# Prognostic Capacity of Oxidative Biomarkers in Paroxysmal Atrial Fibrillation

**Mariya Negreva, Krasimira Prodanova, Katerina Vitlianova, Albena Alexandrova**

***Abstract- Background:*** ***In our previous studies on the oxidative status of patients with paroxysmal atrial fibrillation (PAF) we found eight oxidative biomarkers - plasma malondialdehyde (Pl-MDA), erythrocyte malondialdehyde (Er-MDA), plasma glutathione (Pl-GSH), erythrocyte glutathione (Er-GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glucose-6-phosphate dehydrogenase (Glu-6-PhD) - that changed significantly still in the first twenty-four hours of the arrhythmia clinical presentation. It is exactly their early changes that suggest a correlation of these biomarkers with the trigger mechanisms of the rhythm disorder which then raise the question of how efficiently they can predict PAF occurrence. Aim: To analyse the changes in these oxidative biomarkers as predictive for PAF development. Place and duration of study: The participants were recruited in 1st Cardiology Clinic of St Marina University Hospital, Varna, Bulgaria, between October 2010 and May 2012. Patients and methods: The oxidative indicators were measured in 51 patients (26 men; mean age 59.84 ± 1.60) and 52 controls (26 men; mean age 59.50 ± 1.46) matched in age, sex, concomitant diseases, harmful habits and body mass index. Blood samples were collected once. A dichotomous logistic regression analysis was performed to identify the oxidative biomarkers (explanatory variables) independently associated with PAF appearance. Eight logistic models with a single explanatory variable were considered to find statistically significant predictors for PAF. A multiple logistic model was used to assess simultaneously the predictive value of all statistically significant explanatory variables. Results: The logistic regression models with a single explanatory variable showed that six of the eight indicators were associated with PAF development: Pl-MDA (P=0.03), Er-MDA (P<0.001), Pl-GSH (P< 0.001), SOD (P< 0.001), CAT (P< 0.001), GSH-Px (P< 0.001). The multiple logistic model using all six explanatory variables confirmed the results (P=0.006). Constructed models were used to obtain adjusted estimate of odds and a prediction success matrix. It was found that the multiple logistic model could measure the PAF probability using values of these six markers. Conclusion: Pl-MDA, Er-MDA, Pl-GSH, SOD, CAT and GSH-Px were found to be oxidative biomarkers with predictive value for PAF occurrence. In clinical practice for each measured value of these biomarkers, the probability of the arrhythmia manifestation could be calculated.***

***Keywords: atrial fibrillation, oxidative markers, prediction, occurrence.***

## I. INTRODUCTION

Biomarkers have emerged recently as invaluable tools for modern medicine. They play a crucial role in the detection, prognosis and treatment of many diseases. They have also been increasingly used for predictive purposes to assess the probability of developing a disease, which is of essential importance for clinical practice and especially for primary prophylaxis [1]-[3]. Atrial fibrillation (AF) is the most common arrhythmia disorder, often labeled as the “new non-infectious epidemic" [4]. Paroxysmal atrial fibrillation (PAF) represents about 25% of all registered cases of AF [5]. Since the disorder is known to have a great recurrence rate and the thromboembolic events related to it become increasingly frequent, the possibility to predict its occurrence could be of great significance for clinical practice. In this aspect, there have been only a few studies on PAF and oxidative biomarkers and it is usually the persistent and the permanent type of AF that are investigated [6]-[9]. In our research on oxidative status of PAF patients, we have found that oxidative stress develops very soon after the onset of the arrhythmia [10]-[12]. Eight biomarkers of oxidative stress were significantly changed compared with those measured in the controls. The levels of plasma malondialdehyde (Pl-MDA) (P=0.02) and erythrocyte malondialdehyde (Er-MDA) (P<0.001) were elevated while the concentrations of plasma glutathione (Pl-GSH) (P<0.001), erythrocyte glutathione (Er-GSH) (P<0.001), the activity of glutathione peroxidase (GSH-Px) (P<0.001) and glucose-6-phosphate dehydrogenase (Glu-6-PhD) (P=0.03) were decreased (Table 1). The antioxidant activity of superoxide dismutase (SOD) (P<0.001) and catalase (CAT) (P<0.001) increased in a compensatory response (Table 1). Because of the early changes of these biomarkers we can assume that PAF initiation is closely connected with oxidative stress and its indicators can bear upon the occurrence of the arrhythmia. Therefore we set out to conduct the present study with the purpose of investigating the changes of these indicators as predictive for the occurrence of the rhythm disorder.

