Novel Biomarker for Diagnosis of Kawasaki Diseases

2015 Feb 4th

Tai-Ming Ko

Jer-Yuarn Wu, Yuan-Tsong Chen
• DISCLOSURES: None
What “biomarker” can do for Kawasaki disease (KD)?

Biomarkers for human disease

- **Sensitivity**
  - AUC=0.5, no discrimination
  - 0.7 ≤ AUC < 0.8, acceptable discrimination
  - 0.8 ≤ AUC < 0.9, excellent discrimination
  - AUC ≥ 0.9, excellent discrimination

- **Specificity**
  - 0% to 100%

**Biomarker**

- **Differential diagnosis**
- **Stage Disease**
- **Monitor Progression/Recurrence**
- **Determine Treatment Efficacy**
- **Predict Response to Treatment**
Diagnosis of Kawasaki disease is currently based on clinical features

Suspected KD Pts → Differential diagnosis 

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>IVIG (intravenous immunoglobulin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever &gt; 5d</td>
<td></td>
</tr>
</tbody>
</table>

Diagnostic Guidelines: ≥ 4 of the following 5 principal features

- Erythema and edema of hands and feet
- Polymorphous exanthema
- Bilateral bulbar conjunctival injection
- Erythema of lips, strawberry tongue
- Cervical lymphadenopathy (≥1.5 cm in diameter)

(Circulation. 2011)
Delay diagnosis of KD results in poor outcome with IVIG treatment

Suspected KD Pts

Fever > 5d

Delay diagnosis (>10 days)

Differential diagnosis

clinical features

IVIG (intravenous immunoglobulin)

recovery

15% ~ 25% of untreated Pts
Coronary Artery Aneurysm (CAA)

1. myocardial infarction
2. ischemic heart disease
3. sudden death

(Circulation. 2004)
(Am Fam Physician. 2006)
Major Problems of KD diagnosis

- Kawasaki disease is an acute, difficult-to-recognize, pediatric vasculitis that often remains undiagnosed.
- Kawasaki disease clinically mimics other common conditions.
- No diagnostic test currently exists.

*Nature Reviews Cardiology 9, 375 (2012)*
Challenge in KD diagnosis

iKD
(incomplete presentation)

non-KD
(fever & inflammatory diseases)

15 % of KD Pts

• Unexplained fever for ≥5 days
• ≤ 3 of the principal features

• Diseases With Similar Clinical Findings
  – infections (eg, enterovirus, EBV)
  – Drug hypersensitivity reactions
  – Stevens-Johnson syndrome
  – Juvenile rheumatoid arthritis
  – Bacterial cervical lymphadenitis
  – Scarlet fever
  – Staphylococcal scalded skin syndrome
  – Toxic shock syndrome
  – Rocky Mountain spotted fever
  – Leptospirosis
  – Mercury hypersensitivity reaction

(Circulation. 2004)
(Nihon Rinsho. 2008)
(Pediatr Cardiol. 2012)

How to develop a simple diagnostic test for KD?
What’s the way to identify biomarkers for KD diagnosis?

- gDNA from blood
- RNA from blood
- Protein from plasma
- Clinical parameters
Kawasaki disease and immune system (genetic studies)

- **ITPKC** (T cell, activation pathway)

- **COPB2** (T cells) & **IGHV** (B cells)

- **FCGR2A** (B cells, Fc fragment of IgG)

- **BLK** (B cells, B-cell lymphocyte kinase)

- **CD40** (T cells, interaction between B cells and T cells)
The diagram illustrates the interaction between BCR and CD40 pathways in B-cell activation, and the TCR pathway in T-cell activation. Key components include FCGR2A, BLK, STAT3, PI3K, MAPK, NF-κB, Ca\(^{2+}/NFAT\) pathways, and IP3. The B-cell activation pathway is activated by FCGR2A, leading to BLK, STAT3, PI3K, and MAPK pathways, ultimately resulting in B-cell activation. The T-cell activation pathway is activated by TCR, involving PLC, IP3, IP4, and Ca\(^{2+}/NFAT\) pathways, resulting in T-cell activation.
ROC (Receiver Operating Characteristic) curve for predictive model of KD with 6 SNPs

Table 2. Susceptibility Genes for KD Identified With Association at Genome-Wide Significance (P<5.0x10^{-8})

