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Predictive value of coagulation profiles for both initial and repeated immunoglobulin resistance in Kawasaki disease: A prospective cohort study

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Abstract

Background: Intravenous immunoglobulin (IVIG) resistance prediction remains substantial in Kawasaki disease (KD), with limited data on the predictive value of coagulation profile for IVIG resistance, particularly for repeated IVIG resistance. Therefore, the aim of our study was to testify the predictive validity of coagulation profile for both initial IVIG resistance and repeated IVIG resistance in KD.

Methods: A total of 385 KD patients were prospectively recruited between April 2015 and May 2019. Coagulation and other profiles were evaluated between the IVIG-responsive and IVIG-resistant groups. Multivariate logistic regression analysis was applied to determine the association between coagulation profiles and IVIG resistance. ROC curves analysis was further performed to assess the validity of coagulation profiles in predicting both initial IVIG resistance and repeated IVIG resistance.

Results: Prothrombin time (PT), activated partial thromboplastin time (APTT), international normalized ratio (INR), fibrinogen degradation products (FDPs), and D-dimer were significantly increased in the initial IVIG-resistant group with antithrombin III

(ATIII) and thrombin time (TT) significantly reduced. Meanwhile, ATIII was declined markedly in repeated IVIG-resistant patients. Multivariate logistic regression analysis showed that PT, APTT, D-dimer, and ATIII were independent risk factors for predicting initial IVIG resistance and ATIII for predicting repeated IVIG-resistant patients with KD. PT, APTT, D-dimer, and ATIII cutoff values of 13.95 s, 41.15 s, 1.48 mg/L, and 89.5% yielded sensitivities of 73%, 32%, 71%, and 81%, and specificities of 55%, 88%, 62%, and 51% for predicting initial IVIG resistance, respectively. The cutoff value of ATIII for predicting repeated IVIG resistance was 68.5%, with sensitivity of 71% and specificity of 55%.

Conclusions: KD patients who have hypercoagulation during the acute phase might be at higher risk of developing IVIG resistance.

KEYWORDS

Antithrombin III, coagulation profile, immunoglobulin resistance, kawasaki disease, prediction

Key Message

Currently, studies elucidating the association between the coagulation profile and Kawasaki disease (KD), especially with regard to IVIG resistance, are limited. To the best of our knowledge, this is the first study to clarify the association between coagulation and Intravenous immunoglobulin (IVIG) resistance in patients with KD based on a relatively large clinical dataset. Our results suggest that patients with impaired coagulation who are resistant to IVIG (initial and/or repeated) may need more aggressive treatment to reduce the likelihood of developing coronary artery lesions (CALs).

1 | INTRODUCTION

Kawasaki disease (KD) is an acute vasculitis that is the leading cause of acquired heart disease in children, with approximately 15%-20% of patients with KD suffering intravenous immunoglobulin (IVIG) resistance.[1] For children who do not respond to initial IVIG treatment, repeated IVIG infusion is recommended by many experts.[2] However, approximately 10% of patients are resistant to both initial IVIG therapy and repeated IVIG therapy,[3] and thus have a higher risk of coronary artery lesions (CALs). Therefore, early identification of both initial IVIG resistance and repeated IVIG resistance is of great importance to reduce CALs, and, most importantly, lower medical costs.

It has long been known that inflammation triggered by acute infection can lead to the activation of the coagulation system by upregulating the expression of cytokines.[4] In an investigation of critically ill patients, Ogura et al[5] found that abnormal coagulation was associated with increased systemic inflammatory response syndrome scores. In addition, the relationship between the activation of the immune system and coagulation system is evident in systemic autoimmune or immune-mediated diseases.[6] Therefore, the balance between coagulation and the fibrinolytic system may be disturbed during the acute stages of KD, particularly in patients with IVIG resistance, since KD vasculitis is accompanied by an increase in inflammatory cells and cytokines, and IVIG resistance may reflect a more severe inflammatory immune condition. Indeed, several studies have previously explored the coagulation profiles in patients with KD.[7-12] However, most of them focused on the changes in coagulation profiles before and after IVIG in KD,[10] the role of coagulation profiles in distinguishing atypical KD from bacterial cervical lymphadenitis,[12] and in predicting the occurrence of CALs or cardiac complications[9-11]. The role of coagulation profile in initial IVIG resistance prediction was only explored in two studies.[7,8] Nevertheless, both of them were limited by small sample size and retrospective nature. Most importantly, the results from them were inconsistent and the predictive value of ATIII has never been explored before. Furthermore, data on the validity of coagulation profiles in repeated IVIG response prediction are lacking. Therefore, a large prospective cohort study was conducted to assess the predictive validity of the serum coagulation profile, including prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), international normalized ratio (INR), and fibrinogen, D-dimer, fibrin degradation products (FDPs), and antithrombin III (ATIII) levels for identifying patients with KD at risk of both initial IVIG resistance and repeated IVIG resistance.

# 2 | MATERIALS AND METHODS

2.1 | Subjects

Patients with KD were prospectively recruited between April 2015 and May 2019 at our hospital. A diagnosis of KD relied on

the standards recommended by the American Heart Association's Scientific Statement for the diagnosis, treatment, and long-term management of KD[13] and was confirmed by two experienced pediatricians (at least one was a KD specialist). Informed written consent was obtained from the parents after the nature of the study had been fully explained to them. The University Ethics Committee on Human Subjects at Sichuan University approved the study.

