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EXTENT OF SINOATRIAL AND ATRIOVENTRICULAR NODAL DEPOSITS IN CARDIAC AMYLOIDOSIS : A CORRELATIVE INVESTIGATION

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Abstract : Objectives. We morphometrically determined the relative area of amyloid deposition (%D) in sinoatrial and atrioventricular nodes in cardiac amyloidosis.

Materials and Methods. We divided 13 hearts with amyloidosis and arrhythmia (arrhythmia group) and 4 hearts with amyloidosis and no arrhythmia (controls) into subgroups. The arrhythmia group included 3 patients with sick sinus syndrome (SSS), 3 with atrioventricular (AV) block, 9 with bundle branch block, 7 with atrial fibrillation, and 4 with ventricular arrhythmias. Among all 17 cases, 14 represented primary (AL) amyloidosis and 3 represented secondary (AA) amyloidosis. We selected five microscopic fields for each case and node for quantitative analysis with an image analyzer to determine %D.

Results. The %D in both nodes was similar between control and arrhythmia groups. Only in the AV block subgroup was the %D in the sinoatrial node significantly greater than controls ($p<0.05$), although %D in the SSS subgroup showed some tendency to be greater than in controls. In the atrioventricular node, %D in the AV block subgroup tended to be greater than in controls. The %D was similar between the two nodes for groups with AL λ, AL κ, and AA amyloid, while %D tended to be greater in the AL group than in the AA group.

Conclusion. Although a close relationship was not decisively demonstrated between arrhythmias and extent of amyloid deposition in sinoatrial or atrioventricular nodes, SSS might be caused by amyloid deposition in the sinoatrial node and AV block might be caused by amyloid deposition in the atrioventricular node.

Key words : cardiac amyloidosis, arrhythmias, morphometry

INTRODUCTION

Amyloidosis represents a heterogeneous group of disorders resulting from extracellular deposition of amyloid fibrils, composed of low-molecular-weight protein subunits derived from a variety of normal or aberrant serum proteins[1,2]. The common feature of this diverse group of serum proteins is a predilection to assume a fibrillar structure of linear,

nonbranching fibril aggregates in a crossed β-pleated sheet conformation[3]. This conformational structure confers well-known characteristics including an affinity for alkaline Congo red stain, apple-green birefringence when Congo red-stained fibrils are exposed to polarized light, and resistance of fibrils to proteolytic degradation.

Amyloidosis becomes apparent clinically when extracellular amyloid deposition disrupts normal tissue architecture sufficient to upset normal structure-function relationships. Clinical manifestations of amyloidosis therefore are protean, depending upon organs involved and extent of infiltration.

The heart commonly is infiltrated by amyloid in primary or myeloma-associated amyloidosis (AL amyloidosis), certain familial amyloidoses, and several age-related amyloidoses. Cardiac involvement is rare in secondary amyloidosis (AA amyloidosis). Crotty et al.[4] have recommended immunohistochemical staining of amyloid in endomyocardial biopsy specimens to distinguish between AL and senile amyloidoses in older patients without light chains in urine or serum. This is prognostically important since cardiac involvement from AL amyloidosis is rapidly fatal[5]. According to Chau et al.[6], endomyocardial biopsy is useful not only for confirming the diagnosis but also for differentiating between cardiac amyloid derived from nonsecretory immunoglobulin and senile cardiac amyloid. Further, Arbustini et al.[7] have found endomyocardial biopsy specimens to be extremely useful for identification and characterization of amyloid fibrils as well as evaluation of myocyte damage and atrophy in cardiac AL amyloidosis.

On the other hand, magnetic resonance imaging (MRI) appears to show promise for noninvasive diagnosis of cardiac amyloidosis by identifying typical morphologic markers and by showing tissue characteristics suggestive of infiltrative disease[8]. In addition, Hamer et al.[9] considered echocardiography to be the best method for estimating severity of cardiac involvement in amyloidosis, as an increasing degree of echocardiographic change parallels deterioration in myocardial function and patients' clinical condition.

When amyloid fibrils are deposited in the heart, clinical manifestations depend upon the site of intracardiac deposition. Fibrils tend to be deposited between myocytes, which atrophy as the extent of amyloid deposition increases[10]. Such interstitial fibrillar deposition reduces ventricular compliance to result in a classic "stiff heart syndrome", and symptomatic congestive heart failure (CHF). M-mode echocardiography is particularly helpful in recognition of cardiac amyloidosis[11]. While median survival in 229 patients with primary systemic amyloidosis (AL) was 12 months after onset of symptoms, median survival in the 77 of these patients who had CHF was 6 months[12]. In AL amyloidosis, the major risk factor affecting survival during the first symptomatic year is the presence of CHF[13].

