Electrophysiology

Plasma Atrial Natriuretic Peptide Concentration Inversely Correlates With Left Atrial Collagen Volume Fraction in Patients With Atrial Fibrillation

Plasma ANP as a Possible Biochemical Marker to Predict the Outcome of the Maze Procedure

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OBJECTIVES	We hypothesized that the plasma atrial natriuretic peptide (ANP) level reflects atrial degenerative change and may predict the outcome of the maze procedure.
BACKGROUND	Although a larger preoperative left atrial dimension and longer duration of atrial fibrillation (AF) have been reported in patients with persistent AF than in those with sinus rhythm (SR), these individual factors were not enough to predict the outcome of the maze procedure.
METHODS	Preoperative plasma ANP levels were measured in consecutive 62 patients who underwent the Kosakai's modified maze procedure. Moreover, we performed histological and molecular biological examinations in the resected left atrial tissues.
RESULTS	The preoperative plasma ANP was lower in the AF group (n = 13) than it was in the SR group (n = 49) (p < 0.001). Multiple logistic regression analysis revealed that duration of AF and plasma ANP were independently associated with postoperative cardiac rhythm. Among 41 patients with a higher plasma ANP or shorter duration of AF than the median value, SR was restored in 95% of patients. In contrast, in 21 patients with a lower plasma ANP and a longer duration of AF than the median value, SR was restored only in 48% of patients. Histological examination revealed that the collagen volume in the left atrial tissue was higher in AF than it was in SR and inversely correlated with plasma ANP. In addition, the messenger RNA expressions of ANP, collagen type I and type III were lower in AF than they were in SR.
CONCLUSIONS	These results suggest that a combination of plasma ANP and/or duration of AF may predict the success rate for the maze operation. Advanced atrial degenerative change may result in a decrease of atrial ANP secretion. (J Am Coll Cardiol 2002;39:288–94) © 2002 by the American College of Cardiology

The maze procedure effectively restores atrial fibrillation (AF) to sinus rhythm (SR) and to atrial systole resulting in the disappearance of palpitation, an improvement in hemodynamics and the inhibition of thromboembolism (1–5). However, recent studies revealed that the maze procedure has several problems, which include larger operative invasion due to longer aortic crossclamp time and cardiopulmo-

nary bypass time (6), body fluid retention due to reduced atrial natriuretic peptide (ANP) secretion (7,8) and sick sinus syndrome (9,10). Indeed, there are some patients whose SR is not restored after the maze operation (6). Thus, the time has come to discuss whether there is a desirable indication for the maze procedure before a heart operation.

Previous studies have demonstrated that there might be a low restore rate in patients who have a larger left atrial systolic dimension (LAD) on echocardiography and longer duration of AF (6). However, these individual parameters were not enough to evaluate whether there is a desirable indication for the maze procedure. A noninvasive biochemical marker would be beneficial for this purpose. Atrial natriuretic peptide is mainly synthesized in the atrium, and a previous report suggested that ANP secretion becomes insufficient in remarkable fibrosis in the atrium (11). In

Abbreviations and Acronyms

AF	=	atrial fibrillation
ANP	=	atrial natriuretic peptide
cDNA	=	complementary DNA
CTR	=	cardiothoracic ratio
LAD	=	left atrial dimension
mRNA	=	messenger RNA
SR	=	sinus rhythm

addition, a recent study reported that the patients who had a longer duration of AF were reported to have lower plasma ANP levels (12), suggesting that patients with a longer duration of AF may have reduced ANP secretion presumably due to the advanced atrial degenerative changes. We hypothesized that the plasma ANP levels reflect the atrial qualitative changes and may be a biochemical marker for predicting the outcome of the maze procedure. To test this hypothesis, in the present study we measured the preoperative plasma ANP level in the patients who underwent the maze procedure. Moreover, to explore the underlying mechanism of reduced ANP secretion, we performed histological and molecular biological examinations in the resected left atrial tissues.

