# Serum tenascin-C predicts resistance to steroid combination therapy in high-risk Kawasaki disease: A multicenter prospective cohort study

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## Abstract

# Background

Tenascin-C (TN-C) is an extracellular matrix glycoprotein related to tissue inflammation. Our previous retrospective study conducted in 2016 revealed that the serum TN-C level was higher in patients with Kawasaki disease (KD) who were resistant to intravenous immunoglobulin (IVIG) and developed coronary artery lesions (CALs). The present study is a prospective cohort study to assess if the serum level of TN-C could be used as a novel biomarker to predict the risk of resistance to initial treatment for high-risk patients.

# Methods

A total of 380 KD patients were registered and provided serum samples for TN-C measurement before commencing their initial treatment. Patients who did not meet the inclusion criteria were excluded from analysis; of the 181 remaining subjects, there were 144 low-risk patients (Kobayashi score: ≤4 points) and 37 high-risk patients (Kobayashi score: ≥5 points). The initial treatments for low-risk patients and high-risk patients were conventional therapy (IVIG with aspirin) and prednisolone combination therapy, respectively. The patient clinical and laboratory data, including the serum TN-C level, were compared between responders and non-responders to initial treatment.

# Results

In the low-risk patients, there was no significant difference in the median levels of serum TN-C between responders and non-responders to initial therapy. However, in the high-risk patients, the median serum TN-C level in non-responders was significantly higher than that in responders (175.8 ng/ml vs 117.6 ng/ml).

# Conclusions

Serum TN-C could be a biomarker for predicting the risk of high-risk patients being non-responsive to steroid combination therapy.

# Trial registration:

This study was a prospective cohort study. It had been performed in accordance with the Declaration of Helsinki and had been approved by the ethics committee in each institute.

## Background

Kawasaki disease (KD) is an acute systemic vasculitis of unknown etiology that occurs in early childhood [1]. Coronary artery lesions (CALs) are the most critical complication of KD; they can lead to myocardial ischemia, infarction, or even sudden death in adulthood. Treatment with a high dose of intravenous immunoglobulin (IVIG) is the most effective evidence-based therapy for the acute phase of KD, and it significantly reduces the rate of CALs [2, 3]. However, approximately 20% of patients with KD still have persistent or recrudescent fever after initial IVIG treatment [4]. IVIG resistance is a major risk factor for the development of CALs [5, 6]. There are some scoring systems that predict initially IVIG-resistant patients at the time of KD diagnosis [7–9]. Specifically, the Kobayashi scoring system predicts patients at high risk of IVIG resistance with 76% sensitivity and 80% specificity among Japanese individuals, and it is widely used in Japan [8]. Steroid combination therapy is recommended for these high-risk patients by Japanese guidelines [10]. The RAISE study showed that additional prednisolone reduced the non-response to IVIG and decreased the occurrence of CALs in high-risk KD patients [11, 12]. Intravenous methylprednisolone plus IVIG also had a positive effect [13, 14]. However, according to a Japanese national survey, the CAL rate has remained at around 2.5% for the past several years [15]. This means that the stratification of severe cases who do not respond to initial steroid combination therapy is not still good enough. In addition, the accuracy of scoring systems are not high in other countries [16, 17]. Therefore, efforts are being made to find a simple biomarker with which to stratify patients with KD [18, 19].

Tenascin-C (TN-C) is a large extracellular matrix glycoprotein that belongs to matricellular proteins [20], sparsely expressed in normal tissue, but upregulated associated with tissue injury and inflammation [21–23]. It has diverse functions to regulate cell behavior in inflammation and tissue repair in many pathological processes [24–26]. Taking advantage of on the specific expression, serum TN-C are used as a biomarker for assessing of disease activity and predicting prognosis in various cardiovascular diseases such as dilated cardiomyopathy [27], acute myocardial infarction [28], aortic aneurysm/dissection [29, 30] and coronary atherosclerosis [31]. In 2015, we have shown that TN-C had been highly expressed in vessel walls of the Candida induced KD vasculitis mouse model [32]. We proposed that TN-C may be involved in the process of CALs formation and serum level of TN-C may be a new biomarker for predicting the risk of CALs of KD.

Our previous retrospective study has demonstrated that the serum level of TN-C can be a promising biomarker for predicting the risk of CALs and IVIG resistance in patients during the acute phase of KD, whose accuracy is comparable to that of Kobayashi score [33]. In the present study, we examined whether serum TN-C can be used as a biomarker to predict resistance to the first line therapy even in KD patients stratified as high risk.