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Methods</th>
<th>Original reports</th>
<th>Replication studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCG2RA*</td>
<td>1q23</td>
<td>GWAS</td>
<td>42</td>
<td>45</td>
</tr>
<tr>
<td>CASP3</td>
<td>4q34–35</td>
<td>Linkage analysis – positional</td>
<td>21</td>
<td>25**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>candidate gene study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA*</td>
<td>6p21.3</td>
<td>GWAS</td>
<td>45</td>
<td>–</td>
</tr>
<tr>
<td>BLK*</td>
<td>8p23–p22</td>
<td>GWAS</td>
<td>45, 46</td>
<td>–</td>
</tr>
<tr>
<td>ITPKC</td>
<td>19q13.2</td>
<td>Linkage analysis – linkage</td>
<td>20</td>
<td>23, 42, 22, 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>disequilibrium mapping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD40*</td>
<td>20q12-q13.2</td>
<td>GWAS</td>
<td>45, 46</td>
<td>–</td>
</tr>
</tbody>
</table>

Sensitivity: 55.6%
Specificity: 65.6%
Positive predictive value: 56.7%
Negative predictive value: 64.5%

AUC = 0.643

(Circ J. 2012)
Specific Aim:

Identify biomarkers that can be used to facilitate diagnosis of KD
Specific Aim:
Identify biomarkers that can be used to facilitate diagnosis of KD

“Systematic / Unbiased / High-throughput screening” Approach:

• Potential protein targets in blood
  – Protein array

• Gene expression profile of PBMCs
  – Deep sequencing of RNA
Specific Aim:
Identify biomarkers that can be used to facilitate diagnosis of KD

Study design for biomarkers evaluation
- Replicable phenotype: multiple stages
  - Discovery (protein array → EIA)
  - Replication
  - Blinded Validation

- Suitable comparison: specified control
  - KD (Acute & Convalescent)
  - KD & Non-KD with fever only
  - KD & Non-KD with fever and clinical features suggestive of KD

- General pattern: adequate sample size
  - Study subjects: 214 children (100 KD, 114 non-KD)
Plasma Profile: The Discovery Study

A. IL-1β
B. IL-4
C. IL-6
D. IL-10
E. IL-17A
F. IL-17F
G. IL-21
H. IL-22
I. IL-23
J. IL-25
K. IL-31
L. IL-33
M. IFN-γ
N. sCD40L
O. TNF-α
P. E-selectin
Q. MPIF-1
R. IP-10
Plasma Profile: The Discovery Study

A. IL-1β
B. IL-4
C. IL-6
D. IL-10
E. IL-17A
F. IL-17F
G. IL-21
H. IL-22
I. IL-23
J. IL-25
K. IL-31
L. IL-33
M. IFN-γ
N. sCD40L
O. TNF-α
P. E-selectin
Q. MPIF-1
R. IP-10
IP-10 Levels: The Replication Study

*** (p = 2.2 × 10^{-11})

IP-10 pg/mL

febrile control       KD

0 1000 2000 3000 4000 5000 6000 7000 8000 9000 10000 pg/ml
IP-10 Levels: Combined Studies
(Discovery & Replication)

**AUC=0.94**

**Sensitivity= 100%**

**Specificity= 77%**

**AUC=0.94**

---

**Cut-off: 1,318 pg/ml**

*(IP-10 Levels)*
IP-10 Levels: Blinded Validation Study

Suspected KD children (n=60)

Medical Center

KD - clinical features - non-KD

Central Lab

IP-10 levels cut-off: 1,318 pg/ml (IP-10 Levels)
IP-10 Levels: Blinded Validation Study

Sensitivity, 96% [22/23]
Specificity, 81% [30/37]
Increase of IP-10 levels in the early stage of KD (< 4 days)

- 81% (30/37) KD Pts

- 1318 pg/ml (cut-off value)
IP-10 Levels in KD in Relation to IVIG Treatment

(n=45) (n=45)
Cell Surface Chemokine Receptor CXCR3 in T Cells of Patients with Acute KD
A potential **upstream** signaling pathway for **IP-10**
Summary

• During the discovery phase, the expression of IL-17F, sCD40L, E-selectin, MPIF-1, and IP-10 were upregulated during the acute phase in KD patients compared to that in the controls.

