Platelet Count and Erythrocyte Sedimentation Rate are Good Predictors of Kawasaki Disease: ROC Analysis

Song Xiu-Yu,* Huang Jia-Yu, Hong Qiang, and Dai Shu-Hui

Clinical Laboratory Department, The First Hospital Affiliated to Xiamen University, Xiamen, China

Background and Aims: Kawasaki disease (KD) is the leading cause of acquired pediatric cardiac disease and requires a timely diagnosis. Available effective therapy is ideally administered within 10 days of illness diagnosis. Recent reports of several laboratory tests in KD have been published. In this study, we aimed to evaluate the sensitivity and specificity of several laboratory tests. **Methods:** We performed a retrospective study of consecutive patients diagnosed with KD from January to December 2008. We studied the sensitivity and specificity of several different tests [T-cell subgroups, platelet count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP)] to predict KD using receiver

operator characteristic curve analysis. **Results:** No significant difference was demonstrated in T-cell subgroups between patients with KD and referent patients ($P > 0.05$). However, platelet count, ESR, and CRP were significantly higher in patients with KD than in referent patients ($P < 0.05$). ESR showed a sensitivity of 93.9% and specificity of 83.3% with a cut-off of 15 mm/hr (area under the curve [AUC], 89.1%; $P = 0.03$). Platelet count showed a sensitivity of 70.6% and specificity of 75% with a cut-off of $336.5 \times 10^9/l$ (AUC, 71.2%; $P = 0.03$). **Conclusions:** These results indicate that platelet count and ESR are good predictors of KD. *J. Clin. Lab. Anal.* 24:385–388, 2010. © 2010 Wiley-Liss, Inc.

Key words: Kawasaki disease; T-cell; platelet count; erythrocyte sedimentation rate; C-reactive protein

INTRODUCTION

Kawasaki disease (KD) is an acute febrile illness of childhood characterized by clinical laboratory and histopathological features of systemic vasculitis (1). Because of its potential to cause coronary aneurysm, KD is the leading cause of pediatric acquired cardiac disease worldwide, especially in developed countries (2). It is now recognized that patients with KD may present in a variety of ways, and in recent years, more children have been diagnosed with “incomplete” KD (3).

Even though they are nonspecific, laboratory tests can provide diagnostic support in patients with clinical features. Recently, several clinical findings have been reported that support this assertion (4,5). However, sensitivity and specificity of laboratory tests are not well known. This study was designed to evaluate the sensitivity and specificity of several of the most commonly reported tests using receiver operating characteristic (ROC) curve analysis. Specific tests were for T-cell subgroups, platelet count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).

MATERIALS AND METHODS

KD Patients

We performed a retrospective study of consecutive patients diagnosed with KD from January to December 2008. Initial clinical examination and all laboratory investigations were reviewed. Diagnosis of KD was confirmed by a pediatric rheumatologist based on the presence of ≥ 5 days of fever plus $\geq 4/5$ KD criteria: bilateral nonpurulent conjunctival injection, oral mucosal changes (strawberry tongue and red cracked lips), cervical lymphadenopathy (≥ 1.5 cm), polymorphous skin rash, and peripheral changes (puffy hands/feet and palmar/plantar erythema) (6).

*Correspondence to: Song Xiu-Yu, Clinical Laboratory Department, The First Hospital Affiliated to Xiamen University, Xiamen, China. E-mail: songxyxm@hotmail.com

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Referent Patients

Referent patients had some of the same symptoms as KD patients, such as the presence of ≥ 5 days, bilateral nonpurulent conjunctival injection, polymorphous skin rash, oral mucosal changes, and so on. The referent people were 34 patients, whose symptoms were most closely with Kawasaki patients, but who were not diagnosed with KD. Diagnosis of referent patients included bronchopneumonia, bronchitis, bronchial asthma, respiratory infections, and anaphylactic purpura. Characteristics of 33 patients with KD and 34 referent patients are shown in Table 1.

Detection of T-Cell Subgroups

Peripheral venous blood was drawn into sterile tubes containing heparin. One hundred microliters of whole blood was mixed with 10 μ l of appropriate monoclonal antibody conjugates for 30 min (4°C in darkness). The following antibodies were used for staining: anti-CD3 fluorescein isothiocyanate, anti-CD4 and anti-CD8 phycoerythrin (Becton Dickinson, San Jose, CA). After incubation, red cells were lysed by adding 400 μ l lysing solution (Immunotech, Marseille Cedex, France) for 10 min at room temperature. Cells were washed once in sheath buffer (Beckman Coulter, Fullerton, CA) and the pellet was resuspended in 1% paraformaldehyde in PBS while being vortexed. Five-color flow cytometric analysis was done on a Beckman Coulter FC500 flow cytometer.

