



Brief communication

Immunoglobulin free light chains as an inflammatory biomarker of heart failure with myocarditis



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ABSTRACT

Background: In this study, we measured immunoglobulin free light chains (FLC), a biomarker of inflammation in the sera of patients with heart failure due to myocarditis.

Methods: FLC kappa and FLC lambda were assayed in stored serum samples from patients with heart failure with myocarditis from the US myocarditis treatment trial by a competitive-inhibition multiplex Luminex[®] assay.

Results: The median concentration of circulating FLC kappa/lambda ratio was significantly lower in the sera from patients with heart failure with myocarditis than in healthy controls, and FLC kappa/lambda ratio had good diagnostic ability for identification of heart failure with myocarditis. Further, FLC kappa/lambda ratio was an independent prognostic factor for overall survival, and allowed creation of three prognostic groups by combining with N-terminal pro-B-type natriuretic peptide.

Conclusions: This study suggests that FLC kappa/lambda ratio is a promising biomarker of heart failure with myocarditis.

1. Introduction

Myocarditis contributes to the global burden of cardiovascular disease primarily through sudden death and dilated cardiomyopathy [1]. Previous studies have clarified the roles played by several cytokines in various cardiovascular disorders [2] and have found increased levels of circulating tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-6, and other proinflammatory cytokines in the patients with myocarditis, cardiomyopathy, heart failure, and acute myocardial infarction [3,4].

Nuclear factor-kappa B (NF- κ B) was originally identified as a family of transcription factors that bind the enhancer of the immunoglobulin kappa light chain gene. NF- κ B plays critical roles in the development, survival, and activation of B lymphocytes [5]. NF- κ B regulates the expression of a large number of genes that are involved in important physiological processes, including survival, inflammation, and immune responses [6].

We found that circulating immunoglobulin free light chains (FLC)

were increased in mice with heart failure due to viral myocarditis, and the increased expression of FLC was seen in mouse heart tissue as well [7]. As FLC could be a biomarker of activation of NF- κ B, inflammation, and immune responses, we measured FLC in the patients with heart failure with myocarditis.

Our goal was to test the hypothesis if there are any differences in concentrations of FLC between patients with heart failure and myocarditis and a healthy control group, and whether FLC concentrations are prognostic factors for overall survival.

2. Methods

2.1. Study design and participants

For heart failure patients with myocarditis group from the US Myocarditis Treatment Trial, the design and patient characteristics in the trial of immunosuppressive therapy for myocarditis have been

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Table 1
Comparison of FLC variables between patients of heart failure with myocarditis versus healthy controls.

FLC variable	Myocarditis (N = 111)	Controls (N = 75)	P-value of Mann-Whitney U test	ROC-AUC [95% CI]
	Median (interquartile range)			
Kappa (mg/L)	13.97 (11.81–18.34)	20.43 (16.97–23.74)	< 0.001	0.80 [0.73–0.86]
Lambda (mg/L)	10.42 (7.14–13.07)	8.63 (7.80–9.86)	0.033	0.59 [0.51–0.67]
Kappa/Lambda	1.45 (1.17–1.79)	2.26 (1.87–2.71)	< 0.001	0.87 [0.82–0.92]
Kappa + Lambda (mg/L)	24.07 (20.05–30.79)	29.31 (25.32–34.37)	< 0.001	0.68 [0.60–0.75]

described previously [8,9]. Briefly, consenting patients were enrolled in the trial if they had 1) suffered from heart failure of undetermined etiology for up to 2 years, and 2) a left ventricular ejection fraction < 45% by radionuclide left ventriculography. Frozen blood samples from all enrollees of the clinical diagnosis of myocarditis stored at -80°C were used for the investigations in this report. As the healthy control group, healthy volunteers in Japan were included.

