

Catheter Ablation of Atrial Fibrillation Raises the Plasma Level of NGF- β Which Is Associated with Sympathetic Nerve Activity

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Purpose: The expression of nerve growth factor- β (NGF- β) is related to cardiac nerve sprouting and sympathetic hyper innervation. We investigated the changes of plasma levels of NGF- β and the relationship to follow-up heart rate variability (HRV) after radiofrequency catheter ablation (RFCA) of atrial fibrillation (AF).

Materials and Methods: This study included 147 patients with AF (117 men, 55.8 \pm 11.5 years, 106 paroxysmal AF) who underwent RFCA. The plasma levels of NGF- β were quantified using double sandwich enzyme linked immunosorbent assay method before (NGF- β_{pre}) and 1 hour after RFCA (NGF- $\beta_{post-1hr}$). HRV at pre-procedure (HRV $_{pre}$), 3 months (HRV $_{post-3mo}$), and 1 year post-procedure (HRV $_{post-1yr}$) were analyzed and compared with plasma levels of NGF- β .

Results: 1) The plasma levels of NGF- β significantly increased after RFCA (20.05 \pm 11.09 pg/mL vs. 29.60 \pm 19.43 pg/mL, p <0.001). The patients who did not show increased NGF- $\beta_{post-1hr}$ were older (p =0.023) and had greater left atrial volume index (p =0.028) than those with increased NGF- $\beta_{post-1hr}$. 2) In patients with NGF- β_{pre} >18 pg/mL, low frequency components (LF)/high-frequency components (HF) (p =0.003) and the number of atrial premature contractions (APCs, p =0.045) in HRV $_{post-3mo}$ were significantly higher than those with \leq 18 pg/mL. 3) The LF/HF at HRV $_{post-3mo}$ was linearly associated with the NGF- β_{pre} (B=4.240, 95% CI 1.114–7.336, p =0.008) and the NGF- $\beta_{post-1hr}$ (B=7.617, 95% CI 2.106–13.127, p =0.007). 4) Both NGF- β_{pre} (OR=1.159, 95% CI 1.045–1.286, p =0.005) and NGF- $\beta_{post-1hr}$ (OR=1.098, 95% CI 1.030–1.170, p =0.004) were independent predictors for the increase of LF/HF at HRV $_{post-3mo}$.

Conclusion: AF catheter ablation increases plasma level of NGF- β , and high plasma levels of NGF- β_{pre} was associated with higher sympathetic nerve activity and higher frequency of APCs in HRV $_{post-3mo}$.

Key Words: Atrial fibrillation, catheter ablation, nerve growth factor, sympathetic nerve

INTRODUCTION

Previous studies have demonstrated that myocardial injuries, such as myocardial infarction or radiofrequency catheter ablation (RFCA), lead to cardiac nerve sprouting and sym-

thetic hyper innervation in animal models.¹⁻⁴ Such cardiac nerve sprouting and cardiac sympathetic hyper innervation have been documented to be related to sudden cardiac death^{5,6} or atrial fibrillation (AF).⁷ The nerve regeneration is triggered by the expression of nerve growth factor- β (NGF- β) gene in the non-neuronal cells around the injury site, consequently raising plasma levels of NGF- β .⁸ Therefore, the increase of the plasma level of NGF- β may indicate active nerve sprouting and generation. However, the relationship between the plasma level of NGF- β and cardiac autonomic nerve activity or arrhythmia in human heart has not yet been evaluated. Heart rate variability (HRV) is a measurement of the cyclic variation of the time intervals between consecutive normal heart beats, and has been widely used to assess cardiac autonomic activity, and may be considered as a marker of sympathetic and parasympathetic influence on the modulation of heart rate (HR). Therefore, HRV is one of the integral compo-

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nents of autonomic nervous system assessment.⁹ Extensive but titrated atrial tissue damage during catheter ablation of AF was known to increase trans-cardiac NGF concentration in patients with AF.¹⁰ However, it has not been elucidated whether the plasma level of NGF increases after RFCA and it may reflect post-procedural cardiac autonomic activity in patients who underwent AF catheter ablation. Therefore, we hypothesized that RFCA for AF changes the plasma concentration of NGF-β, which is associated with 3rd month HRV or frequency of arrhythmias. We also tested the feasibility of double sandwich enzyme linked immunosorbent assay (ELISA) technique for detection of the minimal change of NGF-β in the peripheral blood of patients with AF.

MATERIALS AND METHODS

Study population

The study protocol adhered to the Declaration of Helsinki and was approved by the Institutional Review Board of Yonsei University Health System. All patients provided written informed consent. This study initially included consecutive 229 patients with AF who underwent RFCA guided by computed tomography (CT) merged 3D NavX electroanatomical map.