• ROC analysis of the combined discovery and replication data \([n(KD)=77, n(control)=77]\) showed that the IP-10 level had high AUC values (0.94; sensitivity, 100%; and specificity, 77%).

• With 1,318 pg/mL as the optimal cut-off, the blinded validation study confirmed that the IP-10 levels were a good predictor of KD.

• With IVIG treatment, the IP-10 levels returned to normal.

• The downstream receptor of IP-10, CXCR3, was activated in the T cells of acute KD patients.
Improve differential diagnosis of KD by IP-10

Suspected KD Pts

Fever > 5d

Differential diagnosis

Clinical Features + IP-10 Levels

Delay diagnosis (>10 days)

IVIG (intravenous immunoglobulin)

15% ~ 25% of untreated Pts
Coronary Artery Aneurysm (CAA)

1. myocardial infarction
2. ischemic heart disease
3. sudden death

(Circulation. 2004)
(Am Fam Physician. 2006)
CXCL10/IP-10 is a Biomarker and Mediator for Kawasaki Disease

Tai-Ming Ko¹, Ho-Chang Kuo², Jeng-Sheng Chang³, Shih-Ping Chen¹, Yi-Min Liu¹, Hui-Wen Chen¹, Fuu-Jen Tsai⁴, Yi-Ching Lee⁵, Chien-Hsiun Chen¹, Jer-Yuarn Wu¹ and Yuan-Tsong Chen¹*
Differential diagnosis algorism of iKD based on IP-10 Levels and other parameters

**diagnosis of iKD by clinical features**

1. Fever ≥ 5 days and 2 or 3 clinical criteria
2. Assess Patient Characteristics
   - Consistent with KD
   - Inconsistent with KD
   - Persistent Fever
3. Assess Laboratory Tests
   - CRP < 3.0 mg/DL and ESR < 40 mm/hr
   - CRP ≥ 3.0 mg/DL and/or ESR ≥ 40 mm/hr
4. Follow Daily
   - Fever continues for 2 days
   - Fever resolves
   - No Peeling
   - Typical Peeling
5. Echo
   - Echo –
   - Echo +
   - Fever Persist
   - Fever Abates
   - Treat
6. Repeat Echo Consult KD Expert
7. KD Unlikely

**diagnosis of iKD by lab parameters**

Suspected KD children

- KD/iKD
- non-KD

**Plasma IP-10 levels**

- Urine proteomics
- RNA expression profile
- DNA Genotyping
- other parameter (eg., ESR)

(Circulation. 2004)
**Hypothetical model: IP-10/CXCR3 axis in KD**

- Endothelial cells
- B cells
- T cells
- MΦ
- Plasma cells

**CXCR3**

**IP-10 (CXCL10)**

IP-10/CXCR3 axis

B cells

T cells

(T\textsubscript{H}1 cells)

IL-17

IL-6

(J Immunol. 2010)

(J Exp Med. 2012)
Hypothetical model: IP-10/CXCR3 axis in KD

What’s the pathogenic role of IP-10/CXCR3 axis?

What’s the interplay between IP-10 and susceptibility gene?
Acknowledgment

Academia Sinica
• Yuan-Tsong Chen
• Jer-Yuarn Wu
• Yi-Ching Lee
• Chien-Hsiun Chen
• Shih-Ping Chen
• Chia-Jung Chang
• Yi-Min Liu
• Hui-Wen Chen

Chang Gung Memorial Hospital
• Ho-Chang Kuo

China Medical University Hospital
• Jeng-Sheng Chang
• Fuu-Jen Tsai

Taiwan Kawasaki Disease Genetic Consortium
Thank you
The Multifaceted Functions of CXCL10 in Cardiovascular Disease

Mouse

- CXCL10
- T lymphocytes (Th1)
- NK cells
- Monocytes
- VSMCs
- ECs

CXCR3
- Chemotaxis
- Cell proliferation
- Cell migration
- Cell survival

Human

- CXCL10
- T lymphocytes (Th1)
- NK cells
- Monocytes
- ECs
- VSMCs

CXCR3-A
- Chemotaxis
- Cell proliferation
- Cell migration
- Cell survival

CXCR3-B
- Apoptosis
- Inhibition of:
  - Cell proliferation
  - Cell migration

(BioMed Research International, 2014)