Exclusion criteria included known patients with congenital or chronic hematologic disease affecting the coagulation cascade; patients with end-stage renal disease requiring dialysis, acute or chronic liver failure, and autoimmune disease; patients who had undergone surgery recently; patients with infectious or inflammatory diseases; and patients who received oral anticoagulant or heparin therapy. A total of 520 patients diagnosed with KD were initially screened for participation in this study. Of these, patients who had received initial IVIG treatment at other medical facilities (n = 87), received IVIG treatment within 10 days of fever onset (n = 12), or cases where IVIG treatment was initiated before blood sampling (n = 17) were excluded. Another 19 patients were excluded because of incomplete laboratory data or lack of follow-up results (Figure 1). Finally, data from 385 patients were analyzed.

All patients received 2 g/kg of IVIG for 24 hours and 30-50 mg/kg/day of aspirin until they were afebrile. Initial IVIG resistance was defined as recurrent or persistent fever or other clinical signs
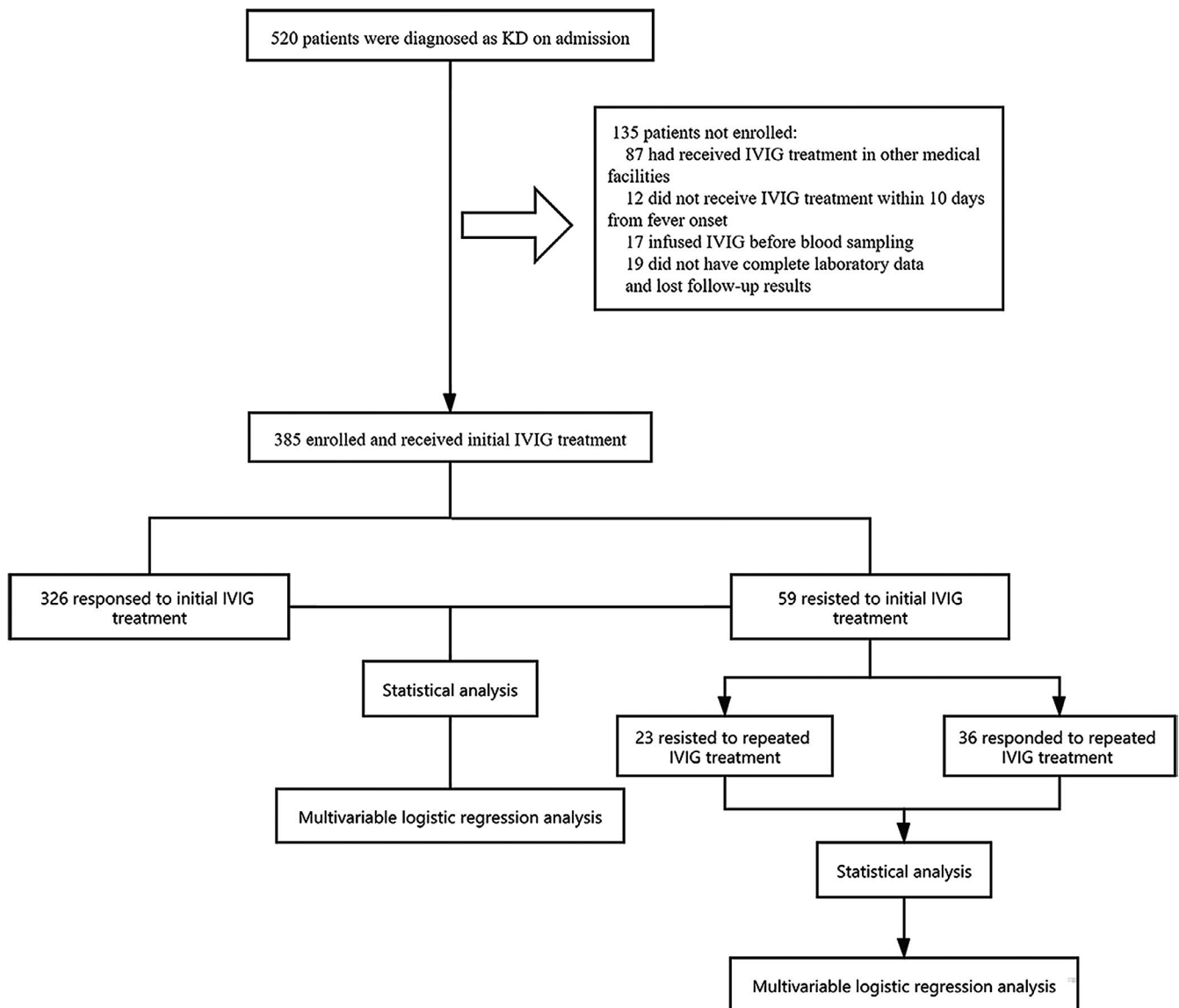
FIGURE 1 The flowchart of our prospective study. A total of 520 patients diagnosed with KD were initially screened for participation in this study. Of these, patients who had received initial IVIG treatment at other medical facilities (n = 87), received IVIG treatment within 10 days of fever onset (n = 12), or cases where IVIG treatment was initiated before blood sampling (n = 17) were excluded. Another 19 patients were excluded because of incomplete laboratory data or lack of follow-up results. Finally, data from 385 patients were analyzed. Of the 385 patients, 326 (84.7%) responded to initial IVIG treatment, whereas 59 (15.3%) did not respond. Of the 59 patients with initial IVIG resistance, 23 did not respond to repeated IVIG treatment and received pulse intravenous methylprednisolone infusion. Then, data analysis and multivariate logistic regression analysis were performed in these groups

of KD for at least 36 hours but not longer than 7 days after initial IVIG treatment. For patients with initial IVIG resistance, the second IVIG dose (2 g/kg given as a single intravenous infusion) was administered according to expert consensus on the diagnosis and treatment of KD in China. Furthermore, pulse intravenous methylprednisolone (10-30 mg/kg/day for three consecutive days) followed by oral prednisone (2 mg/kg/day) tapered over seven days was additionally administered if the patient had recurrent or persistent fever even after the second IVIG administration. No patients received any additional treatment such as infliximab, plasma exchange, or cytotoxic agents.

