Optimization of hypomagnesemia diagnostics as an integral element in paroxysmal atrial fibrillation management strategy

Mikhail Arkhipov, *Yakov* Bozhko, *Nadejda* Beloconova, *Valentina* Kochmasheva, and *Oksana* Chromtsova

Ural State Medical University, 620028, Repina Str., 3, Yekaterinburg, Russian Federation

Abstract. *Purpose.* To study magnesium status of patients having paroxysmal atrial fibrillation (AF) based on the use of an integrated clinical and laboratory approach. *Methods.* A prospective cohort study included 58 patients of the cardiology department of New Hospital Medical Association. The main group consisted of 32 patients having frequently recurring paroxysmal AF, the control group consisted of 26 patients without paroxysmal rhythm disturbance. The clinical status, Holter ECG monitoring data, the test results for magnesium deficiency (MD) clinical evidence, laboratory evidence of calcium, magnesium in blood plasma and formed elements, magnesium in whole blood, free fatty acids (FFA) and osmolality in blood plasma were assessed. *Results.* The score obtained when assessing MD clinical evidence was significantly higher in the main group patients compared with the control group (16.5 (11÷21) vs. 13 (8÷15), p<0.001). A statistically significant magnesium decrease in whole blood was revealed in patients having paroxysmal AF (0.54 (0.46÷0.60) vs. 0.61 (0.59÷0.64), p<0.001) and inside formed elements (0.68 (0.53÷1.07) vs. 1.31 (1.07÷1.44), p<0.001), which reflected changes in their magnesium status to a greater extent than the measured plasma cation concentrations. A close correlation between magnesium content in formed elements (intracellularly) and AF paroxysms frequency (Spearman's rank correlation -0.51, p<0.001) was established. A violation of calcium to magnesium ratio in blood plasma (2.6 (2.5÷2.9) vs. 3.0 (2.8÷3.1), p=0.004) and intracellularly (4.85 (2.62÷9.3) vs. 1.85 (1.57÷2.07), p<0.001) was revealed in patients having AF. It has been shown that complex forming interactions with free fatty acids may affect intracellular calcium and magnesium content.

1 Introduction

Currently, hypomagnesemia is being considered as an important clinical and pathogenetic aspect in the early debut of cardiovascular disease [1]. A number of large foreign studies have demonstrated the interdependencies of hypomagnesemia and coronary heart disease development [2], chronic heart failure [3], atherosclerosis [4] and cardiac rhythm disturbance [5,6,7], moreover a significant amount of studies indicate the need for

hypomagnesemia correction for cardiovascular mortality reduction and pathological conditions prevention.

Clinicians pay special attention to the study of atrial fibrillation (AF) development, which is due not only to this pathology prevalence in the population [8], but also to the high incidence of serious complications leading to life quality decrease, disability, and patients' mortality [9, 10]. Ion channels dysfunction, changes in calcium and magnesium ions content, atria structural remodeling (fibrosis), as well as autonomic nervous system disorganization [11] are actively discussed in AF pathogenesis concept, when considering substrates for a focal ectopic focus formation and re-entry loop subsequent functioning. Hypomagnesemia in this pathology can be of fundamental importance, since intracellular magnesium regulates Na+-K+-ATPase activity, sodium transmembrane gradient regulates calcium physiological stress [12].

Sub-analyses of a large population-based ARIC study results and Framingham cohort demonstrated a convincing interdependencies between low serum magnesium and an increased AF risk [6, 7]. In addition, in a number of studies, intracellular magnesium low content before cardiac surgery closely correlated with an increased AF risk in postoperative period [13], moreover, Henyan et al. have found that low doses of administered magnesium turned out to be the most effective and safe from the point of view of AF prevention, in comparison with moderately high doses [14].

THE STUDY PURPOSE – to study magnesium status of patients having paroxysmal atrial fibrillation based on the use of an integrated clinical and laboratory approach.

2 Materials and study methods

A prospective cohort study included 58 patients of the cardiology department of New Hospital Medical Association. The main group consisted of 32 patients having frequently recurring paroxysmal AF, the control group consisted of 26 patients without paroxysmal rhythm disturbance. Statistical differences in the studied groups were not obtained for the main clinical and demographic indicators when analyzing the clinical and demographic characteristics of patients (Table 1). The study did not include patients with glomerular filtration rate less than 60 ml/min/1.73 m2 in order to minimize the effect of significantly reduced renal function on electrolyte balance. Moreover, magnesium and loop diuretics administration for 3 months before the study was indicated as an additional exclusion criterion.

