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# Predictors of Intravenous Immunoglobulin Resistance in Chinese Children with Kawasaki Disease

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**Abstract** ***Objectives:*** To investigate predictors of intravenous immunoglobulin (IVIG) resistance in children with Kawasaki Disease (KD). ***Methods:*** A retrospective cohort study of children, divided into IVIG-responsive and IVIG-resistant groups, diagnosed with KD and treated with IVIG at Tseung Kwan O Hospital. Demographic, clinical and laboratory data before IVIG treatment and coronary abnormalities were analysed. ***Results:*** 82 children, all were Chinese, with KD were studied. IVIG-resistant rate was 19.5%. Univariate analysis revealed that the IVIG-resistant group had higher neutrophil percentage (p=0.015) and serum total bilirubin level (p=0.033) but lower serum albumin level (p=0.026) and lymphocyte percentage (p=0.024). Multivariable logistic regression analysis revealed that the IVIG-resistant group had lower serum albumin level (odds ratio (OR) 0.776, 95% confidence interval (CI) 0.618-0.975, p=0.03) and higher neutrophil percentage (OR 1.169, 95% CI 1.017-1.344, p=0.029). ***Conclusions:*** Low serum albumin level and high neutrophil percentage were independent predictors of IVIG resistance in Chinese children with KD.

**Key words** Immunoglobulin, intravenous; Mucocutaneous lymph node syndrome

## Introduction

Kawasaki Disease (KD) is an acute multi-organ vasculitic syndrome of unknown aetiology, primarily affecting infants and children. Reported by Ng et al, KD is the leading cause of acquired heart disease in Hong Kong children at the incidence of 39/100 000 among Hong Kong children under five-year-old.[1] Initial high-dose intravenous immunoglobulin (IVIG) (2 grams/kg dose) and aspirin resolved the clinical symptoms and signs in most of the cases, with the rate of coronary artery abnormalities reduced from 20% to 5%.[2] Unfortunately, 7.5% to 38% of children from different studies still have persistence or recrudescence of fever despite the initial treatment; even worse, IVIG resistance has been known to be a major risk factor for coronary complications.[1,3-5] The aim of our study was to identify any clinical or biochemical predictors applied at diagnosis for refractory KD, allowing these patients to receive additional or innovative anti-inflammatory therapies earlier, through which cardiac morbidities could be minimised.

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## Methods

A retrospective cohort study was conducted by studying all children diagnosed with KD and treated with IVIG at Tseung Kwan O Hospital, among whom their clinical details were retrieved from the Clinical Data Analysis and Reporting System, from 1 January 2000 to 31 December 2010.

Diagnosing KD was based on the Japanese criteria (fifth revised edition) by fulfilling at least five principle symptoms: fever persisting for at least five days, changes in peripheral extremities, polymorphous exanthema, bilateral conjunctival congestion, changes in the lips and oral cavity (reddening of lips, strawberry tongue, diffuse injection of oral and pharyngeal mucosa) and acute non-purulent cervical lymphadenopathy.[6] Nevertheless, patients with four of the principle symptoms could be diagnosed to have KD when coronary aneurysm was recognised using the two-dimensional echocardiography or coronary angiography, under the criteria defined by the Japanese Ministry of Health.[6,7] We confirmed resolution of KD according to Tremoulet et al and Kuo et al studies.[3,4] IVIG-resistant patients were classified when having persistence or recrudescence of fever 48 hours after completion of IVIG infusion and the presence of one or more of the initial symptoms within two to seven days of treatment.[4]

Demographic data of age, sex and illness days of IVIG, clinical features of conjunctival injection, mucosal change, extremity change, skin rash and lymphadenopathy and laboratory findings of total white cell count, neutrophil percentage, lymphocyte percentage, haemoglobin level, platelet count, levels of sodium, alanine aminotransferase (ALT), total bilirubin, albumin, C-reactive protein (CRP) and erythrocyte sedimentary rate (ESR) were recorded. Coronary complications, including pericardial effusion and coronary artery abnormalities determined by the two-dimensional echocardiography at the onset of disease, 2nd, 4th, 8th weeks and one year afterward, were reported as well.