METHODS

Patients. Sixty-two patients who underwent Kosakai's modified maze procedure were studied. Each subject provided informed consent for participation. All patients with AF, as a result of organic heart disease, underwent the maze procedure as previously reported (6). On the basis of the postoperative rhythm (duration after surgery: 31.2 ± 20.7 months), the subjects were divided into the SR group (n = 49) or the AF group (n = 13).

Measurements of plasma ANP levels. Venous blood was withdrawn at one day before operation. Immunoradiometric assay was performed to measure plasma ANP levels (Shiono RIA ANP kit; Shionogi Co., Ltd., Osaka, Japan) as previously reported (13).

Histological examination. Sections 5 μm thick of paraffinized left atrial appendage tissues were stained with Masson's trichrome to highlight collagen fibers. The collagen volume fraction was determined by measuring the area of stained tissue within a given field (14). In the resected left atrial appendage, 10 fields were analyzed. We evaluated 30 randomly selected patients (SR group: n = 24, AF group: n = 6).

Messenger RNA (mRNA) expressions of ANP, collagen type I and type III in left atrial appendage tissue. To obtain molecular biological evidence regarding the atrial qualitative differences between the AF and SR groups, resected left atrial tissues were frozen in liquid nitrogen and stored at −80°C for Northern blot analysis in 31 patients (SR group: n = 26, AF group: n = 5). A Pst I restriction fragment of human ANP complementary DNA (cDNA) corresponding to nucleotides −100 to 589 (15) was used as

the human ANP cDNA probe. The human collagen type I and type III cDNA probes were synthesized by polymerase chain reaction using the primers: collagen type I sense, 5′-CAAGGTGTTGTGCGATGACG-3′ collagen type I antisense, 5′-ATTCCTCCGGTTGATTTCTC-3′; collagen type III sense, 5′-ATCCGTTCTCTGC-GATGACATAATA-3′ collagen type III antisense, 5′-GCCTGCGAGTCCTCCTACTGCTACT-3′. RNA extraction and Northern blot analyses were done using a procedure previously described (15–17).

Statistics. Values are the mean ± SD. Differences in proportions were evaluated by chi-square analysis, unpaired t test for continuous normally distributed variables and Mann-Whitney U test for nonnormally distributed variables. The factors that related at the $p < 0.1$ level were selected as independent variable candidates for multiple logistic regression analyses, which were used to evaluate the independent contribution of clinical parameters to cardiac rhythm after the operation. Differences were considered to be statistically significant when the p value was < 0.05. Correlation coefficients were calculated using linear regression analysis.

RESULTS

Plasma ANP levels in the AF and SR groups. There were no significant differences in age, gender, body surface area, underlying heart diseases, operations or cardiothoracic ratio (CTR) between the two groups. The LAD, the duration of AF and preoperative plasma ANP level were significantly larger, longer and lower in the AF group than they were in the SR group (Table 1).

Histological examination data. The collagen volume fraction in the left atrial appendage tissue was higher in the AF group than it was in the SR group (Fig. 1). The collagen volume fraction correlated negatively with the plasma ANP levels (R = −0.39, p < 0.05) (Fig. 2A) and positively with the duration of AF (R = 0.62, p < 0.001) (Fig. 2B).

The effects of plasma ANP and duration of AF on the postoperative cardiac rhythm. Table 2 shows the results of the multiple logistic regression analyses to assess the factors independently contributing to the cardiac rhythm after the operation (mean duration after surgery: 31.2 ± 20.7 months). The LAD, duration of AF, CTR and plasma ANP levels were added to this model. According to the multiple logistic regression analyses, only the duration of AF and plasma ANP levels were independent predictors of cardiac rhythm after the maze operation. In 31 patients with a higher plasma ANP than the median value (>40 pg/ml), SR was restored in 30 (97%) (Fig. 3). In 28 patients with a shorter duration of AF than the median value (<4.0 years), SR was restored in 27 (96%) (Fig. 3). In contrast, in 21 patients with a lower or equal plasma ANP (≤40 pg/ml) and with a longer or equal duration of AF (≥4.0 years), SR was restored only in 10 (48%) (Fig. 3).