Table 1. Clinical and demographic characteristics of patients included in the study

Characteristic	Main group	Control group	P
Sex, f/m, n/n (%/%)	24/8 (75/25)	14/11 (53/47)	0.09
Age, years, M±m	67.2±3.5	65.3±4.6	0.12
Smoking «+», n (%)	5 (15.6)	5 (20)	0.72
Hypertensive disease «+», n (%)	32 (100)	25 (100)	1
CHD «+», n (%)	0 (0)	0 (0)	1
Diabetes «+», n (%)	5 (15.6)	5 (19.2)	0.72
Hypothyreoidism «+», n (%)	12 (37.5)	10 (38.4)	0.398
Alcohol «+», n (%)	4 (12.5)	2 (7.6)	0.398
KK ml/min, M±m	75.8±12.5	75.3±7.2	0.43
GFR, ml/min/1,73м2, M±m	71.95±16.2	72.15±14.3	0.85
CHA2DS2-VASc, score, M±m	4.45±1.43	-	-
HAS-BLED, score, M±m	2.18±0.66	-	-

Paroxysmal AF presence was recognized based on the patient medical history, electrocardiographically confirmed paroxysms presence, as well as daily monitoring results of Holter electrocardiogram (HM-ECG) performed using Cardiotechnics 4000 system (Incart, St. Petersburg).

A clinical assessment of magnesium deficiency evidence consisted in conducting a modified magnesium deficiency (MD) assessment test for all patients according to Ye. A. Tarasov et al. [16], which included an analysis of the most common MD evidence and its progression contributing factors. The initial MD evidence was recognized with a total score of 10 to 15, expressed evidence – above 15. Further, in patients of the main and control groups a venous blood sampling fasted was performed into standard EDTA tubes for trace analysis (Becton Dickinson Intertational, USA) in order to determine calcium and magnesium content in blood plasma and blood cells, magnesium in whole blood, free fatty acids in blood plasma and osmolarity.

From the obtained blood samples, 1 ml was sampled for magnesium analysis in whole blood by inductively coupled plasma mass spectrometry (ICP-MS) method at the premises of Common Use Center of the Ural Branch of RAS «Geoanalyst». Samples were decomposed in 20 ml Teflon weighting bottles with caps. 1 ml of 14M nitric acid was poured onto whole blood weighed samples and kept in the cold for 30 minutes. In this case, rapid gas emission and dark foam formation on the solution surface were observed. After the reaction completion, the inner walls of the weighting bottles were carefully washed off with water and 0.1 ml of H_2O_2 was added. After 15 minutes, the white foam and brown flakes formed in the solution gradually gravitated to the bottom, upon which the reaction was completed. Next, the weighting bottles with sample were kept on a stove at 80°C until the flakes were dissolved, and then they were covered with caps and, adding 1 ml of 14M HNO_3 and 0.1 ml of H_2O_2, reheated at the same temperature for 15 minutes to homogenize the solutions. The cooled solutions were quantitatively transferred into 50 ml polypropylene containers and 10 µg/L of indium (internal standard element) was added, and then diluted to the mark with ultrapure water. The trace composition was measured on NexION 300S quadrupole ICP mass spectrometer (Perkin Elmer). All measurements were performed in a quantitative analysis mode with calibration curves plotting (multi-element standard solutions Perkin Elmer Instruments).

The remaining 3.5 ml of blood were centrifuged for 5 minutes at 3000 rpm, and then blood plasma was sampled with a syringe for subsequent analysis of extracellular magnesium and calcium content using standard reagents Magnesium-Novo and Calcium-Novo (Vector -Best, Novosibirsk) with the help of «Leki» ultraviolet spectrophotometer (Finland). Free fatty acids (FFA) content in blood plasma was determined by spectrophotometric method [17]. OSCR-1M cryoscopic medical osmometer was used (Christmas Center, Moscow) to measure plasma osmolarity.

The blood cells separated by centrifugation were calcined for 2 hours in an incinerator at 1000°C. The calcined residue was dissolved by heating in concentrated hydrochloric acid, and then the resulting solution was used to determine intracellular magnesium and calcium using the previously mentioned standard reagents Magnesium-Novo and Calcium-Novo.

After determining the trace content, calcium-magnesium index (CMI) was calculated as the quotient of dividing intracellular or extracellular calcium content by intracellular or extracellular magnesium content, respectively.

The results statistical processing was performed in SPSS 16.0 software package. Descriptive statistics included the median (Me) calculation, 25th and 75th percentiles (25%÷75%). Mann-Whitney U-test was used to assess intergroup differences reliability in independent samples. Correlation between pairs of quantitative characters was evaluated

using Spearman's rank. For comparisons, a type one error was recognized as statistically significant at p<0.05.