# Methods

# Subjects

We conducted a multicenter prospective study and enrolled 380 KD patients who were hospitalized at 17 hospitals in Japan (Japan Community Health Care Organization Hokkaido Hospital; KKR Sapporo

Medical Center; NTT East Sapporo Hospital; Tenshi Hospital; Teine Keijinkai Hospital; Japanese Red Cross Kitami Hospital; Nikko Memorial Hospital; Kushiro Red Cross Hospital; National Center for Global Health and Medicine; Japanese Red Cross Medical Center; Toho University Medical Center Oomori Hospital; Showa University Hospital; Tokai University Oiso Hospital; Gunma Children's Medical Center; Japanese Red Cross Nagoya Daiichi Hospital; Fukuoka Children's Hospital; and Fukuoka University Chikushi Hospital) between April 2011 and March 2015. We diagnosed KD in accordance with the Japanese diagnostic guidelines for KD [34]. The study design and written consent were approved by each institution's ethics committee. Written informed consent was obtained from the participants or their parents/guardians.

# Protocols

The patients were categorized into two groups based on their Kobayashi score: the group of low-risk patients who had ≤ 4 points, and the group of high-risk patients who had ≥ 5 points at the time of diagnosis. All KD patients were treated in accordance with the guidelines from the Pediatric Cardiology and Cardiac Surgery 2012 [35].

Low-risk patients received the conventional treatment consisting of 2 g/kg of IVIG with aspirin. High-risk patients, in accordance with the RAISE study protocol, received conventional treatment plus 2 mg/kg of prednisolone per day [11]. Prednisolone was administered by intravenous injection in three divided doses for more than 5 days. If fever resolved, the route of administration was changed to oral. When the concentration of C-reactive protein normalized (≤ 5 mg/L), the prednisolone dose was taperd off over 15 days. During the administration of prednisolone, famotidine, a histamine-2 receptor antagonist, was co-administered (1 mg/kg/day). Aspirin was started at a dose of 30 mg/kg per day. After patients became afebrile, the aspirin dose was reduced to 3–5 mg/kg per day; aspirin was administered for over 2–3 months after fever onset. For non-responders to initial therapy (persistent fever for over 24 hours after the initial treatment was terminated), an additional treatment was added in accordance with the strategy of each institute.

Two-dimensional echocardiograms were performed upon admission, on day 10–14, and on day 30–40 from the onset of KD, and the intraluminal diameters of coronary artery segments were measured at each institute. The presence of CALs was diagnosed based on the z-scores of the left main trunk coronary artery (LMT), the proximal left anterior descending coronary artery (LAD), and the right coronary artery (RCA) [36]. CALs were defined based on the z-scores as follows: no involvement (z-score: <2.0), dilation (z-score: ≥2.0 to < 2.5), aneurysm (z-score: ≥2.5 to < 10), and giant aneurysm (z-score: ≥10).

# Measurement of serum TN-C levels

Serum samples were obtained to measure the serum TN-C levels before initial treatment and two or three days after initial treatment was begun. Blood samples were sent to the National Center for Global Health and Medicine, where serum TN-C was measured by enzyme-linked immunosorbent assay using the Human TN-C Large (FN -Japan). Medical, demographic, and laboratory data were collected upon admission in all cases.

# Statistical analysis

All analyses were performed using SPSS software, version 20 (SPSS Japan, Tokyo, Japan). Data are presented as the median with the data range (minimum to maximum) for continuous variables or as a percentage of the patients in a given categorical variable. A series of group comparisons were conducted using the *t*-test

for numerical data with a normal distribution or the Mann-Whitney U test for data that did not have a normal distribution. We used the Kolmogorov-Smirnov algorithm to identify whether variables had a normal distribution. For all comparisons, differences with a *p*-value of < 0.05 were considered to be statistically significant.

## Results

# Patients characteristics

In total, 380 patients were diagnosed with KD upon admission. We first excluded the patients for whom there was no available TN-C data from either before or after their initial treatment (n = 103), patients who had a recurrent case (n = 12), those with a concurrent infection (n = 3), those with underlying congenital heart disease (n = 2), those who were not finally diagnosed with KD (n = 2), and those who did not receive IVIG treatment (n = 36) (Fig. 1). The remaining 222 patients were categorized into two groups according to whether their Kobayashi score was ≤ 4 points (low-risk patients, n = 162) or ≥ 5 points (high-risk patients, n = 60) at the time of diagnosis (Table 1).

Table 1
Scoring systems that predict initially IVIG-resistant patients

| **Kobayashi Score (Cut off: ≥5 points;Sensitivity 76%、Specificity 80、)** | | |
|---|---|---|
| Risk Factor | | Points |
| Illness days at diagnosis | < 4 days | 2 |
| Serum sodium level | < 133 mmol/l | 2 |
| AST | ≥ 100 IU/l | 2 |
| Neutrophil rate | ≥ 80 % | 2 |
| CRP | ≥ 10 mg/dl | 1 |
| Platelet count | ≤ 30.0 × 104/mm3 | 1 |
| Age at diagnosis | ≤ 12 month | 1 |

Regarding the low-risk patients, we further excluded those whose treatment included added ulinastatin (n = 11) or steroids (n = 7). This left 144 enrolled patients who were administered IVIG as a first-line therapy.

