

Changes in serum cardiac myosin light chain 1 levels in children with fulminant myocarditis during continuous blood purification

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SUMMARY

Objective: To investigate the changes in serum cardiac myosin light chain 1 (CMLC-1) levels in children with fulminant myocarditis (FM) during continuous blood purification (CBP), as well as to analyze its correlation with other laboratory indexes.

Method: Twenty-four (24) children with FM who underwent CBP were enrolled. Before and during treatment (48 and 72 hours after treatment, or death), the optical density value of serum CMLC-1 was measured using enzyme-linked immunosorbent assay, and then the serum CMLC-1 concentration was calculated. The correlations between CMLC-1 OD value change and laboratory indexes including creatine kinase-MB (CK-MB), troponin, myohemoglobin and N-terminal pro-brain natriuretic peptide (NT-proBNP) were analyzed.

Results: The serum CMLC-1 concentration significantly increased in the children with FM and decreased obviously during CBP therapy. In the same period, the change of CMLC-1 concentration were positively correlated with creatine kinase-MB ($r=0.528$), troponin ($r=0.726$), myohemoglobin ($r=0.702$), and NT-proBNP levels ($r=0.589$).

Conclusion: The serum CMLC-1 concentration increases significantly in children with FM, but CBP therapy can effectively control this increase.

Keywords: myocarditis, child, myosins/immunology/metabolism, hemofiltration.

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INTRODUCTION

Currently, fulminant myocarditis (FM) is a critical disease that causes death in children and lacks sufficient specificity for early diagnosis.¹⁻³ The pathogenesis of FM is generally believed⁴ to involve infiltration of a large number of inflammatory cells and destruction of myocardial cells in the early stages. Then, allergic reaction or autoimmune involvement can lead to further damage to cardiac muscle cells, resulting in localized or diffuse myocarditis.⁵⁻⁷ The pathogenesis of anti-cardiac antibodies in post-viral autoimmune cases may start with direct viral-induced myocyte damage, with associated release of intracellular proteins.

Autoantibodies potentially pathogenic to various cellular components are found in a high percentage of patients with myocarditis. Autoantigens include alpha and beta cardiac myosins. The damage caused by the release of cardiac myosin was included in the course of autoimmune injury. It has been successfully used in the domestic and

foreign application of cardiac myosin for creating a model of autoimmune myocarditis on which to apply treatment interventions.^{8,9} This shows that cardiac myosin plays an important role in the damage process. The composition of cardiac myosin includes two heavy chains and two light chains. One essential isoform of myosin light chain is called cardiac myosin light chain (CMLC-1). In recent years, sustained release of CMLC-1 can be detected in patients with myocardial injury. This has aroused our interest; thus, in this study, we focused on CMLC-1.

We believe that at an early stage, decreasing the concentration of CMLC-1 can decrease the extent of immune damage. So we thought of physical method: CMLC-1 belongs to the category of middle-sized molecular substances (molecular weight, 22-27 kD), which can be effectively removed through hemofiltration during CVVH or CVVHDF (CVVHDF/CVVH is the standard mode of CBP for children). Therefore, we measured and analyzed

the serum CMLC-1 level in children with FM before and after CBP treatment. In addition, we investigated the therapeutic effect of CBP on CMLC-1 and found its importance in the diagnosis and treatment of FM in children.

METHOD

Clinical data

The children (n=24) were recruited from a study conducted by the Department of Pediatric Intensive Care Unit, The First Hospital of Jilin University, during the time period of June 2012 to December 2014. The diagnostic criteria used were based on details in previous literature.¹ Children with congenital cardiovascular malformations were excluded.

Our study was conducted in accordance with the declaration of Helsinki, after approval from the Ethics Committee of Jilin University. Written informed consent was obtained from all participants' guardians.

Material preparation and experiments

All of the children were treated with CBP.¹⁰ We select CVVHDF/CVVH as the treatment mode since it is the standard mode of CBP for children.¹¹ Fresenius polysulfone membrane transfusion filters (Fresenius Medical Care AG, Frankfurt, Germany) were used in the continuous veno-venous hemodiafiltration/continuous veno-venous hemofiltration (CVVHDF/CVVH) mode. Then the filters and mode were selected according to the patient's weight, as shown in Table 1. Blood samples (3 mL) were collected in coagulation sterile tubes at the initial stage of hospitalization and during CBP treatment (48 and 72 h after treatment, or death).