Table 1. Comparison of Clinical Characteristics of Patients, Underlying Disease, Operation, Left Atrial Dimension, Cardiothoracic Ratio, Duration of AF and ANP

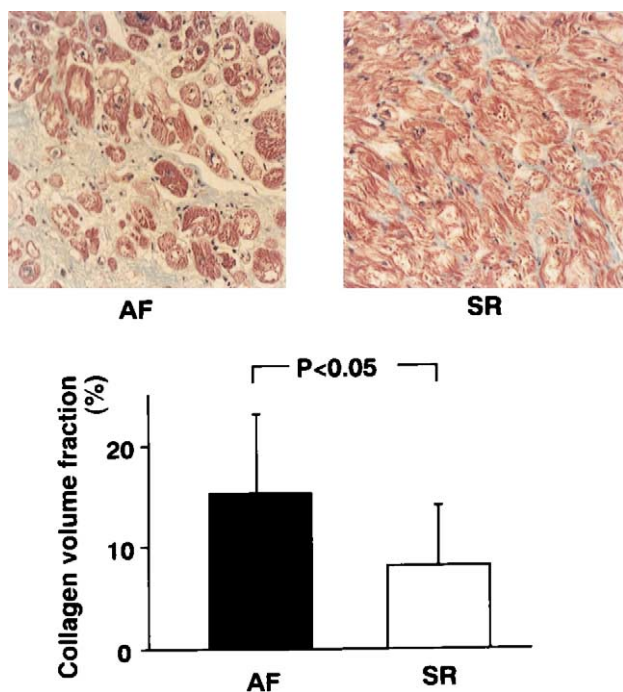|	AF (n = 13)	SR (n = 49)	p Value
Age (yrs)	57.9 ± 8.3	56.7 ± 8.3	0.644
Gender (male/female)	8/5	28/21	0.775
BSA (m²)	1.52 ± 0.14	1.60 ± 0.17	0.118
Underlying disease (No. of patients)			
MS/MR/MSR/VSD/ASD (A)	3/2/8/0/0 (9)	9/17/19/1/3 (14)	0.455
Operation (No. of patients)			
OMC/MVR/MVP/VSD/ASD closure	3/9/1/0/0	3/26/16/1/3	0.140
Medication (No. of patients)			0.358
Digoxin	13	37	
Furosemide	10	37	
Spironolactone	5	9	
Nitrate	3	3	
ACE inhibitor	2	13	
Calcium antagonist	1	9	
Beta-blocker	0	3	
LAD (mm, preop)	63.2 ± 10.7	56.3 ± 8.2	0.014
CTR (%, preop)	62.5 ± 7.8	58.7 ± 6.0	0.068
Duration of AF (yrs, preop)	8.1 ± 3.6	3.6 ± 2.8	<0.0001
Plasma ANP (pg/ml)	24.2 ± 9.6	71.3 ± 55.3	0.0009

Values are the mean ± SD.

(A) = with aortic valve disease; ACE = angiotensin-converting enzyme; AF = atrial fibrillation; ANP = atrial natriuretic peptide; ASD = atrial septal defect; BSA = body surface area; CTR = cardiothoracic ratio; LAD = left atrial dimension; MR = mitral regurgitation; MS = mital stenosis; MSR = mitral stenosis and regurgitation; MVP = mitral valvuloplasty; MVR = mitral valve replacement; OMC = open mitral commissurotomy; preop = preoperative; VSD = ventricular septal defect.

Combined assessment of plasma ANP level and duration of AF versus duration of AF alone: prediction of persistent AF after the maze operation. Table 3 shows the sensitivity, specificity, accuracy and positive and negative predictive value of the combined assessment of plasma ANP level and duration of AF and the duration of AF alone for predicting persistent AF after the maze operation. The combined assessment had a significantly greater specificity and accuracy than the duration of AF alone (80% vs. 55%, 81% vs. 63%, p < 0.05, respectively), and there was a trend toward an increase in positive predictive value for predicting persistent AF after the operation.

