Clinical Study

Can Serum Tenascin-C Be Used as a Marker of Inflammation in Patients with Dilated Cardiomyopathy?

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Received 23 May 2013; Revised 31 July 2013; Accepted 3 August 2013

Academic Editor: Lavjay Butani

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Background. Tenascin-C (TN-C) is an extracellular matrix glycoprotein that appears at sites of inflammation in cardiac pathologies.

Aim of the Work. To evaluate the role of TN-C as a marker for active inflammation in children with dilated cardiomyopathy (DCM).

Subjects and Methods. 24 consecutive patients with primary nonfamilial DCM aged 6–72 months (mean 45.19 ± 11.03) were divided into group I, twelve patients with acute onset DCM (<6 months duration), and group II, twelve patients with chronic DCM (>6 months duration), and compared to 20 healthy age- and sex-matched controls. Investigations included estimation of serum TN-C and echocardiographie evaluation using M-mode and 2D speckle tracking echocardiography (STE).

Results. Serum TN-C showed a higher significant statistical elevation among patients than controls (P < 0.001) and in group I than group II (P < 0.001). EF was significantly decreased, and LVEDD and EDV increased in patients than controls and in GI than GII. STE showed a statistically significant difference in global peak strain longitudinal (GPSL) average in patients than controls (P < 0.05) and between GI and GII (P < 0.001). STE wall motion scoring showed normokinesia (33.5%), hypokinesia (8.33%), and akinesia (50%) in GI and hypokinesia (100%) in GII. There was a statistically significant positive correlation between serum TN-C and GPSL average.

Conclusions. Increased serum TN-C can be used as a marker of inflammation in DCM and is associated with the severity of heart failure and LV dysfunction as detected by STE.

1. Introduction

Myocardial inflammatory diseases are an important cause of dilated cardiomyopathy (DCM) in children. Epidemics of viral myocarditis have been reported, particularly Coxsackie B virus, and the enteroviruses which are considered to be the most common cause of viral myocarditis [1]. Thus infants and young children may be more prone to develop myocarditis, due to the higher rate of entero viral and adenoviral infections in this age group [2]. Although myocarditis has previously been speculated to account for most instances of DCM, it is now clear that 20–30% may be due to familial or genetic forms of DCM [3]; yet myocarditis might still be implicated in the initiation of the process.

There are few biomarkers for myocarditis. Full blood count and erythrocytic sedimentation rate are not usually helpful. Troponin I has a high specificity for diagnosing myocarditis but a sensitivity of only 34%, and creatine kinase and its cardiac isofrom CK-MB are less sensitive and specific than troponin [4]. Increased levels of autoantibodies against myocardial proteins have also been reported [5].

Tenascin-C (TN-C) is an extracellular matrix glycoprotein [6] that is not expressed in normal adult hearts but is expressed in various myocardial diseases such as acute myocarditis [7–9], dilated cardiomyopathy [10], myocardial infarction [11], and myocardial hibernation [12].

Speckle tracking echocardiography (STE) is a noninvasive ultrasound imaging technique that allows for an objective and quantitative evaluation of global and regional myocardial function independently from the angle of insonation and from cardiac translational movements [13]. Its advantage over conventional echo warrants its use in the assessment of the global and regional myocardial performance in acute and chronic DCM.

We hypothesized that serum TN-C might serve as a marker of active myocardial inflammation in children with new onset DCM.
2. Subjects and Methods

This case controlled study was conducted in the Pediatric Cardiology Clinic and Echocardiography Laboratory, Children's Hospital, Ain Shams University. The study included 24 consecutive patients with DCM aged 6–72 months with a mean age of 45.19±11.03 months and 20 healthy children. The subjects were divided into 3 groups: group I included twelve patients with acute DCM of less than 6 months duration; group II included twelve patients with chronic DCM for more than 6 months; control group included twenty (20) healthy age- and sex-matched children. The control group was chosen from healthy children who came with their sick siblings to the outpatient clinic of the children's hospital.

Clinical diagnosis of DCM was based on the WHO [14] and American Heart Association [15] criteria. At time of diagnosis all patients had an ejection fraction <25% and/or a fractional shortening of <25% and a left ventricular end diastolic dimension (LVEDD) of >112% of the predicted value corrected for age and body surface area [14]. All patients received antifailure measures in the form of inotropes (lanoxin), diuretics (furosemide and spironolactone), and angiotensin enzyme inhibitor (captopril). Patients with familial, genetic, or secondary cardiomyopathy were excluded. A written informed consent was given by parents of patients and controls, and the study protocol was approved by the institutional review board.