Exclusion criteria were as follows: 1) permanent AF refractory to the electrical cardioversion, 2) left atrial (LA) anterior posterior dimension >55 mm measured on echocardiogram, 3) uncontrolled thyroid disease, 4) aortic aneurysm or dissection, 5) intracardiac thrombi detected by transesophageal echocardiography, 6) significant rheumatic valvular disease, or 7) previous AF ablation or maze surgery. Among 229 patients, 82 patients were excluded because one of HRV data (pre-RFCA, post-RFCA 3rd months, and post-RFCA 1 year) was not available due to frequent AF or other arrhythmias. We did not include the patients with cardiac implantable electronic device in this study. Finally, 147 patients with acceptable 3 times of HRV data were included for data analysis. The baseline characteristics of the patients are summarized in Table 1. All patients maintained optimal anticoagulation (target international normalized ratio 2.0–3.0) before the procedure and antiarrhythmic drugs (AADs) were discontinued for at least five half-lives of each drug and for at least 4 weeks especially in amiodarone. We examined all patients with 3D-spiral CT (64 Channel, Light Speed Volume CT, Philips, Brilliance 63, the Netherlands) in order to visually define the anatomy of LA.

Table 1. Patient Characteristics and Comparisons Based on the Median Value of NGF-β_{pre} and Whether Increase of NGF-β_{post-1hr}

	Overall (n=147)	NGF-β _{pre} >18 pg/mL (n=73)	NGF-β _{pre} ≤18 pg/mL (n=74)	p value	Increase of NGF-β _{post-1hr} (n=127)	No increase of NGF-β _{post-1hr} (n=20)	p value
Male (%)	117 (79.6)	57 (78.1)	60 (81.1)	0.652	101 (79.5)	16 (80.0)	0.961
Age, yrs	55.8±11.5	54.2±12.2	57.4±10.6	0.099	54.9±11.4	61.2±10.8	0.023
PAF (%)	106 (72.1)	52 (71.2)	54 (73.0)	0.814	90 (70.9)	16 (80.0)	0.397
AF duration, months	27.2±6.1	26.4±6.2	28.1±6.0	0.119	27.1±6.4	28.2±4.8	0.457
CHADS ₂ score	0.80±1.03	0.82±1.00	0.77±1.07	0.763	0.76±1.04	1.00±0.97	0.344
Heart failure (%)	0 (0.0)	0 (0.0)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	1.000
Hypertension (%)	62 (42.2)	31 (42.5)	31 (41.9)	0.944	52 (40.9)	10 (50.0)	0.446
Age >75 yrs (%)	4 (2.7)	3 (4.1)	1 (1.4)	0.304	3 (2.4)	1 (5.0)	0.500
Diabetes mellitus (%)	20 (13.6)	9 (12.3)	11 (14.9)	0.654	17 (13.4)	3 (15.0)	0.845
Prior stroke or TIA (%)	15 (10.2)	8 (11.0)	7 (9.5)	0.764	12 (9.5)	3 (15.0)	0.446
Body mass index (kg/m ²)	25.0±2.5	24.9±2.7	25.0±2.4	0.887	25.1±2.5	23.9±2.6	0.048
Echocardiography							
LA size, mm	41.3±5.4	41.6±5.9	41.1±5.0	0.646	41.0±5.3	43.4±5.8	0.067
LVEF, %	63.8±6.9	63.5±7.3	64.2±6.6	0.559	63.8±7.1	64.0±5.8	0.926
E/Em	10.1±3.6	10.0±4.0	10.3±3.2	0.604	10.0±3.6	10.6±4.1	0.506
CT & NavX							
LA volume index, mL/m ²	64.0±19.2	66.6±19.5	61.3±18.8	0.114	62.5±18.6	73.2±21.2	0.028
LA voltage, mV	1.23±0.56	1.23±0.58	1.22±0.53	0.912	1.21±0.56	1.30±0.56	0.534
Clinical outcome							
Ablation time, min	88.0±26.7	88.3±26.6	87.7±26.9	0.891	89.0±26.6	81.9±27.1	0.272
Early recurrence (%)	39 (26.5)	19 (26.0)	20 (27.0)	0.891	35 (27.6)	4 (20.0)	0.477
Clinical recurrence (%)	43 (29.3)	20 (27.4)	23 (31.1)	0.624	38 (29.9)	5 (25.0)	0.653
Post-RFCA antiarrhythmic drugs (%)	42 (28.6)	24 (32.9)	18 (24.3)	0.251	35 (27.6)	7 (35.0)	0.494

NGF-β, nerve growth factor-β; AF, atrial fibrillation; LA, left atrial; RFCA, radiofrequency catheter ablation.