The mRNA expression of ANP and collagen. As the result of plasma ANP levels, the mRNA expression of ANP in the left atrial appendage tissues was also significantly lower (p < 0.05) in the AF than it was in the SR (Fig. 4). In contrast with the higher collagen volume fraction in AF than in SR, the mRNA expressions of type I and III collagen in the left atrial appendages were lower (both: p < 0.01) in the AF group than they were in the SR group (Fig. 4).

DISCUSSION

Comparison with other studies. An earlier study reported that there were significant differences in LAD as determined by echocardiography and duration of AF between the SR and AF groups after the maze procedure (6). In addition, recent studies also demonstrated that duration of AF, LAD and CTR were preoperative risk factors for persistent AF after the maze operation (18–20). In the present study, the patients with persistent AF after the maze procedure had a longer history of AF and larger LAD compared with the SR group. These results were consistent with the previous studies and suggest that these parameters might be useful for predicting the outcome of the maze procedure. However, since these parameters had considerable overlap between the AF and SR groups and none had
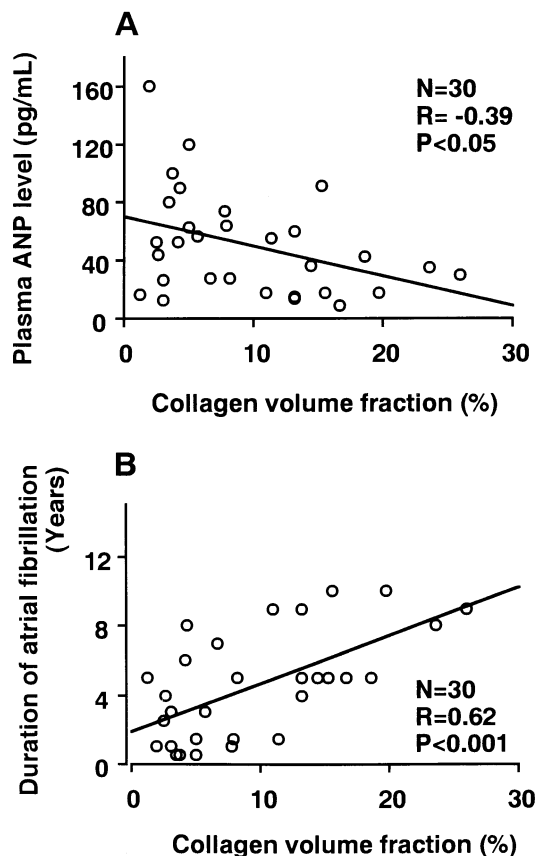

Figure 1. Masson's trichrome staining of the excised left atrial appendages under light microscopy and quantification of the collagen volume fraction in the atrial fibrillation (AF) and sinus rhythm (SR) groups.

Figure 2. Correlations between collagen volume fraction and plasma atrial natriuretic peptide (ANP) level **(A)** and duration of AF **(B)**.

cut-off points, it seems to be difficult to determine a preoperative indication for the maze procedure using these individual parameters.