Among them, 116 (80.6%) patients responded to the IVIG-treatment and did not require a second-line therapy (low-risk initial treatmeant-responsive group), while 28 patients were resistant to the IVIG and did require a second-line therapy (low-risk initial treatment-resistant group). Regarding the high-risk patients, we excluded those who were not administered steroids (n = 23). This left 37 patients who were administered IVIG + steroid as a first-line therapy. Among them, 27 (73.0%) patients responded to the IVIG + steroid and did not require a second-line therapy (high-risk initial treatment-responsive group), while 10 patients did not respond to the IVIG + steroid and did require a second-line therapy (high-risk initial treatment-resistant group).

The baseline characteristics of the high-risk patients and low-risk patients are shown in Tables 2 and 3, respectively. In both the low- and high-risk groups, there were no significant differences in terms of age, sex, and laboratory data between the initial treatment-responsive group and the initial treatment-resistant group.

Table 2
Characteristics and data of high-risk patients in the initial treatment (IVIG + steroid)-responsive and initial treatment-resistant groups

| | IVIG + steroid responder group | IVIG + steroid resistant group | *p*-value |
|---|---|---|---|
| Number | 27 | 10 | |
| Age (months) | 42 [11–80] | 44 [9–86] | 0.880 |
| Male gender, n (%) | 17 (63.0) | 8 (80.0) | 0.285 |
| Kobayashi score | 6 [5–10] | 6 [5–10] | 0.216 |
| < Laboratory data before 1st line therapy > | | | |
| TN-C, ng/mL | 117.6 [35.0–324.8] | 175.8 [80.4–380.9] | 0.037 |
| WBC, $\times 10^3/\mu L$ | 14.8 [6.6–33.2] | 18.6 [6.9–36.8] | 0.242 |
| Neutrophils, % | 83 [60–95] | 88 [68–94] | 0.191 |
| Platelets, $\times 10^4/mL$ | 26.2 [13.1–59.4] | 23.9 [13.5–36.6] | 0.555 |
| CRP, mg/dL | 10.0 [2.5–24.0] | 10.1 [5.2–21.7] | 0.853 |
| Albumin, g/dL | 3.6 [2.7–4.1] | 3.6 [2.8–4.4] | 0.801 |
| T-bilirubin, mg/dL | 0.7 [0.3–5.5] | 1.4 [0.5–4.6] | 0.391 |
| AST, IU/L | 57 [20–787] | 551 [25–2725] | 0.013 |
| ALT, IU/L | 83 [8–937] | 518 [9–1435] | 0.067 |
| Sodium, mEq/L | 133 [127–137] | 132 [128–135] | 0.578 |

TN-C: tenascin-C, WBC: white blood cell, CRP: C-reactive protein, AST: aspartate aminotransferase, ALT: alanine aminotransferase.

* The Kobayashi score was ≥ 5 points in all cases.

* In all cases, the first-line therapy was IVIG, prednisolone, and aspirin.

Table 3
Characteristics and data of low-risk patients in the initial treatment (IVIG)-responsive and initial treatment-resistant groups

| | IVIG-responder group | IVIG-resistant group | *p* value |
|---|---|---|---|
| number | 116 | 28 | |
| Age in month | 29.5 [4–130] | 22 [4–107] | 0.352 |
| Male gender, n (%) | 56 (48.3) | 13 (46.4) | 0.861 |
| < Laboratory data before IVIG > | | | |
| TN-C, ng/mL | 106.6 [29.1–449.6] | 113.5 [46.6–483.4] | 0.432 |
| WBC, $\times 10^3/\mu L$ | 13.0 [6.1–32.3] | 12.7 [6.3–22.0] | 0.435 |
| Neutrophil, % | 66 [24–91] | 67 [26–88] | 0.418 |
| Platelet, $\times 10^4/mL$ | 33.3 [16.6–53.3] | 32.9 [19.4–62.5] | 0.612 |
| CRP, mg/dL | 6.7 [1.3–31.4] | 8.8 [1.5–20.0] | 0.190 |
| Albumin, g/dL | 3.6 [2.6–4.8]<br>n = 115 | 3.6 [2.6–4.3] | 0.998 |
| T-bilirubin, mg/dL | 0.5 [0.1–3.1]<br>n = 114 | 0.6 [0.2–3.4] | 0.064 |
| AST, IU/L | 34 [15–298] | 33 [18–236] | 0.677 |
| ALT, IU/L | 19 [5–442] | 24 [8–237] | 0.608 |
| Sodium, mEq/L | 136 [127–143] | 135 [127–138] | 0.093 |
| TN-C: tenascin-C, WBC: white blood cell, CRP: C-reactive protein, AST: aspartate aminotransferase, ALT: alanine aminotransferase. | | | |
| * The Kobayashi score was < 5 points in all cases. | | | |
| * In all cases, the first-line therapy was IVIG and aspirin. | | | |

# Serum TN-C levels

First, we compared the serum TN-C levels on admission between the high-risk patients and the low-risk patients. The median level of TN-C for the high-risk patients was significantly higher than that of the low-risk patients (median: 121.6 [35.0–380.9] ng/ml vs 110.2 [29.1–293.6] ng/ml, $p = 0.028$) (Fig. 2).