**Table 1. Oxidative stress markers in patients with PAF and controls**

| Oxidative stress biomarkers | Patients with PAF | Controls |
|---|---|---|
| Pl-MDA (nmol/mg protein) | 0.143±0.007 | 0.125±0.004 |
| Er-MDA (nmol/mg Hb) | 1.368±0.069 | 0.386±0.027 |
| Pl-GSH (ng/mg protein) | 64.966±1.281 | 75.001±1.392 |
| Er-GSH (ng/mg Hb) | 997.004±32.603 | 1347.002±32.613 |
| GSH-Px (nmol/min/mg Hb) | 25.004±0.814 | 30.114±0.854 |
| Glu-6-PhD (nmolNADP$^+$min/mg) | 2.499±0.102 | 2.903±0.149 |
| SOD (U/mg Hb) | 8.463±0.255 | 5.808±0.135 |
| CAT ($E_{240}$/min/mg Hb) | 7.362±0.251 | 4.760±0.121 |

**Manuscript Received on May 07, 2015.**

**Mariya Negreva**, MD, PhD, working as Asst. Prof., in Intensive Care Unit at University hospital of Varna, Bulgaria.

**Krasimira Prodanova**, B.Sc (Faculty of Mathematics, Informatics and Mechanics) University of Sofia, Bulgaria.

**Katerina Vitlianova**, PhD, DSc, Master of Phylosophy degree in Epidemiology and Biostatistics - Cambridge University, UK.

**Albena Alexandrova**, PhD, DSc, Master of Phylosophy degree in Epidemiology and Biostatistics - Cambridge University, UK.



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## II. MATERIAL AND METHODS

### A. Study Population

For the investigation of these oxidative biomarkers, 338 patients with PAF (episodes lasting < 48 hours) were screened to the study. The arrhythmia episode duration until hospitalization was precisely determined for each patient by his medical history. The diagnosis was made on the base of ECG performed immediately after hospitalization. The exclusion criteria eliminated 282 patients from the study (see exclusion criteria). The sinus rhythm in the remaining 56 patients (31 men, 25 women) was restored after they received propafenone according to the established regimen [13]. After balancing the gender structure of study patients, 51 patients (26 male, 25 female) with mean age of 59.84 ± 1.60 years (31-77 years) were selected for the study. A total of 169 subjects were screened as controls and 52 of these were included in the study (26 men, 26 women, mean age 59.50±1.46 years, range 30-76 years). The exclusion criteria were the same for both patients and controls. The controls had no evidence for AF in their history or in their ECG studies until the performance of the study.

***Exclusion criteria:***

1. Cardiovascular diseases: ischemic heart disease, chronic heart failure, refractory hypertension, implanted devices to treat arrhythmias, inflammatory or congenital heart diseases, moderate or severe acquired valve diseases, cardiomyopathies;
2. Other diseases – renal or hepatic failure, diseases of the central nervous system, history of inflammatory or infectious diseases three months prior to the study, neoplastic or autoimmune disorders, chronic lung diseases, diseases of the endocrine system (except type 2 diabetes mellitus, noninsulin dependent, well controlled);
3. Hormone replacement therapy, receipt of contraceptives, systematic receipt of analgesics;
4. Patients unable to determine the onset of the arrhythmia;
5. Persistent rhythm disorder after the 24$^{th}$ hour scheme of propafenone; restoration of sinus rhythm by means of electrical cardioversion (only for the patients);
6. Evidence of AF episode (only for the controls).