Plasma ANP as information for predicting the outcome of the maze procedure. In the present study, we measured preoperative plasma ANP levels in patients who underwent the maze procedure. Atrial natriuretic peptide is mainly synthesized in the atrium, and its secretion is reduced in remarkable fibrosis in the atrium (12). Therefore, we hypothesized that plasma ANP reflects the atrial myocardial qualitative change and might be beneficial for predicting the outcome of the maze procedure. In the present study, patients with a higher preoperative plasma ANP level ($>$40
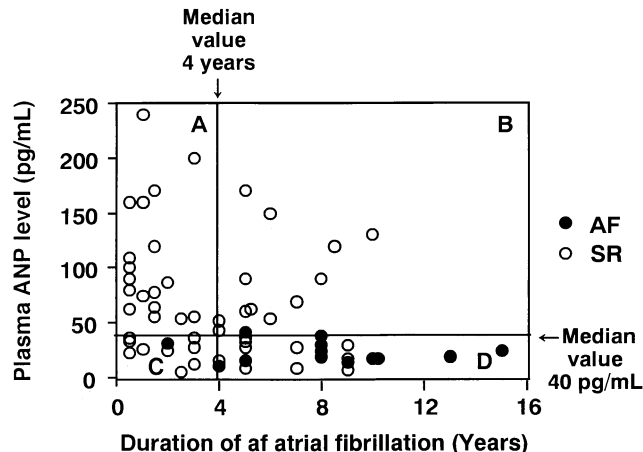
Figure 3. Combined assessment of plasma atrial natriuretic peptide (ANP) level and duration of atrial fibrillation (AF). SR = sinus rhythm.

pg/ml) than the median value showed a high SR restoration rate (97%), suggesting that high plasma ANP levels may be a noninvasive biochemical marker for success in the maze operation. In addition, to assess the factors independently contributing to the cardiac rhythm after the operation, multiple logistic regression analyses were performed. According to these analyses, only duration of AF and plasma ANP level were independent predictors of cardiac rhythm after the maze operation. The significant relationship between the outcome of the maze procedure and the duration of AF was consistent with previous reports (6,20). In fact, patients with a shorter duration of AF ($<$4.0 years) than the median value, irrespective of their ANP levels, showed a high SR restoration rate, suggesting that a short duration of AF is also a marker for success in the maze operation. Furthermore, the combined assessment of plasma ANP level and duration of AF had a significantly greater specificity and accuracy and a trend toward an increase in positive predictive value compared with the duration of AF alone for predicting the outcome of the maze operation. In fact, patients with lower plasma ANP levels ($\leq$40 pg/ml) and a longer duration of AF ($\geq$4.0 years) showed a low restoration rate (48%), suggesting that the combination of a low plasma ANP level and long duration of AF is predictive of a low success rate for the maze procedure. Specifically, in the patients with high ANP levels ($>$50 pg/ml), SR was completely restored in 29/29 (100%). Furthermore, among the patients with a lower ANP ($\leq$40 pg/ml) and with a longer duration of AF history ($\geq$8.0 years), SR was restored only in 3/12 (25%). Thus, a high ANP level or short duration of AF may predict a good outcome for the maze procedure, whereas a low ANP with long duration of AF may predict a lower rate of recovery to SR after the maze procedure. Since recent studies revealed that the maze procedure has several problems, which include greater operative invasion (6), body fluid retention and sick sinus syndrome (9,10), these parameters may help to determine an indication for the maze procedure.

Table 2. Associations Between Cardiac Rhythm After the Operation and Clinical Parameters by Multiple Logistic Regression Analyses

	Cardiac Rhythm After the Operation		
	Chi-Square	df	p Value
LAD	1.190	1	0.275
Duration of AF	4.717	1	0.030
CTR	0.0002	1	0.988
Plasma ANP	8.300	1	0.004

AF = atrial fibrillation; ANP = atrial natriuretic peptide; CTR = cardiothoracic ratio; df = degrees of freedom; LAD = left atrial dimension.

Table 3. Predictive Values Associating the Combination of ANP and Duration of AF and the Duration of AF Alone With the Outcome of the Maze Operation

	Sensitivity	Specificity	Accuracy	Positive Predictive Value	Negative Predictive Value
Duration of AF	12/13 (92%)	27/49 (55%)	39/62 (63%)	12/34 (35%)	27/28 (96%)
Plasma ANP and duration of AF	11/13 (84%)	39/49 (80%)	50/62 (81%)	11/21 (52%)	39/41 (95%)
Fisher's p value	>0.9999	0.0171	0.0452	0.2658	>0.9999

Cut-off values were 4.0 years and 40 pg/ml for AF duration and plasma ANP, respectively.
AF = atrial fibrillation; ANP = atrial natriuretic peptide.

