Supplemental Data

Case 1

Patient 1 was a 23 year old male admitted with several days of fevers, rash, strawberry tongue, painful swelling of hands/feet, and non-exudative conjunctivitis, preceded by diarrhea and abdominal pain beginning about 1 week prior to admission (physical findings; Figure 1). Admission evaluation included new diagnoses of HCV and AIDS. He was treated with multiple antibiotics without improvement and after 1 week, he remained febrile (103.9°F), with episodes of hypotension, and was transferred to the ICU for impending vascular collapse. His CXR showed increased interstitial markings; a bronchoscopy/BAL with negative stains for PCP, yeasts, molds, viral, or bacterial pathogens; cultures grew a few Candida albicans. Evaluation for other infectious agents was negative (Supplemental Table 1). On day 9 he became severely hypotensive requiring fluid resuscitation and high dose pressors while on empiric high dose hydrocortisone, vancomycin, meropenem, doxycycline, amphotericin, and metronidazole. An echocardiogram showed hyperdynamic ventricles with preserved function. Ultimately, the diagnosis of KLS was considered. Without other changes he received 2 gm/kg of IVIG. Aspirin was not initiated due to platelet count of 33; methylprednisolone was added to hydrocortisone. Hypotension and fevers rapidly responded to the IVIG with resolution of fevers and tapering pressors to off within 24 h. Four days later fevers relapsed on methylprednisolone without clinical deterioration, and resolved with initiation of aspirin therapy and discontinuation of antimicrobials. He developed desquamation of the fingers and toes, and around the eyes, with sloughing of his tongue epithelium. He was discharged home on day 11 after IVIG#2 on 165 mg aspirin daily, a rapid prednisone taper, and prophylaxis for PCP/MAC. The patient had follow up visits at one, five, and thirteen weeks. He started cART with subsequent immune reconstitution (absolute CD4 293; HIV viral load <50 copies/ml). He had a negative cardiac stress thallium at 6 months and discontinued ASA. With the patient's permission serum collected immediately prior to IVIG#1 and 13 weeks post discharge was stored at -80° C. The patient did not have a KLS recurrence during the next 5 years.

Case 2

Patient 2 was a 31 year old male recently diagnosed with HIV (CD4 count 19, viral load 139,000 copies/ml) and idiopathic eosinophilia, admitted with ten days of fever (102.7° F), myalgias, mild abdominal pain with diarrhea and painful swelling of the hands and feet coincident with 1st cART regimen. His exam was remarkable for non-exudative conjunctivitis,

cracked lips, mild thrush, non-tender cervical lymphadenopathy, impressive painful swelling of the hands and feet, and a rash. cART was held. The working diagnoses were drug reaction *versus Chlamydia*-associated reactive arthritis. On day 5 the patient remained febrile (103° F); all testing was negative including *Chlamydia* (Supplemental Table 1). He was treated with 2 gm/kg IVIG plus ASA 325 mg qid. During the IVIG infusion he defervesced and had no further fevers, felt remarkably better and was discharged the following day on fluconazole, prior cART, ASA 325 mg bid, MAC prophylaxis with plan to initiate PCP prophylaxis during follow up. He returned to ID clinic at 2 and 5 weeks and was well on both visits. He had periungual desquamation of the hands at the 2 week visit as typically seen in KD. With the patient's permission, serum collected immediately prior to IVIG and 5 weeks post discharge were stored at -80° C. He had no KLS recurrences in the subsequent 5 years.

Statistical Analyses

For the HIV KLS study, data was analyzed as individual comparisons of patient 1 and patient 2 acute and convalescent values to the combined analyte data from the three asymptomatic HIV⁺ control subjects with a Student's t-test. Results from the seronegative control "normal serum" were not included in the analysis. For within-the-study statistical comparisons, *p values* of <0.05 are considered statistically significant. Correction for multiple comparisons were not performed because analytes in the panel had previously been reported to be elevated in KLS or KD, or were logically linked with KLS pathogenesis (*Ccl1*, IL-13, and *Cxcl11*). A power calculation was performed for analytes of interest (elevated in both KLS patients with *p values* <0.05) to estimate the sample size in a follow up study to confirm/refute the preliminary data. Power calculation results are included in the figure legends for analytes of interest. Power analyses were performed with an alpha level of 0.05 and power level of 0.80 (beta level = 0.20), with means and standard deviations based off of values derived from this pilot study with three controls and one case (thus weighted at a three to one ratio). As the standard deviation of the case arm could not be estimated based on a single typical KLS case, it was assumed to be the same as the standard deviation as the HIV control arm for power analysis purposes, an assumption not likely valid for all analytes, and likely problematic for analytes that do not approximate zero in asymptomatic HIV⁺ individuals.

