DIAGNOSTIC VALUE OF DISARRAY IN ENDOMYOCARDIAL BIOPSY SPECIMENS IN HYPERTROPHIC CARDIOMYOPATHY

—A Critical Report Based on Distribution of Disarray in the
Subendocardial Region of Autopsied Hearts—

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Myocardial fiber disarray in endomyocardial biopsies is considered to be a histologic feature of hypertrophic cardiomyopathy (HCM). To determine whether this disarray is specific for HCM, we examined the distribution of disarray in the subendocardial region in 11 autopsied hearts from patients with HCM and 41 autopsied control hearts. The percent area of disarray in the subendocardial region of the right ventricular side of the septum with maximum septal hypertrophy was $9 \pm 3\%$ in HCM and $4 \pm 2\%$ in the controls ($p < 0.001$). The frequency of occurrence of disarray in this area was $38 \pm 10\%$ in HCM and $20 \pm 7\%$ in the controls ($p < 0.001$). Disarray covering over 76% of a visual field, although extremely rare, was specific for HCM. In the right ventricular side of the septum in the lower half and in the left ventricular free wall, there was no significant difference between HCM and controls in regard to the percent area of disarray and its frequency of occurrence.

In conclusion, although disarray in right ventricular endomyocardial biopsies is considered to be more frequent in HCM than in the controls, too much emphasis should not be placed on disarray in endomyocardial biopsy specimens as a diagnostic criterion for HCM.

SINCE the first report by Teare,¹ myocardial fiber disarray has been described as a histologic feature of hypertrophic cardiomyopathy (HCM).²⁻⁶ However, it has been found, albeit to a lesser extent, in many patients with or without heart disease.⁵⁻¹⁰

Endomyocardial biopsy, the technique for which was developed by Sakakibara and Konno in 1962,¹¹ has been performed on many patients with cardiac diseases including HCM,¹²⁻¹⁷ and disarray is considered to be one of the diagnostic criteria for HCM.^{3,15-17} However, Noda¹⁸ has reported that there was no significant difference between HCM and congestive cardiomyopathy in regard to the disarray seen in endomyocardial biopsy specimens.

Key Words:

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TABLE I SUMMARY OF CASES

	No. of cases	Sex (m/f)	Age (Y)		H.W. (g)	
			Range	Mean	Range	Mean
HCM	11	9/2	20-76	46	350-970	623
Normal adult	13	12/1	21-81	52	200-350	261
Normal infant	11	7/4	2M-2	10M	30-80	45
Hypertension	7	5/2	45-68	58	460-600	516
MI	10	6/4	48-80	68	210-520	363

f = female; HCM = hypertrophic cardiomyopathy; H.W. = heart weight; *m* = male; *M* = months; MI = myocardial infarction; *Y* = years

In our autopsy studies,⁹ disarray was frequently observed in the right ventricular side of the septum and the inner third of the left ventricular free wall in normal hearts and in those with various diseases. On the other hand, disarray was seen chiefly in the middle three fifths of the ventricular septum and in the outer and middle thirds of the left ventricular free wall in hearts with HCM.¹⁰

The present study was undertaken to examine the distribution of disarray in the subendocardial region, which might be expected to be obtained by endomyocardial biopsies, in hearts with HCM, normal adult and infant hearts, and diseased hearts from patients with hypertension and myocardial infarction, and to determine whether or not disarray in endomyocardial biopsies can be considered a diagnostic criterion for HCM.

MATERIALS AND METHODS

Cases

Fifty-two hearts were studied: 11 with HCM, 13 normal adult hearts, 11 normal infant hearts, 7 hearts with concentric hypertrophy due to hypertension and 10 hearts with old or fresh myocardial infarctions. The age and sex of these subjects and the heart weights are listed in Table I.