Underlying mechanism for reduced ANP in patients with AF. A previous study reported that plasma ANP levels were increased due to an atrial pressure elevation in patients with supraventricular tachycardia and AF (21) and promptly decreased after electrical cardioversion (22). Furthermore, patients who had an increase in the plasma ANP levels from rest to peak exercise before defibrillation had a better outcome of electrical cardioversion (23). In addition, atrial mechanical overload leads to an atrial qualitative change resulting in the initiation and perpetuation of AF (24–26). These findings suggest that patients with maintaining SR after electrical cardioversion may have less atrial qualitative change and that the ability of ANP secretion was reserved in these patients. In addition, previous study demonstrated that the plasma ANP levels were lower among patients with AF of longer duration (11). This finding suggests that the plasma ANP level also reflects the qualitative change of atrial myocardium. However, there was no direct evidence, which clarified the relationship between plasma ANP levels and histological findings. In the present study, we performed a histological examination in the excised left atrial appendage tissues and demonstrated for the first time that the collagen volume fraction was significantly increased in the AF group compared with the SR group and that the
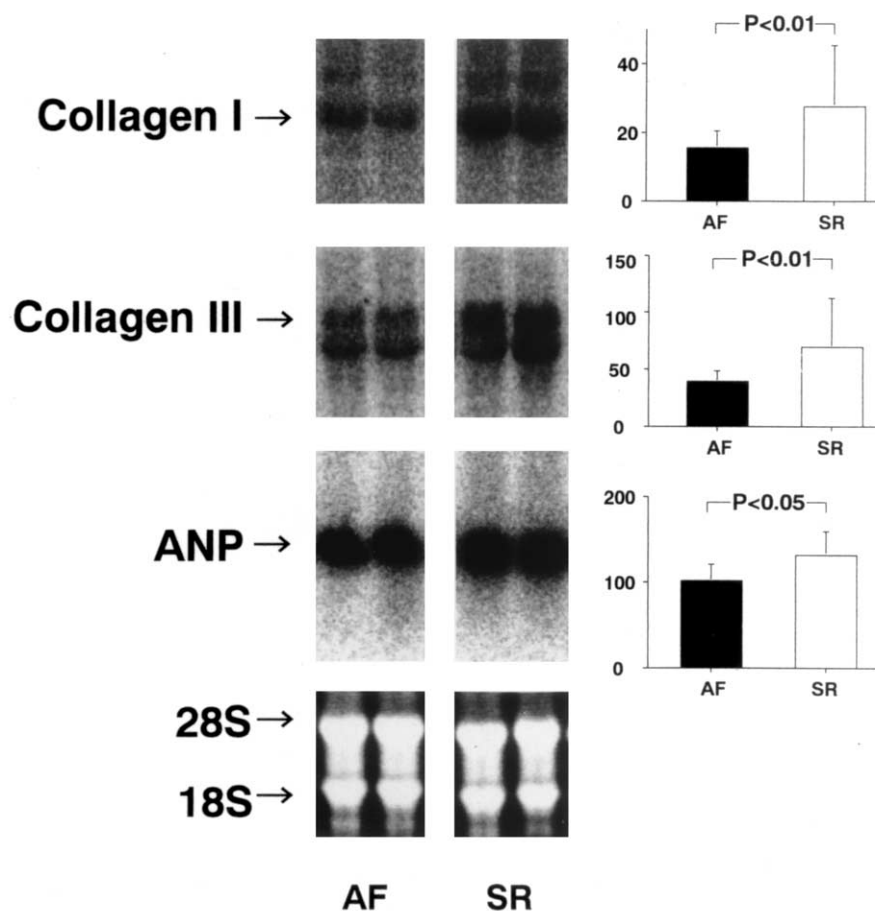
Figure 4. Representative Northern blots showing collagen type I, type III and atrial natriuretic peptide (ANP) in the excised left atrial appendages in patients with persistent atrial fibrillation after the maze procedure (AF group) and patients with sinus rhythm (SR group). Bar graph shows the summary of findings of Northern blot analysis of atrial collagen type I, type III and ANP messenger RNA in the AF and SR groups.