Among the high-risk patients, the median TN-C level on admission for the first-line treatment-resistant group (IVIG + ASA + steroid) was significantly higher than that of the first-line treatment-responsive group (median: 175.8 [80.4–380.9] ng/ml vs 117.6 [35.0–324.8] ng/ml, $p = 0.037$) (Fig. 2a). After the first line-

treatment was initiated, the level of TN-C was significantly reduced in the initial treatment-responsive group (median: 117.6 [35.0–324.8] ng/ml to 88.7 [23.8–263.3] ng/ml, $p = 0.011$), whereas no significant change was found in the initial treatment-resistant group (median: 175.8 [80.4–380.9] ng/ml to 166.1 [86.2–696.2] ng/ml, $p = 0.878$). Hence, the median TN-C level after the first treatment of the patients who required a second-line treatment was significantly higher than that of the patients who did not need additional treatment (median: 166.1 [86.2–696.2] ng/ml vs 88.7 [23.8–263.3] ng/ml, $p = 0.004$).

Among the low-risk patients, no significant difference in the level of TN-C upon admission was found between the initial treatment-responsive group and the initial treatment (IVIG + ASA)-resistant group (median: 106.6 ng/ml [29.1–293.6] vs 113.5 [46.6–277.4] ng/ml, $p = 0.432$) (Fig. 2b). As in the high-risk patients, the first-line treatment significantly reduced the level of TN-C in the initial treatment-responsive group (median: 106.6 [29.1–293.6] ng/ml to 81.1 [22.4–181.4] ng/ml, $p < 0.001$), whereas, no significant change was found in the initial treatment-resistant group (median: 113.5 [46.6–277.4] ng/ml to 107.3 [35.1–218.5] ng/ml, $p = 0.212$). Aagin, the TN-C levels after the first-line treatment of the initial treatment-resistant patients were significantly higher than those of the group who did not require additional treatment (median: 107.3 [35.1–218.5] ng/ml vs 81.1 [22.4–181.4] ng/ml, $p = 0.016$).

# Coronary artery lesions

In the high-risk patients, there were three patients who had coronary aneurisms (z-score: ≥2.5) out of 37 patients (8.1%). The serum TN-C level upon admission was not significantly different between the CAL-positive group and the CAL-negative group (119.5 ± 33.0 ng/ml vs 120.4 ± 75.3 ng/ml, $p = 0.291$). In the low-risk patients, there were seven patients who had coronary aneurisms (z-score: ≥2.5) out of 144 patients (4.9%). The serum TN-C level upon admission was not significantly different between the CAL-positive group and the CAL-negative group (150.4 ± 67.5 ng/ml vs 110.2 ± 43.6 ng/ml, $p = 0.835$).

# Discussion

The present multicenter prospective study has shown that serum TN-C can be a useful biomarker for treatment selection in the acute phase of KD. The serum level of TN-C upon admission of the patients who were categorized as high-risk according to their Kobayashi score was significantly higher than that of the low-risk patients. This finding is consistent with the results of our previous retrospective study, suggesting that serum TN-C alone could be a biomarker for identifying high-risk patients that is comparable with the Kobayashi score [8].

Histologically, coronary arteritis begins 6–8 days after KD onset and is characterized by inflammation consisting of a marked accumulation of monocytes/macrophages [37]. TN-C is expressed in the areas where inflammatory lesions form on the coronary arteries during the acute stage of KD, and the intensity of its expression correlates with the degree of inflammation [38]. Therefore, a high serum level of TN-C may reflect the severity of inflammation.

Japanese guidelines allow the stratification of KD patients by prediction scoring systems of IVIG resistance, treating the low-risk patients with IVIG and treating the high-risk patients with a steroid combined with conventional IVIG as the first-line therapy. However, unresponsiveness to this treatment protocol has become a major problem recently. In our present study, 28 out of the 114 low-risk patients were not responsive to the IVIG, and 10 out of the 37 high-risk patients were not responsive to the steroid combination therapy, consequently needing additional therapies. Importantly, in the high-risk patients, the serum TN-C levels upon admission for the initial treatment-resistant patients were significantly higher than those of the initial treatment-responsive patients. This finding suggests that the stratification of KD severity by Kobayashi score has limitations and that TN-C could be used as a biomarker to identify patients at greater risk of resistance to first-line treatment among the patients classified as high-risk based on their Kobayashi score.

Jone et al. demonstrated that the use of IVIG plus infliximab as initial therapy reduces the need for additional therapy in KD patients presenting with CALs [39]. Hamada et al. demonstrated that treatment with IVIG plus cyclosporine was safe and effective for favorable coronary artery outcomes in high-risk KD patients [40]. Notably, KD patients who are predicted to be at high risk require the use of a more potent treatment in addition to or instead of treatment with steroids/IVIG.

In the low-risk patients, the TN-C level upon admission was not significantly differenent between the initial treatment-responsive and initial treatment-resistant patients, suggesting that TN-C may not be a predictor of IVIG-resistance in this group. Thus, the TN-C level may be useful as a predictive biomarker in only the high-risk cases.

