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MAJOR ARTICLE

Pediatric Kawasaki Disease and Adult Human Immunodeficiency Virus Kawasaki-Like Syndrome Are Likely the Same Malady

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Background. Pediatric Kawasaki disease (KD) and human immunodeficiency virus (HIV)$^+$ adult Kawasaki-like syndrome (KLS) are dramatic vasculitides with similar physical findings. Both syndromes include unusual arterial histopathology with immunoglobulin (Ig)A$^+$ plasma cells, and both impressively respond to pooled Ig therapy. Their distinctive presentations, histopathology, and therapeutic response suggest a common etiology. Because blood is in immediate contact with inflamed arteries, we investigated whether KD and KLS share an inflammatory signature in serum.

Methods. A custom multiplex enzyme-linked immunosorbent assay (ELISA) defined the serum cytokine milieu in 2 adults with KLS during acute and convalescent phases, with asymptomatic HIV$^+$ subjects not taking antiretroviral therapy serving as controls. We then prospectively collected serum and plasma samples from children hospitalized with KD, unrelated febrile illnesses, and noninfectious conditions, analyzing them with a custom multiplex ELISA based on the KLS data.

Results. Patients with KLS and KD subjects shared an inflammatory signature including acute-phase reactants reflecting tumor necrosis factor (TNF)-α biologic activity (soluble TNF receptor I/II) and endothelial/smooth muscle chemokines *Ccl1* (Th2), *Ccl2* (vascular inflammation), and *Cxcl11* (plasma cell recruitment). *Ccl1* was specifically elevated in KD versus febrile controls, suggesting a unique relationship between *Ccl1* and KD/KLS pathogenesis.

Conclusions. This study defines a KD/KLS inflammatory signature mirroring a dysfunctional response likely to a common etiologic agent. The KD/KLS inflammatory signature based on elevated acute-phase reactants and specific endothelial/smooth muscle chemokines was able to identify KD subjects versus febrile controls, and it may serve as a practicable diagnostic test for KD.

Keywords. Kawasaki disease; Kawasaki-like syndrome; KD; KLS.

Kawasaki disease (KD) is diagnosed based on a constellation of findings including fever of at least 5 days plus at least 4 of the following 5: nonexudative conjunctivitis, rash, changes of the oropharynx, erythema and/or painful swelling of hands and feet, and sentinel lymph node. Kawasaki disease has a predilection for coronary arteries, causing aneurysms with residual risk for cardiovascular morbidity or mortality [1]. Intravenous immunoglobulin (IVIG) reduces aneurysm formation from 20% to 4% [2, 3]. Coronary arteries in fatal KD show a mixed inflammatory pattern that includes immunoglobulin (Ig)A$^+$ plasma cells [4, 5]. Immunoglobulin A$^+$ plasma cell infiltration occurs in vascular and nonvascular tissues, which suggests an infectious agent invading via respiratory tract or gut mucosa [6]. Periodic outbreaks suggest a ubiquitous infectious etiology [7, 8] with an atypical presentation in susceptible individuals. Diagnosing KD is problematic because children with atypical presentations may not receive IVIG but can develop aneurysms and sudden death [9].

In the 1980s, an adult syndrome resembling KD, termed Kawasaki-like syndrome (KLS), came to medical attention in human immunodeficiency virus (HIV)$^+$ adults with advanced disease [10]. Patients with KLS are treated with IVIG like KD patients, and they have similar responses (see reviews in Johnson et al [11] and Stankovic et al [12]). In a setting of limited evaluations, no aneurysms have been documented in KLS; however, aneurysms occur in HIV-seronegative adults with KD [13, 14]. In 2003, a KLS conjunctival biopsy showed IgA$^+$ plasma cells infiltrating arterial walls, linking KLS to KD by histopathology. The same study showed elevated soluble tumor necrosis factor receptor (sTNFR)II levels, suggesting a role for TNF-α in pathophysiology [15].

Understanding pathophysiology may improve KD/KLS diagnosis and treatment. Inflammatory responses can be categorized by cytokine patterns of activated CD4 T cells. In broad terms, responses to intracellular pathogens (eg, viruses) feature

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interferon gamma ([IFN-γ] labeled Th1) to (1) parasites and allergens interleukin (IL)-4 and IL-13 (Th2) and to (2) some bacterial infections and autoimmune diseases IL-17 (Th17) (reviewed in [16]). Chemokines recruit specific cell types to inflamed tissues, they are cell lineage specific, and they are differentially regulated in Th1/Th2 cytokine milieus (reviewed in [17]). We used this immunobiology as the framework to design (1) a multiplex ELISA investigation of cytokine milieu in 2 KLS cases and (2) a subsequent KD investigation that allowed us to compare the KD and KLS inflammatory signatures.

Case 1

Patient 1 is 23-year-old male with fevers, rash, strawberry tongue, painful swelling of hands and feet, and nonexudative conjunctivitis, preceded by diarrhea and abdominal pain (Figure 1). Admission evaluation revealed HIV/acquired immune deficiency syndrome (CD4 = 3; viral load 180 000 copies/mL).

