Baseline serum globulin as a predictor of the recurrence of lone atrial fibrillation after radiofrequency catheter ablation

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ABSTRACT

Objective: Inflammation and autoimmune responses play an important role in recurrence of atrial fibrillation (AF). Serum globulin levels are a commonly used clinical index that represents inflammation and autoimmune response. This study aimed to determine the relationship between baseline serum globulin levels and the risk of recurrence after ablation in lone AF patients.

Methods: We enrolled 348 lone AF patients undergoing radiofrequency catheter ablation for the first time for whom complete follow-up data were available. Pre-ablation peripheral venous blood samples were obtained for measurement of serum globulin levels.

Results: During the follow-up period of 22 months (range, 6–62), AF recurred in 129 patients (37.1%). Recurrence was associated with a low level of pre-ablation serum globulins. Multiple Cox proportional hazard regression analysis showed that persistent AF, AF duration, left atrial diameter, no amiodarone after ablation, and the serum globulin level in particular were independent predictors of AF recurrence. According to receiver operating characteristic curve analysis, the best diagnostic cut-off serum globulin level was 25.4 g/L, which showed 74.4% sensitivity, 71.3% specificity, and 73.3% accuracy.

Conclusion: The baseline low serum globulin level is associated with AF recurrence after first-time ablation in lone AF patients. Therefore, it may be used as a predictor of AF recurrence in these patients. *(Anatol J Cardiol 2017; 17: 381-5)*

Keywords: lone atrial fibrillation, globulin, radiofrequency catheter ablation, serum, recurrence

Introduction

Atrial fibrillation (AF) is a common supraventricular arrhythmia that is associated with an increased risk of ischemic stroke and contributes to the disease burden in heart failure (1). Radiofrequency catheter ablation (RFCA) is a common treatment option for patients with symptomatic AF in hospitals throughout the world (2). However, it is associated with a high risk of late recurrence of AF (2), and its mechanism is not fully understood. Atrial arrhythmogenic remodeling is considered as the essential pathological mechanism underlying AF (3), with inflammatory and autoimmune reactions playing an important role in the progress of remodeling (4, 5).

Serum globulins are synthesized and secreted by mononuclear phagocytes. Inflammatory cytokines and antibodies are the two major components of serum globulins. Thus, serum globulin levels are a good biomarker of inflammation and the immune status of the body. Inflammatory cytokines as well as autoantibodies are involved in the pathogenesis and recurrence of AF (6, 7).

Therefore, serum globulin levels may be a serological marker for predicting the risk of AF recurrence after RFCA.

To test this hypothesis, we performed a retrospective study to investigate the role of serum globulin levels in predicting AF recurrence after RFCA in lone AF (LAF) patients.

Methods

Patient enrollment

We consecutively recruited 1676 nonvalvular symptomatic AF patients who were admitted to the Affiliated Drum Tower Hospital of Nanjing University Medical School between May 2009 and January 2014, after obtaining their written informed consent for participation. We only included patients with LAF who had no comorbid conditions that could predispose them to AF recurrence; thus, the number of patients included was decreased to 532 patients who were diagnosed with LAF based on the following criteria (8): no past history of cardiovascular disease, no evidence of ischemic heart disease, no cardio-

myopathy, no valvular heart disease, no heart failure, no diabetes, no hypertension, and no hyperthyroidism. All the patients were genetically unrelated and were from the ethnic Han Chinese population. The study was conducted according to the Helsinki Declaration and approved by the ethics committee of Nanjing University (2009-NJEA-10).

Preprocedural 7-day Holter electrocardiography (ECG) was performed to establish the preexisting type and burden of AF. Paroxysmal and persistent AF was defined according to the international consensus on the definition of AF (8). A baseline physical examination, two-dimensional transthoracic and transesophageal echocardiogram, and computed tomography/magnetic resonance imaging were performed to exclude significant structural cardiac disease, left atrial (LA) thrombus, and coronary artery disease, as well as to establish the anatomy of the pulmonary veins (PVs). The exclusion criteria were (1) age ≥60 years (2), a history of cardiac arrhythmia other than AF (3), previous catheter ablation (4), a history of hepatitis and cirrhosis (5), right heart failure (6), diseases of the immune system (7), current chemotherapy treatment for cancer (8), current use of immunosuppressants, and (9) failure of ablative treatments or severe postprocedural complications.

