months daily consumers, while ex-smoker have quit since 6 months minimum.

- *Type of tobacco product* (knowing that certain products are more harmful than others, for example risk of oral cancer is higher in cigars and pipes vs. cigarette smokers [6])

- *Intensity of tobacco consumption*, as quantified by *number of packs-years* for cigarette smokers, respectively number of pipes, cigars, amount of tobacco chewed –in grams-, etc. Number of packs-years (PY) is calculated as number of cigarettes smoked daily/ 20 (a standard cigarettes pack has 20 cigarettes) x number of years of smoking.

- *Nicotine dependence score* is resulting from the Fagerstrom nicotine dependence test - a sum of 6 items questionnaire, most importantly querying about time of first cigarette smoked at awakening. The dependence score is calculated based on answers to all 6 questions, like mild (0-3), moderate (4-6) or severe (>7) addiction to nicotine.

- *Medical history* of a smoker is relevant generally, but especially for any tobacco related disorder imposing immediate cessation or any concomitant disorder like anxiety, depression, myocardial infarct, stroke, anorexia, skull and brain injury, dermatological affections, etc., that might ask cautions of the tobacco cessation pharmacotherapy.

- *Analysis of previous attempts to stop smoking* is crucial to prevent smoking relapse and to identify risk factors for a severe nicotine dependence or to find explanations for unsuccessful treatment. In this view, one should expect smokers feel more difficult to quit during the first 6-8 weeks after stopping tobacco use. Due to absence of nicotine provided to the body via tobacco smoke, patients might describe a “*nicotine withdrawal syndrome*”, consisting of: acute/uncontrollable need to smoke (craving), irritability, restlessness, depressive mood, increased appetite (especially for sweets), trouble to concentrate and focus memory, headaches, insomnia and sometimes dizziness.
When available, clinical evaluation of smokers should be supplemented with a psychological evaluation, aiming diagnosis of behaviour disorders, but also an inventory of depressive symptoms and a motivational interview about personal perception of the quitting smoking process.

RESULTS AND DISCUSSIONS

Laboratory diagnosis of tobacco exposure

Smoking status established on basis of the above mentioned clinical criteria, when also declared by the patient, must be objectively validated by biochemical tests to determine biomarkers of tobacco exposure. These ones were developed as specific tools to assess human body exposure to tobacco products. They offer a possibility to measure components and toxicants inside tobacco smoke, independently of smoking behaviour, which means even if a subject is not a smoker, but is passively exposed to smoking by others, tobacco traces can be detected into the body of the non smoker. It seems use of only one such biomarker won’t completely satisfy the smokers’ evaluation, thus several biological assessments are recommended, in current clinical practice. The main biomarkers utilized by practitioners are: carbon monoxide in expired air and cotinine (a metabolite of nicotine) that can be measured in plasma, saliva, urine, hair and intranasally. Actually, researchers have studied also the role of some new biomarkers: anatabine, anabasine, thiocyanate, uric acid and nitric oxide. Same time, biomarkers of tobacco carcinogenesis, like: 1 hydroxypyrene (1-HOP), 4-(methylamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (NNAL-Gluc), 4-(methylamino)-1-(3-pyridyl)-1-butanolone (NNK) and 8-Oxo-7,8-dihydro-2’-deoxyguanosine (8-oxo-dG) in urine, were approached in various studies. [7,8,9].

Carbon monoxide (CO) can be most easily monitored and represents an indicator of certain tobacco consumption. CO concentration in a smoker’s body is determined if the patient exhales in a carbon monoxide analyzer. The CO unit is ppm (parts per million), a parameter that can be converted to equivalent % CO Hb carboxyhemoglobin reading, by a 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 interpreting CO values, yet active smoking remains the major cause to increase CO levels. In normal conditions, in non-smokers, exhaled CO is < 4 ppm.

The European Smoking Cessation guideline of the European Network for Smoking Prevention (ENSP, www.ensp.org) has stated the 7 ppm limit of CO level to distinguish active smokers from non smokers [4]. Also, cautious interpretation of CO is required in some special situations, when CO levels may register higher than estimated values, such as in smokers with COPD, 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 usually is seen in this category of patients [10].