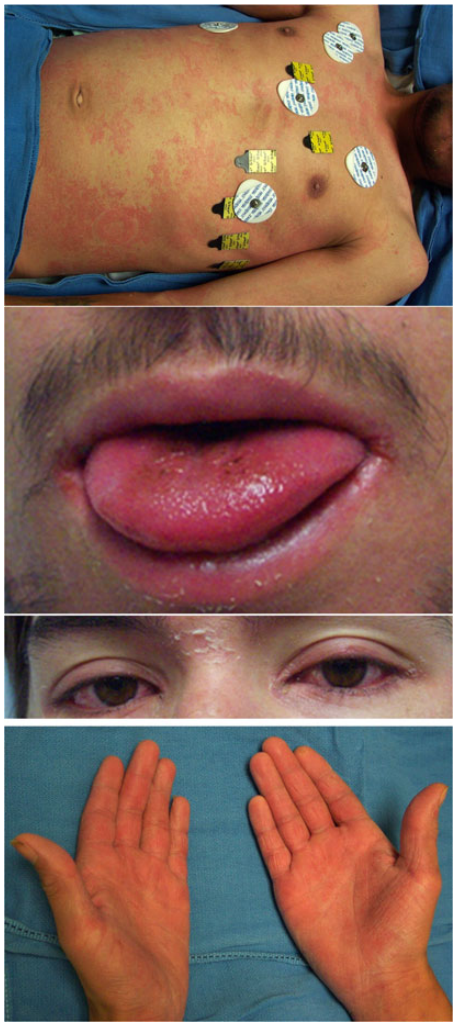

Figure 1. Physical findings in patient no. 1. Sequential panels showing rash, changes of the oropharynx, nonexudative conjunctivitis, and erythema with mild swelling of hands.

On hospital day 9, the patient had persistent fevers (103.9°F), and developed severe hypotension (maximum fluid resuscitation and pressors) in spite of empiric antimicrobials plus hydrocortisone; the workup for infectious etiologies was entirely negative (Supplementary Table 1). In the intensive care unit, he was diagnosed with KLS and treated with IVIG without aspirin due to thrombocytopenia, with a rapid response including fever resolution and pressor discontinuation within 24 hours. Without deterioration, fevers relapsed 4 days after IVIG no. 1, which led to a second infusion (IVIG no. 2). The relapsed fever only resolved with subsequent initiation of aspirin therapy enabled by improved platelet counts. With permission from the patient, serum was collected before IVIG no. 1 and 13 weeks postdischarge.

Case 2

Patient 2 is a 31-year-old HIV^+ male (CD4 = 19, viral load 139 000 copies/mL) with idiopathic eosinophilia who presented with 10 days of fever (102.7°F), myalgia, mild abdominal pain and diarrhea, painful swollen hands and feet. Findings included nonexudative conjunctivitis, cracked lips, mild thrush, cervical lymphadenopathy, impressive painful swelling of hands and feet, and rash. On hospital day 5, the patient remained febrile (103°F) but testing was negative (Supplementary Table 1). He was treated with IVIG and aspirin, defervesced during the infusion, and felt remarkably better. He was discharged the following day on previously prescribed antiretroviral medication plus aspirin. He returned to clinic at 2 and 5 weeks, and he appeared well on both visits. With permission serum was collected before IVIG and 5 weeks postdischarge.

Both patients had desquamation involving the hands at approximately 2 weeks, as typically seen in KD. Neither patient had a recurrence in the following 5 years; detailed clinical summaries are included in the Supplementary Data.

METHODS

Kawasaki-Like Syndrome Investigation

A case-control study was performed with approval from the Indiana University Institutional Review Board (IRB). A custom multiplex ELISA with analytes representing cytokine polarization, tissue/cell-type specific inflammation, and/or KD/KLS association was designed (Supplementary Table 2). Analyte levels in acute and convalescent KLS serum were compared with levels in asymptomatic HIV^+ male serum (CD4 > 350 not taking combination antiretroviral therapy) leftover from an unrelated study [18]. Serum from a healthy female was included to preliminarily identify analytes abnormally low in HIV^+ individuals. A commercial laboratory routinely servicing large clinical trials ([19, 20]) (Aushon Biosystems, Woburn, MA) performed multiplex ELISA on blinded samples. Samples 1 and 2 comprised acute/convalescent sera from patient 1; samples 3 and 4 comprised acute/convalescent sera from patient 2; samples 5–7 comprised

HIV controls; and sample 8 comprised a seronegative female (dataset in Supplementary Table 3). An in-house ELISA was performed to quantify IFN-γ (human IFN-γ ELISA kit; Pierce-Endogen, Rockford, IL).

Kawasaki Disease Study

Based on our KLS investigation, we hypothesized that IL-6, IL-13, and sTNFRI/II along with chemokines *Ccl1*, *Ccl2*, and *Cxcl11* would be elevated in KD and that elevated sTNFRI/II plus *Ccl1*, *Ccl2*, and *Cxcl11* would selectively identify KD cases. A custom multiplex ELISA was designed for the KD study including IL-1β (anticipated negative acute-phase reactant), IL-4 (Th2), IL-13 (Th2), *Ccl1* (Th2 chemokine), *Ccl2* (vascular inflammation), *Ccl11* (Th2 chemokine), *Ccl4* (anticipated negative Th1 chemokine), *Cxcl9* (neutrophil recruitment), and *Cxcl11* (plasma cell recruitment). In the context of a small study, paired serum and plasma samples (pseudo-duplicates) were obtained to address outliers and determine optimal sample type. Blood sampling was done before IVIG for KD subjects.