According to institutional protocol, standardized echocardiograms were obtained from patients by the same experienced pediatric ultrasonologists during the acute/subacute phase and 6-8 weeks later in the cardiology clinic during follow-up evaluations until the coronary artery abnormalities were resolved. CALs were defined based on the normalization of dimensions for body surface area as Z-scores (standard deviation units from the mean, normalized for body surface area): no involvement (Z-score <2.0), dilation (Z-score ≥2.0 to <2.5), and aneurysm (Z-score ≥2.5; Z-score ≥10 for giant aneurysms) of the coronary arteries depending on the maximal internal diameters of the right coronary, left anterior descending, and left circumflex coronary arteries.

Patients were subsequently categorized into two groups depending on whether they responded to the initial IVIG treatment (initial IVIG-responsive group, n = 326; initial IVIG-resistant group, n = 59). The initial IVIG-resistant group was further divided into two subgroups based on the effectiveness of repeated IVIG treatment [repeated IVIG-responsive group (n = 36) and repeated IVIG-resistant group (n = 23)].

2.2 | Laboratory measurements

Coagulation analyses of the patient's plasma, including PT, APTT, TT, fibrinogen, D-dimer, FDP, international normalized ratio, and ATIII activity, were performed before initial IVIG within 10 days from fever onset. Standard coagulation measurements were performed with a CoagXL-automated coagulometer (Diagon Ltd.) using reagents from Diagon.

2.3 | Statistics

Data analyses were performed using SPSS version 21.0 (SPSS Inc.). Quantitative data are presented as the medians with the 25th and 75th percentiles in square brackets, while qualitative data are expressed as the number or percentage as appropriate. A chi-squared test and unpaired Student's *t* test or Mann-Whitney *U* test were used to compare the demographic characteristics, clinical manifestations, and laboratory data. Significant indicators from univariate analysis were then subjected to multivariate logistic regression analysis to identify independent predictors of IVIG resistance. Receiver operating characteristic (ROC) curve analysis was used to evaluate the

value of coagulative biomarkers for predicting the development of initial and repeated IVIG resistance. A P value <.05 was considered statistically significant.

3 | RESULTS

3.1 | Subjects

CALs were observed in 47 patients (12.2%), while transient pericardial effusion, valve regurgitation, cardiac enlargement, and ventricular systolic dysfunction were noted in 11, 40, 38, and 2 children, respectively. No significant difference was found in serum coagulation profiles between the CAL and non-CAL groups. (Supplemental material 1).

3.2 | Serum coagulation levels for predicting initial IVIG resistance

As shown in Table 1, there were no significant differences in age, sex, the day of illness before IVIG treatment, sampling day of illness, and typical clinical manifestations between the initial IVIG-responsive and IVIG-resistant groups. As for other cardiac complications, the percentage of patients with cardiac enlargement and pericardial effusion was found to be significantly higher in the initial IVIG-resistant group (P = .003 and P = .016, respectively). Initial IVIG-resistant patients had much higher incidence of CALs, with substantial higher level of serum C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), serum total bilirubin (TB), serum alanine aminotransferase (ALT), creatinine, and urea nitrogen, but with lower hemoglobin, platelet count, albumin, sodium, and potassium before the initial IVIG treatment (all P < .05). No significant differences were found in the white blood cell count, erythrocyte sedimentation rate, and aspartate aminotransferase (AST) between the two groups.

The effect of KD on the coagulation system was evaluated by assessing the coagulation profiles of initial IVIG-responsive and IVIG-resistant subjects. The PT (14.3 [13.6-15.5] s vs 13.8 [13-14.4] s, P = .005) and APTT (35.3 [31.9-44.7] s vs 34.2 [30.7-38.1] s, P = .006) were significantly longer, and TT (16.0[15.8-16.4]s vs 16.5[16-16.9]s, P < .001) was significantly shorter in the initial IVIG-resistant group, with significantly higher INR (1.19[1.13-1.30] vs 1.13[1.06-1.20], P < .001), FDP (8.7[6.2-12.5]ug/mL vs 5.9[3.8-9.8] ug/mL, P < .001), D-dimer levels (2.1 [1.20-2.82] mg/L vs 1.2 [0.8-2.07] mg/L, P = .020), and ATIII activity (81% [65%-88%] vs 89% [81%-100%], P < .001) (Table 1).