In the tobacco cessation center of the Clinic of Pulmonary Diseases of Iasi, routinely determination of CO in exhaled air of smokers attending our unit has been introduced since 2000, with various equipments, continuously improving our technique and resources until nowadays (fig.1). Usually, in our current practice of assisting smokers to quit, we correlate CO assessments with “lung life” small instrument results. This is an easy to manipulate device, very useful for an interactive manner to
involve patients in the process of stopping smoking. After blowing into the “lung life” device, it will display lung function (FEV) values, together with estimated age of the patient’s lung, adjusted with age, gender, race and height. If a patient smokes, his/her age will appear older, in a dose-relationship with number of packs-years of cigarette consumption. This diagnostic tool is currently in use in the practice of our smoking cessation center, since 2009 (fig.2).

**Figure 1. Micro Smokerlyzer – Smoking Cessation Center of the Clinic of Pulmonary Diseases Iasi**

**Figure 2. The “lung-life” device - Smoking Cessation Center of the Clinic of Pulmonary Diseases Iasi**

**Thiocyanate** is another marker of tobacco exposure as tobacco smoke contains significant amounts of hydrocyanic acid (hydrogen cyanide), which will be converted in the body into thiocyanate; it can be measured in serum, urine and saliva. Thiocyanate lacks specificity as a marker of involuntary smoking, primarily because of dietary contributions from cyanide-containing foods, such as almonds, or from the presence of thiocyanate itself in certain cruciferous vegetables such as cabbage, broccoli, cauliflower, horseradish, garlic and also in beer. Still, it is good to know that much smaller amounts are found in food compared to tobacco smoke. Serum level of thiocyanate is also influenced by industrial exposure to cyanides in metallurgy, by electrolysis, precious metal refining, case hardening of steel and gas and in the chemical industry, also by processing of the photos.

Although most recent research reported significantly increased levels of thiocyanate in active vs. passive smokers, respectively vs. non smokers, yet, its lack of specificity limits routine use of this lab test for detecting aggression from tobacco smoke[11]. It is unanimously recommended a threshold of > 60μmol / L for distinguishing smokers from non smokers[12].

**Cotinine** is considered the „gold standard”biomarker to prove contact with tobacco products and the most useful in dividing smokers from non smokers, also to check level of inhaled nicotine and any passive ( „second-hand”) tobacco smoke presence. Nicotine enters the body at skin level, also by respiratory and by digestive way. Besides tobacco, another source of nicotine may be delivered in some vegetables (potatoes, tomatoes, cauliflower and cabbage, as well as black tea and coffee). So, confusion may intervene with false tobacco exposure; it was estimated that a medium daily consumption of tomatoes, potatoes, cauliflower together with black tea could bring 8.8 μg nicotine/day, which equals 0.7 ng/ml, thus less than the recognized level of exposure to nicotine from tobacco [13].

Cotinine is a metabolite of nicotine, to be used as an indicator of exposing to the drug delivery via tobacco, because it stays more than 24 hours in the blood. While nicotine has a half-life of approximately 2 hours, in turn,
cotinine has 15-20 hours [14]. Cotinine can be determined in blood, saliva, hair and urine[15].

a) Hair cotinine is a good, cheap, non-invasive indicator of long term tobacco use, in both smokers and non-smokers. Benefits and disadvantages of using hair versus salivary cotinine or salivary versus urinary cotinine were compared in various studies. When it comes to hair versus salivary cotinine, researchers have shown superior long term outcomes for hair samples: allowing 1-3 months exposure estimates, it is easier to collect and manipulate the hair samples, it needs only 30 mg of hair to detect cotinine as 0.05 ng/mg hair. In exchange, it takes 0.5 ml of saliva to evidence cotinine in 0.05 ng/ml, but salivary test of cotinine is interpreting a short term (1-2 days) exposure and saliva samples are more difficult to handle. Yet, some cautions should be taken in relation to any hair treatment or colour [16,17].

b) Salivary cotinine was studied to link cigarette smoking to cotinine levels in saliva. A Romanian study done in Constanta analyzed correlation between concentration of salivary cotinine and parodontal health status in smokers of 15 to 19 years old. By using the NicAlert™ Saliva tests, saliva samples from 362 active smokers were tested to evaluate cotinine fraction, based on last 48 hours tobacco consumption. This was a significant determination of the salivary cotinine. The “cut-off” level for the NicAlert™ assessment is of 10 ng/mL [18]. It is a rapid method; interpretation of results is done after 20 minutes, but quite expensive (39$ for only two determinations). A subject is considered as a non smoker or passive smoker if salivary cotinine ranges 0-10 ng/ml; as an occasional/moderate smoker when a 10-100 ng/ml salivary cotinine range and as an active smoker if saliva cotinine concentration is 100-1000 ng/ml [19].