Amyloid fibril deposition may occur within intramyocardial vessels[14-16] and may be sufficiently extensive to result in localized ischemic injury. Such intramyocardial vascular infiltration by amyloid may cause angina[15-17] even when epicardial coronary vascular disease does not limit blood flow. Amyloid fibril deposition also has been associated with fibrosis of specialized cardiac conduction tissue[18]. Consequently, almost every arrhythmia has been reported in association with cardiac amyloidosis, although atrial fibrillation is most common. Amyloid deposits in conduction tissue appear to be responsible for sudden death and atrioventricular block[19]. Finally, amyloid deposits can occur on cardiac valves, where they

have been associated with both regurgitant and stenotic valvular dysfunction.

In this study we morphometrically determined the extent of amyloid deposition in sinoatrial and atrioventricular nodes in cardiac amyloidosis to identify relationships of arrhythmias and conduction system dysfunction in cardiac amyloidosis to the distribution of amyloid deposition in sinoatrial and atrioventricular nodes.

MATERIALS and METHODS

1. Materials

Twenty-three hearts of patients with amyloidosis that had been obtained at autopsy between August 1977 and July 1999 were studied at the National Cardiovascular Center, Osaka, Japan. In 17 of the 23 hearts, sinoatrial and atrioventricular nodes were examined. The 17 patients ranged in age at death from 44 to 82 years (mean 65.4±11.1), and included 12 men and 5 women. Four of them had neither arrhythmias nor disturbances of cardiac conduction (control group), and while others had either arrhythmias or disturbances of cardiac conduction (arrhythmia group). The arrhythmia group included 3 patients with sick sinus syndrome (SSS), 3 with atrioventricular (AV) block, 9 with bundle branch block, 7 with atrial fibrillation, and 4 with ventricular tachycardia or ventricular fibrillation. We also divided the 17 patients into 14 with AL amyloidosis (AL group) and 3 with amyloid A-related amyloidosis (AA group) on the basis of pathologic diagnosis. Clinical backgrounds of the 17 patients are summarized in Table 1. We should present the details of how the type of amyloid protein was determined in these cases, but we were not able to do so.

Table 1. Clinical backgrounds and the relative area of amyloid deposition (%D) in sinoatrial and atrioventricular nodes of the 17 patients

Case	Age (years)	Sex	Clinical diagnosis	Pathologic diagnosis (Amyloid protein)	Arrhythmias or disturbances of cardiac conduction	Amyloid deposition in sinoatrial node (%)	Amyloid deposition in atrioventricular node (%)
1	76	M	Multiple myeloma (IgGλ)	ALλ		33.9	51.9
2	60	F	Chronic renal failure	AA	VF, VT	13.4	60.4
3	77	M	Multiple myeloma (IgAλ)	ALλ		25.6	44.7
4	60	F	Reumatoid arthritis	AA	AF	26.9	48.2
5	68	F	Cerebral embolism, AF	ALκ	AF, CRBBB	27.5	36.5
6	67	M	Multiple myeloma (IgAλ)	ALλ	LBBB (ant)	18.0	46.9
7	77	F	Sick sinus syndrome (SSS)	ALλ	SSS	54.6	42.0
8	80	M	Hypertrophic cardiomyopathy	ALλ		42.7	57.1
9	82	M	Restrictive cardiomyopathy	ALκ	AF, AVB, LBBB (ant), SSS, VT	69.9	79.9
10	52	F	Multiple myeloma (IgD)	ALλ	AF, VF	53.6	54.3
11	44	M	Hypertrophic cardiomyopathy	ALλ	LBBB (ant)	37.9	39.7
12	58	M	Primary amyloidosis	ALλ	AF, AVB, LBBB (ant), SSS, VT	54.3	50.7
13	71	M	Restrictive cardiomyopathy	ALλ	AF, CRBBB	17.0	42.2
14	66	M	Reumatoid arthritis	AA		24.7	21.1
15	55	M	Cardiac amyloidosis	ALλ	LBBB (ant)	60.9	44.4
16	67	M	Primary amyloidosis	ALκ	AF, LBBB (post)	60.9	48.5
17	51	M	Primary amyloidosis	ALλ	AVB, LBBB (post)	67.2	45.3

AF, atrial fibrillation; AVB, atrioventricular block; CRBBB, complete right bundle branch block; LBBB(ant), left anterior hemiblock; LBBB(post), left posterior hemiblock; VF, ventricular fibrillation; VT, ventricular tachycardia.