The prospective KD study was performed between September 2014 and July 2015. Children >6 months and <8 years were enrolled into 3 groups: (1) KD, (2) febrile illness (febrile controls [FC]), and (3) noninfectious illness (healthy controls [HC]). Inclusion criteria included formal KD diagnosis (KD), fever >38.2°C during evaluation, a non-KD working diagnosis without rash (FC), and admission for noninfectious condition (HC). Exclusion criteria included known HIV^+ status or genetic disorder (all) and absence of a working diagnosis or presence of rash (FC). Serum and plasma datasets are in Supplementary Tables 5 and 6. The IRBs at Indiana University Riley Children's Hospital and Children's Hospitals and Clinics of Minnesota approved this study.

Statistical Analysis

For KLS, data were analyzed as individual comparisons of patient 1 and 2 acute and convalescent values to the combined analyte data from 3 HIV^+ control subjects with a Student's *t* test. "Normal serum" was not included in analyses. For within-study comparisons, *P* values of <.05 were considered significant. Power analyses to guide follow-up studies were performed, and these results are included in the legend of Figure 3.

Based on our KLS data and the literature [15, 21], we postulated that KD would be inextricably linked to a marked elevation in sTNFRI/II or IL-6 and that elevations in *Ccl1*, *Ccl2*, and w *Cxcl11 w*ould identify KD versus FC. Elevations in sTNFRIl/II were confirmed during preliminary examination of data. One-way analysis of variance with Tukey post hoc tests and receiver operator curve (ROC) analyses were performed on sTNFRI/II and IL-6 to determine optimal cutoffs. An sTNFRII cutoff of >1900 pg/mL eliminated all HC and 3 FC, step 1 of a 2-step algorithm. We then performed *t* tests with Welch correction and ROC analyses on subjects with sTNFRII levels >1900 pg/mL to determine whether remaining analytes were uniquely elevated in KD versus FC. For pathogenesis-specific chemokines *Ccl1* (*P* value = .036), *Ccl2* (*P* value = .024), and *Cxcl11* (*P* value = .13) identified in our KLS study as possible "KD predictors", a ROC analysis was performed to find optimal cutoffs; *Ccl1* (>3.55 pg/mL), *Ccl2* (>715 pg/mL), and *Cxcl11* (>39.4 pg/mL). Educated by samples 12 (KD) and 26 (FC) with similar measurements (Table 1), we found that at least 2 KD predictors were needed to appropriately identify these subjects; a comprehensive statistical analysis description is in Supplementary Data.

RESULTS

Kawasaki-Like Syndrome Study

Kawasaki-like syndrome results can be grouped into 3 categories. In the first category, analytes not elevated in KLS were compared with HIV^+ controls. This category includes IL-17 and *Ccl5* (Supplementary Figure 1) and IL-1β (below limit of detection).

The second category includes analytes related to KLS severity. Of these, IFN-γ, *Cxcl10*, osteoprotegerin, TNF-α, IL-1ra, and IL-10 were elevated only in patient 1 with shock (Figure 2). Although the results were statistically significant, *Cxcl10* is included in this category because the level in patient 2 was only 30% higher than HIV^+ controls; IL-1ra was included here because the "elevated" level in patient 2 was lower than the level found in normal serum. Macrophage colony-stimulating factor was elevated in typical KLS and persistently elevated in severe KLS. Interferon-α (Figure 2, top right panel) was elevated in typical KLS and absent in KLS shock (KLSS). Although conclusions cannot be drawn based on 2 patients, an inadequate IFN-α response may be a marker for severe disease.

The third (interesting) category includes analytes elevated in acute KLS that, during the convalescent phase, decreased to or toward levels seen in HIV^+ controls. The category includes IL-6, sTNFRII, IL-13, *Ccl1*, *Ccl2*, and *Cxcl11* (Figure 3). As previously reported in KD [22, 23], IL-6 and *Ccl2* were elevated in KLS. As previously reported in KLS [15], sTNFRII was markedly elevated in both KLS patients. Particularly interesting were elevations in IL-13 (Th2), and chemokines *Ccl1* (Th2), and *Cxcl11*, a plausible link to pathognomonic IgA^+ plasma cell infiltration of arterial walls in KD/KLS. Supplementary Table 4 summarizes the KLS results versus published data for KD and non-KD illnesses.

Kawasaki Disease Study Results

Sixteen KD, 13 FC, and 10 HC subjects were enrolled. Two FC were excluded before analysis: subject-2101 was 9 years old (too old), and subject-2102 had a prominent rash of unknown etiology (a predefined exclusion criterion). Sixteen KD (1 with aneurysms, 1 incomplete, 1 IVIG-resistant), 11 FC, and 10 HC were included in the final analyses; demographics and clinical findings are in Supplementary Tables 7–9.