2.2. FLC and other biomarkers

FLC kappa and lambda were assayed by a competitive-inhibition multiplex Luminex[®] assay that uses mouse monoclonals directed against light chain epitopes that are hidden when light chains are bound in whole antibody [10–13]. We have measured serum FLC in samples freeze thawed up to 3 times and seen no difference in levels. We have measured serum FLC levels in MRC Myeloma clinical trial samples stored from as early as the 1990s and seen no problems from storage.

2.3. FLC kappa/lambda ratio and the sum of kappa and lambda were calculated

C-reactive protein (CRP), N-terminal pro-B-type natriuretic peptide (NT-proBNP), troponin I, and troponin T were measured at Roche Diagnostics, Tokyo, Japan.

2.4. Ethics

The Institutional Review Boards of each enrolling medical center reviewed and approved the protocol of US Myocarditis Treatment Trial, and all study participants granted their consent to the investigative use of blood specimens.

2.5. Statistical analysis

Comparisons between the groups were made using the Mann-Whitney *U* test. The results were expressed as median and interquartile ranges. Receiver operating characteristic (ROC) curve analysis was performed to distinguish significant differences between patients with heart failure with myocarditis and healthy controls for the FLC biomarker. The area under the curve (AUC) for ROC (ROC-AUC) was calculated as the predictive performance measure of the biomarker. The optimal cutoff value was determined as that with the minimum chi-squared test p-value. The sensitivity, specificity, positive predictive value, and negative predictive value with an optimal cutoff value were calculated. The odds ratio and the 95% confidence interval (CI) were also calculated by logistic regression.

The associations between the biomarkers and overall survival were analyzed by the Cox proportional hazard models. The hazard ratio (HR), the 95% CI, and p-value (based on the Wald test) were calculated. The variables with significance in univariate analysis were selected as candidate predictive factors. Using multivariate analysis, the predictive factors among these candidates ($p < .05$) were determined by backward variable selection. Further, based on a conditional tree model analysis with the predictive factors as covariates, we classified survival

prognostic groups. Kaplan-Meier curves stratified by the groups were calculated, and a log-rank test was used for comparison among them.

All statistics were performed with the use of Stat Flex software version 6 (Artek, Osaka, Japan) and R 3.5.0 (R core team, Vienna, Austria). For all the analyses, a p-value of $< .05$ was considered to be statistically significant.

3. Results

3.1. Participants characteristics

The background characteristics of 111 patients of heart failure with myocarditis have been reported in the US Myocarditis Treatment Trial [8,9]. The age range of the 75 healthy control participants was 25 to 72 years (median 51, interquartile range 41–57). The diagnostic accuracy of FLC variables was evaluated between healthy controls and the patients with myocarditis. Among them, data of only 107 patients were available for the survival analysis. Twenty-seven patients (25.2%) died by the end of the study, and the 3-year and 5-year survival rates were 73.7% [95% confidence interval (CI): 65.1–83.6] and 70.5% [61.3–81.1], respectively.

3.2. Difference of FLC variables between myocarditis patients and healthy controls

The median concentration of circulating FLC kappa and the sum of kappa and lambda was significantly lower in the sera from patients with heart failure with myocarditis than in healthy volunteers ($p < .001$, Table 1). In contrast, FLC lambda was significantly higher ($p = .033$ and $p < .001$), and the kappa/lambda ratio was lower in heart failure patients with myocarditis than in healthy volunteers ($p < .001$, Table 1 and Fig. 1).

The area under the curve (ROC) analysis showed that FLC kappa/lambda ratio is a specific and sensitive biomarker of heart failure with myocarditis (Table 1). The AUC for ROC (ROC-AUC) of the FLC kappa/lambda ratio showed the largest AUC (0.87, 95% CI: 0.82–0.92) compared with other FLC variables. With an optimal cutoff value of 1.86 for the FLC kappa/lambda ratio, the diagnostic performance for distinguishing between myocarditis and healthy control were a sensitivity of 0.82, a specificity of 0.77, a positive predictive value of 0.74, and a negative predictive value of 0.84. The corresponding OR [95% CI] with > 1.86 as a reference was 15.5 [7.51–32.10].