Electrophysiological mapping and 3D voltage mapping

Intracardiac electrograms were recorded using a Prucka CardioLab™ Electrophysiology system (General Electric Health Care System Inc., Milwaukee, WI, USA). Double trans-septal punctures were performed and multi-view pulmonary venograms were obtained. After obtaining trans-septal access, systemic anticoagulation was achieved with intravenous heparin to maintain an activated clotting time of 350–400 sec. We generated 3D-spiral CT merged 3D electroanatomical mapping (NavX system, St. Jude Medical Inc., Minneapolis, MN, USA). We generated a LA 3D voltage map by obtaining contact bipolar electrograms from 350–500 points of the LA endocardium during high right atrial pacing (pacing cycle length: 500 ms) using a multi-polar ring catheter (Lasso, Johnson & Johnson Inc., Diamond Bar, CA, USA). The bipolar electrograms were filtered from 32 to 300 Hz. Color-coded voltage maps were generated by recording bipolar electrograms and measuring peak-to-peak voltage. The percentage of color-coded areas of voltage maps was analyzed by customized software (Image Pro software 6.0, Media Cybernetics Inc., Silver Spring, MD, USA), referenced to the color scale bars, and utilized for the calculation of the mean and regional endocardial voltages.¹¹

AF ablation techniques

We used an open irrigated-tip catheter (Celsius, Johnson & Johnson Inc., Diamond Bar, CA, USA; irrigation flow rate 20 to 30 mL/min; 30 W; 47°C) to deliver RF energy for ablation (Stockert generator, Biosense Webster Inc., Diamond Bar, CA, USA). Patients with both PAF and PeAF initially underwent circumferential pulmonary vein isolation (CPVI) and cavotricuspid isthmus block. Following CPVI in PeAF patients, we generated an LA roof line, a posterior inferior line, and an LA anterior line, and confirmed bidirectional blocks by differential pacing.¹² Depending on the operator's decision, additional ablations for superior vena cava, non-PV foci or complex fractionated electrogram were conducted. If AF persisted beyond the aforementioned ablation protocols for PAF or PeAF, we stopped the procedure after internal cardioversion. The end point of our procedure was the point of no immediate recurrence of AF after cardioversion with isoproterenol infusion (5 μ g/min). If there were non-PV foci under isoproterenol infusion, we ablated them all.

Post-ablation management and follow-up schedule

Patients were asked to visit the outpatient clinic 1 week, 1 month, 3 months, 6 months, and 12 months after RFCA. AADs were stopped in all patients after procedure, but AAD was prescribed for patients with AF recurrence or highly symptomatic ECG-documented frequent atrial premature beats. Warfarin was maintained for at least 2 months after RFCA. The Holter monitoring (24 hr or 48 hr) were evaluated at pre-RFCA and 3, 6, 12, 18, and 24 months after RFCA following the

HRS/EHRA/ECAS Expert Consensus Statement guidelines.¹³ Patients were also advised to call a clinician or visit the outpatient clinic if they experienced symptoms suggestive of an arrhythmia and Holter (24- or 48-h) or event recorder was performed to document ECG in those symptomatic patients. Patients with any documented AF episode lasting longer than 30 sec after the 3-month follow-up were deemed as having clinical recurrence and AADs were prescribed.

HRV analyses

All patients had analyzable HRV data in Holter monitoring taken at 3 different periods (pre-RFCA, post-RFCA 3rd month, and post-RFCA 1 year) by utilizing a GE Marquette MARS 8000 Holter analyzer (GE Medical System, Milwaukee, WI, USA). We excluded the patients whose HRV was not analyzable due to sinus node dysfunction, high number of AF or other arrhythmia episodes. Premature ventricular contractions (PVCs), atrial premature contractions (APCs), and electrical artifacts were also excluded from the analysis. Only high-quality recordings were considered for analysis. All recordings were converted to a digitized format and reviewed by an experienced operator. HRV parameters were obtained and used as an indicator of autonomic activity according to the guidelines previously published.¹⁴ The mean HR, time-domain HRV parameters [mean RR interval (mean NN interval), the standard deviation of NN intervals (SDNN), the standard deviation of 5-minute means of NN intervals (SDANN), the root-mean square of differences between successive NN intervals (rMSSD), the proportion of adjacent NN intervals differing by >50 ms (%) (pNN50)], frequency domain parameters [very-low-frequency components (<0.04 Hz), low frequency components (LF; 0.04–0.15 Hz), high-frequency components (HF; 0.15–0.40 Hz), and the ratio of LF/HF] were analyzed, respectively. The HF and rMSSD served as an indicator of parasympathetic nervous activity, and the LF and LF/HF ratio reflected sympathetic nervous activity.