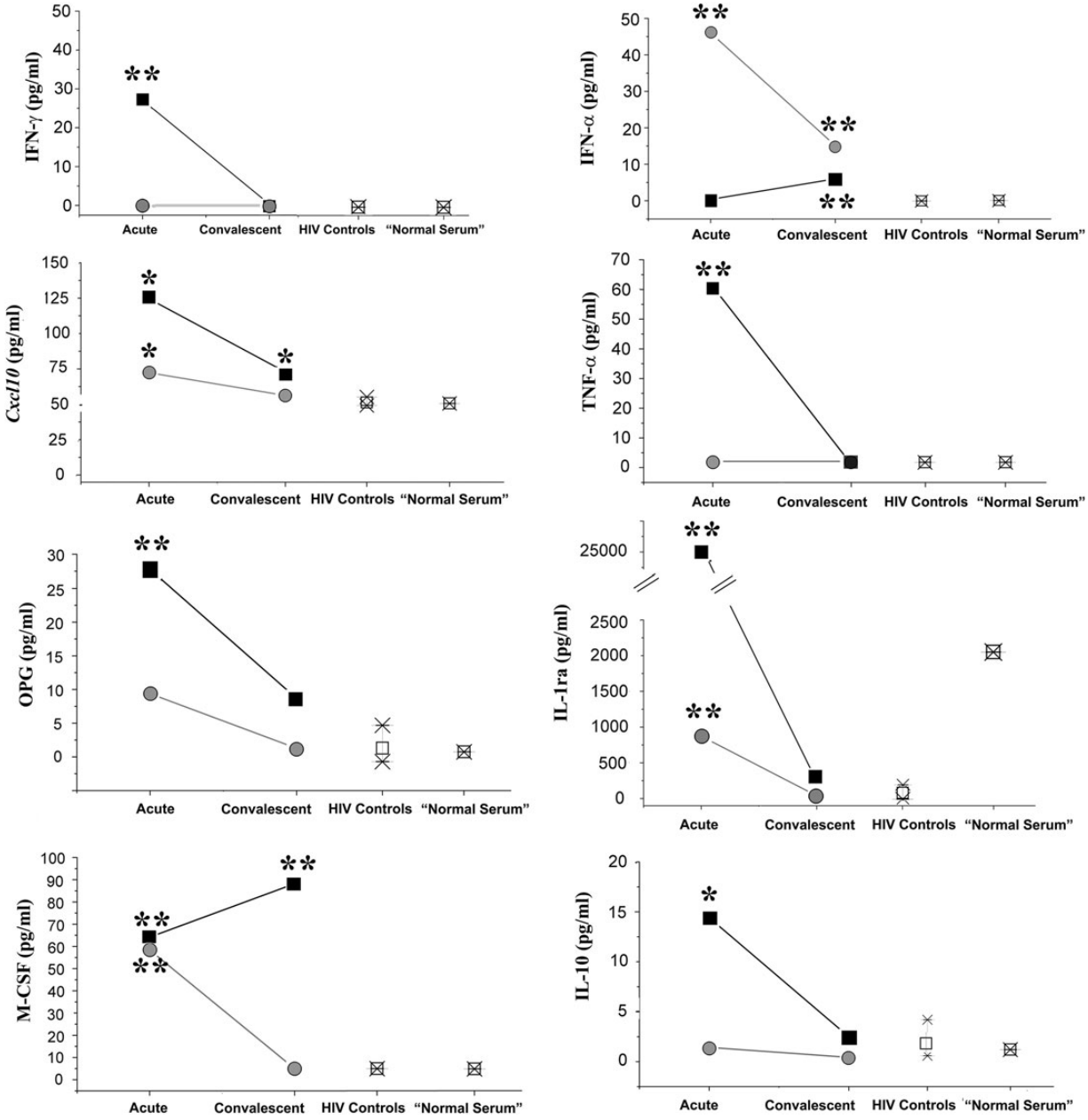

Figure 2. Analytes reflecting severity of Kawasaki-like syndrome (KLS). Patient 1 (severe KLS shock), black squares; patient 2 (typical KLS), gray circles. The control human immunodeficiency virus (HIV) subjects' mean (open square) and range of analyte values are indicated in the third column. The level of analyte in a single HIV-negative "normal serum" is shown as a square in the final column. For interleukin (IL)-1ra, note (1) the break in the scale and (2) that the level of IL-1ra in HIV-negative normal serum is higher than in HIV^+ control subjects. Interferon (IFN)-γ and tumor necrosis factor (TNF)-α for the HIV^+ controls and normal serum were below the limit of detection and were plotted as "0". **P* value <.05; ***P* value <.01. OPG, osteoprotegrin.

Multiplex ELISA testing (Figure 4) showed that KD universally included marked elevations in acute-phase reactants IL-6 or TNF-α surrogates TNFRI/II but usually in both. There were significant differences or trends in KD versus FC in KLS-implicated pathogenesis-chemokines *Ccl1*, *Ccl2*, and *Cxcl11*. *Cxcl11* did not reach significance ($P = .13$) due to a high level (3488 pg/mL) in KD subject-1101. Subject-1101 was arguably the sickest subject with an echocardiography left anterior descending z-score of +2.69, marked perivascular brightness around the coronaries, and the highest C-reactive protein (CRP) in the study at 34.4 mg/dL (see Supplementary Table 8). The markedly elevated *Cxcl11* may have reflected the severity of the vasculitis. The high *Cxcl11* level for subject-1101 did not appear to be erroneous because it was similarly elevated in paired-plasma (pseudo-duplicate as per study design). The KD *Cxcl11* mean without subject-1101 was 163