Methods. All studied patients were subjected to full medical history, thorough clinical examination, and investigations that included the following:

(i) Laboratory. Serum TN-C was measured. Serum levels of TN-C with the large subunit containing the C domain of FITIII repeats were determined using an ELISA technique with 2 monoclonal antibodies, 19C4MS and 4F10TT (IBL, Gunma, Japan) [16].

(ii) Echocardiography. Echocardiography was done. M-mode, two dimensional, continuous, pulsed colors Doppler and Speckle track echocardiography (STE) transthoracic echocardiography was done using device model GE medical system VIVID7 dimension N-3190, Horten, Norway. STE was performed according to the methods recommended by the American Society of Echocardiography [17]. Longitudinal strain (LS) was assessed in standard four-chamber, three-chamber (apical long-axis), and two-chamber apical views. The images were obtained at end-expiratory phase using a 5 MHz center frequency phased-array probe with second-harmonic imaging. The settings were configured to obtain optimal quality images. Frame rates were kept between 60 and 90 frames/sec. They were stored in cine loop format for offline analysis by vendor customized software (Echo PAC PC-2D Strain; GE Medical Systems). LS was measured in 16 LV segments by tracing the endocardial contour on an end-systolic frame that allowed the software to automatically place the contour on subsequent frames by temporally tracking the “natural acoustic speckle” in the B-mode images. Full thickness of myocardium from endocardial to epicardial borders was covered. Adequate tracking for the study was verified in real time, and in segments with poor tracking, the endocardial trace line was readjusted until a better tracking score was achieved. A suboptimally tracked segment was excluded from further analysis. LS curves reflected the average value of all of the acoustic markers in each segment [17].

(iii) Statistical Analysis. Data were expressed as means ± SD for continuous variables and as numbers (percentages) for categorical variables. Continuous variables were analyzed by the unpaired Student's t-test. Pearson's or Spearman's correlation analysis was performed to estimate correlations between variables. A P value <0.05 was considered statistically significant.

3. Results

The levels of TN-C of all patients (groups I and II) and controls are shown in Tables 1 and 2.

M-mode echocardiographic data of the patients in comparison to the control group are shown in Table 3 and those of group I in comparison with group II are shown in Table 4. 2D STE showed that there was a highly statistical significant difference between patients and controls as regard global peak strain longitudinal apical long axis (G SL ap lax), G peak SL apical 4 chambers (a4c), G peak SL apical 2 chambers (a2c), and G peak SL average. STE of groups I and II is shown in Table 4. STE wall motion score was normokinetic in all controls while data of groups I and II are shown in Table 3. Correlation between serum TN-C level and the echocardiographic findings showed a statistically significant positive correlation between serum TN-C level and LVEDD and enddiastolic volume in patients (Figure 2) and in acute cases.

| Tenascin-C (ng/mL) | DCM patients (groups I and II) | Controls |
|-------------------|-------------------------------|----------|
| Range             | 18–90                         | 4.5–12.5 |
| Mean              | 47.45                         | 6.50     |
| ±SD               | 15.89                         | 1.67     |
| t-test            | 11.472                        |          |
| P value           | <0.001 highly significant     |          |

| Tenascin-C (ng/mL) | Group I | Group II |
|-------------------|---------|----------|
| Range             | 42.5–90 | 18–47.5  |
| Mean              | 59.25   | 35.83    |
| ±SD               | 11.82   | 9.45     |
| t-test            | 5.358   |          |
| P value           | <0.001 highly significant     |          |
Table 3: M-mode echocardiographic data of patients compared to controls.

| M-mode             | N  | Mean ± SD | t-test | P value |
|--------------------|----|-----------|--------|---------|
| LVIDD (cm)         |    |           |        |         |
| Patients           | 24 | 3.84 ± 1.18 | 4.860  | 0.001** |
| Control            | 20 | 2.52 ± 0.29  |        |         |
| LVIDS (cm)         |    |           |        |         |
| Patients           | 24 | 3.13 ± 1.24  | 4.223  | 0.001** |
| Control            | 20 | 1.95 ± 0.06  |        |         |
| EDV (mL)           |    |           |        |         |
| Patients           | 24 | 79.16 ± 55.25 | 3.645  | <0.05*  |
| Control            | 20 | 33.90 ± 2.26 |        |         |
| ESV (mL)           |    |           |        |         |
| Patients           | 24 | 50.79 ± 42.29 | 3.806  | 0.001** |
| Control            | 20 | 14.70 ± 1.78 |        |         |
| EF%                |    |           |        |         |
| Patients           | 24 | 45 ± 17.46 | 4.385  | 0.001** |
| Control            | 20 | 62.25 ± 2.44 |        |         |
| FS%                |    |           |        |         |
| Patients           | 24 | 22.91 ± 10.72 | 3.855  | 0.001** |
| Control            | 20 | 32.25 ± 1.40 |        |         |
| LA/AO              |    |           |        |         |
| Patients           | 24 | 1.78 ± 0.88  | 2.006  | >0.05   |
| Control            | 20 | 0.88 ± 0.30  |        |         |