3 Study results and discussion

In 32% of the examined patients of the main group, the incidence of symptomatic AF paroxysms was 1-2 times a month, in 34% – 2-3 times a week, in 16% – once a week. 18% of the patients experienced daily symptoms associated with paroxysmal AF, which significantly reduced their life quality.

The clinical test results for magnesium deficiency clinical evidence assessment are shown in Figure 1. According to the total score determined when processing the test results, the patients having paroxysmal AF had more pronounced MD clinical evidence 16.5 (11÷21), compared with the control group patients 13 (8÷15), p<0.001.
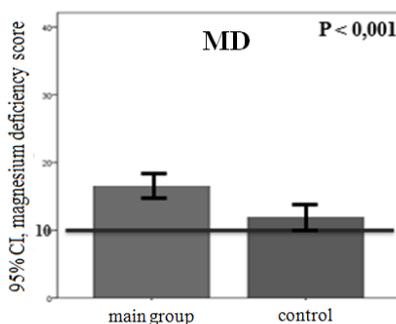

Fig. 1. MD evidence assessment using a clinical test

Table 2 shows the results of blood plasma osmolarity identification, plasma concentrations of FFA, calcium, magnesium (extracellular), calcium and magnesium content in formed elements (intracellular), as well as magnesium in whole blood.

**Table 2.** Laboratory blood counts of the main and control groups patients

Character	Group		P
	Main	Control	
Blood plasma (extracellular)			
Osmolarity, mmol/kg	258 (238.2÷271.7)	284.5 (280÷289.2)	0.001
Free fatty acids, μmol/L	655 (580.5÷823)	416.5 (329.2÷522)	0.001
Magnesium, mmol/L	0.83 (0.82÷0.84)	0.85 (0.83÷0.87)	0.124
Calcium, mmol/L	2.18 (2.1÷2.43)	2.54 (2.39÷2.77)	0.001
CMI	2.6 (2.5÷2.9)	3 (2.8÷3.1)	0.004
Whole blood			
Magnesium, mmol/L	0.54 (0.46÷0.60)	0.61 (0.59÷0.64)	<0.001
Formed elements (intracellular)			
Magnesium, mmol/L	0.68 (0.53÷1.07)	1.31 (1.07÷1.44)	<0.001
Calcium, mmol/L	3.9 (3.11÷4.87)	2.39 (2.12÷2.56)	<0.001
CMI	4.85 (2.62÷9.3)	1.85 (1.57÷2.07)	<0.001

An analysis of electrolytes plasma concentrations indicates a significant CMI decrease in patients having paroxysmal AF, which is explained by a detected extracellular calcium concentration decrease against significantly reduced plasma osmolarity. Calcium to magnesium ratio in blood plasma of patients without pathology tends to 3:1. When intracellular CMI value in patients without AF was close to 2:1, this indicator in the main group patients was significantly higher due to calcium content increase in formed elements and intracellular magnesium decrease.

The total magnesium content in whole blood according to ICP-MS was also significantly lower in patients having paroxysmal AF, compared with patients without this pathology. It seems interesting that the traditional method for magnesium plasma concentrations determining did not show a significant difference in the groups, in contrast to studying magnesium content inside formed elements and in whole blood. According to the correlation analysis, the score obtained using MD assessment test closely correlated with magnesium content in whole blood (Spearman's rank correlation -0.65, $p<0.001$), as well as with intracellular magnesium content (Spearman's rank correlation -0.44, $p=0.007$). Moreover, it was magnesium content in formed elements that showed a close correlation with AF paroxysms frequency (Spearman's rank correlation -0.51, $p<0.001$), which is important to take into account when determining magnesium status of patients. Intracellular CMI is somewhat less closely related to AF paroxysms frequency (Spearman's rank correlation 0.43, $p=0.002$).

In the study conducted, we have found that free fatty acids content in blood plasma significantly affects magnesium content inside formed elements: the higher is FFA plasma concentration, the lower is intracellular magnesium content (Spearman's rank correlation -0.57, $p<0.001$). The same significant trend was found for magnesium content in whole blood, but to a lesser extent (Spearman's rank correlation -0.38, $p=0.02$).

The revealed dependences allow us suggesting that the following interactions may influence the redistribution into the cell of both FFA and calcium and magnesium cations:

$$Mg2+ (Ca2+) + L- -> [Mg L]+, \tag{1}$$

$$Mg2+ (Ca2+) + 2L- -> [Mg L2], \tag{2}$$

$$Mg2+ (Ca2+) + 4L- -> [MgL4]2-, \tag{3}$$
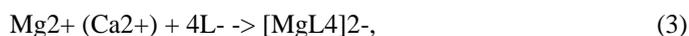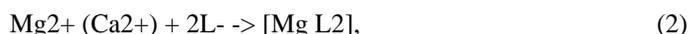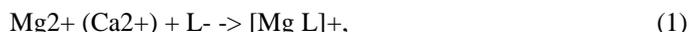
where L is FFA.