The study was conducted in the First Clinic of Cardiology at St. Marina University Hospital in Varna, Bulgaria, between October 2010 and May 2012 after obtaining approval from the University Hospital Research Ethics Committee and in compliance with the Helsinki Declaration [14]. The participants were included in the study after signing a written informed consent.

### A. Sample Collection and Analytic Methods

Blood samples from patients and controls were taken once. For the patients it was immediately after their admission in the hospital. All blood samples were collected and stored in accordance with the requirements of analytical methods that had been described in details elsewhere [10]-[12]. Eight oxidative biomarkers were measured and evaluated in every blood sample: Pl-MDA, Er-MDA, Pl-GSH, Er-GSH, SOD, CAT, GSH-Px and Glu-6-PhD.

### A. Statistical analysis

The logistic regression model was used in the present study to identify the statistically significant, PAF-occurrence related predictive biomarkers among those we measured.

Brief explanation of the model:

Assume that a response (dependent) variable $y$ is a dichotomous categorical variable (presence or absence of PAF) and that, for given values of the explanatory variables (the investigated oxidative markers) the probabilities for the two categories are $p$ and $(1-p)$. The odds of dichotomous response are given by the following equation:

$$odds = p/(1-p).$$

The function $\ln[p/(1-p)]$ (logarithm of odds) is called a logit function. The dichotomous logistic regression model assumes that the logistic function can be modeled as a linear function of a vector of $n$ explanatory variables $x(x_1, x_2, ..., x_n)$:

$$d(x) = \beta_0 + \beta_1 x_1 + ... + \beta_n x_n$$

Given a random sample of $N$ observations $(y_i, x_{i1}, x_{i2}, ..., x_{in}), i = 1, 2, ..., N$, the maximum likelihood estimator $\hat{\beta}(\hat{\beta}_0, \hat{\beta}_1, ..., \hat{\beta}_n)$ of the model parameters vector $\beta(\beta_0, \beta_1, ..., \beta_n)$ can be obtained. The estimator $\hat{\beta}$ can be used to obtain the estimator for the probability of PAF manifestation $\hat{p}_i = \exp\left(x_i\hat{\beta}\right)/\left(1+\exp\left(x_i\hat{\beta}\right)\right)$ for each of the $N$ observations. Testing of hypothesis concerning the regression parameters can include test of single parameter, test involving several parameters from the same regression, and joint tests involving parameters from different regressions. In logistic regression, tests for contribution of one or more parameters from the same regression are usually constructed with a large sample Wald'test [15], with test statistic $Q_W = \hat{\beta}^T [Var(\hat{\beta})]^{-1} \hat{\beta}$, where $Var(\hat{\beta})$ is the estimated covariance sub-matrix for the relevant parameters. This statistics is approximately distributed as a $\chi^2(n)$ random variable with $n$ degrees of freedom under the null hypothesis that $n$-dimensional vector $\hat{\beta}$ is equal $\vec{0}$.

In comparison to the multiple linear regression models, the coefficient vector $\hat{\beta}_i$ must be interpreted differently:

- The coefficients $\hat{\beta}_i$ were interpreted as estimates of *ln odds*.
- A marginal one unit increase in $x_i$ brings an increase in *ln odds* of the amount of $\hat{\beta}_i$.

The confidence intervals were calculated for the odds ratio estimates by taking the exponent of upper and lower endpoints of the asymptotic confidence interval for the log odds. Descriptive statistics were applied for the calculation of mean values, standard error of the mean (SEM) and relative shares. The analysis of the hypotheses for differences of two means and relative share equality was done by Student's t-test at the 0.05 level. The results were presented as mean ± SEM or n(%). Data analysis was carried out by the specialized statistical package STATISTICA, Version 10.0 [16].