Statistical analysis

Continuous data were expressed as mean \pm SD and normality tests were performed for each variable to determine whether or not a data set was well-modeled by normal distribution. The baseline characteristics of the two groups were compared using the Student t-test for continuous variables and the chi-square test and Fisher's exact test for categorical variables. We analyzed HRV parameters in Holter monitoring according to the plasma levels of NGF- β before and after RFCA. Baseline characteristics and clinical variables associated with RFCA were also compared according to the plasma levels of NGF- β . Continuous variables were divided and assessed using the median value as the cut-off points. Statistical significance was established at a value of $p < 0.05$. Statistical analysis was performed by SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

AF ablation raises the NGF- β _{post-1hr}, but not in old patients with significant LA remodeling

The plasma levels of NGF- β significantly increased 1 hour after RFCA (20.1 ± 11.1 pg/mL vs. 29.6 ± 19.4 pg/mL, $p < 0.001$) (Fig. 1A). There was a significant correlation between the plasma levels of pre-procedural NGF- β and post-procedural NGF- β ($R = 0.773$, $p < 0.001$) (Fig. 1B). Table 1 summarized the baseline characteristics of included patients, and there was no significant difference between the patients with NGF- β _{pre} > 18 pg/mL ($n = 73$) and those with ≤ 18 pg/mL ($n = 74$) based on the median plasma level of NGF- β . When we compared the patients who had an increased plasma NGF- β _{post-1hr} ($n = 127$) and those did not ($n = 20$), the group with no increase of NGF- β _{post-1hr} showed older age ($p = 0.023$), lower body mass index ($p = 0.048$), and greater LA volume index ($p = 0.028$) (Table 1). However, higher plasma levels of NGF- β were not related to longer duration of total ablation time. In Post AF ablation, mean heart rate and LF was higher than before ablation. In contrast, rMSSD was higher in pre AF ablation (Fig. 1C, D, and E).

High NGF- β _{pre} and NGF- β _{post-1hr} are associated with high LF/HF and APC frequency in HRV_{post-3mo}

Fig. 2 summarized the changes of mean HR and HRV at pre-RFCA (HRV_{pre}), post-RFCA 3 months (HRV_{post-3mo}), and post-RFCA 1 year (HRV_{post-1yr}), depending on median plasma levels of NGF- β _{pre} and NGF- β _{post-1hr}. After catheter ablation of AF, mean HRs were increased at Holter_{post-3mo} (68.9 ± 12.3 bpm to 72.4 ± 10.3 bpm, $p = 0.010$) and at Holter_{post-1yr} (71.9 ± 10.1 bpm, $p = 0.023$). The rMSSD (27.1 ± 22.9 ms to 20.6 ± 15.8 ms, $p = 0.009$ and 20.7 ± 14.8 ms, $p = 0.010$), LF (16.4 ± 19.3 Hz to 10.6 ± 11.4 Hz, $p = 0.003$ and 11.3 ± 10.9 Hz, $p = 0.010$) were reduced at HRV_{post-3mo} and HRV_{post-1yr}, respectively. We compared HRV parameters depending on the plasma level of NGF- β _{pre} and NGF- β _{post-1hr} using the median values as a cut-off (Table 2). Compared to the patients with NGF- β _{pre} ≤ 18 pg/mL, NGF- β _{pre} > 18 pg/mL group showed higher LF/HF ratio ($p = 0.003$) and higher number of APCs ($p = 0.045$) in HRV_{post-3mo} (Fig. 2E and F). LF/HF ratio was also higher in high NGF- β _{post-1hr} group than in low NGF- β _{post-1hr} group ($p = 0.042$). Because both sympathetic (represented by LF) and parasympathetic (represented by rMSSD) activities were reduced after AF catheter ablation, we might not have found significant change of LF/HF ratio in the whole population (Fig. 2).