If we assume the formation of a complex cationic (1) or anionic (3) type compound, its lipophilic properties should be less pronounced compared to complex compounds formed by reaction (2).

One of the possible methods for compounds lipophilicity assessing is to study their redistribution to a non-polar solvent – chloroform. We have conducted a model experiment to study fatty acids (FA) redistribution in chloroform at various concentrations of FFA, calcium, and magnesium in the initial system (Fig. 2, Fig. 3). FFA determining method was the experiment methodological basis [17]. Solutions of stearic acid in ethanol were used as control solutions of FFA.
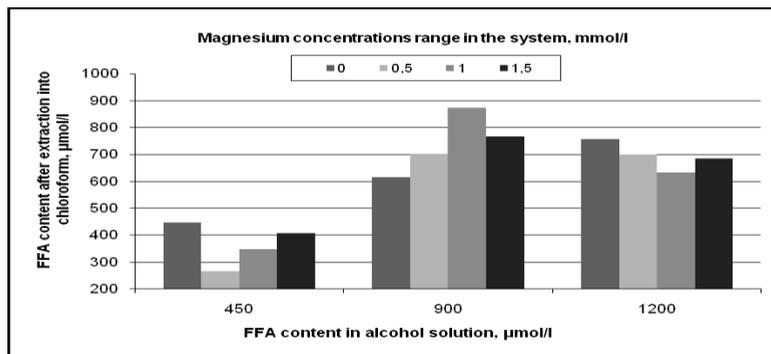
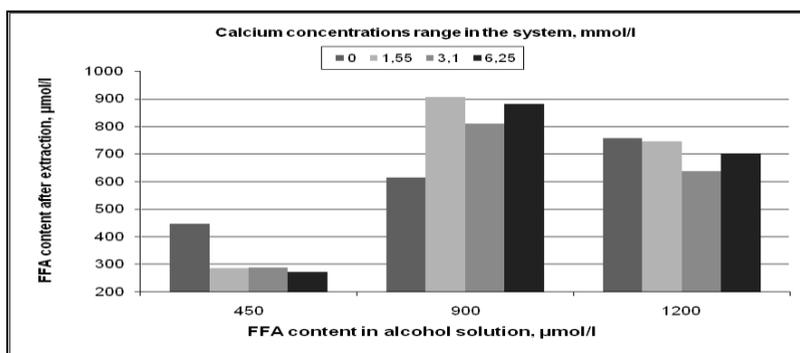**Fig. 2.** FFA redistribution at various magnesium contents in the system

Fig. 3. FFA redistribution at various calcium contents in the system

When FFA content was 900 μmol/L, the addition of magnesium ions in a concentration of 0.5 to 1.5 mmol/L significantly increased fatty acids (FA) redistribution into chloroform medium. FFA content increase to 1200 μmol/L or their concentration decrease to 450 μmol/L in the initial system did not lead to the FA redistribution increase, despite the addition of various magnesium concentrations. A similar dependence was obtained in experiments with calcium – only with FFA content of 900 μmol/L, the addition of calcium ions in a concentration of 1.55 to 6.25 mmol/L led to FA redistribution increase into chloroform.

Therefore, it is possible to conclude that calcium and magnesium redistribution can be affected not only by cations initial concentration, but also by FFA. The best redistribution was obtained with FFA concentration of 900 μmol/L and magnesium content of 1 mmol/L, and calcium content of 3 mmol/L, which is consistent with the optimal CMI value in blood plasma. The revealed interdependencies can be used to further develop measures for MD correction and prevention in cardiologic profile patients.

4 Summary

1. Patients with paroxysmal atrial fibrillation had significantly lower magnesium content in whole blood and inside formed elements, compared with patients without this arrhythmia.

2. In the study, intracellular magnesium content determined by spectrophotometric method and magnesium concentration in whole blood, analyzed by inductively coupled plasma mass spectrometry, were closely correlated with the clinical test results for MD

assessment, in contrast to element plasma concentrations. A close correlation between hypomagnesemia and AF paroxysms frequency has been established for magnesium content indicator in formed elements.

3. Calcium and magnesium redistribution can be affected not only by the initial cations concentration, but also by FFA. The best redistribution was obtained with FFA concentration of 900 μmol/L and magnesium content of 1 mmol/L, and calcium content of 3 mmol/L, which is consistent with the optimal CMI value in blood plasma.

Acknowledgements

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