3.3 | Multivariate logistic regression and ROC curve analysis for predicting initial IVIG resistance

To not affect the prediction efficiency, the correlation between univariate factors and coagulation profiles was tested by Pearson's

TABLE 1 Comparison of clinical data between the initial intravenous immunoglobulin (IVIG)-resistant and IVIG-responsive groups in Kawasaki disease

	IVIG-resistant (n = 59)	IVIG-responsive (n = 326)	P value
Age (mo)	28.0 [17.0-60.0]	25.00 [14.00-43.00]	.396
Male (%)	29 (49.2)	187 (57.4)	.242
Day of illness before IVIG	5.00 [4.00-6.00]	5.00 [5.00-6.00]	.170
Sampling day of illness, days	4.00 [3.00-5.00]	5.00 [4.00-5.00]	.446
Clinical manifestations			
Rash, n (%)	51 (86.4)	262 (80.4)	.271
Bilateral bulbar conjunctive injection, n (%)	52 (88.1)	301 (92.3)	.304
Erythema of oral and pharyngeal mucosa, n (%)	54 (91.5)	296 (90.8)	.858
Edema and erythema of the extremities, n (%)	31 (52.5)	188 (57.7)	.464
Cervical lymphadenopathy, n (%)	31 (52.5)	136 (41.7)	.123
Incomplete KD, n (%)	19 (32.2)	123 (37.7)	.418
Pericardial effusion (%)	5 (8.5)	6 (1.8)	.016[*]
Valve regurgitation (%)	9 (15.3)	31 (9.5)	.183
Cardiac enlargement (%)	12 (20.3)	26 (8.0)	.003[*]
Coronary artery lesions (CALs), n (%)	12 (20.3)	22 (6.7)	.001[*]
Laboratory features			
WBC count (10^9/L)	13.6 [9.8-16.0]	13.30 [10.9-16.53]	.312
NLR	5.2 [3.57-10.83]	2.73 [1.73-4.93]	<.001[a,*]
Hemoglobin (g/L)	104.00 [98.00-114.00]	109.00 [101.00-116.00]	.031[*]
PLT count (10^9/L)	295.00 [233.00-343.00]	318.00 [267.75-395.00]	<.001[*]
AST (IU/L)	37.00 [24.00-73.00]	33.00 [25.00-47.00]	.056
ALT (IU/L)	54.00 [27.0-123.0]	37.50 [21.00-83.00]	.004[*]
ALB (g/L)	33.6 [29.7-38.90]	38.25 [35.30-41.20]	<.001[*]
Total bilirubin (mg/L)	7.7 [4.7-20.3]	5.95 [3.70-8.43]	.002[*a*]
Creatinine (umol/L)	27.00 [24.00-33.00]	24.00 [20.75-29.00]	.002[*]
Urea nitrogen (mmol/L)	3.03 [2.3-4.0]	2.70 [2.11-3.30]	.016[*]
Sodium (mmol/L)	134.00 [131.9-137.00]	137.00 [135.00-139.00]	<.001[*]
Potassium (mmol/L)	3.78 [3.40-4.2]	4.17 [3.78-4.51]	<.001[*]
ESR (mm/h)	67.00 [46.0-82.00]	66.00 [46.25-83.75]	.820
CRP (mg/L)	96.00 [62.00-153.00]	73.70 [43.75-107.00]	.001[*]
PT (s)	14.3 [13.6-15.5]	13.8 [13-14.4]	<.001[a,*]
APTT (s)	35.3 [31.9-44.7]	34.2 [30.7-38.1]	.015[a,*]
TT (s)	16.0 [15.8-16.4]	16.5 [16-16.9]	<.001[a,*]
D-dimer (mg/L)	2.1 [1.20-2.82]	1.2 [0.8-2.07]	<.001[a,*]
Fibrinogen (mg/dL)	557 [484-632]	561 [469.75-653]	.800
FDP (ug/mL)	8.7 [6.2-12.5]	5.9 [3.8-9.8]	<.001[a,*]
ATIII (%)	81 [65-88]	89 [81-100]	<.001[a,*]
INR	1.19 [1.13-1.30]	1.13 [1.06-1.20]	<.001[a,*]

The data are presented as the median with the 25th and 75th percentiles in square brackets for continuous variables and as the percentage for the categorical variables.

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; AT, antithrombin; CALs, coronary artery lesionsCRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FDP, fibrin degradation products; INR, international normalized ratio; NLR, neutrophil-to-lymphocyte ratio; PLT, platelet; PT, prothrombin time; TT, total thrombin time; WBC, white blood cell.

[a]Variables between the two groups were compared by the Mann-Whitney U test due to abnormal data distribution.

*Statistically significant (P < .05)

TABLE 2 A multivariate logistic regression model for initial and repeated intravenous immunoglobulin (IVIG) resistance in Kawasaki disease