## III. RESULTS AND DISCUSSION

### A. Clinical Characteristics of Patients and Control Group



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The patients were statistically matched to the controls in the following parameters: number of participants in a group, mean age, gender structure, concomitant diseases, dyslipidemia, received treatment (until hospitalization), frequency of harmful habits and body mass index (BMI) (Table 2). Most of these have been found to affect the oxidative status of patients [17], [ 18]. We considered therefore that matching the two groups by these parameters made the comparison between them as objective as possible and would be a good basis for getting correct results from the logistic regression analysis.

**Table 2. Clinical characteristics of patients with PAF and the control group**

| | Patients with PAF | Control group | *P* |
|---|---|---|---|
| Number in group | 51 | 52 | 0.89 |
| Age (years) | 59.84±1.60 | 59.50±1.46 | 0.87 |
| Men/Women | 26/25 | 26/26 | 1/0. 93 |
| Concomitant diseases<br>Hypertension<br>Type 2 diabetes mellitus | 37 (72.54%)<br>3 (5.88%) | 34 (65.38%)<br>2 (3.84%) | 0.44<br>0.62 |
| Dyslipidemia | 4 (7.84%) | 3 (5.77%) | 0.69 |
| Drugs for hypertension and dyslipidemia<br>Beta blockers<br>ACE inhibitors<br>Sartans<br>Statins<br>Metformin | 19 (37.35%)<br>15 (29.41%)<br>11 (21.57%)<br>4 (7.84%)<br>3 (5.88%) | 17 (32.69%)<br>14 (26.92%)<br>9 (17.31%)<br>3 (5.77%)<br>2 (3.84%) | 0.62<br>0.78<br>0.58<br>0.69<br>0.62 |
| Harmful habits<br>Smoking*<br>Drinking alcohol** | 8 (15.69%)<br>7 (13.72%) | 7 (13.46%)<br>6 (11.53%) | 0.75<br>0.74 |
| BMI (kg/m²) | 23.85±0.46 | 24.95±0.45 | 0.09 |

* No more than half a box of cigarettes per week. Hospitalized patients had not smoked at least 24-48 hours before onset of arrhythmia. The lab tests for the controls were done after a 48-hour non-smoking interval.

** No more than 1-2 drinks/week. Hospitalized patients had not drunk alcohol at least 48 hours before onset of arrhythmia. The lab tests for the controls were done after a 48-hour nondrinking interval.

We found from the case history that patients had been admitted to the ward very soon after the onset of rhythm disorder. The statistical analysis showed that until admission the AF episodes lasted 8.14 ± 0.76 hours on the average (minimum 2 hours and maximum 24 hours).

### *A. Logistic Regression Data Analysis*

The primary purpose of the constructed logistic models was to study the impact of the oxidative markers on the response variable – the presence or the absence of PAF. To examine the bivariate relationships between PAF and each of the eight markers, single variable logistic regression models were estimated. The analysis showed that six of the oxidative biomarkers were statistically significant for PAF manifestation, namely: Pl-MDA, Er-MDA, Pl-GSH, SOD, CAT and GSH-Px (Table 3). Levels of Er-GSH and Glu-6-PhD activity were not predictive because P was greater than 0.05.