In both high-risk and low-risk patients, the first-line therapy significantly reduced the TN-C levels in the initial treatment-responsive patients but did not in the initial treatment-resistant patients. The patients with higher TN-C levels after the initial treatment needed a second-line therapy. These findings suggest that the TN-C level reflects the effectiveness of the treatment and could be used as a rationale for commencing additional therapy. Because steroids often mask the symptoms of inflammation, their use can make it difficult for physicians to accurately judge whether the inflammation has decreased or if further intervention is needed. Based on the findings of the present study, the TN-C level might be useful as an indicator of whether additional treatment is needed.

In this study, there was no difference in TN-C levels of patients with or without CALs. This could be because the number of CALs in our enrolled patients was too small for accurate evaluatation. However, it is known that treatment resistance is closely related to CAL onset [6–8]. If some biomarkers, such as TN-C, could predict a severe course of disease, it would be expected that more aggressive initial/additional treatment might reduce the incidence of CALs.

There are some limitations of this study. A sample size that might not be large enough to determine severe cases who need additional treatment. Among our enrolled subjects, there were 37 cases with high-risk patients, of which only 10 cases (5% of all subjects) did not respond to steroid combination therapy. Because only 5% of our subjects had such refractory cases, more subjects should be recruited for future

studies. Second, a total of 199 patients (52%) were excluded because of not meeting the criteria. However, there were no significant differences in age, Kobayashi score and labolatory data between all 380 patients and 181 subjects. It seems no bias in patient distribution.

# Conclusions

The serum level of TN-C could be used as a biomarker for predicting KD severity. The early identification of severe cases who are resistant to steroid combination therapy among high-risk patients could help to prevent CALs through the application of an aggressive treatment strategy. It is expected that severity diagnosis using biomarkers such as TN-C will be added in the treatment guidelines for acute KD worldwide.

# Abbreviations

TN-C
Tenascin-C; KD:Kawasaki disease; IVIG:intravenous immunoglobulin; CALs:coronary artery lesions

# Declarations

*Ethics approval and consent to participate:* This study had been approved by the ethics committee in each institute. Written informed consent was obtained from the participants or their parents/guardians.

*Consent for publication:* Not applicable.

*Availability of data and materials:* Not applicable.

*Competing interests:* The authors declare that they have no competing interests.

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*Authors' contributions:* YY drafted the manuscript and contributed to the data collection. YO and TM (No 9 out of 11) performed the TN-C measurements, interpreted the statistical analysis and contributed the data collection. TM (No 3 out of 11), JH, TK and TA contributed to the data collection. FR contributed to the study design, analysis and the data collection. SK, HM and KIY contributed to the study design and analysis. KIY and YY are project leaders. All authors read and approved the final manuscript.

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# References

1. Burns JC, Glode MP. Kawasaki syndrome. Lancet. 2004;364:533–44.
2. Furusho K, Kamiya T, Nakano H, Kiyosawa N, Shinomiya K, Hayashidera T, et al. High-dose intravenous gamma globulin for Kawasaki disease. Lancet. 1984;2:1055–8.
3. Newburger JW, Takahashi M, Beiser AS, Burns JC, Bastian J, Chung KJ, et al. A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. N Engl J Med. 1991;324:1633–9.
4. Makino N, Nakamura Y, Yashiro M, Ae R, Tsuboi S, Aoyama Y, et al. Descriptive epidemiology of Kawasaki disease in Japan, 2011–2012: From the results of the 22nd nationwide survey. J Epidemiol. 2015;25:239–45.
5. Burns JC, Capparelli EV, Brown JA, Newburger JW, Glode MP., US/Canadian Kawasaki Syndrome Study Group. Intravenous gamma-globulin treatment and retreatment in Kawasaki disease Pediatr Infect. Dis J. 1998;17:1144–8.
6. Durongpisitkul K, Soongswang J, Laohaprasitiporn D, Nana A, Prachuabmoh C, Kangkagate C. Immunoglobulin failure and retreatment in Kawasaki disease. Pediatr Cardiol. 2003;24:145–8.
7. Egami K, Muta H, Ishii M, Suda K, Sugahara Y, Iemura M, et al. Prediction of resistance to intravenous immunoglobulin treatment in patients with Kawasaki disease. J Pediatr. 2006;149:237–40.
8. Kobayashi T, Inoue Y, Takeuchi K, Okada Y, Tamura K, Tomomasa T, et al. Prediction of intravenous immunoglobulin unresponsiveness in patients with Kawasaki disease. Circulation. 2006;113:2606–12.
9. Sano T, Kurotobi S, Matsuzaki K, Yamamoto T, Maki I, Miki K, et al. Prediction of non-responsiveness to standard high-dose gamma-globulin therapy in patients with acute Kawasaki disease before starting initial treatment. Eur J Pediatr. 2007;166:131–7.
10. Saji T, Ayusawa M, Miura M, Kobayashi T, Suzuki H, Mori M, et al. The clinical guideline for medical treatment of acute stage Kawasaki disease from the scientific committee, the Japanese society of pediatric cardiology and cardiac surgery. Pediatric Cardiology and Cardiac Surgery. 2012 (28) Suppl. 3:s1–s28.
11. Kobayashi T, Saji T, Otani T, Takeuchi K, Nakamura T, Arakawa H, et al. Efficacy of immunoglobulin plus prednisolone for prevention of coronary artery abnormalities in severe Kawasaki disease (RAISE study): a randomized, open-label, blinded –endpoints trial. Lancet. 2012;379:1613–20.

