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Histological features of endomyocardial biopsies in patients undergoing hemodialysis: Comparison with dilated cardiomyopathy and hypertensive heart disease

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ABSTRACT

Background: Heart failure is a frequently occurring complication in patients on maintenance hemodialysis (HD). However, the histological features of right ventricular endomyocardial biopsy (RVEMB) samples remain unclear.

Methods: The clinical characteristics and histological findings of consecutive patients undergoing HD with available RVEMB samples (HD group; n=28) were retrospectively compared with those of patients with dilated cardiomyopathy (n=56) and hypertensive heart disease (n=15).

Results: The mean myocyte diameter was significantly larger in the HD group than in the other groups (P<.001), whereas the mean percent area of fibrosis did not differ among the three groups. Immunohistochemical analysis revealed that the capillary density was significantly lower in the HD group compared with the other groups (P<.001), and it was positively associated with left ventricular ejection fraction (P=.014). The number of CD68-positive macrophages, which was significantly higher in the HD group compared with the other two groups (P<.001), was associated with cardiovascular mortality (P=.020; log-rank test).

Conclusions: Myocyte hypertrophy, macrophage infiltration, and reduced capillary density were characteristic histological features of the RVEMB samples in patients undergoing HD, which may be related to the pathogenesis of cardiac dysfunction.

1. Introduction

The number of patients receiving maintenance hemodialysis (HD) treatment annually is increasing globally, with an estimated prevalence of nearly 3.2 million [1]. The risk of cardiovascular disease is higher in patients undergoing HD than in the general population [2]. In particular, heart failure is responsible for the high mortality of these patients [3]. In Japan, where the prevalence of HD-treated end-stage renal disease is very high (2688 per million population), the major cause of death in patients undergoing HD is heart failure (23.5%), followed by infections (21.3%), malignancies (8.4%), cerebrovascular disease (6.0%), and myocardial infarction (3.6%) [4]. Various factors have been implicated in the pathogenesis of cardiomyopathy in patients undergoing HD, including left ventricular (LV) pressure overload secondary to hypertension and arteriosclerosis, volume overload caused by arteriovenous shunt and anemia, inflammation, and uremic toxins, with profibrotic and prohypertrophic effects [5,6]. However, the precise mechanism underlying cardiac dysfunction remains unclear.

Cardiomyopathy in patients undergoing HD is clinically diagnosed as HD-related cardiomyopathy or uremic cardiomyopathy. In these patients, the predominant pathological features in the heart include concentric hypertrophy and LV dilatation, which are associated with pressure and volume overload, respectively [7,8]. The frequently observed cardiac histological findings include myocyte hypertrophy and interstitial fibrosis, which may be observed in other cardiomyopathies as well. In particular, cardiomyopathy associated with HD and dilated cardiomyopathy (DCM) may be his-

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tologically indistinguishable based on their shared histological features.

Endomyocardial biopsy (EMB) was pioneered in 1962 by Drs. Sakakibara and Konno at our institution [9,10]. It has been used worldwide to monitor rejection following heart transplantation and diagnoses of cardiac diseases, such as myocarditis, cardiomyopathy, drug toxicity, and secondary cardiomyopathy caused by systemic diseases [11]. Conversely, EMB is rarely performed in patients undergoing HD who experience cardiac dysfunction because their histological findings are expected to be nonspecific. Consequently, after excluding autopsy cases, data regarding the histological features of EMB samples from patients undergoing HD are limited. Furthermore, numerous pathological studies have analyzed the histological features of only the LV without considering the distinction between the right ventricle (RV) and LV. The only study that examined biopsy samples from the LVs of patients undergoing HD found that myocyte hypertrophy was more severe in these patients than in those with DCM and that severe fibrosis was associated with poor prognosis [12]. However, to date, no study has focused on the histological features of the RV in patients undergoing HD.

At many institutions, EMB samples are usually obtained from the RV owing to the low incidence of major complications [13]. Therefore, we investigated the characteristic histological features of RV biopsy samples and their correlation with the clinical features of patients undergoing HD.