**Table 3. The parameters of estimated single variable logistic regression models**

| **Oxidative Biomarker** | $\hat{\beta}_0$ | $\hat{\beta}_1$ | ***P*- level (Wald's $x^2$)** | **Odds Ratio-unit change (OR)** | **Odds Ratio 95%Confidential Interval (CI)** |
|---|---|---|---|---|---|
| **Pl-MDA** | -1.70 | 12.63 | ***0.033*** | 2.14 | 1.33-30.23 |
| **Er-MDA** | -6.84 | 9.42 | ***0.000004*** | 23.28 | 6.31-73.71 |
| **Pl-GSH** | 7.89 | -0.11 | ***0.00003*** | 4.39 | 1.37-17.68 |
| ***Er-GSH*** | 0.38 | -0.00 06 | *0.37* | 0.89 | |
| **SOD** | -10,07 | 1,47 | ***0.0000005*** | 24.92 | 13.16-49.9 0 |
| **CAT** | -10,09 | 1,74 | ***0.0000002*** | 50.53 | 29.34-110. 16 |
| **GSH-Px** | 3,911 | -0,14 | ***0.0002*** | 4.50 | 1.51-48.18 |
| ***Glu-6-Ph D*** | -0,04 | 0,007 | *0.97* | 0.99 | |

To illustrate the information presented in Table3, let examine in details the relationship between PAF and the variable Er-MDA. The fitted logistic regression model has the equation

$$\ln[p/(1-p)] = -6.84 + 9.42\,\text{Er-MDA}$$

The fitted equation indicated ($\hat{\beta}_1 > 0$) that the higher the levels of Er-MDA, the greater the probability *p* for PAF presence. The range of values of Er-MDA in the sample were 0.63 (nmol/mg Hb) to 2.7 (nmol/mg Hb). The range of values for probability were therefore given by

$$p[d(0.63)] = \frac{\exp(-6.84 + 9.42 * 0.63)}{1 + \exp(-6.84 + 9.42 * 0.63)} = 0.288,$$

$$p[d(2.7)] = \frac{\exp(-6.84 + 9.42 * 2.7)}{1 + \exp(-6.84 + 9.42 * 2.7)} = 0.998.$$

Constructed model was used to obtain prediction success matrix for presence (code "1") and absence (code "0") of PAF in our sample:



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**Table 4. The prediction success matrix for the oxidative biomarker Er-MDA**

| Classification of cases: Er-MDA Percent correct: 93.14% | | | |
|---|---|---|---|
| Observed | Predicted - 0 | Predicted - 1 | Percent correct |
| **0** | **48** | **4** | **92.30769** |
| **1** | **3** | **47** | **94.00000** |

In conclusion, the marker MDA investigated in erythrocytes (Er-MDA) was a predictor for the development of the rhythm disorder and the correctly predicted cases by the statistical model were 93.14%. Data from Table 3 show that Pl-MDA was also a predictive factor for the development of PAF (P=0.03), and the probability of the arrhythmia manifestation grew with the increase of its values ($\hat{\beta}_1$=10.26). The logistic regression model correctly classified 59.22% of the observed cases in our sample (OR 2.14, 95%CI 1.33-30.23). It is well known that MDA is one of the main products of oxidative degradation of lipids. The high levels of this aldehyde are indicative of enhanced lipid biomolecules oxidation. It can serve as an indirect sign of increased levels of reactive oxygen species [19], [ 20]. MDA is frequently used as a biomarker in assessing the in vivo oxidative stress [21]. Considering the above facts, we could draw the conclusion that the intensified oxidative processes, and accordingly the elevated levels of oxidative damage indicators, could be associated with increased probability of developing PAF. GSH is a "key" non-enzymatic antioxidant agent in the living; it is a compulsory co-factor of GSH-Px, an important antioxidant enzyme [22]. The GSH/GSH-Px system is of crucial importance for the antioxidant defense system [23]. The lower levels of Pl-GSH indicated an increased probability for the development of PAF (P<0.001; $\hat{\beta}_1$ = -0.11). The statistical model made accurate predictions in 67.65% of the cases in the study sample (OR 4.39, 95%CI 1.37-17.68). A decrease in the activity of GSH-Px was associated with increased probability of the arrhythmia occurence (P<0.001; $\hat{\beta}_1$ = -0.14) and 67.96% of the study cases were correctly classified by the logistic model we used (OR 4.50, 95%CI 1.51-48.18). The exhaustion of GSH/GSH-Px system potential made occurrence of PAF more likely to happen. The enzymes SOD and CAT are the first and most important line of enzymatic antioxidant protection in the human body [24], [ 25]. Their increased activities are indirect indicators of wide pro-oxidant damage [26], [27]. Changes in their activity were also predictive for the development of the arrhythmia (P<0.001). Increased values led to increased probability of the rhythm disorder ( $\hat{\beta}_1$ =1.47; $\hat{\beta}_1$ =1.74 respectively). The logistic model we used correctly predicted respectively 82.52% and 87.38% of the diagnosed cases (OR = 24.92, 95%CI 13.16-49.90; OR = 50.53, 95%CI 29.34-110.16). Single variable logistic regression models determined Er-MDA, Pl-GSH, SOD and CAT as the predictors of PAF (OR=23.28, 95%CI 6.31-73.71; OR=4.39, 95%CI 1.37-17.68; OR=24.92, 95%CI 13.16-49.90; OR=50.53, 95%CI 29.34-110.16). To determine how the explanatory variables (oxidative biomarkers) together predict the probability, a multiple logistic regression model was fitted using all outlined above six predicative markers. The fitted model was given by