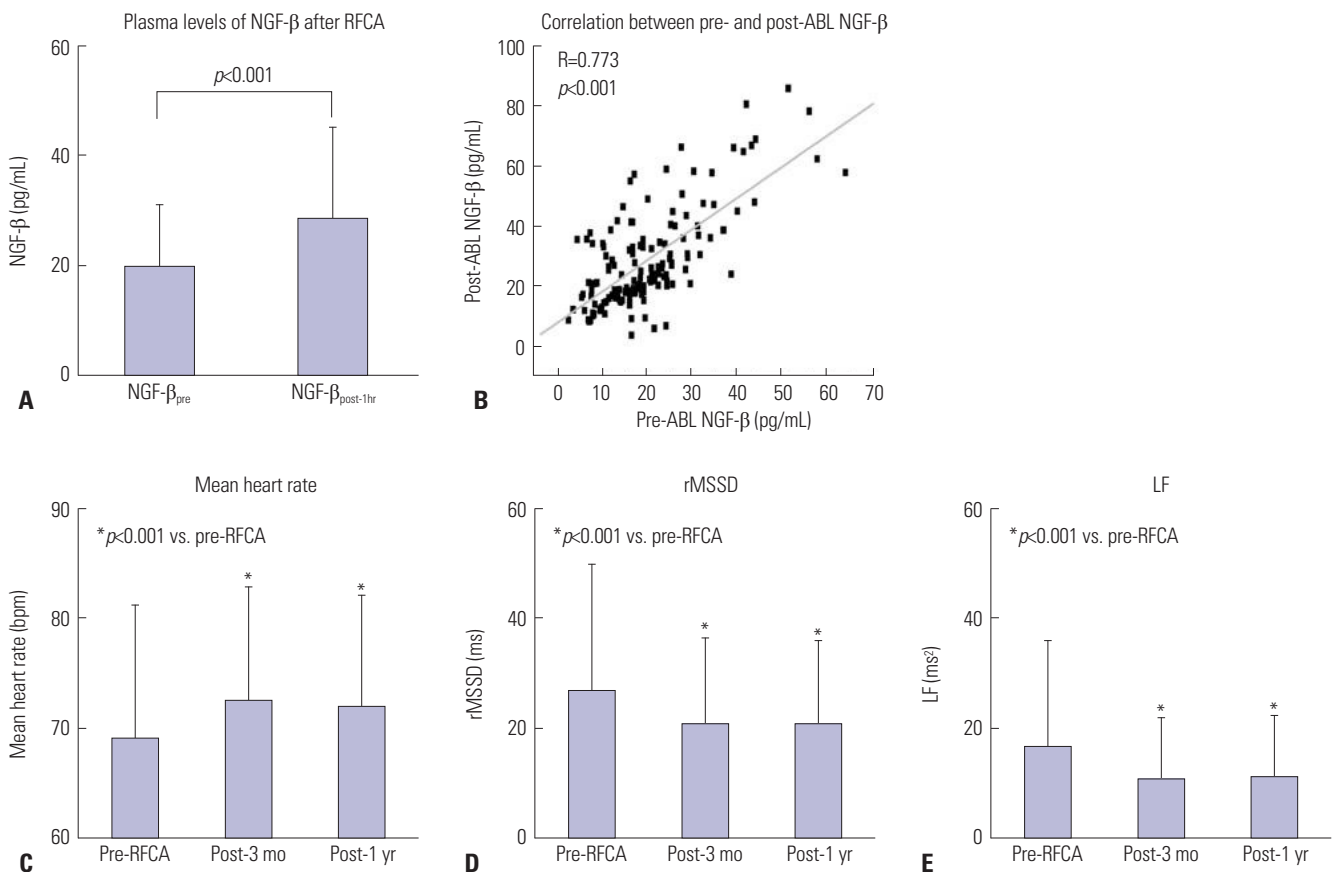


Fig. 1. (A) Plasma levels of NGF- β before and after AF ablation. (B) Correlation between pre- and post-ablation NGF- β s. (C) Mean heart rate in pre AF ablation, post 3 month AF ablation, and post 1 year AF ablation. (D) rMSSD in pre AF ablation, post 3 month AF ablation, and post 1 year AF ablation. (E) LF in pre AF ablation, post 3 month AF ablation, and post 1 year AF ablation. NGF- β , nerve growth factor- β ; RFCA, radiofrequency catheter ablation; LF, low frequency components.