2. Materials and methods

2.1. Study design

A total of 1632 patients underwent EMB of the RV at Tokyo Women's Medical University Hospital between January 1996 and December 2013. Among these, we retrospectively reviewed the clinical characteristics and histological features of 28 consecutive patients on chronic HD (HD group) and compared to those of age- and sex-matched patients with clinically diagnosed idiopathic DCM (DCM group; n=56) and those with hypertensive heart disease (HHD) who were diagnosed clinically based on long-standing, untreated hypertension (HHD group; n=15). Idiopathic DCM was defined as impaired LV contraction, with an ejection fraction (EF) of $\leq$50% or fractional shortening of $<$25%, and ventricular dilatation. The clinical data including laboratory tests and cardiac catheterization data were collected from the medical records. Patients with valvular heart disease as well as those with a history of cardiovascular ischemic events or coronary stenting were excluded from this study. Patients with DCM or HHD with an elevated creatinine level of $>$2.0 mg/dL were excluded. The data collection protocol was approved by the institutional review board (#3189-R2), and informed consent was obtained in the form of opt-out on the webpage of Tokyo Women's Medical University. The protocol complied with all ethical guidelines outlined by the 1975 Declaration of Helsinki.

2.2. Histological evaluation of biopsy samples

The biopsy samples were routinely obtained from the RV septum using a cardiac bioptome during diagnostic right heart catheterization. The samples were fixed in 10% formalin, dehydrated with a series of increasing ethanol concentrations, and embedded in paraffin. Next, the samples were cut into 4-μm-thick serial sections, deparaffinized, and stained with hematoxylin/eosin or Masson's trichrome.

The mean myocyte diameter was determined by measuring across the nucleus, in 50 randomly selected myocytes from hematoxylin/eosin-stained sections, at 400 $\times$ magnification. Because the number of EMB samples per patient varied from one to five, the percentage of fibrotic areas was evaluated in the sample with the most severe fibrosis for each patient using the Image J software (National Institutes of Health, Bethesda, MD, USA).

Immunohistochemical staining for CD3, CD20, CD34, CD68, and CD206 with appropriate negative and positive controls was performed to evaluate capillary density and extent of inflammatory cell infiltration. The primary antibodies used in this study were as follows: CD3 (1:200, Dako, Santa Clara, CA, USA), CD20 (1:200, Dako), CD34 (1:200, Dako), CD68 (1:300, Dako), CD11c (1:100, Abcam, Cambridge, UK) and CD206 (1:5000, Abcam, Cambridge, UK). Antigen retrieval was achieved by boiling the samples in citrate buffer (pH 6.0). The density of inflammatory cells was measured using digital whole-slide photomicrographs of immunohistochemically stained serial sections of the biopsy samples that were used to determine the extent of fibrosis captured by a BZ-X710 microscope (Keyence, Osaka, Japan). The capillary density was determined in five randomly selected fields per slide using a Leica DMD108 microscope (Leica Microsystems, Wetzlar, Germany).

2.3. Statistical analyses

Data are presented as means$\pm$standard deviation or frequencies with percentages. One-way analysis of variance or Kruskal-Wallis test was used to assess differences among the groups, as appropriate. For multiple comparisons, Tukey's honestly significant difference test or Games-Howell test was performed after the analysis of variance and two-tailed Mann-Whitney U test with Bonferroni's correction was performed after Kruskal-Wallis test. Categorical variables were compared using the Chi-square test or Fisher's exact test, as appropriate. Haberman's analysis of residuals was used as a post hoc analysis of chi-square test with a Pvalue of $<$.05. The correlation between morphometric analysis and EF of LV (LVEF) was explored using Pearson's product-moment correlation coefficient or Spearman's rank-order correlation coefficient, as appropriate. Univariate analysis was performed to determine the morphometric parameters associated with deterioration in the LVEF. The receiver operating characteristic curves were generated to determine the optimal cutoff values. Event-free survival was calculated from the date of biopsy to the date of LV assist device (LVAD) implantation or to the date of cardiovascular death, which included any deaths resulting from acute myocardial infarction, sudden cardiac death, death due to heart failure, death due to stroke, and death due to cardiovascular hemorrhage. Overall survival was calculated from the date of biopsy to the date of death from any cause. Survival was compared using Kaplan–Meier method with the log-rank test. Data were censored on December 31, 2018. All statistical analyses were performed using the SPSS software (version 25.0 for Windows; SPSS, Tokyo, Japan). P values of $<$.05 were considered to indicate statistical significance for all tests except for those used for multiple comparisons in which a P value of $<$.017 was considered to indicate statistical significance.