**Highly significant, * Significant. LVIDD: left ventricular internal diameter in diastole, LVIDS: left ventricular internal diameter in systole, EF: ejection fraction, FS: fraction shortening, AO: aortic annulus, LA: left atrium, EDV: end-diastolic volume, ESV: end-systolic volume, LA: left atrium, and AO: aorta.

Table 4: M-mode echocardiographic data of group I compared to group II.

| M-mode             | N  | Mean ± SD | t-test | P value |
|--------------------|----|-----------|--------|---------|
| LVIDD (cm)         |    |           |        |         |
| Group I            | 12 | 4.62 ± 0.91 | 4.264  | 0.001** |
| Group II           | 12 | 3.06 ± 0.87 |        |         |
| LVIDS (cm)         |    |           |        |         |
| Group I            | 12 | 4.10 ± 0.79 | 5.997  | 0.001** |
| Group II           | 12 | 2.17 ± 0.77 |        |         |
| EDV (mL)           |    |           |        |         |
| Group I            | 12 | 118.6 ± 40.5 | 5.013  | 0.001** |
| Group II           | 12 | 39.66 ± 36.5 |        |         |
| ESV (mL)           |    |           |        |         |
| Group I            | 12 | 84.41 ± 30.9 | 6.528  | 0.001** |
| Group II           | 12 | 17.16 ± 17.8 |        |         |
| EF%                |    |           |        |         |
| Group I            | 12 | 31.66 ± 9.14 | 5.841  | 0.001** |
| Group II           | 12 | 58.33 ± 12.90 |       |         |
| FS%                |    |           |        |         |
| Group I            | 12 | 14.75 ± 4.18 | 5.799  | 0.001** |
| Group II           | 12 | 31.08 ± 8.81 |        |         |
| LA/AO              |    |           |        |         |
| Group I            | 12 | 2.20 ± 1.08  | 2.558  | <0.05*  |
| Group II           | 12 | 0.88 ± 0.26  |        |         |

**Highly significant, * Significant. LVIDD: left ventricular internal diameter in diastole, LVIDS: left ventricular internal diameter in systole, EF: ejection fraction, FS: fraction shortening, AO: aortic annulus, LA: left atrium, EDV: end-diastolic volume, ESV: end-systolic volume, LA: left atrium, and AO: aorta.

Table 5: G-peak sl ap lax, G peak sl a2c, G peak sl a4c, and G peak sl avg in group I and group II.

| Tissue            | N  | Range   | Mean ± SD | t-test | P value |
|-------------------|----|---------|-----------|--------|---------|
| G peak sl ap lax  |    | −7—20   | −12.08 ± 5.36 | 4.922  | 0.001** |
| Group I           | 12 | −16—24  | −20.50 ± 2.50 |        |         |
| Group II          | 12 |         |           |        |         |
| G peak sl a4c     |    | −4—20   | −11.91 ± 5.35 | 2.042  | <0.05*  |
| Group I           | 12 | −10—23  | −15.41 ± 2.574 |       |         |
| Group II          | 12 |         |           |        |         |
| G peak sl a2c     |    | −9—19   | −14 ± 6.53  | 4.227  | 0.001** |
| Group I           | 12 | −15—25  | −24.2 ± 5.27 |        |         |
| Group II          | 12 |         |           |        |         |
| Peak sl avg       |    | −6—19   | −12.25 ± 5.62 | 2.585  | <0.05*  |
| Group I           | 12 | −18—24  | −18.41 ± 6.05 |        |         |
| Group II          | 12 |         |           |        |         |

**Highly significant, * Significant.
Correlation between serum TN-C level and patients, group I and group II, is shown in Figures 3, 4, and 5, respectively.

