formation, anticoagulants (warfarin, heparin) should also be used. Warfarin suppresses the biosynthesis of clotting factors II (prothrombin), VII, IX, and X, thereby inhibiting coagulation.[13] Heparin activates anti-thrombin III, which inhibits thrombin, factor Xa (the active form of factor X), and other serine protease activities, resulting in suppression of coagulation. Upon detection of a thrombus or AMI onset, it is necessary to prevent thrombus reformation and re-infarction by treating with heparin for 3 consecutive days.[14]

Thrombolytic therapy is based primarily on urokinase and t-PA. These drugs convert plasminogen into plasmin within the thrombus, and plasmin decomposes fibrin, resulting in thrombolysis. t-PA and urokinase exert their actions via the same mechanism, and both have a high affinity for solid-phase fibrin and express their enzymatic activities on the thrombus.[15] In Japan, three t-PA preparations are primarily used for thrombolytic therapy in patients with KD: alteplase (recombinant t-PA; Activacin), tisokinase (human tissue type t-PA; Hapase and Plasvata), and monteplase (mutant t-PA; Cleactor). Half-lives differ among these three drugs: several minutes for alteplase, approximately 1 h for tisokinase, and 7.8 h for monteplase. Alteplase is given in two steps (initial rapid injection of 10% of the total dose in 1–2 min and subsequent drip infusion of the remainder over 1 h). At present, monteplase is the only t-PA that can be given via rapid i.v. injection.[16]

As stated here, urokinase is the only officially authorized drug for ICT in patients with KD in Japan, but t-PA is actually used in many cases. In several cases, this drug was used for ICT at doses exceeding those recommended for IVCT or it was used for repeated ICT. Unlike its use in adults, however, no serious hemorrhagic complications associated with use of this drug for ICT have been reported in pediatric cases.[4,5] Reported complications include fever after the use of urokinase at doses recommended for IVCT and nasal bleeding after the use of monteplase in addition to urokinase (both at doses recommended for IVCT), but both were mild. It appears to be reasonable to expand the range of doses of these drugs used for ICT in children, unlike in adults.[17,18]

Selection of treatment methods depends on individual patient status

Although existing guidelines refer to IVCT and ICT for thrombolytic therapy, they do not provide detailed information about these treatment methods or drugs to be selected depending on patient and thrombus status, doses of drugs, or the duration of drug treatment.

Figure 1 shows the results obtained in the secondary survey on the treatment methods selected at each facility depending on individual patient status (asymptomatic thrombus alone or thrombus with AMI) as well as the effects of these methods. In cases in which only thrombus was detected and there were no symptoms, IVCT alone was often selected (63%, 12/19), resulting in a high response rate (75%, 9/12). Among the five patients with AMI, none received IVCT alone and four received ICT alone, including two who responded to ICT. Combined therapy was selected for only one patient with AMI. In that patient, combined therapy was not rated as effective, but the thrombus tended to subside slowly over several days, eventually disappearing completely.

According to the existing guidelines, there is little difference in efficacy between IVCT and ICT. When used in adults, re-canalization is reportedly achieved in 70–80% of all cases after IVCT and the re-canalization rate was 10% higher when IVCT was combined with ICT.

These results suggest that in asymptomatic patients with only a thrombus, efficacy can be expected when treatment is initiated with IVCT which is simple and does not require special facilities or skills. For patients failing to respond to IVCT or having symptoms or signs suggestive of AMI on electrocardiography, echocardiography, hematological tests, and so on, it would be advisable to perform ICT, depending on individual circumstances.

Treatment methods should be selected depending on thrombus status

The present results indicate that ICT is effective for small thrombi, particularly those ≤10 mm, and that IVCT is effective even for giant thrombi >10 mm. Efficacy, however, was evaluated at the specific timing set for the present study (i.e. within 3 days after completion of IVCT and within 6 h after completion of IVCT). Because of this limitation, the long-term course of thrombi after treatment by these methods remains unclear, warranting further study. Furthermore, judging whether or not thrombolysis was successful is difficult in cases of giant thrombi.