$d(x)= – 20,35+ 9.29(Pl\text{-}MDA)+ 13.42(Er\text{-}MDA)– 0.207(Pl\text{-}GSH)+1.51(SOD)+$

$+2.42(CAT) –0.29(GSH\text{-}Px)$

The P-level of $x^2$- Wald'test test statistic for these relevant six parameters was 0.0006. The specific mathematical model we developed for calculating the probability of occurrence of PAF could be used in clinical practice to determine more precisely the need for and duration of antiarrhythmic therapy.

## IV. CONCLUSION

The previously established early change in the oxidative biomarkers Pl-MDA, Er-MDA, Pl-GSH, Er-GSH, SOD, CAT, GSH-Px and Glu-6-PhD in PAF allow us to suggest a close relation between oxidative stress and the arrhythmia occurence. The results of the logistic regression analysis indicate that changes in six of these indicators - Pl-MDA, Er-MDA, GSH, SOD Pl-, CAT and GSH-Px – are independently associated and are reliable predictors for the development of the rhythm disorder. By calculating the values of these biomarkers, we could predict the percentage probability of future occurrence of PAF.

## ACKNOWLEDGMENT

This study was not supported financially by any funds or institutions.

## COMPETING INTERESTS

This study was not supported financially by any funds or institution.

## REFERENCES

[1] Nalejska E, Mączyńska E, Lewandowska MA. Prognostic and predictive biomarkers: tools in personalized oncology. Mol Diagn Ther. 2014;18(3):273-84. doi: 10.1007/s40291-013-0077-9.

[2] Spitzer P, Klafki HW, Blennow K, Buée L, Esselmann H, Herruka SK et al. cNEUPRO: Novel Biomarkers for Neurodegenerative Diseases. Int J Alzheimers Dis. 2010 Sep 19;2010. pii: 548145. doi: 10.4061/2010/548145.

[3] Huo AP, Lin KC, Chou CT. Predictive and prognostic value of antinuclear antibodies and rheumatoid factor in primary Sjogren's syndrome. Int J Rheum Dis. 2010;13(1):39-47. doi: 10.1111/j.1756-185X.2009.01444.x.

[4] Lip GY, Kakar P, Watson T. Atrial fibrillation – the growing epidemic. Heart. 2007;93(5):542-43. PMID: 17435064.

[5] Levy S, Maarek M, Coumel P, Guize L, Lekieffre J, Medvedowsky JL et al. Characterisation of different subsets of atrial fibrillation in general practice in France: the ALFA study. Circulation. 1999;99(23):3028–35. doi: 10.1161/01.CIR.99.23.3028. PMID: 10368121.