Of the 11 cases of HCM, 9 were diagnosed both clinically and pathologically as HCM. In the remaining 2 patients, echocardiogram and cardiac catheterization were not performed, and the diagnosis was based on the pathological findings. The anatomic criteria for HCM have been reported by others.¹⁻⁶ In such cases, there was no more than 50% luminal narrowing in each major coronary artery and no significant valvular, congenital or infectious heart disease. In only one case was there a past history of mild hyperten-

sion. Of the 11 patients with HCM, 8 had obstruction of the left ventricular outflow tract.

During the selection of specimens, a heart was considered normal if there was no evidence of clinical heart disease, the coronary arteries did not show more than 75% luminal narrowing and there was no significant valvular or congenital heart disease. If there was a clear-cut history of hypertension and the heart weighed over 400 g, it was included in the hypertensive group. In the cases of myocardial infarction, luminal narrowing in one or more major coronary arteries was greater than 90%.

Histologic Examination

The heart was sliced serially and transversely at about 1-cm intervals (Fig. 1). The slice with maximum septal hypertrophy was selected in all cases of HCM. Maximum septal hypertrophy was in the upper or middle third of the septum in all but one case, in which it was located in the apical third. A slice from the apical third, where asymmetric septal hypertrophy was not present, was also used in 6 cases of HCM. One slice each from the upper and lower halves of the heart was used in cases other than HCM. All the heart slices were embedded in paraffin and sectioned transversely at 25 μ in adult hearts and 20 μ in infant hearts. These heart slices were then cut longitudinally at the anterior and posterior portions of the ventricular septum and of the left ventricular free wall (Fig. 1). From each heart slice, two tissue blocks 3 mm thick were taken from the ventricular septum and the left ventricular free wall. These tissue blocks were sectioned longitudinally at 25 μ . In our previous study, we found that thick sections (20-25 μ) were adequate for the observation of myocardial fiber disarray.⁹ The sections were stained with hematoxylin and eosin.

Fig.1. Diagram of the method of examination of the heart. The heart was sliced perpendicularly to the long axis of the left ventricle at about 1-cm intervals and one slice each from the upper and lower halves was used (upper left portion of diagram). From these heart slices, transverse plane sections were obtained (upper right portion of diagram). The heart slices were then cut longitudinally at the anterior and posterior portions of the ventricular septum and of the left ventricular free wall and four tissue blocks were obtained from each heart slice (lower left portion of diagram). These tissue blocks were sectioned longitudinally (lower right portion of diagram). The distribution of disarray was examined in the subendocardial region within 2.0 mm from the endocardium (shown by stippled areas in upper and lower right portions of diagram) in transverse and longitudinal planes.

A = anterior; FW = left ventricular free wall; LA = left atrium; LV = left ventricle; P = posterior; RA = right atrium; RV = right ventricle

Definition of Disarray

In the thick sections of the hearts with HCM, fascicular and fiber disarray were observed^{9,10}. Fascicular disarray was classified into poorly-differentiated and well-differentiated fascicular patterns. Fiber disarray comprised mild, moderate and marked fiber disarray. Marked and moderate fiber disarray was seen in the converging

areas of fascicles and in the areas where the fascicle bent at an acute angle. In these areas, adjacent fibers were aligned perpendicularly or obliquely to each other and bizarre shaped cells were seen. In mild fiber disarray, fibers showed minor deviations in alignment from the rigid parallelism, which gave fibers the appearance of branching or Y-shape. Mild fiber disarray was

Fig.2. Various histologic pictures of well-differentiated fascicular disarray. Broad bundles with a clear fascicular structure are bent and cross each other obliquely or perpendicularly, but fibers within these bundles are normally arranged. A, B, C and D are from normal adult hearts. (hematoxylin and eosin stain; A and B, $\times 40$; C and D, $\times 100$)

not considered to represent true abnormalities because it was seen in all areas in hearts with HCM and also in the controls. Therefore, fascicular disarray and marked and moderate fiber disarray were considered abnormal in this study. Examples of abnormal disarray in the subendocardial region are shown in Figs. 2(A–D), 3(A–D) and Fig. 4(C and D).