TABLE 1 Filter and mode selection for different weight ranges.

Weight	Filter	Mode
<3 kg (infants)	AV pead	CWH
3-20 kg	AV 400 s	CWH
>20 kg	AV 600 s	CWH/CVHDF

CWH: continuous veno-venous hemodiafiltration; CVWH/CVHDF: continuous veno-venous hemodiafiltration/continuous veno-venous hemofiltration.

The collected samples were coagulated at room temperature for 10-20 min and then ultracentrifuged (American Backman L8-80M) for 10 minutes at the speed of 3,000 rpm. The supernatant was labeled after centrifugation, and the samples were maintained at a temperature of -70°C. When all specimens were collected completely, the samples were thawed at 37°C. The optical density (OD) value of serum CMLC-1 was measured using enzyme-

linked immunosorbent assay (ELISA; Human CCMLC-1 ELISA Kit, Shanghai Wan Jiang Bio Technology Co., Ltd.), and then the serum CMLC-1 concentration was calculated.

The correlations between CMLC-1 OD value change and laboratory indexes including creatine kinase-MB (CK-MB), troponin, myohemoglobin, and N-terminal pro-brain natriuretic peptide (NT-proBNP) were analyzed.

Result determination

We placed the OD value on the horizontal axis and standard density on the vertical axis, a standard curve was drawn ($y = 9.8584x^3 - 21.182x^2 + 41.098x, t = 0.99931$) on a graph paper (Figure 1). The OD values were calculated by using the aforementioned formula, where a higher value indicates positivity.

Statistical analysis

All data were compared using SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). All normal distribution data were expressed as mean±SD. A paired t-test was used to compare data. Correlation analysis was performed by using the Pearson (normality data) or Spearman correlation coefficient (non-normality data). The significance level was set at < 0.05 for all the tests.

RESULTS

Twenty-four (24) children with FM were enrolled in the study. Length of stay following initiation of treatment ranged from 13 hours to 5 days. Twenty (20) children with FM survived (improvements were seen during treatment in heart rate, respiration, CVP) and four patients died, yielding a mortality rate of 16.67%. Because of the small number of cases, death had no statistical significance. Our purpose is to explore the value of serum CMLC-1 level during CBP therapy.

Change in serum CMLC-1 level during the CBP therapy

During early hospitalization, serum CMLC-1 OD was measured in all children with FM, and then the substitution curve formula was derived. The CMLC-1 OD were all above the standard curve. It means that the serum CMLC-1 concentration significantly increased in these children. In the early stage of admission, the CMLC-1 OD value was comparable between the children who died and those who survived, but because of the limited number of cases, the difference was not statistically significant.

We recorded the serum CMLC-1 OD values before and during treatment (48 hours after treatment, 72 hours after treatment or death). In terms of survival, the CMLC-1 OD value obviously decreased during CBP therapy but