According to the exclusion criteria, 113 patients were excluded, which made the final number of patients 406. All the 406 patients underwent catheter ablation for the first time and were successfully cardioverter to stable sinus rhythm (SR). Of the 406 patients, 58 were lost to follow-up. Therefore, a total of 348 patients completed the follow-up examination, and their clinical data were used for analysis.

RFCA

The RFCA protocol we used has been described in detail previously (2). In brief, the CARTO-guided PV isolation procedure was performed using a deflectable circular PV mapping catheter and an open-irrigated 3.5-mm tip quadripolar ablation catheter (Thermo-Cool Navistar; Biosense Webster Inc., USA). The elimination of all ostial vein potentials and complete block of the entrance to PVs were verified with a Lasso catheter. Additional ablation of the roof line (connecting the two superior PVs) and the mitral line (joining the mitral annulus to the PV either anteriorly or laterally) was conducted in all the patients. The lines were checked for bidirectional block after restoration of SR. Complete block of the mitral and roof lines was confirmed on observation of reversal of the expected activation sequence on one side of the line while pacing from the other side.

Follow-up and grouping

After ablation, administration of oral anticoagulants was resumed and continued for at least 2–3 months. The AF-free period was calculated from the date of ablation to the date of recurrence or last follow-up (6–62 months). Atrial arrhythmias that occurred during the first 2 months after ablation, which is considered as the blanking period (8) were not counted as re-

currences. Antiarrhythmic medications, including amiodarone, metoprolol and propafenone, were generally continued till the end of the third month after ablation, unless recurrent arrhythmia indicated the need for continuation of treatment. Cases of documented arrhythmia and continued administration of antiarrhythmics for control of AF beyond the blanking period were counted as recurrences.

Clinical visits, 12-lead ECG, and 24-h Holter monitoring were conducted at 3, 6, and 12 months after ablation and then yearly after the first year. Moreover, patients underwent ECG monitoring at local clinics if they developed any AF-related symptoms at other time points. AF recurrence was identified by atrial tachyarrhythmia lasting for ≥30 s on a 12-lead ECG or during Holter ECG monitoring. The 348 patients were divided into two groups according to whether AF recurrence occurred (AF group) or not (SR group). AF recurrence during the follow-up was considered censored.

Blood sampling and biochemical assays

When the patients were admitted to the hospitals, venous blood samples were routinely obtained and stored in EDTA-coated tubes for determining the serum globulin level using the same kit (Roche, Switzerland) before RFCA.

Statistical analysis

For comparison between the two groups, Student's t-test (for normally distributed data) or Mann–Whitney U test (for nonnormally distributed data) was used for continuous variables, and the χ^2 test was used for categorical variables. The Cox proportional hazard regression model was used to determine the factors predictive of AF recurrence. Factors that were found to be significant predictors in the univariate analysis (that is, factors for which the p value was ≤0.05) were included in the multiple Cox proportional hazard regression analysis. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. Youden index (Sensitivity + Specificity – 1) of the receiver operating characteristic (ROC) curve was calculated to determine the best cut-off value of serum globulin for predicting AF recurrence. Sensitivity, specificity, and accuracy were determined by Fisher's exact test. Survival curves for the incidence of AF recurrence according to the serum globulin level were calculated with the Kaplan–Meier method, and the log-rank test was used to assess statistical significance. A p value of <0.05 was considered to indicate statistical significance, and all the statistical tests were two-sided. The statistical analyses were performed using the GBSTAT statistical analysis package (version 9.0, Dynamic Microsystems Inc.).