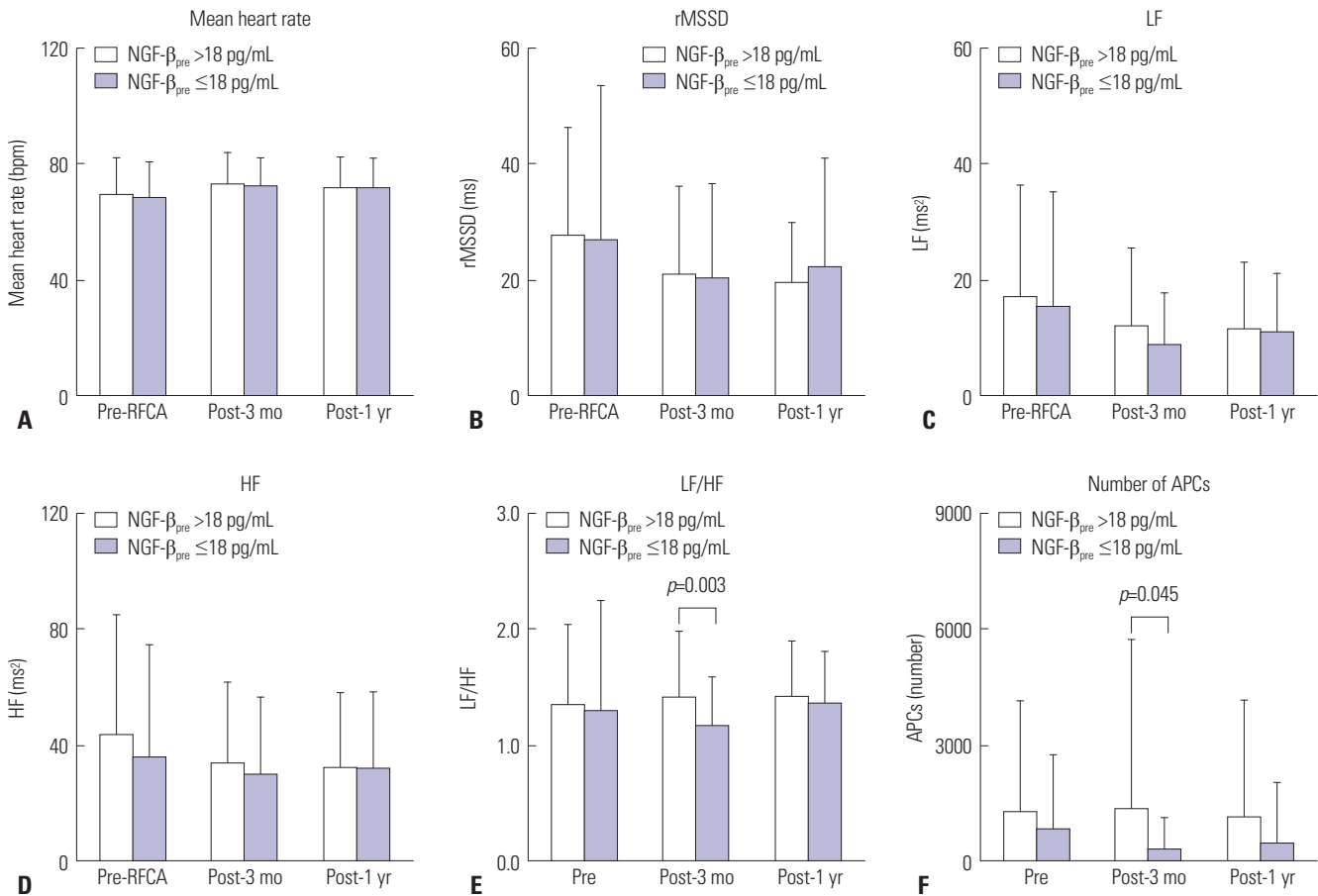


Fig. 2. Changes of mean heart rate and HRV after catheter ablation of AF. We compared the change of Mean heart rate (A), rMSSD (B), LF (C), HF (D), LF/HF ratio (E), and number of APCs (F) depending on the NGF-β_{pre} plasma level. HRV, heart rate variability; AF, atrial fibrillation; HF, high-frequency components; LF, low frequency components; APCs, atrial premature contractions; NGF-β, nerve growth factor-β.

High NGF-β_{pre} and NGF-β_{post-1hr} are independently associated with high LF/HF in HRV_{post-3mo}

In the uni- and multi-variate linear regression analyses, both NGF-β_{pre} (B=4.240, 95% CI 1.114-7.336, *p*=0.008) and NGF-β_{post-1hr} (B=7.617, 95% CI 2.106-13.127, *p*=0.007) were linearly associated with LF/HF ratio at HRV_{post-3mo} (Table 3). Both the plasma levels of NGF-β_{pre} (OR=1.159, 95% CI 1.045-1.286, *p*=0.005) and NGF-β_{post-1hr} (OR=1.098, 95% CI 1.030-1.170, *p*=0.004) were independently associated with the increase of LF/HF at HRV_{post-3mo} (Table 4). However, AF recurrence or ventricular arrhythmic events did not vary depending on the plasma levels of NGF-β.

DISCUSSION

The present study demonstrated that catheter ablation of AF increases the plasma level of NGF-β significantly, and NGF-β was closely associated with high sympathetic nerve activity estimated by HRV and high number of APCs in Holter performed 3 months after the procedure. However, plasma level of NGF-β was not increased after RFCA in patients with old

age and advanced LA remodeling. We also documented the feasibility of double sandwich ELISA technique for detection of the minimal change of NGF-β in the peripheral blood of patients with AF.