The present results additionally confirmed that thrombolytic therapy is more effective as the time from thrombus formation until the start of treatment becomes shorter, as was already well known. When the response rate was analyzed by treatment method, the response rate to IVCT was high even when considerable time (≥1 week) had elapsed since thrombus formation. Because of the small sample size, we cannot draw definite conclusions about the efficacy of ICT, either alone or combined with another therapy, but all responders to ICT, both alone and combined, received treatment within several hours after thrombus formation. Despite these results, it is difficult to conclude that ICT is not effective in cases in which several days or even a week or more has elapsed since thrombus formation. We cannot rule out the possibility that even slight thrombolysis achieved by ICT on the thrombus surface later enhances responses to IVCT. In practice, there were cases in which ICT was rated as ineffective, while subsequent IVCT resulted in gradual thrombus disappearance.

t-PA and urokinase doses

There are no established dosing criteria for t-PA and urokinase in children. Existing guidelines refer to the use of these drugs in children but recommend careful determination of doses tailored to individual cases. The recommended doses are listed in Table 1. The present survey identified cases in which t-PA, which is not authorized for ICT use, was used or urokinase was given at doses or frequencies higher than recommended, but no serious hemorrhagic complications were reported. In adults, the risk for intracranial hemorrhagic complications reportedly rises

approximately 10-fold with t-PA use.[19] In the present study of children, however, no such complications were seen, raising the possibility of expanding the scope of t-PA use in the future.

imitations of the present study

Because of difficulty in visually assessing dramatic changes occurring in cases of giant thrombus, efficacy evaluation at each facility was often dependent on subjective judgment, rather than being based on uniform criteria.

The second point is especially important, in that differences in the length of time from thrombus formation until the start of ICT led to differences in the time needed for ICT to manifest its thrombolytic efficacy, thereby affecting the evaluation of responses to ICT. For this reason, accurate evaluation of responses to ICT was difficult. There may have been cases in which no marked change had been noted during the first 6 h after ICT, but subsequent thrombolysis was induced. How the differences in the setting of the ICT and IVCT endpoints are reflected in the results remain unclear, but, in terms of the ICT method, it could only be distinguished in this manner. And it is desirable to investigate long-term outcomes, that is, the long-term course of thrombosis after treatment.

The present survey was retrospective, covering a 5 year period, involving a small number of patients ($n = 23$), therefore, statistical analysis was not possible, and there were limitations in all the conclusions made. Although coronary aneurysm thrombosis in KD is a rare condition, it is often fatal and a prospective study on this condition is warranted.

Conclusion

At many facilities (11 of 14), IVCT was selected as the initial therapy. IVCT was selected in 12 (63%) of 19 KD patients without AMI, yielding a high response rate (75%). In KD patients with AMI, ICT (either alone or combined with IVCT) was consistently selected.

In some cases, t-PA, despite not being authorized for ICT, was used, or urokinase was given at doses or frequencies higher than recommended, but no serious hemorrhagic complications associated with such unauthorized use were reported.