In a previous study published in 2000, salivary cotinine varied between 0 to 838 ng/ml (with a mean of 166 ng/ml). Every supplemental cigarette/day was associated to an increase of 14 ng/ml in salivary cotinine and established limit to distinguish between smokers and non smokers was 7-13 ng/ml, somehow below other already recognized “cut-off-s” such as 10, 44, or even 100 ng/ml [20].

c) Urinary cotinine determination allows few advantages, compared to serum quantification of cotinine: is not so invasive and shows a significant difference between smokers and non smokers. But, there are also some shortfalls; for instance, during smoking cessation with nicotine substitutes, cotinine from nicotinic pharmacotherapies may add to cotinine provenient from tobacco, so overlapping levels may induce confusion in interpretation of results. As well, collecting and storage of urine sample is more costly and laborious; also lab results are not so immediate as for carbon monoxide, for instance, a fact that might delay therapeutic interventions [21].

Optimal proposed limit to differentiate active smokers from non smokers was 100 μg/g for cotinine in urine [21]. Heinrich-Ramm et al. have demonstrated that urinary cotinine is accumulating in a dose-relationship with number of cigarettes smoked daily (p < 0,0001). Each cigarette is supplementing with approximately 41μg/g of creatinine the level of cotinine in urine. These authors have found a mean urine cotinine in non smokers 200 folds lower, compared to active smokers found with 5.0 μg/g of creatinine [22].

Concentration of urinary cotinine in relation to plasma cotinine may be affected by several factors like urinary flow rate, urine ph and renal function. So, in 2009, Benowitz demonstrates that measuring urine cotinine, after adjustment with creatinine levels,
seems to be the best predictor for plasma cotinine correct values [23].

On the market, there are also available qualitative tests of cotinine in urine, less expensive and more rapid, resembling to pregnancy tests, by also displaying a positive or negative result (read after only 5 minutes). (fig.3).

**Figure 3. Urinary cotinine test- qualitative determination method applied in the Smoking Cessation Center of the Clinic of Pulmonary Diseases Iasi**

d) **Plasma cotinine** can be measured by a quantitative method, yet invasive and sometimes difficult to apply, especially when collecting samples in children. A plasmatic cotinine of less than 15 ng/ml is considered probative for a non-smoking status. In smokers, cotinine level is around 200 ng/ml, but it can reach to 1000 ng/ml, depending on smoking intensity [15].

Numerous studies have tried to find correlations among levels of cotinine in plasma and other tobacco impregnation biomarkers. One of these studies has analyzed CO comparatively to cotinine from plasma in 207 participants and found that it is preferably to use cotinine when assessing long term tobacco abstinence, except in patients treated with nicotine substitution therapy [24]. In a study showing for the first time a significant correlation between plasma cotinine levels and risk of pancreatic cancer (PC), it was observed an increasing risk of PC in subjects with serum cotinine > 1187.8 nmol/L (level corresponding to a number of 17 cigarettes smoked daily), by comparison to subjects with serum cotinine having < 55 nmol/L (OR: 3.66, 95% CI: 1.44-9.26). The highest PC risk was revealed in smokers of more than 30 cigarettes/day versus non smokers (OR: 4.15, 95% CI: 1.02-16.42) [25]

e) **Cotinine determined in nasal lavage fluid**

Nasal mucosa is directly exposed to cigarette smoke in both active and passive smokers. Aggression from tobacco smoke manifests through reduced olfactory function; on the other hand, in smokers treated by nasal spray with nicotine for quitting smoking, side effects like nasal irritation or burning and impaired smell were also described. Only one study exists that determined levels of cotinine in nasal lavage, to estimate exposure to tobacco smoke. It resulted that cotinine levels in nasal lavage were significantly higher in smokers versus non smokers. Optimal " cut-off" limit used by these researchers was of 1.0 ng/ml for distinguishing smokers from non smokers, with a sensibility of 91% and a 99% specificity. Yet, it is to keep in mind that this method of quantifying nasal cotinine concentration indicates furthermore direct injury of smoking on the nasal mucosa [26].