## Statistical Analysis

The statistical analyses were conducted using the Statistical Product and Service Solutions version 17.0 for Window 7. Taking $p<0.05$ as statistically significant, student t-test and Wilcoxon rank-sum test were used to compare continuous data with normal distribution and skewed data respectively while chi-square test or Fisher's exact test whichever appropriate was used to analyse categorical variables. Variables that gave $p<0.2$ from univariate analyses were further evaluated using a multivariable logistic regression model to adjust for confounding factors and to identify any factors that independently predicting IVIG resistance. Risk factors for IVIG resistance as reported by Egami et al, though some factors with $p>0.2$ in our study, were also included in our multivariate analysis.[8]

## Results

Between 1 January 2000 and 31 December 2010, 82 children at the median age of 24 months, diagnosed with KD and treated with IVIG at Tseung Kwan O Hospital, met the inclusion criteria of this study. All of them were Chinese. 16 (19.5%) cases were found to be IVIG-resistant.

As shown in Table 1, no significant differences were identified in the demographic data and clinical features between IVIG-responsive and IVIG-resistant groups. However, the IVIG non-responders had longer median length of stay (12 days vs 6 days) and median duration of fever (13 days vs 6 days). The differences were statistically significant at a p-value <0.001.

Table 2 compares the laboratory data of IVIG-responsive and IVIG-resistant groups. Univariate analysis revealed that the IVIG-resistant group had higher neutrophil percentage ($p=0.015$) and serum total bilirubin ($p=0.033$) but lower serum albumin level ($p=0.026$) and lymphocyte percentage ($p=0.024$). Furthermore, multivariable logistic regression analysis with step-wise procedures carried out using those variables with $p<0.2$ singled out lower serum albumin level (OR 0.776, 95% CI 0.618-0.975, $p=0.03$) and higher neutrophil percentage (OR 1.169, 95% CI 1.017-1.344, $p=0.029$) as the significant independent predictors of non-responsiveness to initial IVIG treatment for KD. Predictors for IVIG resistance reported by Egami et al were also studied in our cohort, yet, no significant differences could be identified in the following data: infants <6-month-old, illness days of IVIG, platelet count, levels of ALT and CRP.[8]

The coronary complication rates between IVIG-responsive and IVIG-resistant groups are compared in Table 3. We found that the incidences of coronary artery abnormalities at 2nd week ($p=0.001$), 4th week ($p=0.007$) and 8th week ($p=0.006$) after onset of disease in the IVIG-resistant group were significantly higher. The differences, however, did not reach the statistically significant level at one-year follow-up. Besides, pericardial effusion was significantly ($p=0.001$) more prevalent in the IVIG-resistant group (25%) whereas there was not a single IVIG-responsive patient (0%) suffering from this complication.

Among the 16 IVIG non-responders, six patients received a second dose of IVIG. There was no mortality. Seven of these non-responders had coronary artery abnormalities, whereas one of them had coronary aneurysm and the other had giant aneurysm. For significant non-cardiac complications, one patient had hydrops of gall bladder and one had intestinal pseudo-obstruction. These two patients however did not have cardiac complication.

**Table 1** Comparison of demographic and clinical data between intravenous immunoglobulin (IVIG) responsive and resistant groups