Subendocardial Regions Obtained by Endomyocardial Biopsies

Endomyocardial biopsy specimens are commonly obtained from the right ventricular side of the septum and sometimes from the anterior portion of the right ventricular free wall near the junction of the ventricular septum in right ventricular biopsies, and from the left ventricular free wall in left ventricular biopsies.^{12, 13, 16–18} In our 40 biopsy specimens, the depth of the specimens from the endocardium averaged 1.4 ± 0.4 mm (range: 0.6–2.5 mm) in right ventricular biopsies and 1.4 ± 0.5 mm (range: 0.6–2.3 mm) in left ventricular biopsies. Therefore, the

subendocardial region within 2.0 mm from the endocardium was selected (Fig. 1).

Quantitation of Disarray in the Subendocardial Region

The subendocardial region within 2.0 mm from the endocardium was observed under a $\times 100$ magnification of a light microscope by moving the visual field successively, and the presence or absence and extent of disarray in each visual field were determined. The extent of disarray in one visual field was graded as 1–25, 26–50, 51–75 and 76–100%. Under a $\times 100$ magnification, an area 2.0 mm in diameter, which nearly corresponds to a medium sized biopsy specimen, could be observed in one visual field. The border between visual fields was observed carefully to exclude overlapping or omission of examined fields. The following three expressions were used for the quantitation of disarray. First, the extent of disarray was calculated from the following formula and called the “percent area of disarray”:

Fig.3. Various histologic pictures of poorly-differentiated fascicular disarray. Each bundle is relatively narrow and various patterns, such as fern-like (A and B) and whorl-like (D) are seen. A, B, C and D are from normal adult hearts. (hematoxylin and eosin stain; A, C and D, $\times 100$; B, $\times 200$)

% area of disarray

$$= \frac{1/8 \times A + 3/8 \times B + 5/8 \times C + 7/8 \times D}{T} \times 100 (\%)$$

where T = the number of all visual fields with and without disarray in the subendocardial region of transverse and longitudinal plane sections; A, B, C, D = the number of visual fields with disarray of 1–25, 26–50, 51–75 and 76–100%, respectively, in the subendocardial region of transverse and longitudinal plane sections. Coefficients of 1/8, 3/8, 5/8 and 7/8 were given for the four grades, respectively, assuming that they were the mean values of the extent of disarray in each grade. Second, the frequency of visual fields with disarray in relation to the total number of visual fields with and without disarray was calculated as follows and called the “frequency of occurrence of disarray”:

frequency of occurrence of disarray

$$= \frac{A + B + C + D}{T} \times 100 (\%)$$

This index represents the probability of detection of disarray in endomyocardial biopsy specimens. Third, according to the grading of the extent of disarray in one visual field, the frequency of occurrence of disarray in each of the four grades was calculated as:

$$A/T, B/T, C/T \text{ or } D/T \times 100 (\%)$$

In this study, the total number of visual fields with and without disarray in the subendocardial region of the transverse and longitudinal plane sections ranged from 19 to 74 (mean 42) in the right ventricular side of the septum and from 28 to 115 (mean 54) in the left ventricular free wall.

Data Analysis

The statistical significance of differences among the groups was determined by the analysis of variance and Duncan's multiple comparison.

RESULTS

Percent Area of Disarray (Fig. 5)

The percent area of disarray in subendocardial region in slices with maximum septal hyper-

Fig.4. Various histologic pictures of fiber disarray. A: normal parallel alignment of fibers. B: mild fiber disarray, in which fibers have a Y-shaped or branching appearance. In marked (D) and moderate (C) fiber disarray, adjacent fibers are aligned perpendicularly or obliquely to each other and bizarre shaped cells are seen. These features are conspicuous in marked fiber disarray. In this study, marked and moderate fiber disarray were considered abnormal. A, B and C are from normal adult hearts, D is from a heart of a patient with hypertension. (hematoxylin and eosin stain, $\times 200$)