3. Results

3.1. Patient characteristics

Patient characteristics are presented in Table 1. Briefly, the mean age of the HD group, comprising approximately 80% males, was 50.9$\pm$14.3 years. Chronic glomerulonephritis was the most common primary disease (32.1%), followed by diabetic nephropathy (21.4%) and polycystic kidney disease (7.1%), excluding cases with unknown etiologies (21.4%). The hemoglobin level was lower in the HD group, and the mean duration of maintenance HD was 97 months. The percentages of patients with hypertension and diabetes were lower in the DCM group than in the HD and HHD groups. There were no significant differences in the hemodynamic

Table 1
Clinical characteristics of the study patients

	HD (n=28)	DCM (n=56)	HHD (n=15)	P value
Age, years	50.9±14.3	51.6±14.0	53.8±10.1	.8
Male (%)	22 (78.6%)	44 (78.6%)	12 (80.0%)	.992
NYHA class ≥ III	6 (21.4%)	15 (26.8%)	6 (40.0%)	.424
Atrial fibrillation	2 (7.1%)	9 (16.1%)	2 (13.3%)	.496
Hypertension	25 (89.3%)[b]	5 (8.9%)[b]	15 (100.0%)[b]	<.001
Diabetes mellitus	7 (25.0%)	5 (8.9%)[b]	6 (40.0%)[a]	.012
Dyslipidemia	6 (21.4%)	13 (23.2%)	6 (40.0%)	.355
Hemoglobin (g/dl)	11.4±1.5	14.4±1.5[c]	14.7±1.4[c]	<.001
Creatinine (g/dl)	9.5±2.7	0.9±0.2[d]	1.0±0.4[d]	<.001
EF (%)	34.9±10.6	29.5±9.7	39.3±11.4	.002
EDVI (ml/mm2)	139.3±57.4	154.8±59.4	116.1±36.5	.078
PCWP (mmHg)	10.8±4.4	11.7±7.5	14.6±6.5	.099
Cardiac index (L/min/mm^2)	3.2±0.9	2.7±0.7[d]	3.1±0.6	<.001
β-blocker	21 (75.0%)	53 (94.6%)[a]	13 (86.7%)	.034
ACE inhibitor/ARB	19 (67.9%)	56 (100%)[b]	15 (100%)	<.001
Follow-up time (years)	8.6±4.9	11.0±5.1	6.9±4.9	.011
Duration of HD (months)	97±109	-	-	

[a] P<.05.
[b] P<.01, based on Haberman's analysis performed after the Chi-square test.
[c] P<.01 vs. HD, based on Tukey analysis performed after ANOVA.
[d] P<.017 vs. HD, based on Mann–Whitney U test with Bonferroni's correction.NYHA, New York Heart Association; EF, ejection fraction; EDVI, end-diastolic volume index; PCWP, pulmonary capillary wedge pressure; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 2
Histological and immunohistochemical analyses

	HD	DCM	HHD	P value
Myocyte diameter (μm)	18.4±2.6	16.6±1.6[b]	16.8±1.2[a]	<.001
Fibrosis (%)	10.6±9.1	11.6±8.9	10.1±5.0	.742
Capillary density(/mm^2)	997±183	1238±261[e]	1162±189[c]	<.001
CD3 (/mm^2)	15.4±17.1	17.3±13.7	19.3±12.0	.229
CD20 (/mm^2)	0.2±0.7	0.1±0.3	0.4±0.7	.323
CD68 (/mm^2)	43.8±26.3	14.1±12.3[e]	19.6±19.4[d]	<.001
CD206 (/mm^2)	17.4±13.2	8.2±6.8[d]	10.3±7.1	.003

[a] P<.05 vs. HD.
[b] P<.01 vs. HD, based on Games-Howell test performed for multiple comparisons after one-way ANOVA.
[c] P<.017 vs. HD.
[d] P<.003 vs. HD.
[e] P<.0003 vs. HD, based on Mann-Whitney U test with Bonferroni's correction performed for multiple comparisons after the Kruskal-Wallis test.

findings such as end-diastolic volume index and pulmonary capillary wedge pressure among the three groups; however, the DCM group included more patients with reduced EF compared with the HHD group and more patients with reduced cardiac index compared with the HD and the HHD groups. In the HD group, β-blockers and angiotensin-converting enzyme inhibitors/angiotensin receptor blocker were administered to 21 (75.0%) and 19 (67.9%) patients, respectively, which were less frequent than those in the other two groups. The follow-up period, which was longer in the DCM group than in the HHD group (P=.018), was comparable in HD group and the other two groups.