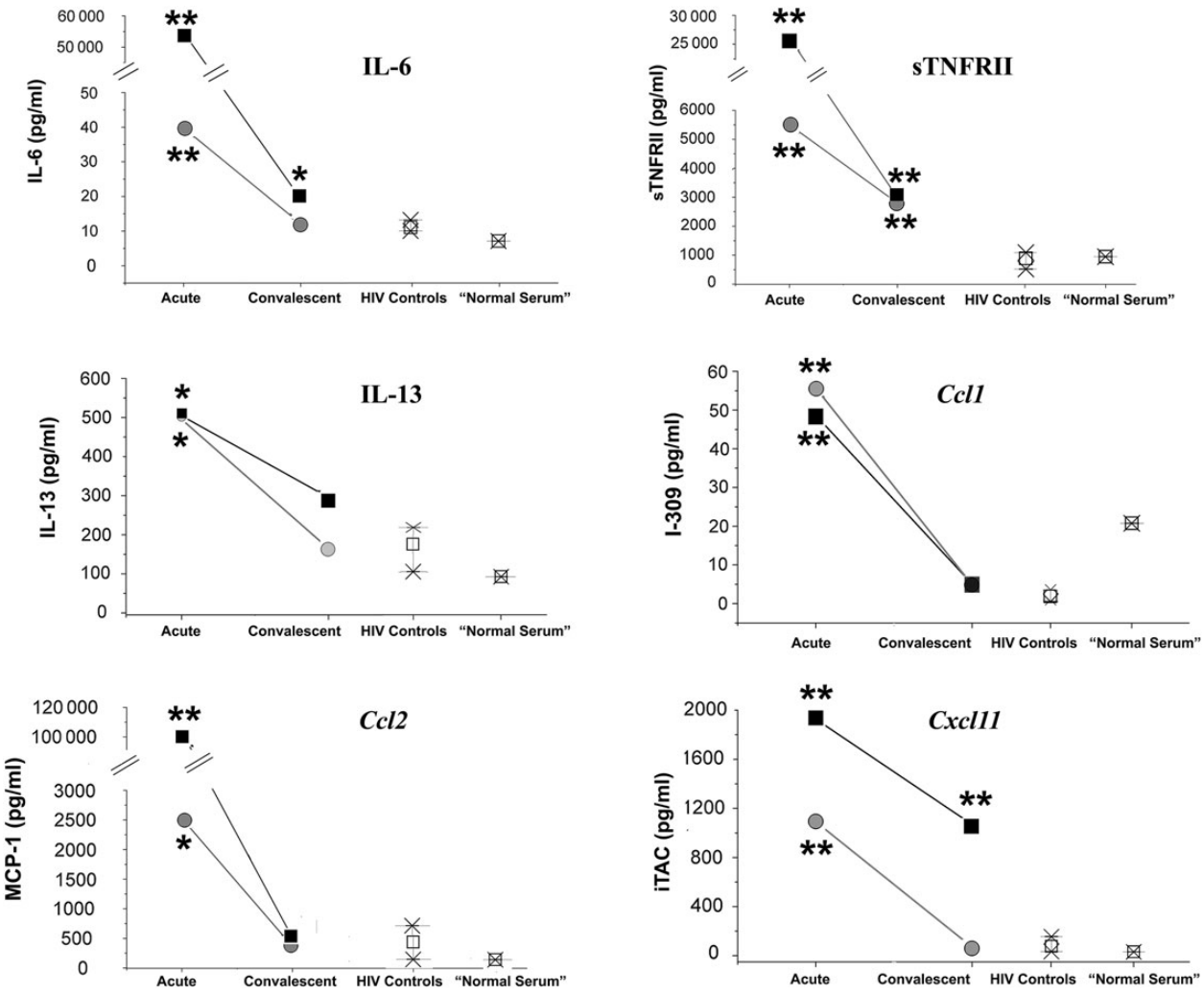

Figure 3. Analytes elevated in Kawasaki-like syndrome (KLS) patients in the acute phase that, during the convalescent phase, return to or toward levels seen in asymptomatic human immunodeficiency virus (HIV)$^+$ control subjects. Patient 1 (severe KLS), black squares; patient 2 (typical KLS), gray circles. For chemokines, the common nomenclature is used on the y-axis; the graph body is labeled with the systematic name. Power analyses of these pilot data suggest a confirmatory sample size as small as 3 controls and 3 cases for interleukin (IL)-6, tumor necrosis factor receptor (TNFRII), *Ccl1*, *Ccl2*, and *Cxcl11*; 6 controls and 3 cases for IL-13. **P* value <.05; ***P* value <.01. Abbreviations: MCP, monocyte chemoattractant protein; sTNFRII, soluble TNFRII.

pg/mL. With subject-1101 absent, the *Cxcl11 P* value based on 15 KD subjects was .008. The acute-phase reactant IL-1β and the Th1 chemokine *Ccl4* were not specifically elevated in KD nor was the Th2 chemokine *Ccl11*. Elevated IL-13 documented in KLS was not seen in KD nor was previously reported elevation in Th2 cytokine IL-4. *Cxcl9* was elevated in KD, but ROC analysis showed that *Cxcl9* did not separate KD from FC. Before the KD study, we hypothesized that elevated sTNFRI/II combined with elevated pathogenesis-chemokines *Ccl1*, *Ccl2*, and *Cxcl11* would be a diagnostic test for KD. Based on that hypothesis, we tested a 2-step algorithm. Using a TNFRII level of >1900 pg/mL as the cutoff for considering a KD diagnosis, secondary ROC analysis showed that elevations in 2 or more pathogenesis-chemokines (*Ccl1*, *Ccl2*, *Cxcl11*) accurately identified all KD versus FC (Figure 5; Table 1).

DISCUSSION

Our investigation shows that KD and KLS share an inflammatory signature including a robust acute-phase response (sTNFRI/II) and elevations in pathogenesis-specific chemokines *Ccl1*, *Ccl2*, and likely *Cxcl11*. We posit that KD and KLS are the same disease based on inflammatory signatures, combined with unusual arterial histopathology (IgA$^+$ plasma cells), similar physical findings, and rapid clinical responses to IVIG. The KD/KLS inflammatory signature is compatible with an infectious agent, perhaps the ribonucleic acid (RNA) virus-like particles visualized in pathology specimens using reverse-engineered KD IgA antibodies [24, 25].