trophy in HCM was compared with that in slices of the upper half of control hearts. In the right ventricular side of the septum, the percent area of disarray was $9 \pm 3\%$ (mean $\pm$ SD) in HCM, $4 \pm 2\%$ in normal adult hearts, $3 \pm 2\%$ in normal infant hearts, $4 \pm 2\%$ in cases of hypertension and $4 \pm 2\%$ in cases of myocardial infarction. The percent area of disarray was significantly greater in HCM than in controls ($p < 0.001$). In the subendocardial region of the left ventricular free wall, the percent area of disarray was $3 \pm 2\%$ in HCM, $3 \pm 2\%$ in normal adult hearts, $3 \pm 2\%$ in normal infant hearts, $2 \pm 1\%$ in cases of hypertension and $4 \pm 2\%$ in cases of myocardial infarction. The difference in the percent area of disarray between HCM and controls was not significant. Among the groups of controls, there was no significant difference in the percent area of disarray in either the right ventricular side of the septum or the left ventricular free wall.

In 6 of the 11 hearts with HCM, the heart

slice at the apical third, in which asymmetric septal hypertrophy was not seen, was also examined. Slices of the lower half of control hearts were examined for comparison. As shown in the lower portion of Fig. 5, there was no significant difference between HCM and controls in the percent area of disarray in either the right ventricular side of the septum or the left ventricular free wall.

Frequency of Occurrence of Disarray (Fig. 6)

In the right ventricular side of the septum in the upper half, the frequency of occurrence of disarray was $38 \pm 10\%$ in HCM, $20 \pm 7\%$ in normal adult hearts, $18 \pm 7\%$ in normal infant hearts, $19 \pm 8\%$ in cases of hypertension and $21 \pm 7\%$ in cases of myocardial infarction. The difference in frequency of occurrence of disarray between HCM and controls was significant ($p < 0.001$). In the left ventricular free wall, there was no significant difference between HCM and

Upper Half

Lower Half

Fig.5. Percent area of disarray in the subendocardial region of the right ventricular side of the septum and of the left ventricular free wall in upper and lower halves of the heart. Open circles represent cases of HCM, solid circles controls. Bars represent mean value. HCM = hypertrophic cardiomyopathy; HT = hypertension; LV = left ventricle; MI = myocardial infarction; NA = normal adult; NI = normal infant; RV = right ventricle

controls in regard to the frequency of occurrence of disarray. In the lower half, there was no significant difference between HCM and controls in regard to the frequency of occurrence of disarray in either the right ventricular side of the septum or the left ventricular free wall.

Frequency of Occurrence of Disarray in Each of Four Grades (Fig. 7)

The frequency of occurrence of disarray of 1–25, 26–50, 51–75 and 76–100% of a visual field in the right ventricular side of the septum with maximum septal hypertrophy was 27 ± 8 , 7 ± 4 , 4 ± 2 and $2 \pm 2\%$, respectively, in HCM.

Fig.6. Frequency of occurrence of disarray in the subendocardial region of the right ventricular side of the septum and of the left ventricular free wall in upper and lower halves of the heart. Symbols and abbreviations are the same as in Fig. 5.

The difference in the frequency of occurrence of disarray of 1–25, 51–75 and 76–100% between HCM and controls was significant. In the left ventricular free wall in the upper half, there was

no significant difference between HCM and controls in regard to the frequency of occurrence of disarray in any of the four grades. Although its frequency of occurrence was as low as 2%,

Fig. 7. Frequency of occurrence of disarray in each of the four grades in the subendocardial region of the right ventricular side of the septum and of the left ventricular free wall in the upper half of the heart. The extent of disarray is graded as 1-25, 26-50, 51-75 and 76-100% of a visual field. Symbols and abbreviations are the same as in Fig. 5.

disarray covering over 76% of a visual field was noted in 7 of the 11 cases of HCM in the right ventricular side of the septum with maximum septal hypertrophy. In contrast, such disarray was detected in only one of the 41 control cases in the same region. This difference between HCM and controls was significant ($p < 0.001$, using χ^2 test). In the left ventricular free wall in the upper half, disarray covering over 76% of a visual field was detected in one of 11 cases of HCM and one of 39 control cases. Disarray covering 51-75% of a visual field was noted in 10 of the 11 cases of HCM and 16 of the 41 control cases in the right ventricular side of the septum, and the difference was also significant ($p < 0.01$, using χ^2 test).