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References

1 Yanagawa H, Nakamura Y, Yashiro M *et al.* Incidence survey of Kawasaki disease in 1997 and 1998 in Japan. *Pediatrics* 2001; **107**: E33.
2 Japan Kawasaki Disease Research Center. *Results of the 20th Nationwide Survey on Kawasaki Disease.* Japan Kawasaki Desease Research Center, Japan, 2009.
3 Duan C, Du ZD. Endothelial progenitor cells and the neovascularization in patients with coronary aneurysms due to Kawasaki disease. *Chin. J. Pediatr.* 2011; **49**: 397–400.
4 Ohkubo T, Fukazawa R, Ikegami E, Ogawa S. Reduced shear stress and disturbed flow may lead to coronary aneurysm and thrombus formations. *Pediatr. Int.* 2007; **49**: 1–7.
5 Kuramochi Y, Takeuchi N, Fukumi D, Uchikoba Y, Ogawa S. Hemodynamic factors of thrombus formation in coronary aneurysms associated with Kawasaki disease. *Pediatr. Int.* 2000; **42**: 470–75.
6 Fukuhara S, Takase E, Fujimoto T *et al.* Coronary ectasia resulting in thrombotic coronary occlusion after warfarin interruption: A case report. *J. Cardiol.* 2005; **46**: 195–200.
7 Suda K, Kudo Y, Higaki T *et al.* Multicenter and retrospective case study of warfarin and aspirin combination therapy in patients with giant coronary aneurysms caused by Kawasaki Disease. *Circ. J.* 2009; **73**: 1319–23.
8 Kadar K, Piskothy A, Bendig L. Giant coronary thrombus as a complication of Kawasaki disease. *Orv. Hetil.* 1993; **44**: 2431–3.
9 Horigome H, Sekijima T, Miyamomto T. Successful thrombolysis with intracoronary administration of tissue plasminogen activator in an infant with Kawasaki disease. *Heart* 1997; **78**: 517–18.
10 Nagasawa M, Watanabe N, Okuno M, Okamoto N, Fujino H. Effects of intracoronary tissue-type plasminogen activator treatment in Kawasaki disease and acute myocardial infarction. *Cardiology* 2000; **94**: 52–7.
11 JCS Joint Working Group. Guidelines for diagnosis and management of cardiovascular sequelae in Kawasaki Disease (JCS 2008): Digest version. *Circ. J.* 2010; **74**: 1989–2020.
12 Majerus PW, Tollefsen DM. Anticoagulant, thrombolytic, and antiplatelet drugs. In: Hardman JG, Limbird LE, Gikman AG (eds). *The Pharmacological Basis of Therapeutics*, 10th edn. McGraw-Hill, New York, 2001; 1519–1538.
13 Takahashi N. Thrombolysis by intravenous tissue plasminogen activator (t-PA): Current status and future direction. *Brain Nerve* 2009; **61**: 41–52.
14 Tsuda E. Q & A on Kawasaki's disease: Treatment and prognosis of sequelae. *Jpn. J. Pediatr. Med.* 2003; **35**: 1592–4 (in Japanese).
15 Motomiya T. Thrombolytic agents, urokinase, tissue-type plasminogen activator and pro-urokinase – their routes of administration and dosage: Comparison between intracoronary thrombolysis and intravenous coronary thrombolysis. *Nippon Rinsho* 2003; **61**: 441–5 (in Japanese).
16 Kawai C, Yui Y, Hosoda S *et al.* A prospective, randomized, double-blind multicenter trial of a single bolus injection of the novel modified t-PA E6010 in the treatment of acute myocardial infarction: Comparison with native t-PA, E6010 Study Group. *J. Am. Coll. Cardiol.* 1997; **29**: 1447–53.
17 Inoue T, Nishiki R, Kageyama M, Node K. Therapeutic potential of monteplase in acute myocardial infarction as a powerful thrombolytic agent for pretreatment of coronary intervention. *Cardiovasc. Drug Rev.* 2004; **22**: 320–33.
18 The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N. Engl. J. Med.* 1995; **333**: 1581–7.

Preliminary study of serum tenascin-C levels as a diagnostic or prognostic biomarker of type B acute aortic dissection

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Aortic dissection (AD) is a rare but clinically relevant disorder, especially for emergency room physicians who are confronted with the challenge of fast diagnosis and prognosis estimation [1]. In particular, the identification of patients at highest risk to die is extremely difficult and likewise important for rapid decision making [2]. Tenascin-C (TN-C), a matricellular protein, is not normally expressed in adult tissues but transiently reappears under various pathologic conditions closely associated with tissue injury and inflammation, and is thought to be playing important roles in cardiovascular tissue remodelling, including coronary atherosclerotic plaque, abdominal aortic aneurysm (AAA), myocardial infarction, myocarditis, as well as acute pulmonary thromboembolism [3–7]. In this report, we evaluated clinical implication of serum TN-C levels in patients with type B acute AD.