This is the first reported case of KLSS. It manifested similar to KD shock syndrome ([26–28]). The mechanism appears to be a cytokine storm with high TNF-α and IL-6 and an ineffective

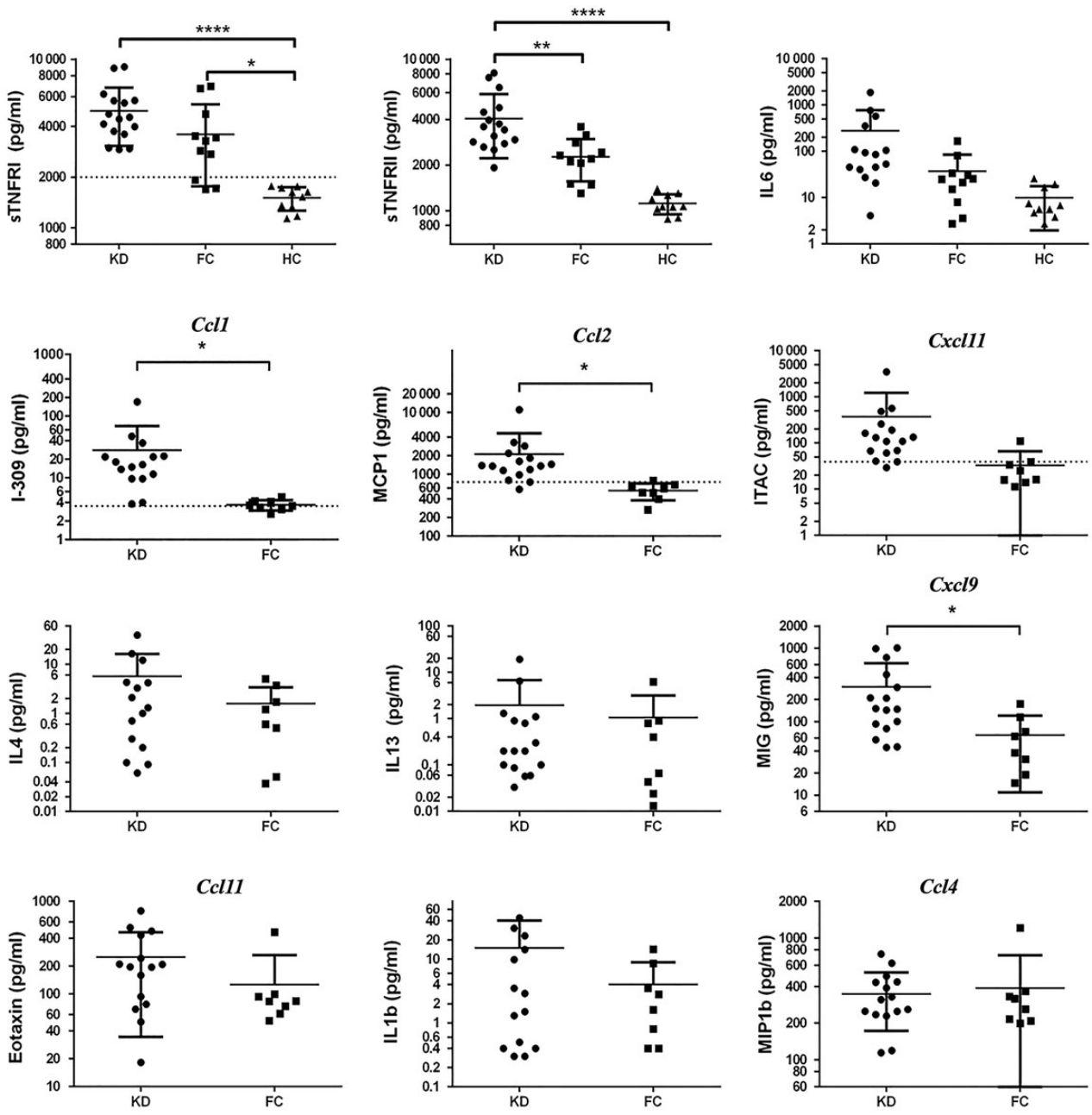

Figure 4. Results of Kawasaki disease (KD) study. Top row shows acute-phase reactants: soluble tumor necrosis factor receptors (sTNFR) and interleukin (IL)-6. Row 2 illustrates pathogenesis-specific chemokines. Rows 3 and 4 show analytes related to polarization of the immune response. For chemokines only, common nomenclature is used on the y-axis and the graph body is labeled with the systematic name. Note that data are plotted on log scale. **P* value <.05; ***P* value < .01; *****P* value < .0001. Abbreviations: ITAC, interferon-inducible T-cell chemokine; MCP, monocyte chemoattractant protein; MIG, monocyte interferon-γ-inducible protein; MIP, macrophage inflammatory protein.

counter-inflammatory response (IL-10/IL-1ra). This case demonstrates that KLS is potentially fatal and that IVIG should be administered beyond the 10-day febrile cutoff used in pediatrics.