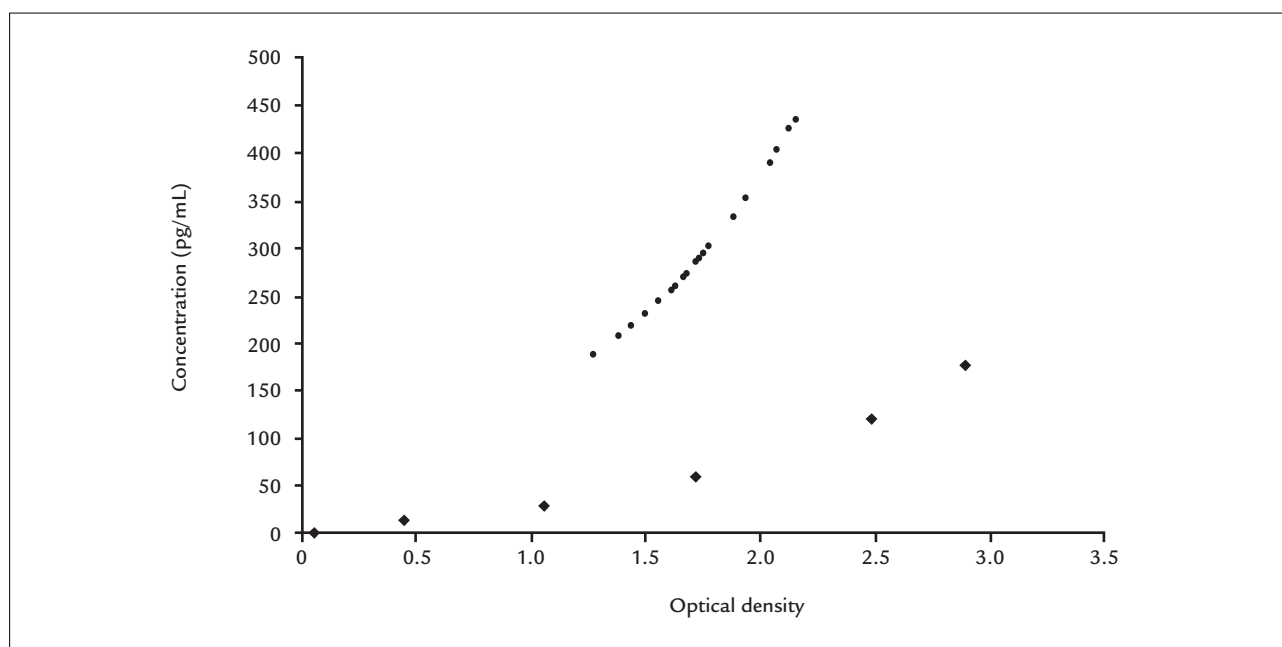


FIGURE 1 CMLC-1 optical density value at early admission.

especially at 48 hours after treatment ($t=19.58$, $p<0.01$; Table 2). This can be observed in Figure 2.

TABLE 2 Change in CMLC-1 OD values in surviving patients after treatment for up to 48 h.

Time	CMLC-1 OD value
Before	1.717±0.25
48 h later	1.309±0.27
t	19.58
p	<0.01

In terms of death, because of the small number of deaths, the difference in the time of death was relatively larger (8-192 hours), so the CMLC-1 OD values of the patients who died were not statistically significant.

Concentration changes within the same period of correlation with laboratory results

In the same period, the change of CMLC-1 concentration were positively correlated with creatine kinase-MB ($r=0.528$), troponin ($r=0.726$), myohemoglobin ($r=0.702$), and NT-proBNP levels ($r=0.589$; Figure 3).

DISCUSSION

Systole-diastole is a complex physiological process that is affected by many factors. With the advances in molecular biology, people have a deeper understanding of

this process at a molecular level and increased awareness of contractile and regulatory proteins. In the present study, we found that myosin is a type of contractile protein. Cardiac myosin is located within the sarcomeres, and it is the main unit of the myofibrillar thick wire, along with ATP enzyme activity. It plays an important role in the regulation of myocardial contractility.^{12,13} Its composition includes two heavy chains (molecular weight, about 200 kDa) and two light chains (molecular weight, 16-27 kDa).¹⁴ The light chains are divided into the basic essential myosin light chain and the phosphorylation regulation light chain. The ventricular muscle consists of an essential myosin light chain isoform called cardiac myosin light chain (CMLC-1).

Normal human serum is free of cardiac myosin.¹⁵ In patients with myocardial edema caused by various factors such as necrosis, apoptosis and cardiac myosin in the protease decomposition, high levels of CMLC-1 are released into the blood through damaged membranes.¹⁶ As a cardiac autoantigen, CMLA-1 antigen presents cells to generate anti-cardiac myosin antibody (AMA),¹⁷ leading to further immune damage.

Myocardial damage is the result of an autoimmune reaction.¹⁸ Cardiac myosin release has now been considered as a cause of secondary autoimmune injury.⁸ Neu et al.¹⁹ found that cardiac myosin-immunized mice had greater genetic predisposition for autoimmune myocarditis and that cardiac myosin can induce autoimmune myocarditis.