4. Discussion

Serum TN-C levels have not been previously studied in pediatric patients with DCM. In the present study, we attempted to investigate the utility of its use as a marker of inflammation in infants and children with DCM. To avoid noninfectious causes of DCM, patients with familial/genetic or secondary cardiomyopathy were excluded. We found serum TN-C levels to be significantly higher in children with DCM than those in controls and in acute than chronic DCM. The significant elevation of TN-C in group I might be due to an inflammatory process since children at this age group are more prone to develop DCM following viral myocarditis [2]. Previous data demonstrated that TNC is a useful marker for evaluation of disease activity in myocarditis [7, 9]. In their study on adult myocardial samples Tsukada et al. [18] found a high prevalence of chronic myocarditis in DCM patients and suggested that TN-C might prove to be a useful marker for distinguishing inflammatory cardiomyopathy from other types of DCM. Researchers found that most of the myocardium in DCM patients shows varying degrees of inflammation and that expression of TN-C is enhanced in the areas of active inflammation with local tissue remodeling [19].
Serum TN-C correlated negatively with the EF and positively with the LVDD and EDV suggesting that a high serum TN-C associated the impaired myocardial functions. Similarly, Aso et al. [10] found that serum TN-C levels were increased in proportion to the severity of left ventricular dysfunction in patients with IDC. The decrease in TN-C that associated the improved EF in group II is suggestive of an improvement but not disappearance of the inflammatory process in chronic DCM. Yet the role of ACEI in blocking vascular TN-C expression cannot be excluded as our patients with chronic DCM were maintained on ACEI. Angiotensin II is a potent inducer of tenascin-C, with drugs such as angiotensin II type 1 receptor (AT-1) antagonists, and angiotensin converting enzyme (ACE) inhibitors potentially block vascular tenasin-C expression in hypertensive patients [20]. On the other hand, we cannot exclude the role of LV dysfunction and heart failure in increasing serum TN-C in our study group. It was suggested that the increase in serum TN-C levels was associated with the severity of heart failure and LV dysfunction and remodeling in patients with DCM [10, 19]. Owing to the significant correlation between the TN-C level and LVDD, it was suggested as a new biomarker for detecting cardiomyopathy in patients with Emery-Dreifuss muscular dystrophy [21].

The recently introduced STE allows easy assessment of segmental and global longitudinal LV function and provides information on top of ejection fraction [13]. The significant positive correlation between serum TN-C and global peak longitudinal strain average in patients indicates that the increase in serum TN-C levels was associated with deterioration in cardiac function as detected by STE. We used the global longitudinal strain since it has been demonstrated that it is a more robust parameter than radial and circumferential strain for the assessment of myocardial function [22]. The STE wall motion scores in group I showed that 33.5% were normokinetic, 8.33% were hypokinetic, and 50% were akinetic. These data are suggestive of the prognosis in this group, where 1/3 of patients with acute DCM are apt to have improved LV functions after the inflammatory process subsides while those with akinsia might either improve or die. STE was more sensitive than conventional echocardiography in detecting wall motion abnormalities in group II, where all the patients had hypokinetic wall motion scoring by STE inspite of the normalized EF. This indicates an ongoing process of myocardial affection in chronic DCM that is associated by a raised serum TN-C.

Drugs targeting the expression or function of tenasin-C or the tenasin-C protein itself are currently being developed [23]. They might help in the better management of acute and chronic cardiomyopathy of infectious origin.

6. Conclusions

Serum-TN-C levels are increased in children with DCM and can be used as a marker of inflammation in acute cases. Its persistence in chronic DCM might lead to progressive myocardial disease and ventricular dilation. STE is more sensitive than conventional echocardiography in the assessment of myocardial performance in DCM. Its values correlate with serum TN-C. Drugs targeting the expression or function of tenasin-C might offer new perspectives for therapeutic approaches in this specific population.