3.2. Histopathological changes in RVEMB samples

Table 2 summarizes the histological and immunohistochemical analyses of the biopsy samples. The mean myocyte diameter was significantly larger in the HD group than in the DCM and HHD groups (P<.001). In contrast, there was no significant difference in the mean percentage of fibrosis among the groups based on the analysis of Masson's trichrome-stained samples (P=.742). The analysis of covariance showed no correlation between the myocyte diameter and hypertension (P=.991). Similarly, the myocyte diameter and the severity of fibrosis were not related to age in the correlation analysis of all three groups.

The immunohistochemical staining for CD34 revealed that the capillary density significantly reduced in the HD group compared with the DCM and HHD groups (P<.001 for all). In the HD group, there was no correlation between the duration of HD and histological parameters such as myocardial diameter (P=.864, r=0.03), degree of fibrosis (P=.29, r=0.21), and capillary density (P=.55, r=0.12). Representative histological and immunohistochemical images are shown in Fig. 1.

3.3. Inflammatory changes in RVEMB samples of patients on maintenance hemodialysis

We examined whether there were differences in the extent of inflammatory infiltration among the three groups using immunohistochemistry (Fig. 1, Table 2). A small number of CD3-positive T lymphocytes were observed in the samples from all groups, whereas the CD20-positive B lymphocytes were rarely observed. The CD68-positive cells were frequently observed in the cardiac interstitium in the HD group, and the number of CD68-positive cells was significantly higher in the HD group compared with the DCM and HHD groups (P<.001). None of the patients exhibited severe inflammatory infiltration accompanied with myocardial damage or necrosis, a typical histological finding of active myocarditis. As most of the CD68-positive cells were considered to be macrophages, we also examined the relative abundance of M1 and M2 macrophage populations by immunohistochemistry for CD11c and CD206, respectively. The CD11c-positive cells were rarely observed in the myocardium in all groups; however, the number of CD206-positive cells was significantly higher in the HD group than in the DCM group (P=.003).

3.4. Relationship between the histological findings and LV function

We assessed whether the changes in specific histological parameters were associated with LV systolic function. The capillary density was positively associated with LVEF in the HD group and was the only parameter associated with EF based on the correlation analysis (r=0.457, P=.014; Table 3). In contrast, only the myocyte diameter was associated with EF in the DCM (r=−0.464,

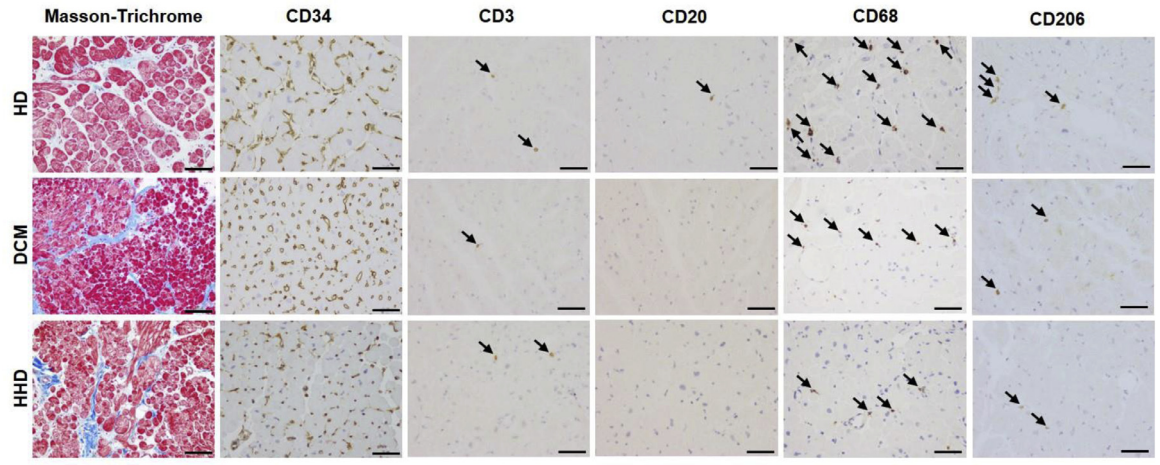

Fig. 1. Representative histological and immunohistochemical images of biopsy specimens. Arrows indicate cells positive for immunohistochemical staining.