**Serum uric acid**

Uric acid is a degradation product from nucleic acids and the final result of the purine oxidation process. Uric acid is transported by the blood plasma from the liver to the kidneys, where it is filtered and then excreted in a percentage of 70%. The rest of uric acid is eliminated and degraded into the gastrointestinal tract. Its normal value in the human body is influenced by food, gender, age, genetic factors, physical effort and physiology [27]. It acts like a valuable antioxidant, including against oxidative stress caused by chronic tobacco smoking.

Literature in the field described 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 (6.18 mg/dL vs. 5.98 mg/dL in current smokers) [28,29]. In a study in 2010, to examine the effect of cigarette smoking on plasma uric acid concentration and to determine the correlation between this parameter and the biological tobacco markers like plasma thiocyanate and urinary cotinine, plasma uric acid concentration was significantly lower in smokers than in nonsmokers. As well, among smokers, authors noted a negative correlation between uric acid and both plasma thiocyanates (r = -0.437; p < 0.05) and urinary cotinine (r = -0.580; p < 0.05). The significant low plasmatic uric acid in smokers was attributed to a reduction of the endogenous production as a result of the chronic exposure to cigarette smoke (a significant source of oxidative stress). Considering these data, it is recommended to stop or to reduce smoking and to introduce plasma uric acid estimation as a routine test, since it is cheap and simple to reflect the antioxidant level [30]. This determination is currently available in the Clinic of Respiratory Diseases of Iasi.

Other data showed increased values of uric acid and reactive protein C in active smokers towards passive smokers and non smokers [31]. Such conflicting evidence suggests more research is needed to define the role of uric acid as a useful biomarker for tobacco use and cessation.

**Expired nitric oxide (NO)**

NO or the endothelium derived relaxing factor is produced in endothelial cells and it is synthesized by the oxidative process of the guanidine of the amino acid L-arginine by a family of enzymes named NO synthases (NOS’S). NO is a potent vasodilator of the smooth muscle. NO diffuses from the alveoli to vascular smooth muscles, it stimulates guanylate-cy clase leading to increased intracellular cGMP, which triggers smooth muscle relaxation and vasodilation. Chronic smoking influences endothelial function, reduces formation of nitric oxide and increases degradation of nitric monoxide by generating reactive oxygene species [32].

Inhaled tobacco smoke has chronic and acute effects on nitric oxide values. Researchers have signaled lower NO levels in smokers [33]. NO level is increasing after stopping smoking [34]. Cigarette smoke has been shown to induce deterioration of the respiratory epithelium (which is responsible for NO production); so decline in NO level can represent a sign of respiratory destruction. Alternatively, cigarette smoke can inhibit NO production by unidentified yet genetic mechanisms. Another proposed explanation is that the existing high CO level in smokers’airways can determine a fall in exhaled NO, as NO synthases is a cytochrome P450 hemoprotein, possibly inhibited by a great CO concentration [35].

Many studies have demonstrated low levels of NO in smokers and high levels of NO after smoking cessation. Travers et al., in 2007, found NO levels with 1.18 (95% CI, 0.92–1.51; p = 0.20) higher in non smokers than in current smokers and with 1.13 (95% CI, 0.97–1.31; p = 0.11) higher than in former smokers. They established as reference NO value in normal subjects a limit of 7.8 - 41.1 ppb [36]. In 2010, Tramontini et al. described twice more high levels of the expired NO in non smokers: 20.1 ppb (17.7-27.8) (p<0.001), towards smokers: 10.8 ppb (7.8-15.3) [37].

Exhaled NO is also a well-studied marker of airway inflammation and of oxidative stress and is increased in lung diseases such as asthma [38] and bronchiectasis [39]. In contrast, exhaled NO is decreased in healthy chronic smokers [40]. Exhaled NO is also increased during COPD exacerbations [41], whereas conflicting results have been reported in stable COPD patients. One study [42] showed higher NO levels in ex-smokers with COPD, compared to healthy nonsmokers.
and in current smokers with COPD, compared to healthy current smokers. Two studies did not show any difference in NO concentrations between subjects with COPD and healthy subjects [43].