We evaluated 42 patients with type B acute AD admitted to Yokosuka Kyosai Hospital between May 2005 and January 2008 (22 men, 20 women; mean age 69.7 ± 12.8 years), and 20 normal controls (14 men, 6 women; 49.0 ± 15.0 years). All patients came to our emergency room with symptom of chest pain, back pain or lumbago and were diagnosed as having type B acute AD by CT. Assay of serum TN-C levels was described previously [8]. Briefly, blood samples were centrifuged at 15000 *g* for 15 min, and the resulting supernatants were stored at −80 °C until analysis. Serum levels of TN-C with the large subunit containing the domain of FNIII repeats were determined using an ELISA kit using two monoclonal antibodies, 4F10TT and 19C4MS (IBL, Gunma, Japan) on admission and at 7 days, 14 days and 28 days after admission. Serum TN-C levels were compared with blood biomarkers such as D-dimer, fibrin degradation products (FDP) and high sensitivity C-reactive protein (hs-CRP). The primary endpoint was defined as all-cause mortality at hospital discharge. Furthermore, immunohistochemistry with anti-TN-C antibody was performed in autospied sample of patient of acute AD and compared to normal aortic walls. Informed consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Patient characteristics, laboratory data, and CT findings are shown in Table 1. All patients had a history of hypertension. The median serum TN-C levels were 66.3 ng/ml. False lumen thrombosis was present in 26 of 42 (62%) patients on CT. Maximum diameter of the dissected aorta on admission was 42.7 ± 8.7 mm. Three patients with AD died due to rupture of the aorta in hospital within 30 days of admission. Serums TN-C levels on admission in AD patients were significantly higher than those of normal controls (median 66.3, range 41.2 to 106.9 ng/ml vs. 33.3, range 24.5 to 39.7 ng/ml, $P = 0.0004$). TN-C levels were significantly peaked at 7 days (median 84.0, range 51.4 to 113.4 ng/ml) and then gradually decreased, although they remained elevated at 28 days (median 45.3, range 29.4 to 68.3 ng/ml). TN-C levels on admission of the death group were significantly higher than those of the survival group (median 176.6, range 66.3 to 263.3 ng/ml vs. 57.4, range 39.9 to 99.4 ng/ml, P = 0.008). TN-C levels at 7 days correlated positively with peak CRP levels (r = 0.52, $P = 0.008$), peak FDP levels (r = 0.48, $P = 0.014$), peak D-dimer levels (r = 0.48, $P = 0.017$), and maximum aortic diameter on admission (r = 0.42, $P = 0.04$) (Fig. 1). Strong expression of TN-C was detected in the aortic wall with dissection at the acute stage, but not in the normal (Fig. 2).

Table 1
Clinical characteristics, laboratory data and CT findings of the patients.

Characteristic	N = 42
Age, yrs	69.7 ± 12.8
Male	22 (52%)
Hypertension	42 (100%)
Dyslipidemia	12 (29%)
Diabetes mellitus	8 (19%)
Current smoker	13 (31%)
Hemodialysis	2 (5%)
Laboratory data	
Creatinine (mg/dl)	0.85 [0.63–1.06]
Total cholesterol (mg/dl)	183.2 ± 40.3
LDL cholesterol (mg/dl)	112.9 ± 39.6
Triglyceride (mg/dl)	116.0 ± 66.4
Glucose (mg/dl)	153.2 ± 48.1
CRP (mg/dl)	0.37 [0.12–1.91]
FDP (μg/ml)	7.30 [4.15–20.75]
D-dimer (μg/ml)	4.51 [1.82–14.62]
Tenascin-C (ng/ml)	66.3 [41.2–106.9]
CT findings	
False lumen thrombosis	26 (62%)
Communicating type	16 (38%)
Maximum diameter of dissected aorta	42.7 ± 8.7 mm
Drugs after admission	
ACE-I/ARB	24 (46%)
Spironolactone/eplerenone	23 (44%)
β-blockers	35 (67%)
Calcium channel blocker	12 (23%)
Diuretics	43 (83%)

Variables are presented as mean ± SD or as number (%).
ACE-I = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker. CRP = C-reactive protein, CT = computed tomography, FDP = fibrin degradation products; LDL = low density lipoprotein.