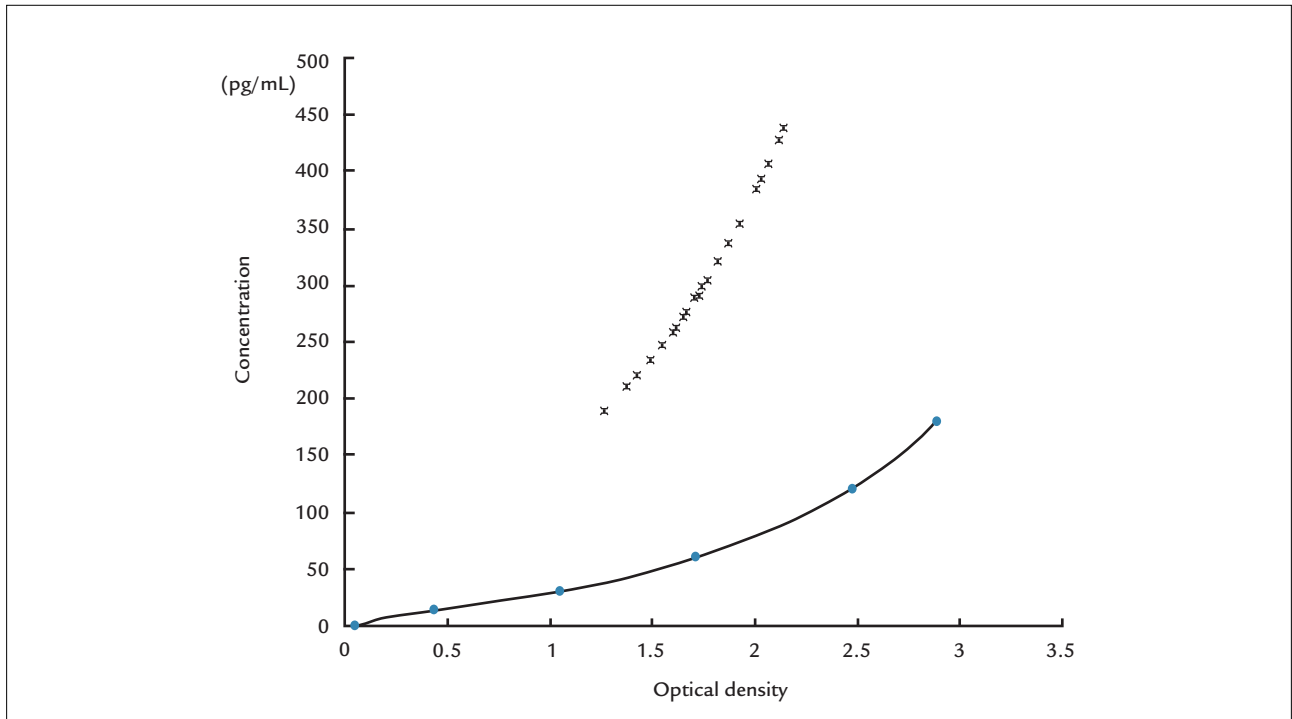


FIGURE 2 Line diagram of CMLC-1 optical density value in survival.