In the lower half, there was no significant difference between HCM and controls in regard to the frequency of occurrence of disarray in each of the four grades in either the right ven-

tricular side of the septum or the left ventricular free wall. Disarray covering over 76% of a visual field was detected in only one of the 23 control cases in the right ventricular side of the septum.

DISCUSSION

Widespread disarray is reportedly a highly sensitive and specific finding in HCM.^{5,6} However, disarray in hearts with HCM is located chiefly in the middle three fifths of the ventricular septum and in the outer and middle thirds of the left ventricular free wall.¹⁰ Isner et al.¹⁹ have reported that the extent of disarray in excised septectomy specimens was significantly less than that in the ventricular septum at necropsy in the same patients with HCM, and examination of septectomy specimens was not a sensitive means of confirming the diagnosis of HCM. On the other hand, disarray in normal

hearts and in those with various diseases is frequently observed in the right ventricular side of the septum and the inner third of the left ventricular free wall, which correspond to the biopsy sites.⁹ In the normal macaque left ventricle, Ross and Streeter²⁹ observed a high degree of disorder in fiber orientation in the subendocardial region. Therefore, the subendocardial region containing mural and luminal trabeculations is the site where disarray is frequently and physiologically present not only in HCM but also in the controls.

The present investigation of the distribution of disarray in the subendocardial region showed that the percent area of disarray in the right ventricular side of the septum with maximum septal hypertrophy was $9 \pm 3\%$ in HCM and $4 \pm 2\%$ in the controls, showing a significant difference. However, the frequency of occurrence of disarray, i.e., the probability of detection of disarray in endomyocardial biopsies, was $38 \pm 10\%$ in HCM and $20 \pm 7\%$ in the controls; hence, both sensitivity and specificity in reaching a diagnosis of HCM from disarray in biopsy specimens are low. Furthermore, in the right ventricular side of the septum in the lower half and in the left ventricular free wall, there was no significant difference between HCM and controls in regard to the percent area of disarray and the frequency of occurrence of disarray. Therefore, disarray in right ventricular endomyocardial biopsies is considered to be more frequent in HCM than in the controls, but it is not pertinent to use this disarray for the diagnosis of HCM.

Van Noorden et al.³ have proposed the histological HOCM index by the use of a combination of characteristics, which composed of short runs of fibers, large and bizarre nuclei, fibrosis, degenerating muscle and disorganized muscle. They reported that using the index a diagnosis could often be made from the myocardial biopsy specimens obtained at open heart operation. Although these histological characteristics are observed frequently in autopsy cases of HCM, any of these are not specific for HCM. They also noted that in about half cases a diagnosis could not be made on histological ground alone. Furthermore, the specimens in their study were obtained from the area of greatest obstruction. From the result in our study, the frequency of occurrence of disarray in these specimens would be higher than that in usual endomyocardial biopsy specimens which can not be obtained selectively.

Disarray covering over 76% of a visual field was detected in 7 of the 11 cases of HCM in the right ventricular side of the septum with maximum septal hypertrophy. In the controls, such disarray was detected in one of 41 cases in the right ventricular side of the septum in the upper half, one of 39 cases in the left ventricular free wall in the upper half and one of 23 cases in the right ventricular side of the septum in the lower half. Therefore, disarray covering over 76% of a visual field is considered specific for HCM, but it would be extremely rare for such disarray to be detected in biopsy specimens, since the frequency of occurrence of such disarray was as low as 2% in HCM.

Our data further revealed that there was no significant difference among the groups of controls in regard to the extent of disarray in the subendocardial region. These findings support the hypothesis that disarray occurs congenitally in the fetus in which the architecture of fascicles is determined.^{2,5,9,10}

In conclusion, disarray in endomyocardial biopsy specimens is of limited value in confirming the diagnosis of HCM.

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