[6] Neuman RB, Bloom HL, Shukrullah I, Darrow LA, Kleinbaum D, Jones DP, et al. Oxidative stress markers are associated with persistent atrial fibrillation. Clin Chem. 2007; 53(9):1652-57. doi: 10.1373/clinchem.2006.083923. PMID: 17599958.

[7] Kim YM, Kattach H, Ratnatunga C, Pillai R, Channon KM, Casadei B. Association of atrial nicotinamide adenine dinucleotide phosphate oxidase activity with the development of atrial fibrillation after cardiac surgery. J Am Coll Cardiol. 2008;51(1):68-74. doi: 10.1016/j.jacc.2007.07.085. PMID: 18174039.

[8] Leftheriotis DI, Fountoulaki KT, Flevari PG, Parissis JT, Panou FK, Andreadou IT et al. The predictive value of inflammatory and oxidative markers following the successful cardioversion of persistent lone atrial fibrillation. Int J Cardiol. 2009;135(3):361-69. doi: 10.1016/j.ijcard.2008.04.012. PMID: 18640731.

[9] Wu Y, Zhang K, Zhao L, Guo J, Hu X, Chen Z. Increased serum HMGB1 is related to oxidative stress in patients with atrial fibrillation. J Int Med Res. 2013;41(6):1796-802. doi:



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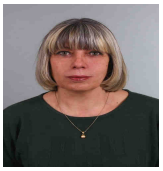
10.1177/0300060513503917. PMID: 24265331.
[10] Negreva MN, Penev AP, Georgiev SJ, Alexandrova AA. Changes in Glucose-6-phosphate Dehydrogenase Activity in Paroxysmal Atrial Fibrillation. J Cardiobiol. 2014;2(1):5.
[11] Negreva MN, Georgiev SJ, Penev AP, Alexandrova AA. Dynamics of oxidative status in patients with paroxysmal atrial fibrillation. Scripta Scientifica Medica. 2014;46(3);33-41. doi: http://dx.doi.org/10.14748/ssm.v46i3.764.
[12] Negreva MN, Penev AP, Georgiev SJ, Alexandrova AA. Paroxysmal atrial fibrillation: dymanics of the main antioxidant enzymes – superoxide dismutase and catalase. Folia Medica. 2014;56(2): 96-101. PMID: 25181846.
[13] Bellandi F, Cantini F, Pedone T, Palchetti R, Bamoshmoosh M, Dabizzi RP. Effectiveness of intravenous propafenone for conversion of recent-onset atrial fibrillation: a placebo-controlled study. Clin Cardiol 1995; 18: 631-634.
[14] World Medical Association Declaration of Helsinki (2008) Ethical principles for medical research involving human subjects. 59th WMA General Assembly. Seoul.
[15] Jobson JD. Applied Multivariate Data Analysis. Berlin: Springer Verlag; 1991; 278-303.
[16] STATISTICA, Statistical Analysis Software, version 10.0, StatSoft Inc. 2010.
[17] Aksnes TA, Schmieder RE, Kjeldsen SE, Ghani S, Hua TA, Julius S. Impact of new-onset diabetes mellitus on development of atrial fibrillation and heart failure in high-risk hypertension (from the VALUE Trial). Am J Cardiol. 2008;101(5):634-38. doi: 10.1016/j.amjcard.2007.10.025. PMID: 18308012.
[18] Lin YK, Chen YJ, Chen SA. Potential atrial arrhythmogenicity of adipocytes: implications for the genesis of atrial fibrillation. Med Hypotheses. 2010;74(6):1026-29. doi: 10.1016/j.mehy.2010.01.004. PMID: 20149554.
[19] Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis. 2005;15:316e328.
[20] Lykkesfeldt J. Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin Chim Acta. 2007;380(1-2):50-58.
[21] Shilpa HD, Bijoor AR. Malondialdehyde as a marker of lipid peroxidation in acute myocardial infarction patients. J Health Sci. 2013;1(1):20-22.
[22] Lushchak VI. Glutathione Homeostasis and Functions: Potential Targets for Medical Interventions. J Amino Acids. 2012; 2012;736837. doi: 10.1155/2012/736837.
[23] Haayes JD, McLellan LI. Glutathione and glutathione-dependent enzymes represent a coordinately regulated defence against oxidative stress. Free Radic Res. 1999; 31(4):273-300.
[24] Zocche SH, Canes SL, Debbas V, Martins Laurindo FR. Cellular prion protein (PrP(C)) and superoxide dismutase (SOD) in vascular cells under oxidative stress. Exp Toxicol Pathol 2011;63(3):229-36. doi: 10.1016/j.etp.2009.12.004.
[25] Mak JC, Ho SP, Yu WC, Choo KL, Chu CM, Yew WW et al. Polymorphisms and functional activity in superoxide dismutase and catalase genes in smokers with COPD. Eur Respir J 2007;30(4):684-90. PMID: 17567676.
[26] Gonzalez-Pinto A, Martinez-Cengotitabengoa M, Arango C, Baeza I, Otero-Cuesta S, Graell-Berna M et al. Antioxidant defense system and family environment in adolescents with family history of psychosis. BMC Psychiatry 2012;12:200. doi: 10.1186/1471-244X-12-200.
[27] Dawn I, Naskar S, Sarkar S, Biswas G, Halder S. A comparative study between synovial superoxide dismutase per oxidation marker and the severity of knee osteoarthritis. Int J Pharm Sci Invent 2013;2(1):01-04.