Our investigation of 2 KLS patients at clinical extremes suggests that IFN-α is important for host protection in KD/KLS. The typical KLS patient (Case 2) had elevated IFN-α (46 pg/mL) with undetectable IFN-γ; the KLSS patient had elevated IFN-γ (27 pg/mL) with undetectable IFN-α, a starkly inverted pattern. For comparison, IFN-γ serum levels during viral illnesses run 8–30 pg/mL [29–31]; IFN-α levels in children with influenza had a mean of 602 pg/mL [32]. Kawasaki disease investigators have documented low to undetectable serum IFN-α levels [33–35]; however, the KD coronary artery transcriptome shows a prominent type I IFN molecular fingerprint; ie, evidence for local IFN activity during KD arteritis [36]. If KD

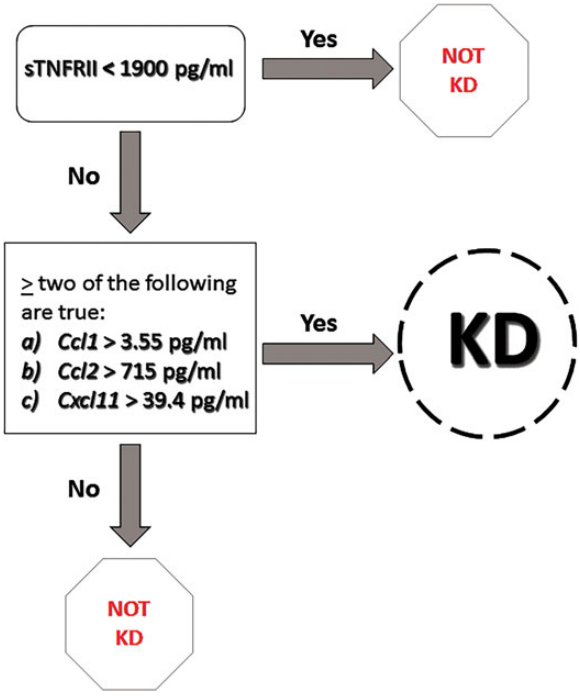

Figure 5. Two-step Kawasaki disease (KD) diagnostic algorithm. Abbreviation: sTNFR, soluble tumor necrosis factor receptor.

susceptibility is determined by suboptimal innate IFN-α responses, then low IFN-α levels do not argue against its role in protection or against an RNA virus-like etiologic agent.

Our KD results are consistent with published data. We found *Cxcl9* elevated in KD, consistent with a recent serum study [37] and the coronary artery transcriptome [36]. Soluble TNFRI/II (TNF-α activity) within the inflammatory signature has been individually reported by others [15, 21] and seems to play a central role in pathogenesis. The mechanism-specific chemokines *Ccl2*, *Cxcl11*, and *Ccl1* investigated in this study are the unique aspect of the KD/KLS inflammatory signature.

Ccl2 (endothelial/smooth muscle cells) is a biomarker for myocardial infarction risk [38] and was previously shown to be elevated in KD [39, 40]. In this study, we identify *Cxcl11* as a likely new KD-associated chemokine (unadjusted *P* value of .13; adjusted *P* value of .008). Its receptor *Cxcr3* is present and functional on human IgA^+ plasma cells [41], consistent with recruitment into arterial walls. Elevated *Cxcl11* is associated with human aortic aneurysms, and *Cxcr3* is required for aneurysm formation in mice [42]. Engagement of *Cxcr3* by *Cxcl9*, *Cxcl10*, and *Cxcl11* results in its removal from the cell surface. Children with KD have reduced *Cxcr3* on circulating T cells attributed to *Cxcl10* [43]; *Cxcl11* is a more potent *Cxcr3*

Table 1. Testing the Kawasaki Disease Algorithm

	pg/mL						
Test ID	hITAC	hMCP1	hI309	ITAC > 39.4	MCP1 > 715	I309 > 3.55	Algorithm Classification[a]
Sample 2	131.2	1156.1	Low sample volume	TRUE	TRUE	TRUE	KD
Sample 4	565.2	11140.2	22	TRUE	TRUE	TRUE	KD
Sample 6	61	1201.8	16.4	TRUE	TRUE	TRUE	KD
Sample 8	39.5	1837	9.7	TRUE	TRUE	TRUE	KD
Sample 10	162.3	804.6	11.5	TRUE	TRUE	TRUE	KD
Sample 12	29.3	752	3.8	FALSE	TRUE	TRUE	KD
Sample 14	67.2	2878.9	169.9	TRUE	TRUE	TRUE	KD
Sample 16	68.9	983.5	36.9	TRUE	TRUE	TRUE	KD
Sample 18	40.7	574.3	15	TRUE	FALSE	TRUE	KD
Sample 60	3487.8	3285.9	47.1	TRUE	TRUE	TRUE	KD
Sample 62	191.4	1360.7	9.7	TRUE	TRUE	TRUE	KD
Sample 64	134.7	1393.6	4	TRUE	TRUE	TRUE	KD
Sample 66	108.7	1624	13.8	TRUE	TRUE	TRUE	KD
Sample 68	257.1	1458.7	21.9	TRUE	TRUE	TRUE	KD
Sample 70	484.9	2189.8	22.6	TRUE	TRUE	TRUE	KD
Sample 72	109.6	1366.2	18.1	TRUE	TRUE	TRUE	KD
Sample 24	14	650.8	4.1	FALSE	FALSE	TRUE	not KD
Sample 26	16	792.3	2.6	FALSE	TRUE	FALSE	not KD
Sample 28	33.7	266.4	3.1	FALSE	FALSE	FALSE	not KD
Sample 30	25	399.7	4.9	FALSE	FALSE	TRUE	not KD
Sample 34	15.9	677.5	3.5	FALSE	FALSE	FALSE	not KD
Sample 36	39.3	499.7	4.2	FALSE	FALSE	TRUE	not KD
Sample 38	11.3	506.1	3.6	FALSE	FALSE	TRUE	not KD
Sample 78	109.7	586.1	3.3	TRUE	FALSE	FALSE	not KD

Samples highlighted in light gray are KD subjects; samples highlighted in red are FC subjects with sTNFRII values >1900 pg/mL.