| | IVIG | | p-values | |
|---|---|---|---|---|
| | **Responsive** | **Resistant** | **Univariate** | **Multivariate** |
| Age (months) | 24 (12-45) | 39 (8.25-56.75)[a] | 0.730[c] | |
| Age (% <6 months) | 4 (6.1) | 2 (12.5)[b] | 0.332[d] | 0.164[f] |
| Sex (% of male) | 56.1 | 56.3 | 0.989[e] | |
| Illness days of IVIG | 5 (5-7) | 5 (4-5.75)[a] | 0.162[c] | 0.464[f] |
| Total duration of fever | 6 (6-8) | 13 (9-19.5)[a] | <0.001[c] | |
| Length of stay | 6 (5-9) | 12 (8.3-19)[a] | <0.001[c] | |
| Conjunctival injection | 63 (95.5) | 14 (87.5)[b] | 0.250[d] | |
| Mucosal change | 62 (93.9) | 14 (87.5)[b] | 0.332[d] | |
| Extremity change | 56 (84.8) | 13 (81.3)[b] | 0.711[d] | |
| Skin rash | 58 (87.9) | 16 (100)[b] | 0.344[d] | |
| Lymphadenopathy | 41 (62.1) | 13 (81.3)[b] | 0.148[e] | 0.156[f] |

[a]Expressed as median±interquartile range; [b]Number of subjects (%); [c]Wilcoxon rank sum test was used; [d]Fisher's exact test was used; [e]Chi square test was used; [f]Multiple logistic regression was used in the full model

**Table 2** Comparison of laboratory data of the IVIG-resistant and responsive groups

| | IVIG[a] | | p-values | |
|---|---|---|---|---|
| | **Responsive** | **Resistant** | **Univariate[c]** | **Multivariate** |
| Total white cells ($10^9$/L) | 15.2 (12.7-17.6) | 13.95 (11.4-17.1) | 0.334 | |
| Neutrophil (%) | 66.6 (59.8-72.4) | 79 (68.1-87.6) | 0.015* | 0.029[f,g]* |
| Lymphocyte (%) | 24.2 (17.6-30.0) | 14 (8.8-23.4) | 0.024* | 0.257[e] |
| Haemoglobin (g/dL) | 11.5 (10.9-12.2) | 11.26 (10.2-12.6) | 0.598 | |
| Haematocrit (%) | 0.34 (0.32-0.36) | 0.33 (0.30-0.36) | 0.535 | |
| Platelets ($10^9$/L) | 391 (331.5-483.8) | 356.5 (296-518) | 0.371 | 0.189[f] |
| Sodium (mmol/L) | 136 (134-138) | 136 (133.3-137.8) | 0.675 | |
| ALT (U/L) | 43 (16.8-149) | 77.5 (30.3-154.3) | 0.311 | 0.792[e] |
| Total bilirubin (umol/L) | 6 (4-11) | 10 (6.3-31.5) | 0.033* | 0.275[e] |
| Albumin (g/L) | 38.05 (±4.26)[b] | 35.38 (±4.01)[b] | 0.029[d]* | 0.03[f,h]* |
| CRP (mg/L) | 100 (47.8-138.5) | 127.8 (98-164.5) | 0.087 | 0.707[e] |
| ESR (mm/hr) | 96.5 (71.3-108.8) | 100 (73-110) | 0.587 | |

[a]Expressed as median±interquartile range; [b]Expressed as mean±standard deviation; [c]Wilcoxon rank sum test was used; [d]Student t-test was used; [e]Multiple logistic regression was used in the full model; [f]Multiple logistic regression was used with albumin, CRP, neutrophil and platelet selected in the reduced model; [g]Odds ratio=1.169 (95% confidence interval: 1.017-1.344); [h]Odds ratio=0.776 (95% confidence interval: 0.618-0.975)

*Statistical significant

IVIG: intravenous immunoglobulin; ALT: alanine aminotransferase; CRP: C-reactive protein; ESR: erythrocyte sedimentary rate

**Table 3** Comparison of coronary complications between IVIG-resistant and IVIG-responsive groups

| | IVIG | | |
|---|---|---|---|
| | **Responsive[a]** | **Resistant[a]** | **p-values[b]** |
| Coronary artery abnormality at 2nd week | 5/64 (7.8) | 7/15 (46.6) | 0.001* |
| Coronary artery abnormality at 4th week | 5/64 (7.8) | 6/16 (37.5) | 0.007* |
| Coronary artery abnormality at 8th week | 3/63 (4.8) | 5/15 (33.3) | 0.006* |
| Coronary artery abnormality at 1 year | 2/44 (4.5) | 2/12 (16.6) | 0.198 |
| Pericardial effusion | 0/66 (0) | 4/16 (25) | 0.001* |