Results

Patient characteristics

The 348 patients were divided into the AF group (n=129) and SR group (n=219). No significant difference between the groups was found in age, body mass index and smoking habit; however, the patients in the AF group were older than those in the

SR group. The incidence of paroxysmal AF was lower in the AF group than in the SR group. Further, the duration of AF was longer in the AF group than in the SR group. Left atrial diameter (LAD) was larger and the ejection fraction was lower in the AF group than in the SR group. Moreover, the number of patients who used propafenone and beta-blockers postoperatively was higher in the AF group than in the SR group, whereas the number of patients who used amiodarone and statins postoperatively was lower in the AF group than in the SR group (Table 1).

Serum level of globulins

The serum level of globulins was lower in the AF group than in the SR group (p<0.001) (Fig. 1).

Predictors of AF recurrence

In the univariate Cox proportional hazard regression analysis, older age, persistent AF, longer AF duration, hyperlipidemia, larger LAD, lower ejection fraction, use of propafenone or beta-blockers after ablation, no amiodarone or statins after ablation, and lower serum globulin levels were found to be significant predictors of AF recurrence (Table 2).

In the multiple Cox proportional hazard regression model, persistent AF, longer AF duration, larger LAD, no amiodarone after ablation, and lower serum globulin levels were found to still be statistically significant predictors of AF recurrence (Table 3).

Predictive value of serum globulin

According to the ROC curve analysis (Table 4), the best threshold value of serum globulin for predicting AF recurrence was 25.4 g/L (AUC: 0.771, 95% CI: 0.718–0.823). This cut-off value

showed 74.4% sensitivity, 71.3% specificity, and 73.3% accuracy.

In addition, Kaplan–Meier survival estimates showed that the incidence of AF recurrence was lower in patients with pre-
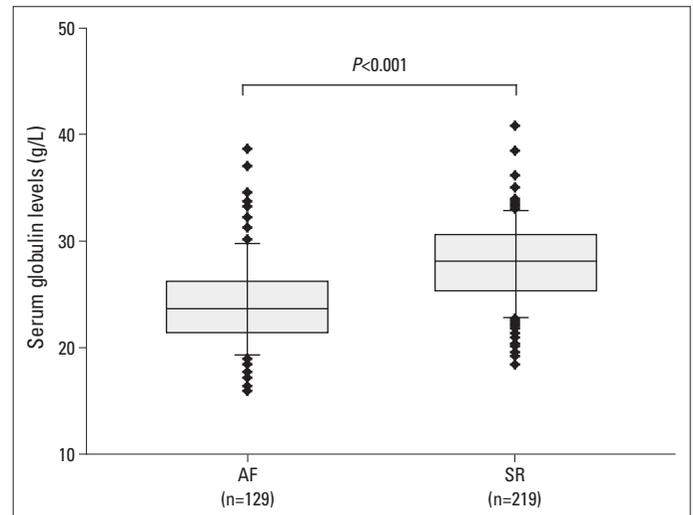

Figure 1. Box plots illustrating the median serum level of globulin in the AF and SR groups. The boxes represent interquartile ranges, and the bars represent the 10th and 90th percentiles

AF - AF group (comprising patients in whom AF recurred); SR - SR group (comprising patients in whom AF did not recur)

Table 1. Clinical characteristics of LAF patient group

Variables	AF	SR	P
Patient number, n	129	219	–
Gender, M/F, n	93/36	156/63	0.864
Age, years	50.5±8.7	47.0±10.1	0.001
Paroxysmal/Persistent AF, n	66/63	189/30	<0.001
AF duration, years	10.8±6.9	3.5±2.9	<0.001
Body mass index, kg/m^2	24.0±2.7	23.9±3.1	0.721
Cigarette smoking, n	25	32	0.246
LAD, mm	42.0±4.7	35.6±4.0	<0.001
Ejection fraction, %	63.1±5.0	64.7±3.9	0.002
Medications after ablation, n			
Propafenone	48	58	0.036
Amiodarone	14	81	<0.001
Beta-blocker	67	80	0.005
ACE-I/ARB	89	133	0.121
Calcium-channel blocker	14	15	0.192
Statins	5	30	0.003