2. Excision of the sinoatrial and atrioventricular nodes

At the time of autopsy the hearts were weighed. Since the sinoatrial node is located along the terminal sulcus between the superior vena cava and the right atrium, we opened the heart from the superior vena cava to the inferior vena cava and excised a rectangular tissue block between the superior vena cava and the right atrial appendage that spanned the terminal sulcus. We then cut this block at 5-mm intervals, again crossing the terminal sulcus. The result was four to six smaller rectangular tissue blocks.

The atrioventricular node is located in the base of the atrial septum at the apex of Koch's triangle; we considered the point of intersection of the Eustachian valve and the Thebesian valve to be the left border of the node, and opened this portion of the heart along a line connecting the posterior leaflet of the tricuspid valve with the oval fossa. Considering the right border of the node to be located 1 cm to the right of the membranous portion of the atrioventricular septum, we cut the heart in an upward direction from the septal leaflet of the tricuspid valve. We took the lower edge of the oval fossa to mark the upper limit and the point 1 cm inferior to the membranous portion of the atrioventricular septum as the lower limit, excising a rectangular tissue block defined by these lines. We cut the block at 5-mm intervals along the long axis of the heart, producing four to six smaller rectangular tissue blocks.

Each block was embedded in paraffin and sectioned at a thickness of 5μm. Sections were affixed to glass slides; some sections were stained with hematoxylin and eosin (H and E). Additional sections from blocks microscopically found to include the greatest extent of the sinoatrial or atrioventricular node were stained with Masson's trichrome (MT), elastica van Gieson (EVG), and Congo red (CR); (Fig. 1).

3. Extent of amyloid deposition

After we microscopically identified the sinoatrial or atrioventricular node in H and E-stained and EVG-stained specimens and verified the distribution of amyloid deposits in CR-stained specimens, we examined the sinoatrial or atrioventricular node in MT-stained specimens at a magnification of $\times$200 to select five fields in each section; (Fig. 2). Consequently, all of these fields lay entirely within the nodal designated area. Each field then was enlarged using an optical enlarger. A square area was defined in each microscopic field with an image analyzer. Because the distribution of amyloid deposits in CR-stained specimens almost corresponded with that in MT-stained specimens, the image analysis was based on the Masson's trichromestain. Within the square, the analyzer was used to determine the area of amyloid deposition. The image analysis system of the specific technique for measurement of the area was the WinROOF. This was done by pixel counting with the computer discriminating based on blue and computer-assisted point counting. We defined the relative extent of amyloid deposition in the five selected fields of each specimen as the extent of amyloid deposition (%D):

%D = (area of amyloid deposition within the 5 squares / total tissue area of the 5 squares) $\times$ 100.