[a]Number of subjects (%); [b]Fisher's exact test was used

*Statistical significant

IVIG: intravenous immunoglobulin

## Discussion

In our cohort study, the 19.5% IVIG resistance rate was comparable to figures from other countries, which ranged from 7.5% to 38%.[1,3-5] As a matter of fact, IVIG-resistant patients have higher chance of developing coronary complications, the incidences of coronary artery abnormalities in the IVIG-resistant group in our cohort were higher at 2nd, 4th, and 8th weeks as well as one year after onset of disease. Pericardial effusion, a potentially serious cardiac complication, was significantly more prevalent in the IVIG-resistant group (p=0.001). Long-term health implications of KD, further illustrated by Cheung et al, pointing out that the adverse cardiovascular profile, as characterised by a pro-atherogenic alteration of the lipid profile and increased arterial stiffness, were worse in Kawasaki patients with transient or persistent coronary artery dilatation than in those without.[9] What's worse, a significant increase in carotid intima-media thickness, a marker of atherosclerosis,[10] was found in KD patients, both with or without coronary artery complications like coronary stenosis or thrombosis late after the acute illness.[11]

To prevent both short-term coronary complications and possible late risk of a cardiac event of KD, there is a need to identify any clinical or biochemical predictors for refractory KD, allowing these patients to receive additional or innovative anti-inflammatory therapies. Various authors reported several distinct predictors of IVIG-resistance for KD children.[3,4,8,12,13] Actually, many study groups had constructed different scoring systems.[3,4,8,12,13] One of these, which we could apply our local data, was devised by Egami et al from Japan.[8] They analysed 320 children and derived a bedside score: one point for (1) infants less than six months old, (2) diagnosed before four days of illness, (3) platelet count ≤300 x $10^9$/L, (4) CRP ≥80 mg/L, and two points for (5) ALT ≥80 IU/L.[8] Using a cut-off point of three, the prediction score could single out IVIG resistance with 78% sensitivity in a group of Japanese children.[8] Incorporating the Egami score into our cohort, nonetheless, the sensitivity was low-44%. Tremoulet et al applied the Egami model to their KD patients in San Diego County of the United States and found that more than 60% of IVIG-resistant patients had been missed.[3] Subsequently these Americans constructed their own scoring system which showed decreasing sensitivity in detecting IVIG resistance when the system was stratified by ethnicity: Caucasians > Hispanics > Asians (81.3%, 68.2% and 66.7% respectively).[3] The racial differences, leading to genetically different responses to injury and illness, may explain the failure of applying our local data to the current international scoring system. As our study was retrospective in nature, only Egami scoring system could be used to validate our exiting available data. A local prospective study therefore is deemed necessary to predict our Chinese KD children at risk of developing IVIG resistance and to verify various scoring systems elsewhere in the world.

Low serum albumin level was proved to be an independent predictor of IVIG resistance in our study (OR 0.776, 95% CI 0.618-0.975, p=0.03). Postulated by Kuo et al, serum albumin was lowered in refractory KD owing to an increase in vascular leakage causing hypoalbuminemia and non-cardiogenic oedema.[4] Terai et al echoed this finding and noticed that an elevation of vascular endothelial growth factors might play a role in the vascular leakage seen in KD, which explained the reduced serum albumin level.[14] Indeed tissue oedema was found during the initial phase of KD, for instance, oedematous changes of extremities occurred within the first five days of illness, and cervical lymph node swelling, which could develop before fever onset in older children.[2] Endothelial gap formation and subendothelial oedema found in the skin suggested the presence of vascular permeability.[14] Our group did demonstrate that the serum albumin level in the IVIG-resistant group was lower and associated with a higher incidence of a potentially serious cardiac complication: pericardial effusion, as seen in four of our IVIG-resistant patients. To sum up, we proposed low serum albumin level to serve as an independent role for predicting the degree of vascular permeability and leakage in refractory KD.