collagen volume fraction negatively correlated with the plasma ANP level. Furthermore, we showed that the ANP mRNA expression was significantly decreased in the AF group compared with the SR group. Thus, the ability to secrete ANP was reduced in the AF group presumably due to the advanced histological and molecular biological changes in the left atrial myocardium. Plasma ANP levels, therefore, may reflect atrial qualitative change and provide information for predicting the outcome of the maze procedure. We also detected the reduced mRNA expressions of collagen type I and III, although the collagen volume fraction was significantly higher in left atrial appendage in the AF group compared with the SR group. The exact mechanism for the discrepancy between the reduced mRNA expression and increased collagen volume is not known at present; however, previous studies have shown that collagen mRNA levels are higher in the active fibrosis tissue than they are in inactive scar tissue (27). In myocardial infarct tissue, mRNA expressions of collagen type I and III reached peak at two to four days after myocardial infarction and gradually decreased thereafter (27). The increase in types I and III procollagen mRNA in infarcted myocardium was followed by an increased collagen deposition, and it reaches peak at day 2 to three weeks (27). Thus, mRNA expression of collagen is often different from collagen volume fraction, and it seems to depend on the time course. These results suggest that atrial myocardium in the SR group is more active in the fibrosis phase than that in AF group and that atrial myocardium of the AF group may be the end stage of AF.

Study limitations. There were relatively small numbers of patients to prove the relationship between atrial degenerative changes and plasma ANP levels in the present study. Furthermore, we could not completely deny the possibility that the reason for remaining AF may not only be due to the advanced atrial degenerative change but also to the technical difficulties of maze procedure in large atria. Further study is required in a large number of patients in the future to resolve these questions.

Conclusions. A high preoperative plasma ANP level or short duration of AF may predict a high success rate for the maze procedure, whereas a low plasma ANP level with longer duration of AF may predict a low success rate.