Variates	β	SE	Walds	P value	OR	95% CI
Initial IVIG resistance-PT						
Platelet	0.005	0.002	7.523	.006*	1.01	1.00-1.01
Creatinine	−0.034	0.020	2.830	.093	0.97	0.93-1.01
Urea nitrogen	−0.168	0.125	1.804	.179	0.85	0.66-1.08
ALT	−0.002	0.001	2.365	.124	0.99	0.99-1.00
PT	−0.091	0.041	5.060	.024*	0.91	0.84-0.99
Initial IVIG resistance-APTT						
Platelet	0.004	0.002	1.323	.038*	1.00	1.00-1.01
Creatinine	−0.019	0.022	0.707	.400	0.98	0.94-1.03
Urea nitrogen	−0.190	0.133	2.017	.152	0.83	0.64-1.07
ALT	−0.002	0.001	2.110	.146	0.99	0.99-1.00
Potassium	0.933	0.291	10.29	.001*	2.54	1.44-4.50
APTT	−0.051	0.019	7.033	.008*	0.95	0.92-0.99
Initial IVIG resistance-D-dimer						
Platelet	0.004	0.002	4.257	.039*	1.00	1.00-1.01
Creatinine	−0.016	0.023	0.532	.466	0.98	0.94-1.03
Urea nitrogen	−0.175	0.133	1.746	.186	0.84	0.65-1.09
ALT	0.000	0.001	0.350	.554	1.00	0.99-1.00
Potassium	0.953	0.300	10.06	.002*	2.59	1.44-4.67
Total bilirubin	−0.021	0.012	3.419	.064	0.98	0.96-1.00
D-dimer	−0.129	0.063	4.237	.040*	0.88	0.78-0.99
Initial IVIG resistance-ATIII						
Creatinine	−0.046	0.021	5.024	.025*	0.96	0.92-0.99
Urea nitrogen	−0.144	0.127	1.293	.256	0.87	0.68-1.11
ALT	−0.002	0.001	2.285	.132	1.00	0.99-1.00
ATIII	0.030	0.008	14.17	<.001*	1.03	1.01-1.05
Initial IVIG resistance-TT						
Hemoglobin	0.026	0.015	3.072	.080	1.023	1.00-1.06
Platelet	0.004	0.002	3.751	.053	1.00	1.00-1.01
Creatinine	−0.027	0.023	1.396	.237	0.97	0.93-1.02
Urea nitrogen	−0.173	0.134	1.667	.197	0.84	0.65-1.09
ALT	−0.001	0.001	0.303	.582	1.00	1.00-1.01
Potassium	0.782	0.306	6.512	.011*	2.19	1.12-3.98
Total bilirubin	−0.021	0.012	2.915	.088	0.98	0.96-1.00
TT	0.287	0.226	1.618	.203	1.33	0.86-2.07
Initial IVIG resistance-INR						
NLR	−0.476	0.491	0.941	.332	0.62	0.24-1.63
C-creative protein	0.000	0.004	0.000	.99	1.00	0.99-1.01
Platelet	0.004	0.002	4.000	.046*	1.00	1.00-1.01
Creatinine	−0.012	0.023	0.287	.592	0.99	0.92-1.03
Urea nitrogen	−0.070	0.133	0.274	.600	0.93	0.72-1.21
ALT	−0.001	0.001	0.160	.689	1.00	1.00-1.01
Potassium	0.706	0.325	4.712	.030*	2.03	1.07-3.83
Sodium	0.086	0.048	3.222	.073	1.09	0.99-1.20

(Continues)

TABLE 2 (Continued)

Variates	β	SE	Walds	P value	OR	95% CI
INR	−0.476	0.491	0.941	.332	0.62	0.24-1.63
Initial IVIG resistance-FDP						
NLR	−0.048	0.034	2.045	.153	0.95	0.89-1.02
C-creative protein	0.001	0.004	0.032	.857	1.00	0.99-1.01
Hemoglobin	0.025	0.015	2.638	.104	1.03	1.00-1.06
Platelet	0.003	0.002	3.400	.065	1.00	1.00-1.01
Creatinine	−0.009	0.004	0.032	.857	1.00	0.99-1.01
Urea nitrogen	−0.156	0.132	1.388	.239	0.86	0.66-1.11
ALT	−0.001	0.001	1.055	.304	1.00	1.00-1.01
Potassium	0.678	0.315	4.634	.031*	1.97	1.06-2.65
Sodium	0.096	0.048	4.028	.045*	1.10	1.00-1.21
FDP	−0.008	0.019	0.172	.679	0.99	0.96-1.03
Repeated IVIG resistance-ATIII						
ATIII	0.068	0.023	8.833	.003*	1.07	1.02-1.11

Abbreviations: ALT, alanine aminotransferaseAPTT, activated partial thromboplastin time; ATIII, antithrombin III; FDP, fibrin degradation products; INR, international normalized ratio; IVIG, intravenous immunoglobulin; PT, prothrombin time; TT, total thrombin time.
*Statistically significant ($P < .05$)

analysis. The strong variables were removed from the model (Supplemental material 2). Multivariate logistic regression analysis showed that longer PT, APTT, higher D-dimer, and lower ATIII activity were independent risk factors for initial IVIG resistance in patients with KD, whereas INR, TT, and FDP failed to reach significant difference in the multivariate logistic regression analysis (Table 2).

The validity of the serum coagulation profile in predicting initial IVIG resistance in patients with KD was assessed using ROC curve analysis. The areas under the curve (AUC) of the various variables for predicting IVIG resistance were 0.662, 0.599, 0.684, and 0.706 for PT, APTT, D-dimer, and ATIII, respectively. The PT, APTT, D-dimer, and ATIII cutoff values of 13.95 s, 41.15 s, 1.48 mg/L, and 89.5% yielded sensitivities of 73%, 32%, 71%, and 81%, and specificities of 55%, 88%, 62%, and 51% for predicting initial IVIG resistance, respectively (Table 3 and Figure 2A).

3.4 | Serum coagulation levels for predicting repeated IVIG resistance

A comparison of clinical data between the repeated IVIG-responsive (n = 33) and repeated IVIG-resistant (n = 26) groups is shown in Table 4. The repeated IVIG-resistant subjects had lower ATIII activity than those of repeated IVIG-responsive subjects (65% [60%-81%] vs 84% [76.23%-89.75%], P = .001). There was no significant difference in any other examined parameters between the two groups. Multivariate logistic regression analysis showed that lower ATIII activity was independent risk factor for repeated IVIG resistance in patients with KD (Table 2).

3.5 | ROC curve analysis for predicting repeated IVIG resistance

ROC curves were used to calculate the AUC and predictive values of each coagulation parameter (Table 3 and Figure 2B). The AUC of ATIII for predicting repeated-KD patients was 0.753. In repeated-KD patients, the cutoff value of ATIII was 68.5%, which yielded a sensitivity of 71% and specificity of 55% for predicting repeated IVIG resistance.