3.3. Predictive performance of FLC variables for mortality

The Cox proportional hazard model was used to determine the association of FLC levels with mortality. Significant associations with mortality were observed for the levels of N-terminal pro-B-type natriuretic peptide (NT-proBNP), FLC lambda, and kappa/lambda ratio (Table 2). The elevated NT-proBNP and FLC lambda levels had significant hazard ratios (HR) and decreased FLC kappa/lambda ratio had an HR 0.31 (0.13–0.73, $p = .007$), indicating that the decrease of FLC kappa/lambda ratio was associated with mortality. Multivariable analysis showed that NT-proBNP and FLC kappa/lambda ratio were independent predictors of survival (Table 3). Further, based on the tree-

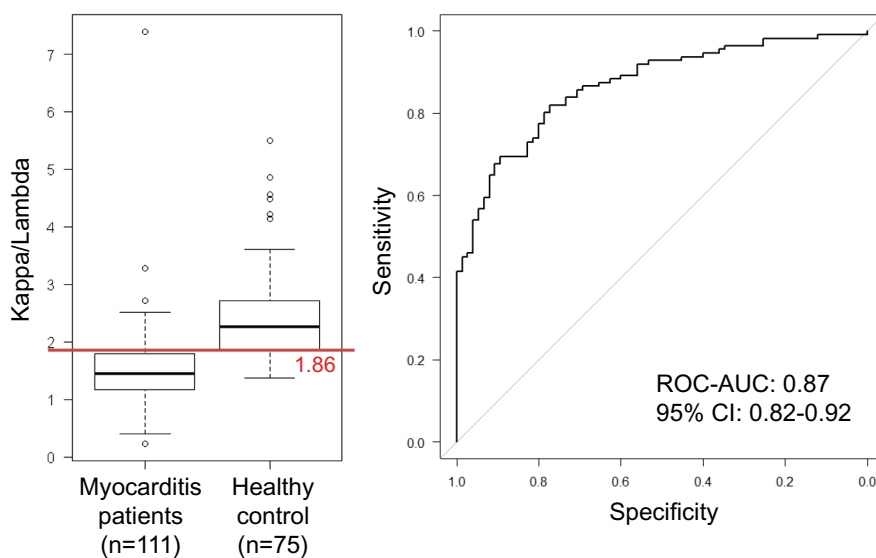


Fig. 1. Ability of immunoglobulin free light chains (FLC) kappa/lambda ratio to distinguish between patients with heart failure with myocarditis and healthy controls.

a: Boxplot for FLC kappa/lambda ratio in myocarditis patients with heart failure and healthy controls. The optimal cutoff with minimum p-value is 1.86. b: ROC-AUC (area under the receiver operating characteristic curve) indicated ability of FLC kappa/lambda ratio to distinguish between myocarditis patients with heart failure and healthy controls.

based analysis, we determined three prognostic groups (NT-proBNP \geq 3250 pg/mL; NT-proBNP < 3250 pg/mL and FLC kappa/lambda ratio \geq 1.5; and NT-proBNP < 3250 pg/mL and FLC kappa/lambda ratio < 1.5). These groups were distinguished according to the prognosis ($p < .001$, Fig. 2).

4. Discussion

Inflammation is increasingly recognized as an important factor in the pathogenesis and pathophysiology of heart failure and other cardiovascular disorders [2–4]. We have previously reported that circulating tumor necrosis factor (TNF)- α levels were increased in mice with heart failure due to viral myocarditis, and that pre-treatment with an anti-TNF- α antibody attenuated myocardial injury and decreased mortality in the acute stage [14]. In addition, our laboratory reported that in the same model, the intracardiac expression of cytokine genes was increased. The degree of their expression correlated with the severity of disease evolution [15].