Abbreviations: I309, inflammatory cytokine 309; ITAC, interferon-inducible T-cell chemokine; MCP, monocyte chemoattractant protein; KD, Kawasaki Disease.

[a]>2 true equals a diagnosis of KD.

internalization trigger than *Cxcl10* [44–46]. *Cxcr3* inhibitors, potentially KD therapeutics, have entered clinical trials for other purposes [47, 48].

Ccl1 was uniquely elevated in KD in this 39-subject study. *Ccl1* is produced by human endothelial cells exposed to atherogenic apolipoprotein A. The *Ccl1* receptor *Ccr8* is found on Th2 cells [49]; however, *Ccr8* is also expressed by human vascular smooth muscle cells. In a mouse model, femoral artery luminal trauma induced *Ccl1* production by smooth muscle cells, suggesting that *Ccl1/Ccr8* is involved in vascular injury repair [50]. *Ccl1* seems to be intimately and uniquely tied to KD/KLS-triggered vascular injury.

The Th1/Th2/Th17 polarization paradigm has been used to interpret KD pathogenesis. Interferon-γ, the prototype Th1 cytokine, is not typically elevated in KD [35, 51]; children with elevated IFN-γ are at higher risk of aneurysms [34]. In this report, the adult HIV^+ patient with detectable IFN-γ went into shock. In contrast, IL-4, the prototypic Th2 cytokine, has previously been reported elevated in children with KD [35], and the Th2 chemokine *Ccl17* recently shown elevated in KD [37]. Idiopathic eosinophilia (Th2 phenomenon) is seen in KD [52–54] and in non-HIV adult KD [14]. KLS patient 2 with typical KLS had idiopathic eosinophilia, a recognized condition in advanced HIV disease [55, 56]. We investigated the Th2 chemokine *Ccl11* (regulated by IL-4), which was not elevated. The Th1 chemokines elevated in KD (*Cxcl9*, *Cxcl10*, *Cxc11*, *Ccl2*) would recruit Th1 cells as available, but those chemokine levels are regulated by IFNs (including IFN-α) and/or TNF-α unrelated to cytokine polarization. We investigated Th1 chemokine *Ccl4* produced by activated cells of several types, which was not elevated. We found that IL-13, a quintessential Th2 cytokine, was elevated in both KLS patients. The IL-13 levels in KD children were low, and differences between KD and FC were not significant, perhaps reflecting the difference between reactivation/recall in KLS versus a primary response in KD. We and others [57–59] have shown that CD8 T cells produce IL-13. CD8 T cells predominate in acutely inflamed KD coronary arteries [60]. It is unclear whether the CD4-based Th1/Th2/Th17 paradigm is a good fit for KD/KLS pathogenesis.

Our results suggest a KD/KLS diagnostic test based on elevated sTNFRI/II combined with elevations in pathogenesis-specific chemokines *Ccl1*, *Ccl2*, and *Cxcl11*. A previously published KD diagnostic algorithm based on 12 conventional laboratory parameters (CRP, total white blood cell count, etc) had a sensitivity of 74.3% and a false-positive KD diagnosis rate of 37.2% for FC [61]. An improved version of this algorithm was published during the revision process for this manuscript [62]. Kawasaki disease pathophysiology has been investigated using advanced transcriptomics, proteomics, and multiplex ELISA. Urine proteomics identified KD biomarkers including meprin A [63], and peripheral blood mononuclear cells (PBMCs) transcriptomics identified KD transcripts, including 130 differing between KD and adenovirus-infected children [64–66]. The analytes in our inflammatory signature are not among the 130 transcripts. It is not clear whether urine protein or circulating PBMC mRNA optimally reflect vascular wall inflammation. Ko et al [43] recently used commercial multiplex ELISA arrays to investigate KD versus FC and demonstrated that *Cxcl10* was differentially elevated in KD. Our custom 12-plex KD ELISA contained 3 analytes investigated in that study with the same results: there was no difference in IL-1β and insignificant trends toward elevated IL-4 and IL-6 in KD versus FC.

CONCLUSIONS

Kawasaki disease and KLS are likely the same disease, based on a shared inflammatory signature. Specific signature components, elevations in sTNFRII, and at least 2 of 3 pathogenesis-associated chemokines (*Ccl1*, *Ccl2*, and *Cxcl11*), were able to identify KD subjects versus FC and healthy children as diagnosed by experienced clinicians at 2 major pediatric tertiary referral hospitals. This was not a definitive study of the KD inflammatory signature's utility in general practice. Children with febrile illness and rash were specifically excluded because incomplete KD is a difficult diagnosis, with existing diagnostic criteria based solely on expert opinion [67]. Inclusion of febrile illnesses with rash as controls (eg, viral syndrome) in this initial investigation would have required study-dictated diagnostic viral testing and echocardiography to ensure that cases of incomplete KD did not get assigned to the febrile control group. Follow-up studies will include controls with defined febrile rashes (eg, adenoviral infections), and an investigation to determine whether KD diagnoses can be made as early as day 3 of fever in the setting of at least 1 of the following: nonexudative conjunctivitis, rash, painful swelling, or erythema of hands or feet. Results of this study also suggest that investigations of type 1 IFNs, *Cxcl11/Cxcr3*, and *Ccl1/Ccr8* will provide new insights into KD and KLS pathogenesis.

Supplementary Data

Supplementary material is available online at *Open Forum Infectious Diseases* online (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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