4 | DISCUSSION

To the best of our knowledge, this is the first study to clarify the association between coagulation and IVIG resistance in patients with KD based on a relatively large clinical dataset. In this prospective study, we support the hypothesis that there are marked differences in coagulation profiles, including longer PT and APTT, higher INR, FDP, D-dimer levels, shorter TT, and lower ATIII activity, that may predict initial IVIG resistance. In addition, using multivariate logistic regression analysis, we found that longer PT, APTT, higher D-dimer, and lower ATIII activity before initial IVIG were significant independent risk factors for initial IVIG resistance. Furthermore, significantly lower ATIII activity was found in patients with repeated IVIG resistance, and multivariate logistic regression analysis showed that lower ATIII activity was independent risk factor for predicting repeated IVIG resistance. Our results suggest that patients with impaired coagulation who are resistant to IVIG (initial and/or repeated) may need more aggressive treatment to reduce the likelihood of developing CALs.

TABLE 3 The validity of coagulation profiles in predicting initial and repeated intravenous immunoglobulin (IVIG) resistance

Initial IVIG resistance	Diagnostic test	Gold standard			Sen	Spe	PPV	NPV	Diagnostic accuracy	OR (95% CI)	P
Total group (n = 385)	PT ≥13.95s	Positive	43	147	0.73	0.55	0.23	0.92	0.58	3.27 (1.77-6.05)	<.001*
		Negative	16	179							
	APTT ≥41.15s	Positive	19	40	0.32	0.88	0.32	0.88	0.79	3.40(1.79-6.43)	<.001*
		Negative	40	286							
	D-dimer ≥1.48mg/l	Positive	42	125	0.71	0.62	0.25	0.92	0.63	3.97(2.17-7.28)	<.001*
		Negative	17	201							
	ATIII ≤89.5%	Positive	48	160	0.81	0.51	0.23	0.94	0.56	4.53(2.27-9.03)	<.001*
		Negative	11	166							
Repeated group (n = 59)	ATIII ≤68.5%	Positive	42	146	0.71	0.55	0.22	0.91	0.58	3.05(1.67-5.57)	<.001*
		Negative	17	180							

Abbreviations: APTT, activated partial thromboplastin time; AT, antithrombin; IVIG, intravenous immunoglobulin;PT, prothrombin time. CI, confidence ratio; NPV: negative predictive value; OR, odds ratio; PPV: positive predictive value; Sen: sensitivity; Spe: specificity
*Statistically significant (P < .05).
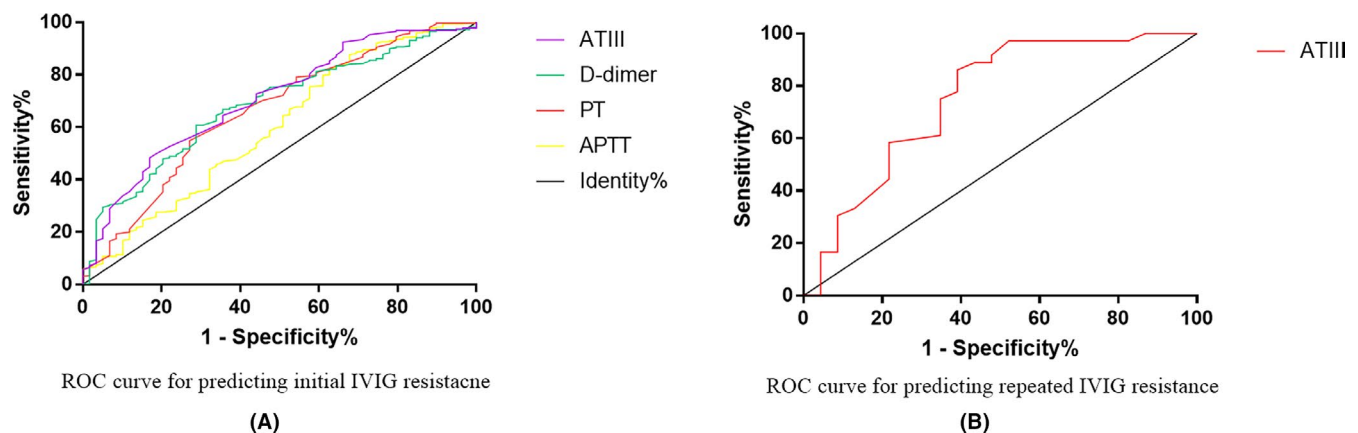
FIGURE 2 The receiver operating characteristic (ROC) curve for coagulation profiles in predicting initial and repeated IVIG resistance. (A) ROC curve for coagulation profiles [prothrombin time (PT), activated partial thromboplastin time (APTT), antithrombin III (ATIII), D-dimer] in predicting initial IVIG resistance. (B) ROC curve for ATIII in predicting repeated IVIG resistance

Although the underlying mechanisms regarding the impaired blood coagulation in KD are not entirely clear, several explanations could be hypothesized. Firstly, the pathophysiologic basis of KD is systemic, medium-sized vasculitis with inflammatory hypercytokinemia.[14] The inflammatory cytokines such as TNF-α and IL-6 would excessively release in the acute phase of KD.[15,16] The cytokine profile may reflect the disease severity and is associated with the development of IVIG resistance.[17] In the present study, higher NLR, AST, ALT, and CRP indicate more severe inflammation of patients in the IVIG-resistant group than those in the IVIG-responsive group. Moreover, vascular inflammation enhances vascular permeability. Higher prevalence of pericardial effusion of the IVIG-resistant group might reflect more severe TNF-α-mediated vasculitis.[15] The clinical importance of the interaction between inflammation and coagulation is widely accepted recently.[18] Higher pro-inflammatory cytokine levels including TNF-α, IL-6, and IL-8 were found in those with higher D-dimer levels. TNF-α, one of the leading cytokines of KD, could

induce vascular endothelial cell injury via oxidative stress, inflammation, and apoptosis.[19] Vascular endothelium is luminary covered by network of membrane-bound proteoglycans and glycoproteins (glycocalyx).[20,21] If glycocalyx is damaged by KD vasculitis, anticoagulant effect of vascular endothelium is impaired followed by abnormal platelet aggregation, hypercoagulable state, and hyperfibrinolysis.[22] Therefore, the impaired blood coagulation could reflect the severity of vascular damage in KD and thereby could be used as useful markers for IVIG resistance prediction.