References

[1] R. E. Berger and D. C. McGillicuddy, “Lyme carditis,” Internal and Emergency Medicine, vol. 4, no. 5, pp. 419–421, 2009.
[2] A. B. Lewis, “Acute myocarditis,” in Heart Failure in Children and Young Adults: From Molecular Mechanisms to Medical and Surgical Strategies, A. C. Chang and J. A. Towbin, Eds., pp. 235–247, Saunders Elsevier, Philadelphia, Pa, USA, 2006.
[3] A. L. P. Cañiero, S. Bottaro, and S. Iliceto, “Dilated cardiomyopathy (DCM) and myocarditis: classification, clinical and autoimmune features,” Applied Cardiopulmonary Pathophysiology, vol. 16, no. 1, pp. 82–95, 2012.
[4] B. Lauer, C. Niederau, U. Kühl et al., “Cardiac troponin T in patients with clinically suspected myocarditis,” Journal of the American College of Cardiology, vol. 30, no. 5, pp. 1354–1359, 1997.
[5] B. Lauer, M. Schannwell, U. Kühl, R. E. Strauer, and H. P. Schulteis, “Antimyosin autoantibodies are associated with deterioration of systolic and diastolic left ventricular function in patients with chronic myocarditis,” Journal of the American College of Cardiology, vol. 35, no. 1, pp. 11–18, 2000.
[6] F. S. Jones and P. L. Jones, “The tenasin family of ECM glycoproteins: structure, function, and regulation during embryonic development and tissue remodeling,” Developmental Dynamics, vol. 218, no. 2, pp. 235–259, 2000.
[7] K. Imanaka-Yoshida, M. Hiroe, Y. Yasutomi et al., “Tenascin-C is a useful marker for disease activity in myocarditis,” Journal of Pathology, vol. 197, no. 3, pp. 388–394, 2002.
[8] M. Sato, T. Toyozaki, K. Odaka et al., “Detection of experimental autoimmune myocarditis in rats by 111In monoclonal antibody specific for tenasin-C,” Circulation, vol. 106, no. 11, pp. 1397–1402, 2002.
[9] S. I. Morimoto, K. Imanaka-Yoshida, S. Hiramitsu et al., “Diagnostic utility of tenasin-C for evaluation of the activity of human acute myocarditis,” Journal of Pathology, vol. 205, no. 4, pp. 460–467, 2005.
[10] N. Aso, A. Tamura, and M. Nasu, “Circulating tenasin-C levels in patients with idiopathic dilated cardiomyopathy,” American Journal of Cardiology, vol. 94, no. 11, pp. 1468–1470, 2004.
[11] K. Imanaka-Yoshida, M. Hiroe, T. Nishikawa et al., “Tenasin-C modulates adhesion of cardiomyocytes to extracellular matrix during tissue remodeling after myocardial infarction,” Laboratory Investigation, vol. 81, no. 7, pp. 1015–1024, 2001.
[12] N. G. Frangogiannis, S. Shimoni, S. Chang et al., “Active interstitial remodeling: an important process in the hibernating human myocardium,” Journal of the American College of Cardiology, vol. 39, no. 9, pp. 1468–1474, 2002.
[13] H. Blessberger and T. Binder, “Two dimensional speckle tracking echocardiography: basic principles,” Heart, vol. 96, no. 9, pp. 716–722, 2010.
[14] P. Richardson, R. W. McKenna, M. Bristow et al., "Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the definition and classification of cardiomyopathies," *Circulation*, vol. 93, no. 5, pp. 841–842, 1996.

[15] B. J. Maron, J. A. Towbin, G. Thiene et al., "Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention," *Circulation*, vol. 113, no. 14, pp. 1807–1816, 2006.

[16] M. Hasegawa, H. Hirata, A. Sudo et al., "Tenascin-C concentration in synovial fluid correlates with radiographic progression of knee osteoarthritis," *Journal of Rheumatology*, vol. 31, no. 10, pp. 2021–2026, 2004.

[17] S. Mondillo, M. Galderisi, D. Mele et al., "Speckle-tracking echocardiography: a new technique for assessing myocardial function," *Journal of Ultrasound in Medicine*, vol. 30, no. 1, pp. 71–83, 2011.

[18] B. Tsukada, F. Terasaki, H. Shimomura et al., "High prevalence of chronic myocarditis in dilated cardiomyopathy referred for left ventriculoplasty: expression of tenascin C as a possible marker for inflammation," *Human Pathology*, vol. 40, no. 7, pp. 1015–1022, 2009.

[19] F. Terasaki, H. Okamoto, K. Onishi et al., "Higher serum tenascin-C levels reflect the severity of heart failure, left ventricular dysfunction and remodeling in patients with dilated cardiomyopathy," *Circulation Journal*, vol. 71, no. 3, pp. 327–330, 2007.

[20] J. W. Fischer, "Tenascin-C: a key molecule in graft stenosis," *Cardiovascular Research*, vol. 74, no. 3, pp. 335–336, 2007.

[21] I. Niebroj-Dobosz, A. Madej-Pilarczyk, M. Marchel, B. Sokolowska, and I. Hausmanowa-Petrusewicz, "Circulating tenascin-C levels in patients with dilated cardiomyopathy in the course of Emery-Dreifuss muscular dystrophy," *Clinica Chimica Acta*, vol. 412, no. 17-18, pp. 1533–1538, 2011.

[22] A. Manovel, D. Dawson, B. Smith, and P. Nihoyannopoulos, "Assessment of left ventricular function by different speckle-tracking software," *European Journal of Echocardiography*, vol. 11, no. 5, pp. 417–421, 2010.

[23] K. S. Midwood and G. Orend, "The role of tenascin-C in tissue injury and tumorigenesis," *Journal of Cell Communication and Signaling*, vol. 3, no. 3–4, pp. 287–310, 2009.