There is increasing evidence that the inflammatory response is associated with an increase in the production of inflammatory mediators. Gene transcription is regulated by transcription factors that are sequence-specific DNA binding proteins capable of modulating the rate of transcription. One such transcription factor, nuclear factor-kappa B (NF- κ B) regulates the expression of a wide range of genes involved in immune and inflammatory responses, including FLC kappa. The expression of interleukin (IL)-1 β , TNF- α , and inducible nitric oxide synthase (iNOS) is regulated at the transcriptional level, and one key factor

Table 2
Univariate Cox regression analysis for overall survival in patients with heart failure with myocarditis.

Variable	Unit	Patients (N)	Univariate Cox regression for OS			
			Hazard ratio (95% CI)	P-value	After log ₁₀ transformation	
					Hazard ratio (95% CI)	P-value
Troponin-I	1	100	4.81 (0.02–1392)	0.587	1.55 (0.66–3.66)	0.318
Troponin-T	1	95	1.88 (0.28–12.79)	0.519	1.54 (0.77–3.07)	0.225
NT-proBNP	1000	100	1.12 (1.06–1.19)	< 0.001	2.75 (1.36–5.54)	0.005
hsCRP	1	96	0.96 (0.71–1.29)	0.775	0.97 (0.47–1.98)	0.924
Kappa	1	107	0.98 (0.93–1.04)	0.469	0.28 (0.03–2.72)	0.272
lambda	1	107	1.03 (1.01–1.05)	0.011	4.29 (0.77–23.90)	0.096
Kappa/Lambda	1	107	0.31 (0.13–0.73)	0.007	0.05 (0.01–0.313)	0.002
Kappa + Lambda	1	107	1.01 (0.99–1.02)	0.452	1.23 (0.15–10.02)	0.845

Table 3
Multivariate Cox regression analysis for overall survival.

Variable	Unit	Multivariate Cox regression for OS (n = 100)	
		Hazard ratio (95% CI)	P-value
NT-proBNP	1000	1.10 (1.04–1.17)	0.002
Kappa/Lambda	1	0.43 (0.19–1.01)	0.051
Variable	Unit	After log ₁₀ transformation (N = 100)	
		Hazard ratio (95% CI)	P-value
Log ₁₀ (NT-proBNP)	1	0.10 (0.01–0.74)	0.024
Log ₁₀ (Kappa/Lambda)	1	2.24 (1.11–4.52)	0.025

in this process is NF- κ B activation [16]. NF- κ B is activated by several factors which increase the inflammatory response, including viral infections, oxidants, and antigens. This activation, in turn, leads to the coordinated expression of several protein-encoding genes, such as cytokines, chemokines, adhesion molecules, and enzymes involved in mediator synthesis, and the further amplification and perpetuation of the inflammatory response.

In our previous study of a murine model of heart failure due to viral myocarditis, FLC kappa was increased, but lambda was lower. Lack of agreement between the viral myocarditis model in mice and the present study in humans may reflect species differences and also variations in the disease pathologies in each.