Secondly, prolonged PT and APTT could result from decreased synthesis and/or increased consumption of coagulation factors.[23] Therefore, the impaired production of coagulation factors due to liver damage in KD might also be involved in abnormal coagulable state since most of the coagulation factors are synthesized in the liver. Indeed, elevated serum liver enzymes, hypoalbuminemia, and hyperbilirubinemia were more severe in the initial IVIG-resistant group in comparison with the initial IVIG-responsive group in our

TABLE 4 Comparison of clinical data between the repeated intravenous immunoglobulin (IVIG)-resistant and IVIG-responsive groups in Kawasaki disease

	IVIG-resistant (n = 23)	IVIG-responsive (n = 36)	P value
Age(mo)	26.0 [17.0-54.0]	36.5 [16.5-60.00]	.474
Male (%)	12 (52.2)	17 (47.2)	.711
Day of illness before IVIG	5.00 [4.00-6.00]	5.00 [5.00-6.00]	.476
Sampling day of illness, days	5.00 [4.00-5.00]	4.00 [3.00-5.00]	.871
Clinical manifestations			
Rash, n (%)	22 (95.7)	29 (80.6)	.133
Bilateral bulbar conjunctive injection, n (%)	21 (91.3)	81 (86.1)	.694
Erythema of oral and pharyngeal mucosa, n (%)	22 (95.7)	32 (88.9)	.639
Edema and erythema of the extremities, n (%)	11 (47.8)	20 (55.6)	.562
Cervical lymphadenopathy, n (%)	13 (56.5)	18 (50.0)	.625
Incomplete KD, n (%)	19 (32.2)	123 (37.7)	.418
Pericardial effusion (%)	3 (13.0)	2 (5.6)	.369
Valve regurgitation (%)	4 (17.4)	5 (13.9)	.725
Cardiac enlargement (%)	3 (13.0)	9 (25.0)	.334
Coronary artery lesions (CALs), n (%)	4 (17.4)	8 (22.2)	.749
Laboratory features			
WBC count (10^9/L)	12.6 [9.3-16.4]	14.65 [10.5-15.8]	.656
NLR	7.42 [2.0-12.86]	4.26 [2.71-9.29]	.494[a]
Hemoglobin (g/L)	104.00 [95.00-113.00]	107.00 [100.25-114.75]	.247
PLT count (10^9/L)	281.00 [233.00-307.00]	308.00 [231.0-369.75]	.171
AST (IU/L)	34.00 [21.00-59.00]	37.50 [25.50-96.00]	.444
ALT (IU/L)	44.00 [27.0-74.0]	80.50 [27.25-131.75]	.370
ALB (g/L)	32.4 [28.1-37.0]	34.85 [31.33-38.98]	.073
Total bilirubin (mg/L)	6.2 [3.0-27.2]	9.2 [5.95-20.03]	.208[a]
Creatinine (umol/L)	29.00 [27.00-38.00]	30.50 [24.0-37.75]	.427
Urea nitrogen (mmol/L)	3.2 [2.3-5.1]	2.99 [2.33-3.88]	.203
Sodium (mmol/L)	133.00 [131.0-135.00]	135.20 [133.00-137.75]	.296
Potassium (mmol/L)	3.89 [3.30-4.3]	3.74 [3.5-4.10]	.720
ESR (mm/h)	65.00 [47.0-78.00]	67.50 [45.25-89.50]	.802
CRP (mg/L)	135.00 [64.00-160.00]	87.50 [62.0-128.75]	.116
PT (s)	14.1 [13.0-15.0]	14.7 [14.0-15.5]	.431[a]
APTT (s)	34.1 [32.3-45.6]	36.6 [30.78-44.05]	.994
TT (s)	16.0 [15.5-16.3]	16.1 [15.83-16.48]	.064[a]
D-dimer (mg/L)	2.3 [1.6-3.35]	2.0 [1.06-2.68]	.132[a]
Fibrinogen (mg/dL)	590 [433-632]	557 [489.75-646.25]	.804
FDP (ug/mL)	9.6 [7.0-12.5]	7.95 [4.95-12.63]	.228
ATIII (%)	65 [60-81]	84 [76.23-89.75]	.001[*]
INR	1.17 [1.13-1.24]	1.22 [1.13-1.31]	.451[a]

The data are presented as the median with the 25th and 75th percentiles in square brackets for continuous variables and as the percentage for the categorical variables.

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; AT, antithrombin; CALs, coronary artery lesions; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FDPs, fibrin degradation products; INR, international normalized ratio; NLR, neutrophil-to-lymphocyte ratio; PLT, platelet; PT, prothrombin time; TT, total thrombin time; WBC, white blood cell.

[a]Variables between the two groups were compared by the Mann-Whitney U test due to abnormal data distribution.