Detection of ESR, CRP, and Platelet Count

Peripheral venous blood was drawn into three sterile tubes, each containing one each of sodium citrate, EDTA-K₂, or coagulant. ESR was measured by using the Micro-Test1 analyzer (Vital Diagnostics Srl, Forli, Italy), serum CRP was measured with rate nephelometry by using IMMAGE analyzer (Beckman Coulter), and

TABLE 1. Clinical Characteristics of KD Patients and Referent Patients

	KD patients (<i>n</i> = 33)	Referent patients (<i>n</i> = 34) ^a
Male, <i>n</i> (%)	22 (66.7)	25 (73.5)
Age (months)		
Median	12	36
Range	4–96	3–156
Admission days		
Mean $\pm$ SD	11.4 $\pm$ 3.8	9.3 $\pm$ 3.1
Range	5–19	4–15

^aThe 34 referent patients had diagnosis of bronchopneumonia (*n* = 12), bronchitis (*n* = 3), bronchial asthma (*n* = 4), respiratory infections (*n* = 8), and anaphylactic purpura (*n* = 7).

platelet count was measured with a Beckman Coulter LH750 hematology analyzer.

Statistical Analysis

All statistical analyses were performed with SPSS v.13.0 for Windows (SPSS Inc., Chicago, IL). For analysis of clinical characteristics, the significance of between-group differences was tested with the independent sample *t*-test. Because CRP and ESR values were skewed, median concentrations were computed for these parameters. Significance of any between-group differences was assessed with Mann–Whitney *U* test. Data are reported as mean $\pm$ SD or median $\pm$ quartile range; a value of *P* < 0.05 was accepted as statistically significant. Cut-off value and sensitivity and specificity of ESR, CRP, and platelet counts were identified by ROC curves.

RESULTS

T-Cell Subgroups

T-cell subgroups are shown in Table 2. CD3⁺, CD3⁺CD4⁺, and CD3⁺CD8⁺ were not significantly different in patients with KD compared with referent patients (*P* > 0.05) as shown by independent sample *t*-test.

ESR, CRP, and Platelet Count

Platelet count was significantly higher in patients with KD than in referent patients (Table 3; *P* < 0.01); ESR was significantly higher in patients with KD than in

TABLE 2. Detection of T-cell Subgroups of KD Patients and Referent Patients

	KD patients	Referent patients	<i>P</i> value
CD3 ⁺ (%)	67.2 $\pm$ 9.42	65.1 $\pm$ 12.6	NS
CD3 ⁺ CD4 ⁺ (%)	39.0 $\pm$ 9.93	38.2 $\pm$ 10.8	NS
CD3 ⁺ CD8 ⁺ (%)	24.8 $\pm$ 7.42	24.3 $\pm$ 8.42	NS

NS, not significant. T cell was classified into Th1 (CD3⁺CD4⁺) and Th2 (CD3⁺CD8⁺). Data were normally distributed and mean $\pm$ SD and independent sample *t*-test were adapted for data analysis.

TABLE 3. Detection of ESR, CRP and Platelet Count of KD Patients and Referent Patients

	KD patients	Referent patients	<i>P</i> *
PLT ($\times 10^9$ /L) ^a	406.2 $\pm$ 167.3	284.7 $\pm$ 105.9	<0.01
ESR (mm/h) ^b	56.0 (59.5)	12.0 (18.75)	<0.01
CRP (mg/L) ^b	21.0 (60.2)	8.0 (10.5)	<0.05

^aData for platelet count were according to normal distribution; mean $\pm$ SD and independent sample *t*-test were adapted for analysis.

^bESR and CRP were according to skewed distribution; median $\pm$ interquartile range and Mann–Whitney *U* test were adapted for analysis.

**P* < 0.05 was accepted as statistically significant.

TABLE 4. Characteristics of ROC Curves of T-cell Subgroups, ESR, CRP and Platelet Count of KD Patients

	AUC (%)	Cut-off value	Sensitivity	Specificity	Youden index(%)
CD3 ⁺	52.4	57.4%	0.929	0.297	22.6
CD3 ⁺ CD4 ⁺	58.1	40.1%	0.536	0.676	21.2
CD3 ⁺ CD8 ⁺	50.4	19.9%	0.821	0.324	14.5
PLT	71.2	$336.5 \times 10^9/l$	0.706	0.750	45.6
ESR	89.1	15.0 mm/h	0.939	0.833	77.2
CRP	70.2	14.5 mg/l	0.690	0.727	41.7

ESR showed the highest AUC, sensitivity, and specificity (89.1%, 0.939 and 0.833, respectively). Platelet counts were lower than ESR in each aspect, although all were >70%. T-cell subgroups were the lowest according to the data, whether regarding sensitivity or specificity.