Table 3
Correlation analysis of left ventricular ejection fraction and histological parameters

	HD		DCM		HHD	
	r	*P* value	*r*	*P* value	*r*	*P* value
Myocyte diameter (μm)	−0.242	.215	−0.464	<.001	−0.721	.002
Fibrosis (%)	−0.309	.11	0.016	.909	−0.327	.234
Capillary density (/mm^2)	0.457	.014	0.197	.145	0.087	.758
CD3 (/mm^2)	−0.144	.466	−0.196	.148	−0.215	.442
CD20 (/mm^2)	−0.16	.417	−0.113	.407	0.248	.372
CD68 (/mm^2)	0.125	.525	0.034	.805	−0.352	.198
CD206 (/mm^2)	−0.083	.673	0.101	.458	−0.327	.234

Table 4
Univariate regression analysis for left ventricular ejection fraction

	HD		DCM		HHD	
	β	Pvalue	β	*P* value	β	Pvalue
Myocyte diameter (μm)	−0.242	.215	−0.464	<.001	−0.721	.002
Fibrosis (%)	−0.195	.32	−0.008	.951	−0.327	.234
Capillary density (/mm^2)	0.463	.013	.163	0.229	0.087	.758
CD3 (/mm^2)	−0.262	.177	−.182	0.181	−0.460	.085
CD20 (/mm^2)	−0.309	.11	−.081	0.551	0.272	.327
CD68 (/mm^2)	0.125	.525	−.071	0.601	−0.200	.475
CD206 (/mm^2)	−0.156	.427	.025	0.855	−0.327	.234

P<.001) and HHD (*r*=−0.721, *P*=.002) groups. Similarly, univariate regression analysis revealed that only the capillary density was associated with EF in the HD group (Table 4). Because of the small number of patients, multiple stepwise regression analysis was not possible in the current study.

3.5. Clinical outcomes

Finally, we assessed the correlation of histological findings and clinical outcomes, which revealed that the rates of mortality due to cardiovascular death and LVAD implantation and the rate of all-cause mortality were not significantly different among the three groups (Fig. 2). We compared the cardiovascular mortality rates among the groups based on the cutoff values for histological parameters, determined by receiver operating characteristic analysis of the HD group. There were no correlations between the histological parameters and prognosis except for the number of CD68-positive cells in the HD group. A CD68-positive cell number of ≥65 was associated with poor prognosis (*P*=.020; log-rank test). Conversely, the number of CD206-positive M2 macrophages did not show an association with cardiovascular mortality (*P*=.181; log-rank test, not shown in the figure).

4. Discussion

The comparative analysis of histological changes in the EMB samples of the RV between the patients on chronic HD and those with DCM or HHD showed that myocyte hypertrophy, macrophage infiltration, and reduced capillary density were more prominent in patients undergoing HD compared to those with DCM or HHD. The reduction in capillary density in the RV was associated with LV systolic dysfunction in patients undergoing HD, whereas the increased myocyte diameter in the RV was correlated with the deterioration of EF in patients with DCM and those with HHD. An increase in the number of CD68-positive cells in EMB samples was associated with poor prognosis in patients undergoing HD.

LV hypertrophy and dilatation, that are well known and prominent gross changes observed in patients undergoing HD, are related to systolic and diastolic dysfunction and are causes of heart failure [14]. Histological examination of autopsy cases has shown that cardiac myocyte hypertrophy is prominent in patients undergoing HD [15]; however, this histological feature is commonly observed in patients with DCM as well. Although few studies have conducted comparative analyses of cardiac histology between patients with HD and those with DCM, one study using EMB samples of the LV reported that the myocyte diameter was larger in patients undergoing HD than in those with DCM [12], in agreement with the results of the present study.