[*]Statistically significant (P < .05).

study. Additionally, a previous study conducted by Wei-Xing Kong[8] in 2019 has found the serum level of cholinesterase, which could reflect the hepatic synthetic capacity, was significantly decreased in IVIG non-responders. Collectively, the prolonged PT and APTT in the initial IVIG-resistant group might also, to some extent, be attributable to the more severe hepatic dysfunction in KD.

Several studies have previously explored the coagulation profiles in patients with KD.[7-12,24,25] However, most of them focused on the changes in coagulation profiles before and after IVIG in KD,[10] the role of coagulation profiles in distinguishing atypical KD from bacterial cervical lymphadenitis,[12] and in predicting the occurrence of CALs or cardiac complications.[9-11]. Chen et al[24] in 2015 noticed that the levels of fibrinogen and D-dimer were significantly elevated in KD patients compared with healthy children. Yoshihiko Sakurai[7] in 2014 found prolonged PT and APTT before IVIG were significantly shortened after IVIG, and elevated levels of FDP and D-dimer were significantly decreased. Similar findings were observed in studies from Michio Sakai et al[10] in 2001 and Goshgar Mammadov et al[26] in 2019. In addition, a previous study conducted by Toshihiko Imamura[12] in 2005 showed that increased D-dimer concentrations are a useful clinical marker to distinguish atypical KD from bacterial cervical lymphadenitis. Moreover, several studies have proved that elevation of D-dimer could be a risk factor for CALs in KD.[8,9] However, to our knowledge, only two studies have previously determined the roles of coagulation profiles in initial IVIG resistance prediction. Yoshihiko Sakurai et al[7] in 2014 failed to find significant differences in coagulation profiles on patients responsive to initial IVIG (n = 13) and those refractory to it (n = 7) except for those with PT-INR. Thereafter, Wei-Xing Kong[8] in 2019 found PT, APTT, TT, and fibrinogen were not significantly different between the IVIG-resistant group and IVIG-responsive group. Nonetheless, the D-dimer was remarkably elevated in the IVIG-resistant group. D-dimer level had a sensitivity of 87.0% and a specificity of 56.3% for predicting IVIG resistance at a cutoff point of 1.09 mg/L. These observations are partly in line with our findings. However, both studies were limited by small sample size and retrospective design. Furthermore, the predictive value of ATIII for IVIG resistance has never been explored before. Given the sufficient number of patients and a prospective approach, the findings in our report might be more conclusive.

In terms of the repeated IVIG resistance prediction in KD, paucity of data was currently available and the role of coagulation profiles in this issue has never been investigated. Although several clinical trials[2,27] from Japan documented the addition of corticosteroid or ciclosporin therapy to standard-dose IVIG and aspirin in the primary therapy of KD reduced the initial non-response rate and decrease the incidence of CALs among high-risk patients for initial IVIG resistance predicted by Kobayashi,[28] Sano,[29] and Egami[30] scores, the non-response rate still remained approximately 10%-20%.[31] These findings suggested that high-risk KD patients for IVIG resistance might mostly benefit from aggressive therapy and prediction of repeated IVIG resistance was equally essential and clinically significant. In the present study, we firstly found the repeated IVIG non-responders presented with a remarkably lower serum ATIII level compared with responders.

A cutoff value of 68.5% for repeated non-responders yielded a relatively moderate sensitivity of 71% and specificity of 55%. Obviously, we could not identify all the non-responders for repeated IVIG by detecting serum ATIII level, and these data, however, may expand the limited information regarding repeated IVIG resistance prediction and provide some references for clinical management.

The strengths of this study were its prospective design and relatively large sample size. However, the present study has several limitations. First, this study was performed at a single institution. Our hospital is the largest pediatric medical center in Southwest China, which may lead to a selection bias due to a higher number of severely ill patients being admitted to this facility. Second, the present study was a prospective cohort study with strict inclusion and exclusion criteria. The findings of this study are, therefore, applicable only to Chinese patients with KD receiving standardized IVIG treatment (2 g/kg) within 10 days of fever onset.

Despite these limitations, this prospective study is the first to report pronounced changes in TT, PT, APTT, FDP, D-dimer levels, INR, and ATIII activity in the acute stage of KD, which may serve as complementary laboratory biomarkers for predicting IVIG resistance. In addition, the predictive validity of ATIII activity as a single biomarker for IVIG resistance may be superior to other coagulation biomarkers with a relatively high sensitivity.

5 | CONCLUSIONS

In summary, the inflammatory process mediated by KD is associated with the cause of impaired coagulation, which supports the notion that patients with KD who also have hypercoagulation during the acute phase could be at higher risk of developing IVIG resistance.

CONFLICT OF INTEREST
The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTION
Shuran Shao: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Resources (equal); Writing–original draft (lead). **Lixia Yang:** Data curation (equal); Investigation (equal); Resources (equal); Supervision (lead); Validation (lead). **Xiaoliang Liu:** Data curation (equal); Investigation (equal); Resources (equal). **Lei Liu:** Data curation (equal); Resources (equal). **Mei Wu:** Data curation (equal); Resources (equal). **Yuxin Deng:** Resources (equal). **Hongyu Duan:** Resources (equal). **Yifei Li:** Funding acquisition (equal); Resources (equal). **Yimin Hua:** Conceptualization (equal); Funding acquisition (equal); Resources (equal). **Lili Luo:** Funding acquisition (equal). **Kaiyu Zhou:** Conceptualization (equal); Methodology (equal); Resources (equal); Supervision (lead); Validation (lead). **Chuan Wang:** Conceptualization (lead); Funding acquisition (lead); Project administration (lead); Supervision (equal); Validation (equal); Writing-review & editing (lead).

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.