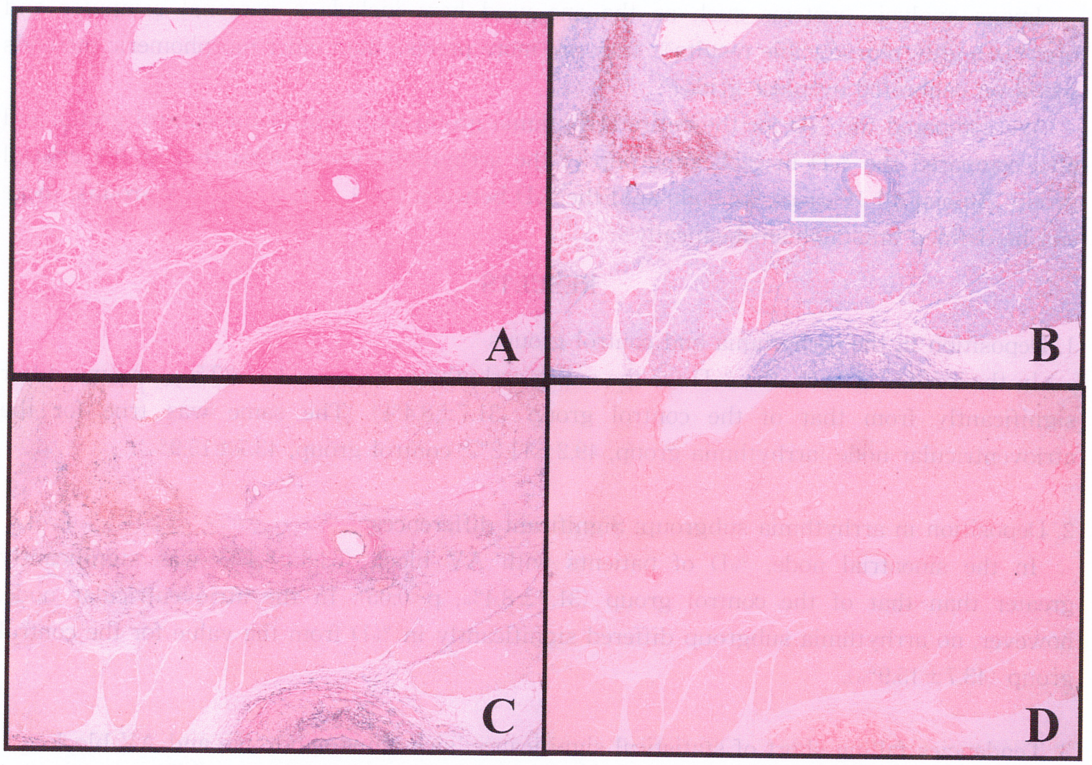

Fig. 1. Sinoatrial node (×40). A: Hematoxylin and eosin stain. B: Masson's trichrome stain. C: Elastica van Gieson stain. D: Congo red stain.

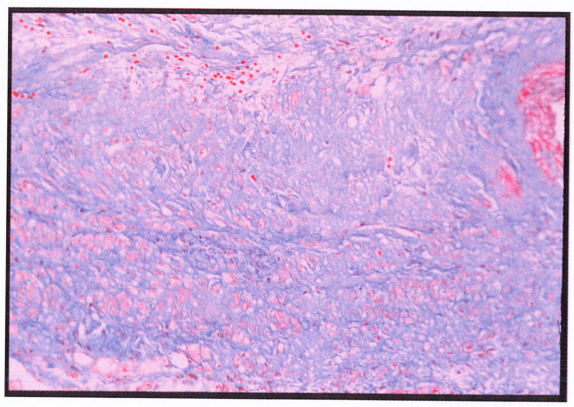

Fig. 2. A portion of the sinoatrial node enclosed by a white line in Figure 1B is shown at a magnification of ×200.

Image analysis systems such as the one used here include a personal computer and provide highly reproducible results[20]; they are more precise than older morphomeric methods based on calibrated eyepiece grids[21].

We compared the %D for the entire arrhythmia group with that of the control group, and also compared the %D in each subgroup of the arrhythmia group with that of the control group. Among all 17 cases, we compared the %D between AL λ, AL κ, and AA groups; these can be defined immunohistochemically[22].

RESULTS

1. Deposition in the arrhythmia and control groups

In the sinoatrial node, the %D for the entire arrhythmia group (43.3±20.5%) did not differ significantly from that of the control group (31.7±8.4%). The same was true for the atrioventricular node (arrhythmia group, 49.2±11.2%; control group, 43.7±15.9%).

2. Deposition in arrhythmia subgroup: Significant differences

In the sinoatrial node, %D of patients with AV block (63.8±8.4%) was significantly greater than that of the control group (31.7±8.4%, $p<0.05$). In the atrioventricular node, however, no arrhythmia subgroup differed significantly in %D from the value for the control group (43.7±15.9%).

3. Tendencies falling short of statistical significance: sick sinus syndrome and AV block

In the sinoatrial node, %D in the patients with sick sinus syndrome (59.6±9.0%) showed a stronger tendency than in the control group (31.7±8.4%; Table 2). In the atrioventricular node, %D in patients with AV block (58.6±18.6%) showed a tendency to be greater than in the control group (43.7±15.9%; Table 3). Neither difference, however, attained statistical significance.

Table 2. The SSS group and the Control group in the Sinoatrial Node

SSS(%)	Control(%)
54.6	33.9
69.9	25.6
54.3	42.7
	24.7
59.6±9.0	31.7±8.4

Table 3. The AV block group and the Control group in the Atrioventricular Node

AV block(%)	Control(%)
79.9	51.9
50.7	44.7
45.3	57.1
	21.1
58.6±18.6	43.7±15.9

4. AL λ, AL κ, and AA groups

Considering all 17 cases, the %D in the sinoatrial node did not differ significantly between the AL λ group (42.4±17.3%), the AL κ group (52.8±22.3%), and the AA group (21.7 ±7.2%). Although %D in the atrioventricular node did not differ significantly between the AL λ group (42.2±5.5%), the AL κ group (55.0±22.4%), and the AA group (43.3±20.2%), the degree of amyloid deposition in the AL group overall showed a strong tendency to be greater than that in the AA group.