The role of plasma NGF-β in cardiac nerve sprouting after cardiac injury

Following a peripheral nerve injury, a complex and finely regulated sequence of events lead to neurilemma cell proliferation and axonal regeneration.¹⁵ Nerve regeneration is triggered by re-expression of NGF or other neurotrophic factors in the non-neuronal cells around the site of injury.⁸ NGF and other neurotrophic factors that are derived from myocardial injuries are transported retrograde to the stellate ganglion, which triggers cardiac nerve sprouting in canine models.¹⁶ Acute myocardial infarction causes an immediate (within 30 minutes) increase of transcardiac NGF concentration, whereas mRNA of NGF in the damaged myocardium begins to increase 3 days after myocardial infarction and peaks 1 week later.¹⁶ NGF over-expression is associated with myocardial hyper innervation and atrial fibrosis,¹⁷ and heterogeneous increases in atrial sympathetic innervation contribute to the

generation and maintenance of AF by exerting significant effects on automaticity, refractoriness, and conduction velocity.¹⁸⁻²⁰ In this study, elevated NGF level was found to be associated with high sympathetic tone and high frequency of APCs after RFCA.

Catheter ablation and NGF-β

RFCA is a kind of necrotic cardiac injury and induces cardiac

nerve sprouting, which has been known to be related to the rises of the level of NGF.^{4,16} Kangavari, et al.¹⁰ demonstrated that RFCA induces over-expression of NGF mRNA, resulting in increased plasma level of NGF. Although study population and detection methods were different, the current study found consistent elevation of plasma level of NGF-β after RFCA, but, it was not the case in patients with old age, low body mass index, and high LA volume index. We applied the

Table 2. Comparisons of HRV_{pre}, HRV_{post-3mo}, and HRV_{post-1yr} Depending on the Median Value of NGF-β_{pre} and NGF-β_{post-1hr} Median Value

	NGF-β _{pre} >18 pg/mL (n=73)	NGF-β _{pre} ≤18 pg/mL (n=74)	p value	NGF-β _{post-1hr} >24 pg/mL (n=74)	NGF-β _{post-1hr} ≤24 pg/mL (n=73)	p value
HRV_{pre}						
Mean HR, beats/min	69.4±12.5	68.5±12.1	0.640	69.2±11.3	68.7±13.3	0.820
APC, N	1300.8±2830.9	833.9±1953.8	0.246	1119.8±2733.6	1010.9±2100.8	0.787
PVC, N	195.3±884.2	216.7±815.6	0.879	232.6±927.5	179.2±763.4	0.704
rMSSD, ms	27.4±18.7	26.7±26.6	0.867	25.9±21.1	28.1±24.7	0.634
HF	10.8±10.4	9.0±9.4	0.358	8.4±5.7	11.3±12.6	0.145
LF/HF	1.36±0.68	1.31±0.94	0.796	1.36±0.72	1.31±0.91	0.732
HRV_{post-3mo}						
Mean HR, beats/min	72.6±11.0	72.2±9.8	0.816	72.2±10.2	72.6±10.5	0.816
APC, N	1368.7±4398.6	319.0±796.5	0.045	1004.3±3863.8	674.0±2315.4	0.531
PVC, N	140.0±487.8	176.6±1196.4	0.809	105.8±469.9	211.7±1208.4	0.484
rMSSD, ms	20.9±15.3	20.3±16.4	0.822	20.2±14.7	21.1±17.0	0.739
HF	8.3±7.1	7.5±6.4	0.492	7.8±6.9	8.0±6.7	0.903
LF/HF	1.42±0.56	1.18±0.41	0.003	1.38±0.52	1.21±0.47	0.042
HRV_{post-1yr}						
Mean HR, beats/min	72.0±10.2	71.8±10.1	0.897	72.7±9.7	71.2±10.5	0.389
APC, N	1145.9±3068.7	490.8±1551.7	0.104	738.6±2473.9	894.7±2420.7	0.700
PVC, N	108.0±343.8	51.9±179.3	0.216	67.7±254.1	92.0±294.3	0.592
rMSSD, ms	19.3±10.4	22.2±18.5	0.273	18.4±7.9	23.2±19.7	0.063
HF	8.1±6.3	8.1±6.5	0.973	7.1±3.9	9.1±8.1	0.074
LF/HF	1.42±0.49	1.37±0.44	0.540	1.42±0.50	1.36±0.44	0.484

HRV, heart rate variability; NGF-β, nerve growth factor-β; APC, atrial premature contraction; PVC, premature ventricular contraction; HR, heart rate; HF, high-frequency components; LF, low frequency components.

Table 3. Uni- and Multivariate Linear Regression Analyses for Pre-NGF-β, Post-NGF-β_{1hr}, and the Increase of NGF-β_{1hr}

	Univariate analysis			Multivariate analysis		
	B	95% CI	p value	B	95% CI	p value
NGF-β_{pre}						
HRV_{pre}						
PVC, N	0.003	0.001–0.005	0.008	0.000	-0.003–0.002	0.871
HRV_{post-3mo}						
LF/HF	4.234	1.150–7.319	0.007	4.240	1.114–7.336	0.008
NGF-β_{post-1hr}						
HRV_{pre}						
PVC, N	0.005	0.001–0.008	0.011	-0.002	-0.007–0.002	0.304
HRV_{post-3mo}						
LF/HF	9.141	3.516–14.765	0.002	7.617	2.106–13.127	0.007
Increase of NGF-β_{post-1hr}						
HRV_{post-3mo}						
LF/HF	4.906	0.235–9.577	0.040			

NGF-β, nerve growth factor-β; HRV, heart rate variability; PVC, premature ventricular contraction; HF, high-frequency components; LF, low frequency components.