The mechanisms underlying myocyte hypertrophy differ between HD-related cardiomyopathy and DCM, which is a primary myocardial disease. In DCM, myocyte hypertrophy of residual myocytes occurs as a compensatory change following myofibrillar loss and myocyte atrophy. Conversely, various factors contribute to myocardial hypertrophic changes in patients undergoing HD. Specifically, hypertension, a well-known complication in these patients, causes hypertrophic remodeling in response to pressure overload [16,17]. Hypertension was identified in nearly 90% of patients undergoing HD in the present study. Therefore, we included the HHD group in this study, although the number of patients in this group was small due to the current standard treatment approaches for hypertension. The myocyte diameter in the RV was larger in the HD group than in the HHD group. Pathophysiologically, histological changes caused by hypertension-related pressure overload commonly occur in the LV rather than in the RV. Therefore, we speculate that the myocyte hypertrophy observed in the RV of patients

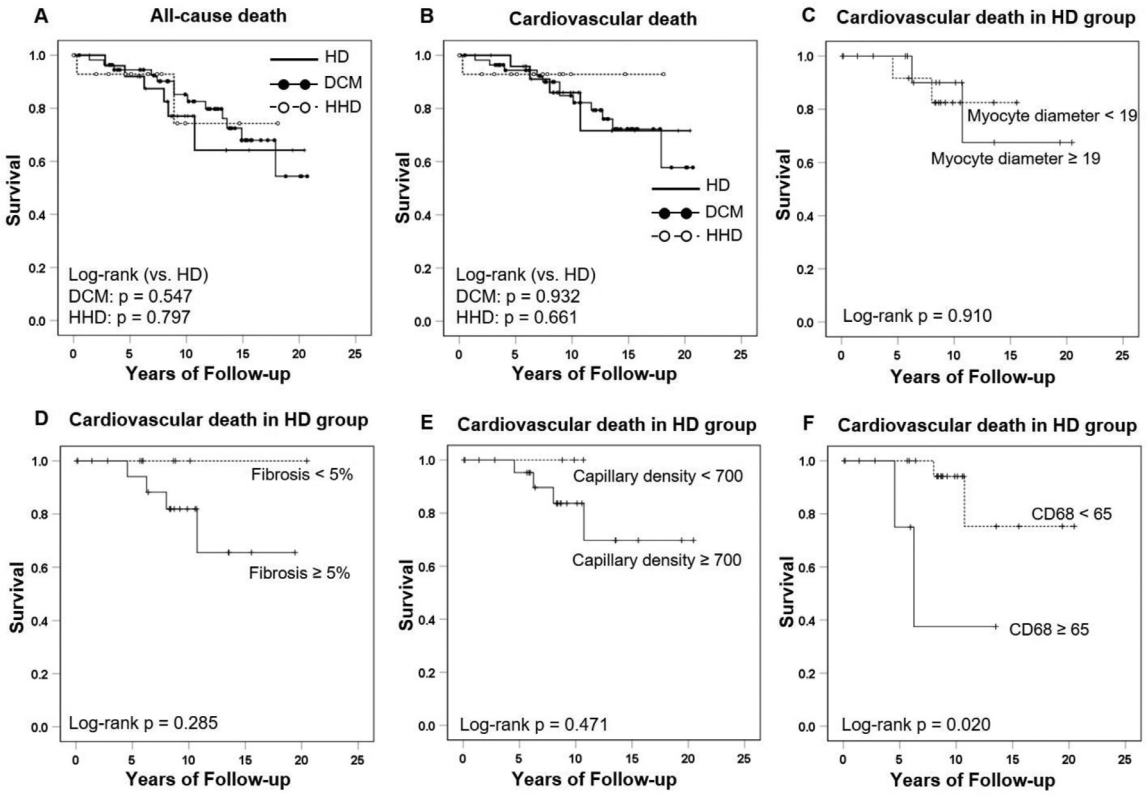

Fig. 2. Kaplan–Meier survival curves for all-cause death (A) and cardiovascular death and left ventricular assist device (LVAD) implantation (B). Kaplan–Meier survival curves for cardiovascular death and LVAD implantation in patients undergoing hemodialysis according to myocyte diameter (C), fibrosis rate (D), capillary density (E) and number of CD68-positive cells (F).

undergoing HD reflects the effects of various factors related to HD. Hypertrophic changes in patients undergoing HD have been reported to occur independent of blood pressure [8,18].

As histological findings related to the uremic state, reduction in capillary density and myocyte/capillary mismatch in the LV have been observed in experimental rat models of renal failure as well as in autopsy studies of uremic patients [15,19,20]. Therefore, we evaluated capillary density via immunohistochemical staining against CD34 and found that the capillary density in the RV of patients undergoing HD decreased compared with that of patients with DCM or HHD. Although the precise mechanism underlying microvascular remodeling in the uremic state remains unclear, the increased expression of vascular endothelial growth factor and the downregulation of its receptor have been demonstrated in uremic rats [20]. Notably, we found that reduced capillary density in the RV was related to the deterioration of LVEF in the HD group, which was not observed in other groups, suggesting that microvascular remodeling might be related to decreased oxygen supply and consequent cardiac dysfunction. Therefore, future studies should examine the histological changes in the LV to test this hypothesis.