DISCUSSION

1. Arrhythmias, conduction system disturbances, and amyloid deposition

Roberts and Waller[23] have stated that although amyloid deposition is always more extensive in "working" than in "conduction" myocardium, arrhythmias and conduction disturbances are common in cardiac amyloidosis (arrhythmias in 73% of cases, heart block in 45%, and complete bundle branch block in 18%). Considering all cases in arrhythmia group, amyloid deposition did not differ significantly from that in the control group for either the sinoatrial or the atrioventricular node. Amyloid deposition in the sinoatrial node in patients with AV block was significantly greater than in the control group ($p<0.05$), but amyloid deposition in the atrioventricular node in any arrhythmia subgroup did not differ significantly from that in the control group. Thus, the study did not definitively show a close relationship between arrhythmias or the conduction system disturbance and extent of deposits in the sinoatrial or atrioventricular node in cardiac amyloidosis; the small number of patients in each group may have been responsible.

2. Sick sinus syndrome, AV block, and amyloid deposition

Amyloid deposition in the sinoatrial node was similar in the patients with sick sinus syndrome and the control group, although the degree of amyloid deposition in patients with sick sinus syndrome tended to be greater. James[24] has pointed out that physicians attempting to restore sinus rhythm in patients with known or suspected cardiac amyloidosis should be aware that the sinoatrial node's pacemaker function may be compromised by amyloid deposition. Similarly, amyloid deposition in the atrioventricular node in patients with AV block was similar to deposition in the control group, even though a tendency to be greater was noted. Therefore amyloid deposition in the sinoatrial node might contribute to occurrence of sick sinus syndrome, and amyloid deposition in the atrioventricular node to occurrence of AV block. According to Song et al.[25], amyloidosis of the cardiac conduction system is seen most prominently in the sinoatrial node; in the atrioventricular system it appears to increase in severity from proximal (atrioventricular node) to distal (left bundle branch) sites.

3. AL and AA amyloidosis

Amyloid deposition did not differ significantly between the AL λ, AL κ, and AA groups in either the sinoatrial or the atrioventricular node, possibly because of the small number of patients in each group. However, amyloid deposition in AL amyloidosis showed a strong tendency to be greater than that in AA amyloidosis in both sinoatrial and atrioventricular nodes. Hamer et al.[9] found heart failure to be rare in AA amyloidosis but more common in the AL type. According to Arbustini et al.[7], the presence of nodular deposits, thick perimyocytic layers of amyloid, and small myocyte diameters in biopsy specimens helped to identify patients with the worst prognosis. Therefore, patient outcome in cardiac amyloidosis may be related to degree of sinoatrial or atrioventricular nodal amyloid deposition.

In conclusion, we could not prove a close relationship between arrhythmias or abnormal

conduction in cardiac amyloidosis and the extent of deposits in sinoatrial and atrioventricular nodes. However, we suspect that with amyloid deposition in the sinoatrial node exceeding 40% to 60%, sick sinus syndrome might occur; with atrioventricular node amyloid deposition exceeding 50% to 60%, AV block might occur. Moreover the degree of amyloid deposition in sinoatrial and atrioventricular nodes in AL amyloidosis showed a strong tendency to be greater than that in AA amyloidosis.

STUDY LIMITATIONS

We analyzed only a relatively small group of hearts from patients with cardiac amyloidosis. We also had to choose multiple sections to sample the sinoatrial and atrioventricular nodes. Moreover, we did not evaluate large portions of the cardiac conduction system including the atrioventricular system, His bundle, and left and right bundle branches. And the descriptive correlations with the types of arrhythmias seen should be considered very tentative, and not clearly demonstrated. For example, only in the AV block group was there a significant difference in the extent of amyloid deposition seen, but this difference was seen in the sinoatrial node, not in the atrioventricular node where one would have expected a causative deposition for AV block. Thus the significance of this finding is not clear. Also no hearts without amyloid were included as controls. Thus the arrhythmia predictive value of finding a certain type of amyloid in either location remains unclear with this study. However, marked amyloid deposition often was seen in the sinoatrial node in patients with sick sinus syndrome, as it was in the atrioventricular node in patients with AV block.

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