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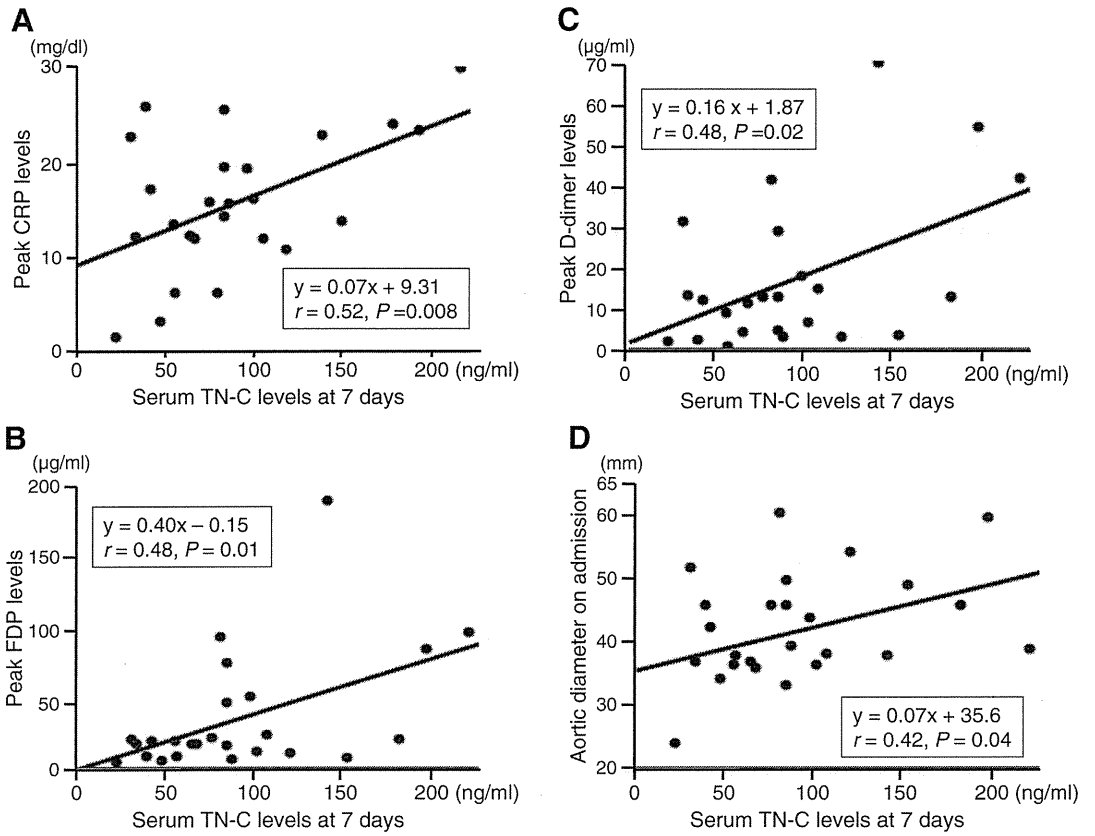

Fig. 1. Regression analysis of correlations between serum TN-C levels at 7 days and peak biomarkers and maximum dissected aortic diameter on admission. (A) Mean serum TN-C levels at 7 days correlated positively with peak CRP levels (y = 9.31 + 0.07 x, r = 0.52, $P = 0.008$). (B) Serum TN-C levels at 7 days correlated positively with peak FDP levels (y = −0.15 + 0.40 x, r = 0.48, $P = 0.01$). (C) Serum TN-C levels at 7 days correlated positively with peak D-dimer levels (y = 1.87 + 0.16 x, r = 0.48, $P = 0.02$). (D) There was a significant correlation of serum TN-C levels at 7 days with maximum aortic diameter on admission (y = 35.67 + 0.07x, r = 0.42, $P = 0.04$).