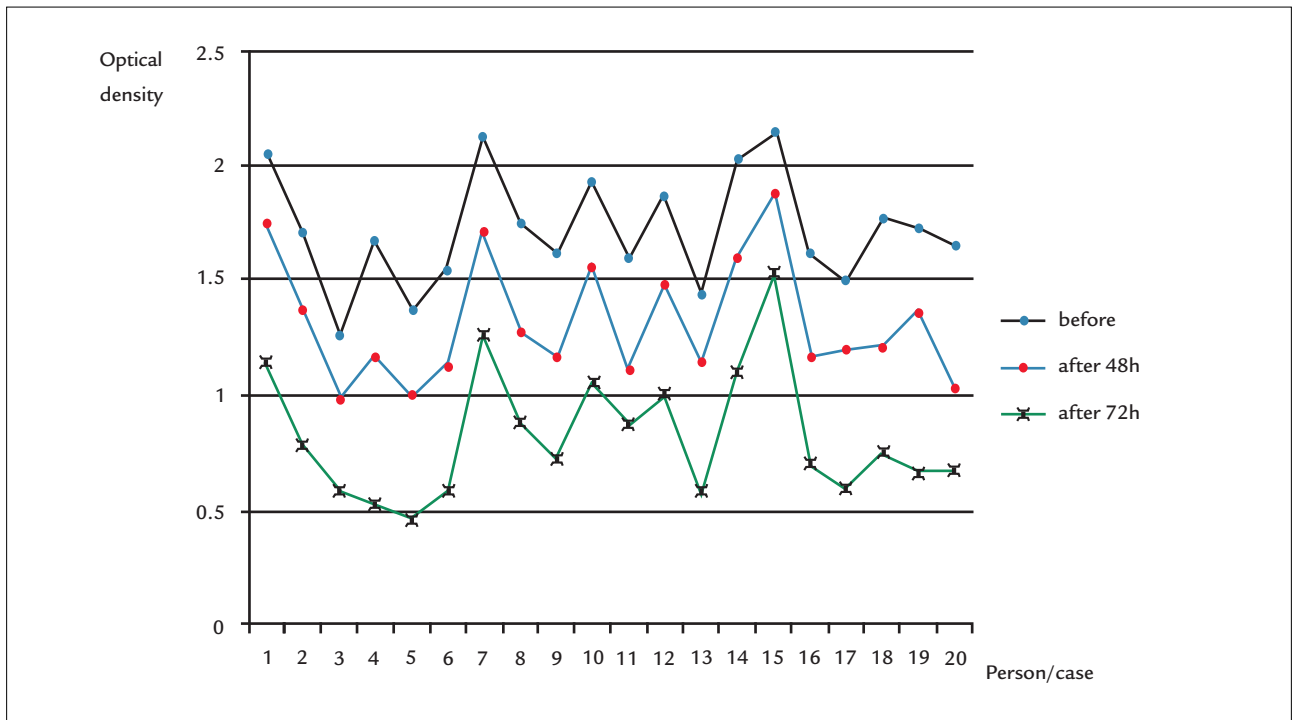


FIGURE 3 CMLC-1 associated with myocardial injury earlier laboratory results.

Considered in the absence of virus and other pathogens of infection injury cases, a simple cardiac myosin can lead to the occurrence of myocarditis. The degree and extent of the myocardial damage were positively correlated. Therefore, we believe the concentrations reflect the degree of myocardial injury and thus can be used as a method to measure the area of myocardial injury.

CMLC-1 belongs to the category of middle-sized molecular substances, which can be effectively removed through hemofiltration during CBP treatment. The CMLC-1 analysis in this study revealed that during CBP therapy, serum CMLC-1 level decreased significantly, particularly 48 hours after treatment ($p < 0.01$). CBP treatment can remove inflammatory mediators, reduce the continuing destruction of myocardial cells, and thereby reduce CMLC-1 release. Reducing antigen presentation and autoimmune damage can effectively remove CMLC-1 from the blood.

In the past, we applied cardiac biomarker elevations (e.g., cardiac troponin and B-type natriuretic peptide hormone [BNP] levels) to reflect myocardial injury and found a prognostic significance.²⁰⁻²² However, these tests cannot be used to establish a diagnosis of myocarditis, as they lack sufficient sensitivity and specificity. Thus, in this study, we focused on the significance of serum CMLC-1 levels in the treatment of children with FM. By monitoring changes in serum CMLC-1, the degree of recovery of myocardial injury and the treatment effects were evaluated. During the early stage of admission, CMLC-1 levels were significantly increased. Moreover, the rate of disease progression, severity of clinical manifestations and CMLC-1 levels positively correlated. The more serious the cardiac injury, the worse the cardiac function, whereas the higher the serum CMLC-1 level in the initial stage, the higher the risk of death.

After CBP treatment, the children showed signs of improvement and their serum CMLC-1 levels decreased gradually and showed positive correlation with laboratory test results. Thus, we considered that serum CMLC-1 may be used as a biological marker to detect the occurrence of diseases such as myocardial infarction or myocarditis, which may have important applications.

However, with the few cases of children with FM in this study and the large difference in the time course of the disease before admission, the correlation between the change in CMLC-1 concentration and deaths was not statistically significant. Further study on the course of treatment is needed.

CONCLUSION

In summary, our study results indicate that the immune system has a great influence on the development of myo-

cardial injury. CMLC-1 levels were significantly increased in the early stage of myocardial injury. CBP treatment effectively decreased these levels and reduced the extent of sustained myocardial damage.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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