12. Miyata K, Kaneko T, Morikawa Y, Sakakibara H, Matsushima T, Misawa M, et al. Efficacy and safety of intravenous immunoglobulin plus prednisolone therapy in patients with Kawasaki disease (Post RAISE): a multicenter, prospective cohort study. Lancet Child Adolese Health. 2018.
13. Ogata S, Ogihara Y, Honda T, Kon S, Akiyama K, Ishii M. Corticosteroid pulse combination therapy for refractory Kawasaki disease: a randomized trial. Pediatrics. 2012;129:e17–23.
14. Ebato T, Ogata S, Ogihara Y, Fujimoto M, Kitagawa A, Takahashi M, et al. The Clinical Utility and Safety of a New Strategy for the Treatment of Refractory Kawasaki Disease. J Pediatr. 2017;191:140–4.
15. Kawasaki Disease National Survey Group. Kosami K, Yashiro M, Makino N, Nakamura Y. 25th Kawasaki Disease National Survey Results. J Pediatr Practice. 2020; 83(2): 269–73.
16. Davies S, Sutton N, Blackstock S, Gormley S, Hoggart CJ, Levin M, et al. Predicting IVIG resistance in UK Kawasaki disease. Arch Dis Child. 2015;100:366–8.
17. Sanchex MJ, Anton J, Bou R, Iglesias E, Calzada HJ, Borlan S, et al. Kawasaki Disease in Catalonia Working Group Role of the Egami score to predict immunoglobulin resistance in Kawasaki disease among a Western Mediterranean population. Rheumatol Int. 2016;36:905–10.
18. Lee SM, Lee JB, Go YB, Song HY, Lee BJ, Kwak JH. Prediction of resistance to standard intravenous immunoglobulin therapy in Kawasaki disease. Korean Circ J. 2014;44(6):415–22.
19. Shao S, Luo C, Zhou K, Hua Y, Wu M, Liu L, et al. Predictive value of serum procalcitonin for both initial and repeated immunoglobulin resistance in Kawasaki disease: a prospective cohort study. Pediatr Rheumatol Online J. 2019;17(1):78.
20. Midwood KS, Hussenet T, Langlois B, Orend G. Advances in Tenascin-C biology. Cell Mol Life Sci. 2011;68:3175–99.
21. Imanaka-Yoshida K. Tenascin-C in cardiovascular tissue remodeling: From development to inflammation and repair. Circ J. 2012;76:2513–20.
22. Imanaka-Yoshida K, Yoshida T, Miyagawa-Tomita S. Tenascin-C in development and disease of blood vessels. Anat Rec (Hoboken). 2014;297:1747–57.
23. Midwood KS, Chiquet M, Tucker RP, Orend G. Tenascin-C at a glance. J Cell Sci. 2016;129(23):4321–7.
24. Giblin SP, Midwood KS. Tenascin-C: Form versus function. Cell Adh Migr. 2015;9:48–82.
25. Spenle C, Saupe F, Midwood K, Burckel H, Noel G, Orend G. Tenascin-C: Exploitation and collateral damage in cancer management. Cell Adh Migr. 2015;9:141–53.
26. Kimura T, Tajifi K, Sato A, Sakai S, Wang Z, Yoshida T, et al. Tenascin-C accelerates adverse ventricular remodeling after myocardial infarction by modulating macrophage polarization. Cardiovasc Res. 2019;115(3):614–24.
27. Fujimoto N, Onishi K, Sato A, Terasaki F, Tsukada B, 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. Jã Cardiac Fail. 2009;15:898–905.