Values are presented as mean±SD or number of patients. ACE-I - angiotensin-converting enzyme inhibitor; ARB - angiotensin receptor blocker; LAD - left atrial dimension

Table 2. Predictors of AF recurrence in univariate Cox proportional hazard regression analysis

Variables	β	SEM	HR	95% CI	P
Age	0.035	0.011	1.035	1.013~1.058	0.002
Persistent vs. Paroxysmal AF	1.152	0.177	3.166	2.239~4.477	<0.001
AF duration	0.129	0.011	1.138	1.114~1.163	<0.001
Hyperlipidemia	-0.838	0.365	0.433	0.211~0.885	0.022
LAD	0.197	0.016	1.218	1.181~1.256	<0.001
Ejection fraction	-0.062	0.019	0.940	0.906~0.976	0.001
Propafenone after ablation	0.553	0.183	1.738	1.214~2.489	0.003
Amiodarone after ablation	-1.476	0.286	0.229	0.131~0.400	<0.001
Beta-blocker after ablation	0.463	0.177	1.588	1.122~2.248	0.009
Statins after ablation	-1.116	0.457	0.328	0.134~0.802	0.015
Serum globulin	-0.168	0.023	0.846	0.809~0.884	<0.001

β - regression coefficient; CI - confidence interval; HR - hazard ratio

Table 3. Predictors of AF recurrence in multiple Cox proportional hazard regression analysis

Variables	β	SEM	HR	95% CI	P
Persistent vs. Paroxysmal AF	0.401	0.195	1.494	1.020~2.189	0.039
AF duration	0.083	0.013	1.087	1.059~1.116	<0.001
LAD	0.149	0.018	1.161	1.120~1.203	<0.001
Amiodarone after ablation	-1.161	0.311	0.313	0.170~0.576	<0.001
Serum globulin	-0.096	0.024	0.908	0.866~0.953	<0.001

Table 4. Predictive value of serum globulin for AF recurrence by ROC curve

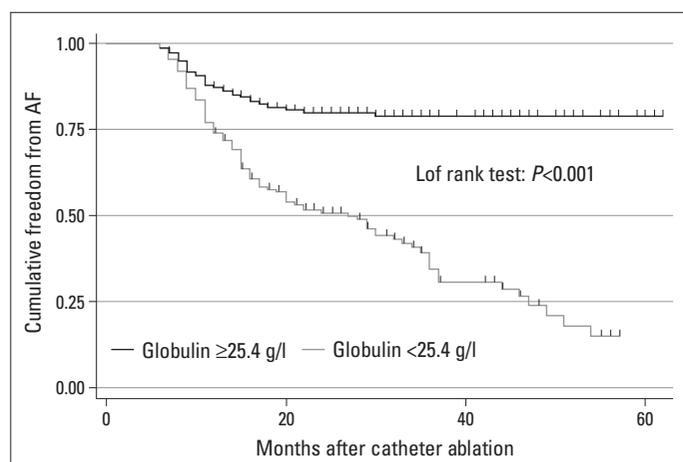|	Best cut-off values	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Accuracy (%)
Serum globulin, g/L	≥25.4	0.771 (0.718~0.823)	74.4	71.3	73.3
Best cut-off value is equal to the largest Youden index. AUC - area under the curve. Youden index= Sensitivity+ Specificity – 1					

Figure 2. Kaplan–Meier survival curves showing freedom from AF recurrence after ablation according to the serum globulin level in LAF patients

operative serum globulin level ≥25.4 g/L than in patients with a globulin level of <25.4 g/L (p<0.001) (Fig. 2).