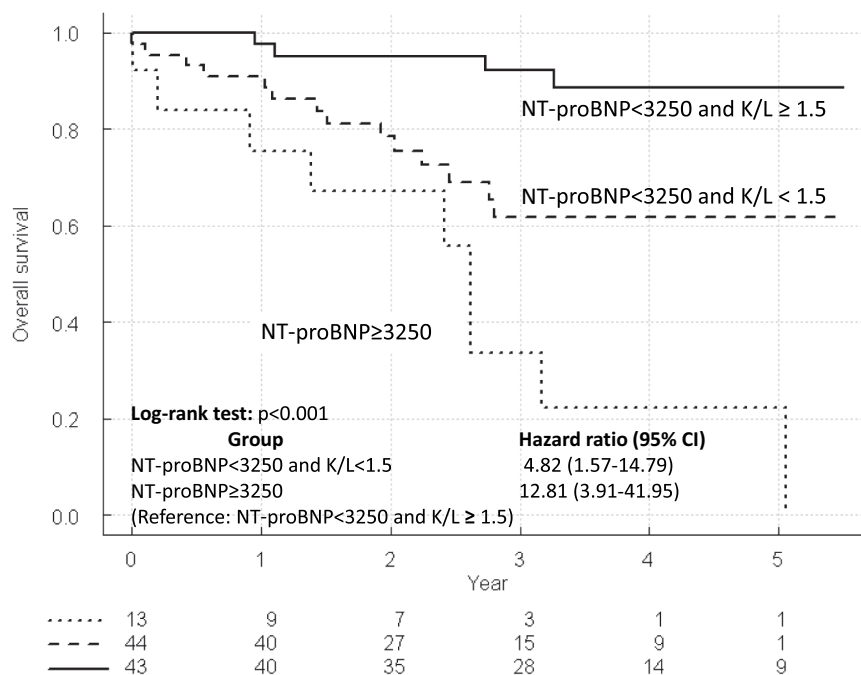


Fig. 2. Prognostic classification by FLC kappa/lambda and NT-proBNP (pg/mL). Kaplan-Meier curve for overall survival was stratified by three groups with NT-proBNP \geq 3250, NT-proBNP < 3250 and K/L \geq 1.5 and, NT-proBNP < 3250 and K/L < 1.5.

NF- κ B was activated in this model, and an NF- κ B inhibitor lowered the mortality of the animals, as well as attenuated myocardial necrosis and cellular infiltration, and decreased the intracardiac production of IL-1 and TNF- α [17]. Therefore, it is possible that the production of FLC may be modulated in viral myocarditis.

The sum of FLC kappa and lambda has been shown to be a significant predictor of worse overall survival in a general population of individuals who do not have any other known plasma cell disorder. However, the increased risk of death was not restricted to any particular cause of death, which suggests that the data may add to the growing body of literature that connects inflammation, aging, and chronic disease [18].

Jackson and his co-workers reported that the patients with heart failure had an elevated sum of FLC kappa and lambda concentration and that the patients with an elevated sum of FLC concentrations were at increased risk of death. The elevated sum of FLC concentration remained an independent predictor of mortality [19]. Elevated kappa and lambda FLC levels can result from renal impairment with reduced glomerular clearance of FLC, by generalized immune system activation and increased immunoglobulin secretion or a more specific inflammatory response, as seen in viral myocarditis [7].

In this study, circulating FLC kappa and the sum of kappa and lambda were significantly lower, and in contrast, FLC lambda were significantly higher, and the kappa/lambda ratio was lower in heart failure patients with myocarditis than in healthy volunteers. The contrasting reduction in kappa light chain levels in our patients compared to elevated kappa FLC levels in Jackson's heart failure patients may partly reflect the different FLC assays used. In our study the FLC kappa/lambda ratio was more specific and sensitive than the sum of kappa and lambda for the diagnosis of heart failure with myocarditis and was an independent predictor of mortality of heart failure with myocarditis, but the sum of kappa and lambda was not. Nonetheless, given the apparent contradictory observations made between the study of Jackson et al. and our study, the readers are encouraged to cautiously interpret these datasets as to the clinical significance of measurement of FLC in heart failure patients until large datasets become available.

These findings suggest that the clones of B lymphocytes and plasma

cells which produce FLC lambda may specifically be activated in heart failure with myocarditis. In B cell progenitors tolerance to self-antigens can be achieved by replacing autoreactive heavy (H) chain and light (L) chain V genes with V genes coding for nonself receptors. This receptor editing at the lambda chain locus may predispose to the development of autoimmunity through multi-reactivity, allelic inclusion, and lambda light chain secretion [20]. The mechanism why FLC lambda is specifically activated is remains to be clarified.

NF- κ B could be a target for new types of anti-inflammatory treatment for heart failure with myocarditis when FLCs are elevated, and FLC could be a surrogate endpoint of mortality from heart failure with myocarditis. Further study is necessary to clarify the role of FLC in heart failure of other etiologies.

Disclosure

None.

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