Increased collagen fiber deposition in the interstitium has been observed in almost all cardiac disease etiologies. In patients undergoing HD, chronic volume overload caused by the arteriovenous shunt may represent a more common cause of fibrosis among the various etiological factors [21]. In the present study, the extent of fibrosis was comparable between the HD and DCM groups, which was in line with the findings of a study on EMB samples from the LV [12].

To the best of our knowledge, this is the first report showing that the number of CD68-positive macrophages exhibiting a shift to the M2 phenotype was higher in the EMB samples of the RV of patients undergoing HD than in those of the RV of patients with DCM or HHD. Infiltration of inflammatory cells and the onset of inflammation are related to cardiac remodeling and systolic dysfunction [22,23]. Experimental studies have demonstrated the pleiotropic effects of macrophages in cardiac remodeling, including the development of myocyte hypertrophy [24], initiation and resolution of fibrosis [25], promotion of wound healing after cryoinjury [26], and protection against myocardial dysfunction caused by hypertension [27].

Our findings regarding macrophages raise the question of whether macrophages are involved in cardiac remodeling in patients undergoing HD. To date, no study has focused on cardiac macrophages in patients undergoing HD except for one study, which reported increased macrophage infiltration of coronary plaques in patients undergoing HD [28]. However, HD can cause sustained systemic inflammation, leading to LV dysfunction and poor prognosis [29–31]. We found that the prognosis in the HD group exhibited a significant association with the number of CD68-positive cells. An increased CD68-positive cell count is associated with poor prognosis in patients with DCM; the same study proposed that immune activation may represent a potential mechanism of cardiac deterioration [22]. Although no study, to date, has assessed the correlation between macrophage infiltration and prognosis in patients undergoing HD, YKL-40, a novel marker of inflammation that is highly expressed in macrophages, was associated with all-cause and cardiovascular mortality in these patients [32]. Although the number of cardiovascular events in the HD group was relatively small in the present study (n=4), the potential effects of chronic inflammatory activation on myocardial dysfunction and prognosis in patients undergoing HD cannot be denied. M2 macrophages express transforming growth factor β and contribute to the development of cardiac fibrosis by acting as anti-inflammatory cells during tissue healing [33,34];

however, we did not observe any correlation between the number of macrophages and the extent of fibrosis. We speculate that the role of M2 macrophages in the cardiomyopathy of patients undergoing HD might be different from their roles during tissue healing following myocardial infarction.

Cardiac hypertrophy and fibrosis have been associated with poor prognosis, including sudden cardiac death of patients undergoing HD [12,35,36]. In contrast, our data showed that neither myocyte diameter nor the extent of fibrosis was associated with prognosis in any study groups. However, for both the DCM and HHD groups, myocyte hypertrophy was associated with the deterioration of EF, resulting in heart failure. In the present study, the cutoff value for fibrosis was 5%, which was lower than that of 30% used in a previous study [12], which may partially explain the differences in the findings between the two studies.

The limitations of the study include the small sample size and the retrospective design of the study, conducted in a single medical center, limiting the generalization of the study findings. The study is further limited by the small size of biopsy samples obtained from the endomyocardium and the limited biopsy sites, which did not allow us to examine the middle layer and the epicardial side of the heart wall. Third, we did not examine the LV histology because LV biopsies are not performed in our institution. Fourth, comorbidities, such as hypertension and diabetes, may affect myocardial pathology. Fifth, the clinical data on RV function were not available. To verify the study results, future studies should include a greater number of biopsy and autopsy samples. Comparison of the RV and LV histology will be informative as well.

5. Conclusions

The present study focused on comparing the histological features of RV biopsy samples of patients undergoing HD with those of samples obtained from patients with DCM or HHD to assess the clinical correlations between histological characteristics and clinical outcomes, which have not been reported to date. Despite the development of dialysis technologies, heart failure remains the leading mechanism of death in patients undergoing HD. Despite the small size of biopsy samples, we believe that EMB is an informative tool in elucidating the pathology underlying cardiomyopathy in these patients.

Coonflicts of Interest

The authors declare no conflicts of interest.

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