Discussion

In the present study, the rate of recurrence after RFCA was 37.1%, which is similar to the results of our previous study (9) and other studies (2, 10). Persistent AF, longer AF duration, and larger LAD were identified as risk factors for AF recurrence after ablation. Atrial arrhythmogenic remodeling, defined as any change in atrial structure or function, is the core mechanism in the pathogenesis of AF (3). In contrast, AF itself may induce further structural remodeling, including atrial fibrosis and atrial dilatation (11). Therefore, atrial fibrosis and dilatation may play a role in the vicious cycle leading to the maintenance of AF. Moreover, we previously found that atrial specimens of persistent AF patients showed a much higher collagen fraction and larger LAD than specimens from patients with paroxysmal AF (12). On the basis of these findings, it was easy to explain our result that longer AF duration and persistent AF are associated with more severe atrial fibrosis and larger LAD, which have been confirmed to be the main causes of AF recurrence (13). In addition, we confirmed the notion that administration of amiodarone after RFCA could prevent AF recurrence to some extent and contribute to the maintenance of restored SR in LAF patients (10).

To date, the pathophysiological mechanisms underlying LAF have not been clearly demonstrated. Therefore, in the present study, we tried to speculate on the mechanism based on the present findings and previously reported ones. Yalçın et al. (14) demonstrated that the levels of serum anti-M2-muscarinic receptor and anti-β1-adrenergic receptor, which are globulin-coupled receptors, were associated with paroxysmal AF without concomitant cardiovascular disease. Zou et al. (15) were the first to report that the preprocedural level of serum anti-M2-muscarinic receptor was an independent predictor of the recurrence of LAF 1 year after RFCA. Moreover, there is increasing evidence to support the association of inflammation with AF. Frustaci et al. (16) were the first to demonstrate the high prevalence of inflammatory infiltrates, myocyte necrosis, and fibrosis in atrial biopsy samples from patients with LAF, which were not in the atrial biopsy samples from control patients. Other papers have reported that an increase in the high-sensitivity C-reactive protein level is an independent risk factor for future AF among patients with SR (17, 18). These findings indicate a mechanistic link between the inflammatory and autoimmune processes and the development and recurrence of AF (4).

In this study, we found for the first time that low levels of serum globulins, particularly serum globulin levels <25.4 g/L, are highly predictive of AF recurrence after RFCA in LAF patients. The evidence supporting the role of autoimmunity and inflammation in the development of AF has stemmed from initial observations indicating the role of autoantibodies as key mediators of atrial electrophysiological processes. In animal studies, a G-protein-coupled receptor called anti-β1-adrenergic receptor was found to promote the passage of calcium through L-type calcium channels by increasing the production of cyclic adenosine monophosphate and protein kinase A (19, 20). Subsequent progressive increase in the amount of intracellular calcium was shown to induce myocyte destruction, fibrotic repair, and electrical instability of the heart (21, 22), causing atrial inflammation and perpetuation of AF (23).

Clinical perspectives

Because AF is a heterogeneous disease, the identification of patients at high risk for the recurrence of AF by simple and objective parameters may be helpful in tailoring therapeutic strategies. According to our previous (9) and present studies, a long history of AF, persistent AF, large LAD and no amiodarone after ablation, particularly low baseline serum globulin levels, were associated with an elevated recurrence rate of LAF. Patients with a high baseline serum globulin (≥25.4 g/L) may be more suitable candidates for AF ablation. This profile evaluation is a routine pre-ablation laboratory assessment without additional intervention and burden. Moreover, identification of immunological and particularly autoantibody-mediated mechanisms of LAF opens new perspectives in the treatment and prevention of this arrhythmia.

Study limitations

There are several limitations to our study that must be acknowledged. First, the serum globulin level after ablation was not

measured; therefore, we were unable to demonstrate the superiority of baseline serum globulin over changes in its level with regard to prediction of AF recurrence. Second, we have only studied the association between serum globulin and AF recurrence, and the pathophysiological mechanism needs to be elucidated in further studies. In addition, because we did not collect the data of the mononuclar number and hepatic function since the beginning of our study, the corrections between the serum globulin level and the number of mononuclar and hepatic function were unknown.

Conclusions

In conclusion, low baseline serum globulin levels (<25.4 g/L) as well as persistent AF, a long history of AF, and a larger LA diameter can be considered as predictors of LAF recurrence after RFCA. This provides new information about treatment strategies and improves our understanding of autoimmunity and the inflammatory process in LAF.

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