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REFERENCES

1. Cox JL, Schuessler RB, D'Agostino HJ, Jr., et al. The surgical treatment of atrial fibrillation: III. Development of a definitive surgical procedure. J Thorac Cardiovasc Surg 1991;101:569–83.
2. Cox JL, Boineau JP, Schuessler RB, et al. Successful surgical treatment of atrial fibrillation. JAMA 1991;266:1976–80.
3. McCarthy PM, Castle LW, Maloney JD, et al. Initial experience with the maze procedure for atrial fibrillation. J Thorac Cardiovasc Surg 1993;105:1077–87.
4. Cox JL, Boineau JP, Schuessler RB, Kater KM, Lappas DG. Five-year experience with the maze procedure for atrial fibrillation. Ann Thorac Surg 1993;56:814–24.
5. McCarthy PM, Cosgrove DM, III, Castle LW, White RD, Klein AL. Combined treatment of mitral regurgitation and atrial fibrillation with valvuloplasty and the maze procedure. Am J Cardiol 1993;71:483–6.
6. Kosakai Y, Kawaguchi AT, Isobe F, et al. Cox maze procedure for chronic atrial fibrillation associated with mitral valve disease. J Thorac Cardiovasc Surg 1994;108:1049–55.
7. Yoshihara F, Nishikimi T, Kosakai Y, et al. Atrial natriuretic peptide secretion and body fluid balance after bilateral atrial appendectomy by the maze procedure. J Thorac Cardiovasc Surg 1998;116:213–9.
8. Yoshihara F, Nishikimi T, Sasako Y, et al. Preservation of the right atrial appendage improves reduced plasma atrial natriuretic peptide levels after the maze procedure. J Thorac Cardiovasc Surg 2000;119:790–4.
9. Cox JL, Boineau JP, Schuessler RB, et al. Modification of the maze procedure for atrial flutter and atrial fibrillation. J Thorac Cardiovasc Surg 1995;110:473–84.
10. Cox JL, Schuessler RB, Lappas DG, et al. An 8 1/2-year clinical experience with surgery for atrial fibrillation. Ann Surg 1996;224:267–75.
11. Maeda K, Tsutamoto T, Wada A, et al. Low dose synthetic human atrial natriuretic peptide infusion in a patient with mitral stenosis and severe pulmonary hypertension. Jpn Circ J 1999;63:816–8.
12. Van Den Berg MP, Crijns HJGM, Van Veldhuisen DJ, et al. Atrial natriuretic peptide in patients with heart failure and chronic atrial fibrillation: role of duration of atrial fibrillation. Am Heart J 1998;135:242–4.
13. Yasue H, Yoshimura M, Sumida H, et al. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. Circulation 1994;90:195–203.
14. Yu HC, Burrell LM, Black MJ, et al. Salt induces myocardial and renal fibrosis in normotensive and hypertensive rats. Circulation 1998;98:2621–8.
15. Horio T, Nishikimi T, Yoshihara F, et al. Production and secretion of adrenomedullin in cultured rat cardiac myocytes and nonmyocytes: stimulation by interleukin-1beta and tumor necrosis factor-alpha. Endocrinology 1998;139:4576–80.
16. Sugo S, Minamino N, Kangawa K, et al. Endothelial cells actively synthesize and secrete adrenomedullin. Biochem Biophys Res Commun 1994;201:1160–6.
17. Ohmichi N, Iwai N, Kinoshita M. Expression of angiotensin converting enzyme and chymase in human atria. J Hypertens 1997;15:935–43.
18. Kawaguchi AT, Kosakai Y, Isobe F, et al. Factors affecting rhythm after the maze procedure for atrial fibrillation. Circulation 1996;94 9 Suppl:II139–42.
19. Isobe F, Kawashima Y. The outcome and indications of the Cox maze III procedure for chronic atrial fibrillation with mitral valve disease. J Thorac Cardiovasc Surg 1998;116:220–7.
20. Ueshima K, Hashimoto K, Chiba M. What are the predictors of restoration of sinus rhythm after combined treatment with surgical repair for organic heart disease and the Maze procedure for atrial fibrillation? J Cardiovasc Surg 1998;40:793–6.
21. Roy D, Paillard F, Cassidy D, et al. Atrial natriuretic factor during atrial fibrillation and supraventricular tachycardia. J Am Coll Cardiol 1987;9:509–14.
22. Mookherjee S, Anderson G, Smulyan H, et al. Atrial natriuretic peptide response to cardioversion of atrial flutter and fibrillation and role of associated heart failure. Am J Cardiol 1991;67:377–80.
23. Theodorakis GN, Markianos M, Kouroubetsis CK, et al. Clinical,

adrenergic and heart endocrine measures in chronic atrial fibrillation as predictors of conversion and maintenance of sinus rhythm after direct current cardioversion. Eur Heart J 1996;17:550–6.

24. Van Wagoner DR, Pond AL, McCarthy PM, et al. Outward K^+ current densities and Kv1.5 expression are reduced in chronic human atrial fibrillation. Circ Res 1997;80:772–81.

25. Ohkusa T, Ueyama T, Yamada J, et al. Alterations in cardiac sarcoplasmic reticulum Ca^{2+} regulatory proteins in the atrial tissue of patients with chronic atrial fibrillation. J Am Coll Cardiol 1999;34: 255–63.

26. Van Wagoner DR, Pond AL, Lamorgese M, et al. Atrial L-type Ca2+ currents and human atrial fibrillation. Circ Res 1999;85:428– 36.

27. Cleutjens JP, Verluyten MJ, Smiths JF, et al. Collagen remodeling after myocardial infarction in the rat heart. Am J Pathol 1995;147: 325–38.