28. Sato A, Aonuma K, Imanaka-Yoshida K, Yoshida T, Isobe M, Kawase D, 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.
29. NozatoT, Sato A, Hirose S, Hikita H, Takahashi A, Endo H, et al. Preliminary study of serum Tenascin-C levels as a diagnostic or prognostic biomarker of type B acute aortic dissection. Int J Cardiol. 2013;168:4267–9.
30. Nozato T, Sato A, Hikita H, Takahashi A, Imanaka-Yoshikda K, Yoshida T, et al. Impact of serum Tenascin-C on the aortic healing process during the chronic stage of type B acute aortic dissection. Int J Cardiol. 2015;191:97–9.
31. Gao W, Li J, Ni H, Shi H, Qi Z, Zhu S, et al. Tenascin C: A potential biomarker for predicting the severity of coronary atherosclerosis. J Atheroscler Thromb. 2019;26:31–8.
32. Yoshikane Y, Koga M, Imanaka-Yoshida K, Cho T, Yamamoto Y, Yoshida T, et al. JNK is critical for the development of Candida albicans-induced vascular lesions in a mouse model of Kawasaki disease. Cardiovasc Pathol. 2015;24(1):33–40.
33. Okuma T, Suda K, Hideyuki N, Katsube Y, Mitani Y, Yoshikane Y, et al. Serum Tenascin-C as a novel predictor for risk of coronary artery lesion and resistance to intravenous immunoglobulin in Kawasaki disease -A multicenter retrospective study-. Circ J. 2016;80:2376–81.
34. Ayusawa M, Sonobe T, Uemura S, Ogawa S, Nakamura Y, Kiyosawa N, et al. Revision of diagnostic guidelines for Kawasaki disease (the 5th revised edition). Pediatr Int. 2005;46:232–4.
35. Saji T, Ayusawa M, Miura M, et al Kobayashi T, Suzuki H, et al. The Clinical Guideline for Medical Treatment of Acute Stage Kawasaki Disease from the Pediatric Cardiology and Cardiac Surgery. 2012 (28) Suppl. 3:s1–s28.
36. Kobayashi T, Fuse S, Sakamoto N, Mikami M, Ogawa S, Hamaoka K, et al. A new Z score curve of the coronary arterial internal diameter using the Lambda-Mu Sigma Method in a pediatric population. J Am Soc Echocardiogr. 2016;29(8):794–801.e29.
37. Takahashi K, Oharaseki T, Yokouchi Y, Naoe S, Saji T. Kawasaki disease: basic and pathological findings. Clin Exp Nephrol. 2013;14(5):690–3.
38. Yokouchi Y, Oharaseki T, Enomoto Y, Sato W, Imanaka-Yoshida K, Takahashi K. Expression of tenascin C in cardiovascular lesions of Kawasaki disease. Cardiovasc Pathol. 2019;38:25–30.
39. Jone PN, Anderson MS, Mulvahill MJ, Heizer H, Glode MP, Dominquez SR. Infliximab plus intravenous immunoglobulin (IVIG) Versus IVIG alone as initial therapy in children with Kawasaki disease presenting with coronary artery lesions: is dual therapy more effective? Pediatr Infect Dis J. 2018;37(10):976–80.
40. Hamada H, Suzuki H, Onouchi Y, Ebata R, Terai M, Fuse S, et al. Efficacy of primary treatment with immunoglobulin plus cyclosporine for prevention of coronary artery abnormalities in patients with Kawasaki disease predicted to be at increased risk of nonresponse to intravenous immunoglobulin (KAICA): a randomized controlled, open-label, blinded-endpoints, phase 3 trial. Lancet. 2019;393(10176):1128–37.