NO is easy to measure and is very useful for clinical assessment of the smoking status, as well in evaluating of the above mentioned chronic airway disorders, by monitoring airway inflammation. Exhaled NO values are more stable to monitor, as they modify every few days, unlike the CO monitorization, as CO values are more dynamic, changing every few hours. NO determination in exhaled breath has been introduced since last decade in the routine practice of the Clinic of the Pulmonary Diseases Iasi, as a valuable biomarker of the airway inflammatory process in asthma and COPD, in particular and for showing effects of chronic exposure to tobacco smoke. The NIOX MINO (Fig. 4) is provided with a filter free of NO and with a sensor especially designed to provide electronic signals to NIOX about exhaled breath samples. Measurements are done by a deep inhalation to total lung capacity through the special filter, followed by an exhalation through the filter, done for 10 seconds. To wait for results, it takes 1:40 min.

**Figure 4. Nitric oxide determination equipment - Smoking Cessation Center of the Clinic of Pulmonary Diseases Iasi**

**Biomarkers of exposure to tobacco smoke involved in carcinogenesis**

Among many known carcinogenic agents in tobacco smoke, the most important are considered: polycyclic aromatic hydrocarbons (PAH), N-nitrosamines, metals, aromatic amines, heterocyclic amines and aldehydes. Those ones more involved in carcinogenesis, being also approached in various studies dedicated to this topic are the following: 1-hydroxypyrene (1-HOP), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNAL) and its glucuronides (NNAL-Gluc), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N′-nitrosonornicotine (NNN) and 8-Oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) in urine. Their use in current practice was rapidly extended abroad in the past few years and so, new data are now available. These are sensible, quantitative and reliable tests, but also costly, unfortunately not available in daily practice in our smoking cessation center; anyway such biomarkers are almost constantly increased in smokers, as majority of study reports. A panel of biomarkers alike was elaborated for routinely evaluation of risk for cancer in both active and passive smokers, here including: NNAL, NNK, NNN, 1-HOP, MHBMA (monohydroxybutyl mercapturic acid), SPMA for S-phenyl mercapturic acid (a metabolite of benzene), HPMA for 3-hydroxypropyl mercapturic acid (a metabolite of acrolein), HBMA for 4-hydroxybut-2-yl mercapturic acid (a metabolite of crotonaldehyde), HEMA for 2-hydroxyethyl mercapturic acid (a metabolite of ethylene oxide), 8-epi-PGF (prostaglandins growth factor) 2αd, cyclooxygenase-derived prostaglandin E2 (PGE2) etc. [7].

Hecht et al. have found considerable differences, by gender (p = 0.003) and race (p = 0.022) in smokers, when studying two such composed biomarkers, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-Nitrosonornicotine involved as causal agents in lung, oral, oesophagus and pancreas cancers [7]. These cancers are well known as certified to
develop due to chronic tobacco consumption [6]. 4-(methylamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (NNAL-Gluc) were identified and quantified in the urine samples from patients in Hecht’s study[7]. Hence, non-hispanics smoking women were found with increased levels of urinary NNAL (353 and respectively 336 pg/mL) [7].

Acrylonitrile is a class 2B carcinogen present in cigarette smoke. Urinary 2-cyanoethylmercapturic acid (CEMA) is an acrylonitrile metabolite and a potential biomarker for acrylonitrile exposure. Actuality research has shown much greater urinary CEMA levels in smokers versus non smokers[44].

CONCLUSIONS
There are many recognized and still developing biomarkers for tobacco exposure assessment. There is a great amount of literature in the field, useful to clarify the multitude of questions arising about role of such biological markers and their concrete utility for daily clinical practice. Indeed, these lab tests offer the opportunity to measure constituents of tobacco smoke and to show exposing to toxicants in tobacco, regardless smoking behavior. More exactly, by the help of tools like this, we can objectively confirm if a person, either active smoker, or non smoker but passively exposed to smoking, was affected by noxious tobacco components.

Only one such biomarker is not enough to validate tobacco exposure, thus associating several tests is more reliable. Among all these biomarkers outlined above, we have been successfully introduced in daily clinical practice of the Clinic of Pulmonary Diseases of Iasi carbon monoxide and nitric oxide in exhaled air, serum uric acid and urinary cotinine determinations. These ones are in use since almost a decade already and are in particular aimed for smoking cessation monitoring and for assessing asthma and COPD status, in our patients, either active, passive or never smokers.

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