Fig. 1. Receiver operating characteristic (ROC) curves for T-cell subgroups. Area under the curve (AUC) indicates that CD3⁺ was 52.4% accurate for diagnosing KD. The 95% confidence interval of the AUC for CD3⁺ ranged from 38.2 to 66.6%, indicating that the null hypothesis (CD3⁺ cannot be used to diagnose KD) could be accepted (a test associated with a 50% AUC; $P = 0.741$). For CD3⁺CD4⁺, the AUC shows 58.1% accuracy. The 95% confidence interval of the AUC for CD3⁺CD4⁺ ranged from 43.6 to 72.5%, indicating that the null hypothesis (CD3⁺CD4⁺ cannot be used to diagnose KD) could be accepted (a test associated with a 50% AUC; $P = 0.269$). For CD3⁺CD8⁺, the AUC shows 50.4% accuracy. The 95% confidence interval of the AUC for CD3⁺CD8⁺ ranged from 36.2 to 64.6%, indicating that the null hypothesis (CD3⁺CD8⁺ cannot be used to diagnose KD) could be accepted (a test associated with a 50% AUC; $P = 0.952$).

referent patients ($P < 0.01$); and CRP levels were also higher in patients with KD than in referent patients ($P < 0.05$).

Characteristics of ROC Curves

CD3⁺, CD3⁺CD4⁺, and CD3⁺CD8⁺ showed a low diagnostic accuracy (Table 4). CD3⁺ showed a higher sensitivity but lower specificity than CD3⁺CD4⁺ and CD3⁺CD8⁺ (Fig. 1). Area under the curve (AUC) of the ESR ROC curve was higher and showed both a higher sensitivity and specificity than that of CRP and platelet counts. Platelet counts showed a lower diagnostic accuracy than ESR (Fig. 2).

Fig. 2. ROC curves of platelet (PLT) counts, ESR and CRP. AUC shows that ESR reported the highest accuracy for predicting KD (89.1%). The 95% confidence interval of the AUC for ESR ranged from 74.2 to 104%. The null hypothesis (ESR is unable to predict KD) was rejected according to the P value ($P = 0.003$). AUC of PLT was 72.8%. The 95% confidence interval of the AUC for PLT ranged from 61.0 to 84.7%, and therefore, the null hypothesis (PLT is unable to predict KD) was rejected (a test associated with a 50% AUC; $P = 0.001$). AUC of CRP was 70.2%. The 95% confidence interval of the AUC for CRP ranged from 55.6 to 84.9%, and therefore, the null hypothesis (CRP is unable to predict KD) was rejected (a test associated with a 50% AUC; $P = 0.014$).

DISCUSSION

Children with KD are at high risk of developing life-threatening coronary complications yet may elude timely diagnosis because they often lack the full complement of classic clinical features (7). Availability of highly effective therapies such as IV immunoglobulin and aspirin, which are ideally administered within 10 days of illness to maximize efficacy, indicates that it is particularly important to diagnose KD as rapidly as possible (8).

Recently, several laboratory tests have been reported in patients with KD (9,10). This study is consistent with earlier reports showing elevated CRP levels, ESR, and platelet counts. Abe et al. showed T-cell repertoire changes in acute KD (11). However, we did not find

any significant differences in *T*-cell subgroups between KD patients and referent patients. Our results showed that *T*-cell subgroups were not changed in the early stage of KD patients, indicating that they cannot be used to predict KD. This finding was also confirmed by ROC curves for the *T*-cell subgroups, which showed a low AUC. We found elevated CRP levels in our study, which is in agreement with an earlier study (12). However, the ROC curve for CRP showed a low sensitivity, illustrating a lower efficiency for diagnosing KD. This may explain why CRP levels, as well as *T*-cell subgroups, changed in the later period of KD. Furthermore, it has been reported that the inflammatory markers CRP and serum amyloid-A are elevated in KD patients with coronary arterial lesions compared with referent patients (13).

Among patients with fever and no definitive alternative diagnosis, patients with an elevated platelet count have a high risk of KD. In our study, platelet count in patients with KD was much higher than in referent patients. ROC curve for platelet count showed a high sensitivity, specificity, and AUC (all >70%), making it possible to diagnose KD. Likewise, ESR showed the highest AUC, sensitivity, and specificity, which indicates a high probability for predicting KD. These data suggest that platelet count and ESR are the best predictors of KD.

This study had several limitations because it was a retrospective study. First, we were unable to determine the influence of the tests on the decision of the treating physician to diagnose KD. If some of these patients were misdiagnosed, the apparent predictive value of the tests would have been exaggerated. Second, even though CRP affects the arteries of healthy children by disturbing endothelial function and promoting intima-media thickening, which supports the hypothesis that CRP plays a role in the pathogenesis of early atherosclerosis, several recent reports in an apparently healthy population demonstrated that CRP levels are significantly associated with several cardiovascular risk factors, such as age, smoking, body mass index, and lipid-based risk factors (10,14,15). Therefore, our laboratory tests may have been influenced by these factors.

Coronary artery damage is seen in 17.0% of KD patients (16). Early diagnosis of KD is the most effective way to protect from those complications. Febrile infants with an extremely high platelet count and ESR, and without any other clinical features which may help determine a clear diagnosis, have a significantly greater

risk of KD compared with infants without such findings. Clinicians should consider the possibility of KD in all febrile children without a clear alternative diagnosis, particularly when the platelet count and ESR are extremely elevated. Echocardiogram may also be useful for identifying early KD.

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