Table 4. Uni- and Multivariate Logistic Regression Analyses for Increase of LF/HF Ratio in HRV_{post-3mo} Compared with HRV_{pre}

	Unadjusted			Adjusted*		
	OR	95% CI	p value	OR	95% CI	p value
NGF-β _{pre}	1.019	0.980–1.059	0.343	1.159	1.045–1.286	0.005
NGF-β _{post-1hr}	1.026	0.998–1.054	0.070	1.098	1.030–1.170	0.004
Increase of NGF-β _{post-1hr}	1.029	0.993–1.066	0.110	1.065	0.998–1.136	0.057

HF, high-frequency components; LF, low frequency components; HRV, heart rate variability; NGF-β, nerve growth factor-β; AF, atrial fibrillation.

*Adjusted for sex, age, AF subtype (PAF vs. PeAF), hypertension, and estimated glomerular filtration rate.

double sandwich ELISA method to enhance detection sensitivity to pg/mL level, and our results of plasma NGF-β level were consistent with other previous reports.²¹⁻²³ Because AF catheter ablation itself has the effects of cardiac autonomic denervation,²⁴ the change of post-ablation HRV cannot be the result of increased plasma level of NGF-β alone. However, the current study found that plasma level of NGF-β was clearly associated with the change of autonomic nerve activity at HRV_{post-3mo} and HRV_{post-1yr}.

AF and HRV

HRV relies on the principle that the pattern of beat-to-beat control of the sinoatrial node provides a reflection of cardiac autonomic nerve activity.¹⁴ Among multiple parameters in HRV, HF components are thought to primarily reflect vagal tone, whereas high LF/HF ratio has been assumed as the index of high sympathetic activity that is known to be pro-arrhythmic and higher frequency of PVCs or APCs.^{14,25} The present study showed that patients with a higher plasma level of NGF-β had a higher LF/HF ratio and a trend of more frequent APCs in Holter monitoring at 3 months after ablation. Although NGF provokes both sympathetic and parasympathetic nerve regeneration, it was associated only with an increased sympathetic nerve activity, but not with an increased parasympathetic nerve activity in this study. This finding may be due to the fact that RFCA destroyed parasympathetic postganglionic cells that are located at or very close to the ablation sites. On the other hand, since sympathetic postganglionic cells are located in the stellate ganglia far from the ablation site, ablation-induced sympathetic nerve axonal damage might recover three months after RFCA. It has been reported that a rapid HR after AF catheter ablation predicts a low recurrence rate of AF.²⁶ Although it might be associated with an appropriate vagal denervation, a high plasma level of NGF-β and an increased post-procedure LF/HF ratio in patients who have less remodeled atrium might contribute to a rapid HR and low recurrence after RFCA.

Study limitations

The present study has several limitations. The patients included in this study were a highly selected group referred for RFCA, and the number of patients was also limited. We excluded patients with LA >55 mm. HRV analysis requires normal sinus rhythm with normal cardiac function, therefore, we

excluded patients whose Holter could not be analyzed for HRV. We did not evaluate the long-term change in plasma level of NGF-β after AF ablation. Although we could not find statistical difference of clinical recurrence rate depending on the plasma levels of NGF-β in this small sample size and short follow-up period study, we found the differences in the LF/HF at HRV_{post-3mo}. Recently, we reported that LF/HF at HRV_{post-3mo} is an independent factor predicting clinical recurrence of AF after RFCA.²⁷ We also found higher LF/HF ratio in HRV_{post-3mo} and higher number of APCs in patients with NGF-β_{pre}>18 pg/mL than those with ≤18 pg/mL, but we do not have direct evidence for the mechanism or causal-results relationship. Further study with a large population may be warranted. We chose post-procedure 1 hour to evaluate the plasma level of NGF-β_{post-1hr}, but it might not be enough time to show increased plasma level of NGF-β after RFCA. Because we used irrigated tip ablation catheter, irrigated fluid volume might affect the plasma concentration of NGF-β.

In conclusion, AF catheter ablation increases plasma level of NGF-β, and high plasma levels of pre- or post-RFCA NGF-β were associated with high sympathetic nerve activity and the presence of high number of APCs in post-RFCA 3 month Holter. Double sandwich ELISA technique was feasible and acceptable for the detection of the minimal change of NGF-β in the peripheral blood of the patients with AF.

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