The major important findings are as follows. First, serum TN-C levels were significantly elevated during the acute stage after AD, and the levels on admission were significantly higher in the patients who died during hospitalization than in the survivors. Secondary, TN-C levels showed good correlation with known biomarkers such as hs-CRP, FDP and D-dimer, and maximum aortic diameter at the level of the dissection. This preliminary study suggests that serum TN-C might be a novel biomarker reflecting active tissue inflammation/remodeling in the aortic wall following AD, and high TN-C levels at acute stages might possibly predicting short-term prognosis.

It was previously reported that immunoreactivity of TN-C showed strong staining in aneurysmal wall of AAA [9,10], and that TN-C may be synthesized medial smooth muscle cells during progression of aneurysm. In the present study, we have found clear TN-C immunostaining in the lesion site of aortic wall of acute AD patients. Therefore, we postulate that this molecule may be synthesized in vascular smooth muscles of the dissected wall and is released into the circulation as a consequence of mechanical disruption of the vessel wall. Recently, the usefulness of D-dimer levels was shown in risk stratifying patients with suspected acute AD within the first 24 h after symptom onset by the International Registry of Acute Aortic Dissection Substudy on Biomarkers (IRAD-Bio study) (2). In the current study, rough but significant correlation was observed between TN-C levels and D-dimer at day 7. Since serum TN-C could elevate in various diseases, including pulmonary embolism or acute myocardial infarction (3–7), it is not specific diagnostic marker for AD. However, our finding that TN-C levels on admission of the death group were significantly higher than those of the survivals suggests that TN-C might be another biomarker for risk stratification of patients with AD.

The current study has some limitations. The sample size was relative small, and prognosis of type B AD in the study cohort was good. Further large-scale prospective investigations and careful comparisons with other clinical parameters are required to confirm the predictive ability of TN-C in the short- and long-term prognosis of type B acute AD.

References

[1] Hagan PG, Nienaber CA, Isselbacher EM, et al. The International Registry of Acute Aortic Dissection (IRAD): new insights into an old disease. JAMA 2000;283:897–903.
[2] Suzuki T, Distante A, Zizza A, et al. Diagnosis of acute aortic dissection by D-dimer: the International Registry of Acute Aortic Dissection substudy on biomarkers (IRAD-Bio) experience. Circulation 2009;119:2702–7.
[3] Imanaka-Yoshida K. Tenascin-C in cardiovascular tissue remodeling: from development to inflammation and repair. Circ J 2012;76:2513–20.
[4] Sato A, Aonuma K, Imanaka-Yoshida K, et al. Serum tenascin-c might be a novel predictor of left ventricular remodeling and prognosis after acute myocardial infarction. J Am Coll Cardiol 2006;47:2319–25.
[5] Sato A, Hiroe M, Akiyama D, et al. Prognostic value of serum tenascin-C levels on long-term outcome after acute myocardial infarction. J Card Fail 2012;18:480–6.
[6] Fujimoto N, Onishi K, Sato A, et al. Incremental prognostic values of serum tenascin-C levels with blood B-type natriuretic peptide testing at discharge in patients with dilated cardiomyopathy and decompensated heart failure. J Card Fail 2009;15:898–905.
[7] Celik A, Kocyigit I, Calapkorur B, et al. Tenascin-C may be a predictor of acute pulmonary thromboembolism. J Atheroscler Thromb 2011;18:487–93.
[8] Hasegawa M, Hirata H, Sudo A, et al. Tenascin-C concentration in synovial fluid correlates with radiographic progression of knee osteoarthritis. J Rheumatol 2004;31:2021–6.
[9] Satta J, Soini Y, Pöllänen R, Pääkkö P, Juvonen T. Tenascin expression is associated with a chronic inflammatory process in abdominal aortic aneurysms. J Vasc Surg 1997;26:670–5.
[10] Kimura T, Yoshimura K, Aoki H, et al. Tenascin-C is expressed in abdominal aortic aneurysm tissue with an active degradation process. Pathol Int 2011;61:559–64.