Another independent predictor of IVIG resistance confirmed in our study was high neutrophil percentage (OR 1.169, 95% CI 1.017-1.344, p=0.029). Apoptosis, or programmed cell death, is a form of cellular physiological death to maintain physiological stability. It was postulated that inhibited apoptosis was involved in the pathogenesis of autoimmune diseases such as systemic lupus erythematosus.[15] Inhibited neutrophil apoptosis in the acute phase of KD resulted in an increase in number of circulating neutrophils.[16] The prolonged life span of activated neutrophils may contribute to the pathogenesis of KD vasculitis in conjunction with the fact that the neutrophils secrete excessive amount of autotoxic mediators into the circulation, further causing severe inflammation.[16] Treated by high-dose IVIG treatment, the number of circulating neutrophils actually decreased by up-regulating their apoptosis.[16] To summarise, we proposed high neutrophil percentage to serve as an independent role for predicting the degree of vasculitis and inflammation in refractory KD.

Although we could identify low serum albumin level and high neutrophil percentage to serve as independent predictors for IVIG resistance, the optimal cut-off value of each predictor could not be determined as our sample size was small.

Our IVIG-resistant group had lower percentage of lymphocytes in our univariate analysis (p=0.024). Do et al explained that firstly possible genetic apoptotic defects and the consequent restriction of effective clonal expansion of lymphocytes against infectious stimuli were present at the acute phase of KD; and secondly, fewer lymphocytes in peripheral blood might represent early tissue infiltration of effector T cells and B cells, which was also related to possible defects in apoptosis of activated immune cells.[17]

Our cohort study also found that serum total bilirubin level was higher in the IVIG-resistant group using univariate analysis (p=0.033), in concord with Sano et al who indeed included total bilirubin concentration as one predictor of IVIG resistance.[12] They found that severe systemic inflammation and vasculitis might have spread to the hepatic sinusoidal circulation including the hepatic reticulo-endothelial system and caused biliary tract inflammation.[12] In fact, up to 10-15% of patients with KD are having acute swelling of the gallbladder on abdominal ultrasound and hyperbilirubinaemia.[18]

In treating IVIG-resistant patients, Ogata utilised the Egami score to identify high-risk IVIG-resistant children in a cohort study who were then randomly assigned to a single-IVIG group or a combination of intravenous pulse methylprednisolone plus IVIG group. Ogata et al found a better response to treatment in terms of resolution of fever and symptoms and less cardiac complication in the combination treatment group.[19] On the other hand, a recent study by Burns et al demonstrated that infliximab, a tumour necrosis factor alpha, could effectively control fever within 24 hours in 13 out of 16 IVIG non-responders of KD.[20] Until further clinical trials could establish a consensus in clinical practice, apart from using repeated doses of IVIG, methylprednisolone plus IVIG and infliximab are both potential treatment modalities for those who are at risk of developing IVIG resistance.

There are limitations in our study. Firstly, as small sample size has limited the power of this study, a territory-wide multi-centered collaboration is therefore necessary to develop a locally applicable scoring system with more significant clinical or biochemical predictors being identified. Refractory KD can then be singled out, allowing these patients to receive additional or innovative anti-inflammatory therapies earlier, through which the immediate or late cardiac morbidities could be minimised. Secondly, the laboratory data and echocardiogram findings were collected retrospectively over a long period of time, and hence there were utilisation of different automated machines for blood counts and calibration methods as well as inter-observer differences by different echocardiographers.

In conclusion, we reported an IVIG resistance rate of 19.5% among our cohort of children, who were all Chinese, diagnosed with KD and treated with IVIG in a general hospital. We proposed low serum albumin level and high neutrophil percentage to be independent predictors for refractory KD.

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