**Mariya Negreva, MD, PhD** graduated from Medical University of Varna, Bulgaria in 2006. Since 2008 Dr. Negreva has been working as assistant professor in Intensive Care Unit at University hospital of Varna, Bulgaria; since 2013 has been a specialist in cardiology and echocardiography. In 2015 – completed her PhD. Her research area is atrial fibrillation and echocardiography. Member Of Bulgarian Society Of Cardiology, Euripean Society of Cardiology.



**Krasimira Prodanova:** Personal Details: 1972 - 1976 BSc (Faculty of Mathematics, Informatics and Mechanics) University of Sofia, Bulgaria; 1976 - 1978 MSc (Differential Geometry) University of Sofia, Bulgaria; 1995 - PhD (Theory of Probability and Statistcs - *thesis title*: Statistical Models in pharmacokinetics) University of Sofia, Bulgaria. Employment history and experiences: 2007 – present: Vice Dean of Faculty of Applied Mathematics & Informatics, Technical University of Sofia, Bulgaria (TU-Sofia). Previous Appointments: 1980-1987 Senior Biostatisticians (Bulgarian Academy of Medicine, Faculty of Pharmacy); 1987-1999: Assistant Professor (TU-Sofia, Faculty of Applied Mathematics&Informatics, Department of Statistics& Optimization); 2000-Present: Professor; 2000 – 2004: Head of Department "Statistics & Optimization". Scientific Publications: 82 with cumulative Impact Factor 8,998 and 7 books. The citation cumulative Impact Factor 15,68. Research Interests include mathematical modeling in pharmacokinetics and medicine, estimation of the parameters of stochastic models, drug response models, survival analysis and optimization problems.

**Katerina Vitlianova, PhD, DSc** Education: Higher medical education - Medical University of Varna, Bulgaria (1992), Master of Phylosophy degree in Epidemiology and Biostatistics - Cambridge University, UK 1993, Specialities in Internal Medicine (2000) and Cardiology (2008) - Medical University of Sofia, Specialisation in Echocardiography and Emergency medicine - Barzilai Medical Center, Ben-Gurion University of Negev, Israel. Research interests mainly in the fields of Heart failure, biomarkers, myocardial infarction ant rrhythm disorders. Memberships: Bulgarian Society of Cardiology, Chairmen of the working group of Women and cardiovascular risk, Bulgarian League of Hypertension, Balkan Medical Union, European Society of Cardiology.



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