# Figures

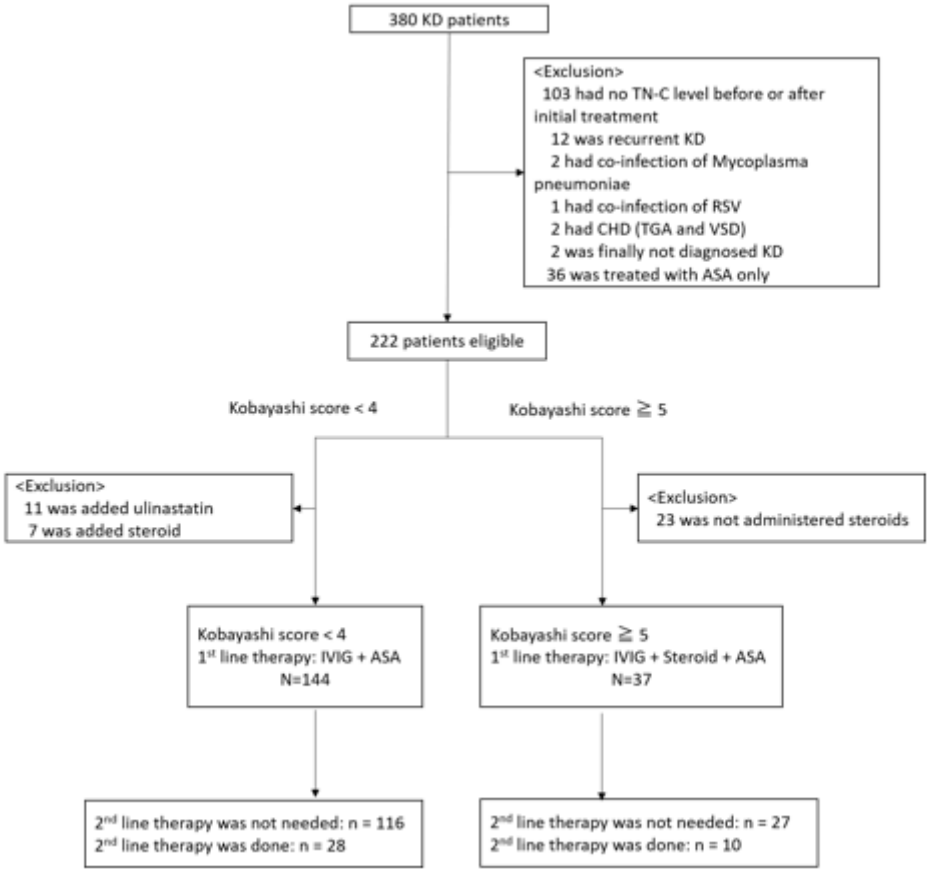
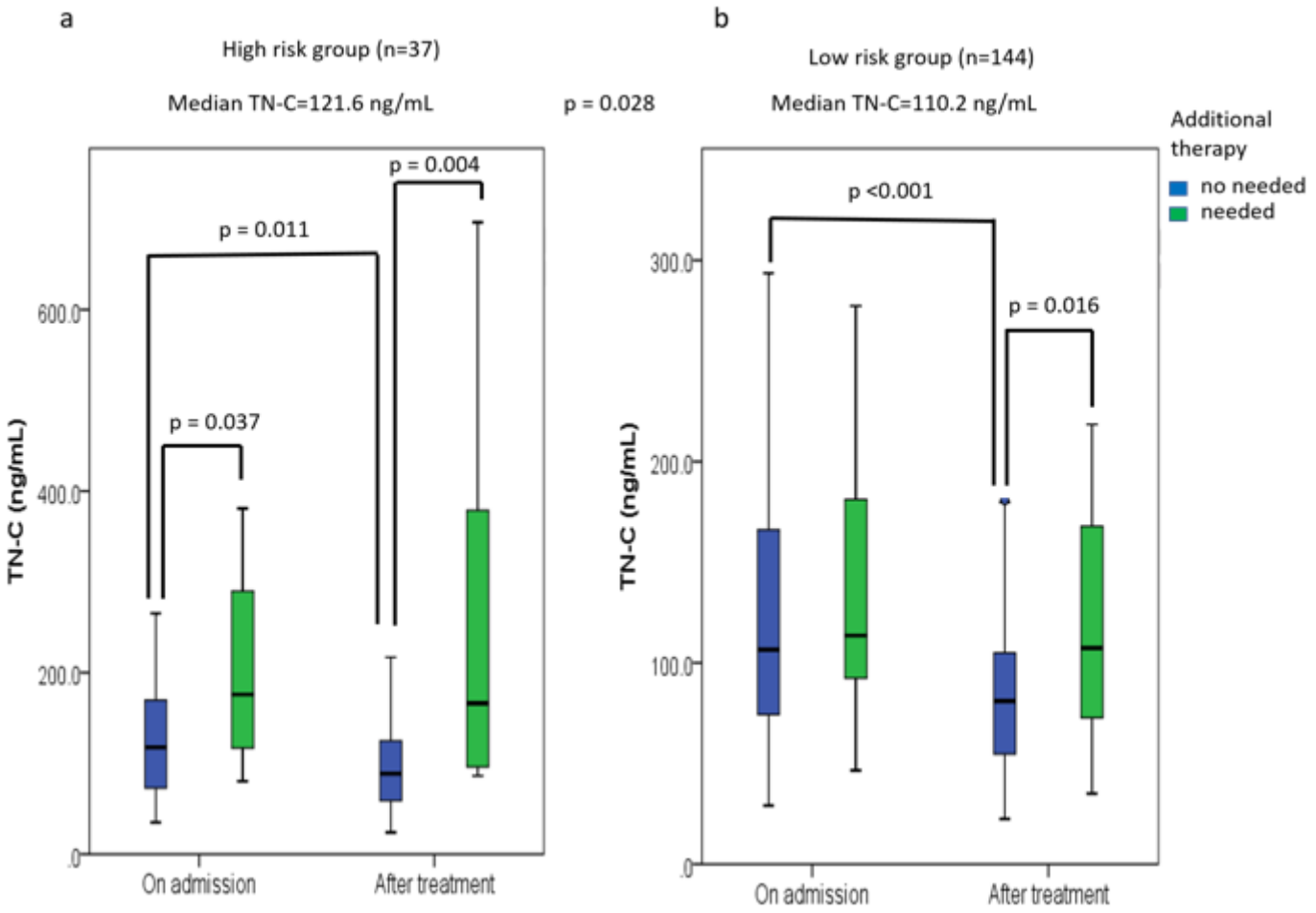



**Figure 1**

Study population. Eligible patients were classified into four groups: low-risk initial treatmeant-responsive group (n = 116), low-risk initial treatment-resistant group (n = 28), high-risk initial treatment-responsive group (n = 27), and high-risk initial treatment-resistant group (n = 10).




## Figure 2

TN-C levels in high- and low-risk patients. (a–b) Median levels of TN-C in high-risk (a) and low-risk (b) patients. “On admission” means “before initial treatment”, and “after treatment” means “after initial treatment”.