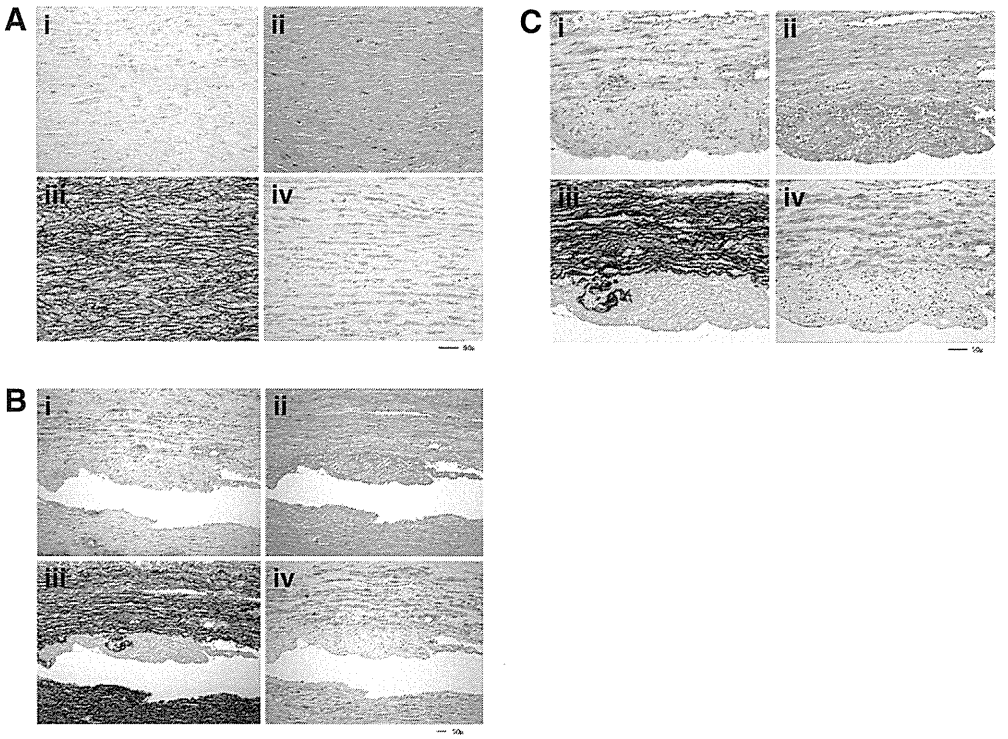

Fig. 2. Expression of TN-C in human aortic wall with dissection. (A) Normal aortic wall obtained from autopsy sample of noncardiac death, (B) dissected wall of aorta, and (C) higher magnification of dissected wall of aorta. (i) Immunostaining with anti-TN-C antibody (4C8), (ii) Hematoxylin–Eosin stain, (iii) Elastica van Gieson stain, and (iv) immunostaining with α-SMA antibody. (A) TN-C was not expressed in normal aortic wall obtained from the autopsy sample of noncardiac death (i). (B, C) However, TN-C was highly expressed in the medial layer of the dissected aortic wall obtained from an autopsy sample of a patient who died due to rupture of aortic dissection (i). TN-C expression was concordant with the area of the vascular smooth muscle cells stained by α-SMA (iv). Scale bars = 50 μm. α-SMA, α-smooth muscle actin.

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