For the pediatric KD study, in Step 1 the acute phase reactants IL-6, sTNFRI, and sTNFRII were analyzed by one-way ANOVA with multiple comparison correction (GraphPad Prism version 5.00) to test if there are differences among KD, FC, and HC samples. A ROC analysis showed that a TNFR1 <2000 and TNFRII <1900 pg/ml removed the same set of samples (all HC and 3 FC). In Step 2, for each of the remaining analytes a T-test with Welch correction was performed to determine whether levels in KD and FC were different, along with an ROC analysis to find optimal cutoffs. ROC analysis showed that *Cc/1, Cc/2*, and *Cxc/11* were useful predictors of KD *versus* FC. Using sample 12 (KD) and sample 26 (FC) to educate, at least two of the predictors needed to be above their cutoffs to classify these two samples correctly. Data points were considered to be outliers if the value was >5-fold higher than the mean of the experimental group, <u>and</u> if the corresponding paired serum or plasma value was not within 5-fold of the questionable value. Of 416 values in the study 3 met outlier criteria (white boxes in Supplemental tables 5 and 6). Two were in the healthy controls (I-TAC) and one in a febrile control (I-TAC), suggesting possible slight technical issues specific to the I-TAC analyte assay. Outlier values were not used during performance of the KD *versus* FC t-tests or for ROC analysis. Including or excluding the outlier values had the possibility of a KD diagnosis rejected at step1 based on sTNFRII levels <1900 pg/ml.

Table 1- Diagnostic Testing

Diagnostic Test	Patient 1	Patient 2
	KLSS (shock)	KLS
Admission WBC	5.8	3.5
CD4 count	3	19
HIV vl	180,000	139,000
HCV studies	1a	neg
HBV sAg	neg	neg
Oral HSV culture	neg	ND
CXR	NAD	NAD
Urinalysis	2 WBC	0 WBC
CSF	ND	1 WBC
ESR	5	87
ANA	ND	<1:40
LDH	1370	ND
lg A level	531	267
ASO titer (ULN <199)	200	200
Strep screen	neg	ND
RPR	neg	neg
Monospot	neg	neg
EBV IgM	neg	neg
CMV IgM	neg	neg
Toxo IgM	neg	ND
Erhlichea pcr	neg	ND
Parvovirus B19 pcr	neg	ND
Parvovirus B19 IgM	ND	neg
HHV6 pcr	neg	ND
Urine Histoplasma ag	neg	neg
Serum cryptococcal ag	ND	neg
Blood cultures	neg	neg
Stool culture/O&P	neg	neg
Chlamydia/GC LCR	ND	neg
Nasopharynx viral cult.	neg	neg
BAL stains	neg	ND
BAL culture	few Candida	ND
BM bx stains & cultures	neg	ND
Skin bx	perivascular lymphoctyes	perivascular lymphocytes, few eosinophils

NAD = no active disease ND = not determined

Table 2- Analyte Panel

Analyte	Cell type origin	Ref.	KD serum	KD ref.
IL-1beta	Multiple	[<u>1</u>]	Elevated	[2]
IL-1ra	Multiple excepting lymphocytes	[<u>3</u>]	No data	NA
IL-6	Multiple including T cells	[<u>4</u> , <u>5</u>]	Elevated	[<u>6-8]</u>
IL-10	Hematopoetic cells including T cells	[<u>9]</u>	Elevated	[<u>10</u> , <u>11</u>]
IL-13	T cells (Th2), eosinophils, mast cells	[<u>12-14]</u>	No data	NA
IL-17	T cells (Th17 & some Th1, rare CD8)	[<u>15</u>]	Elevated	[<u>16</u> , <u>17</u>]
IFN-a	Multiple		Conflicting	[<u>18</u> , <u>19]</u>
IFN-g	T cells (Th1)	[<u>20</u>]	Not elevated	[<u>21</u> , <u>22</u>]
			In majority	
TNFa	Multiple including T cells	[<u>23</u>]	Elevated	[<u>7</u> , <u>24</u> , <u>25]</u>
sTNFRII	Multiple, regulated by TNFa	[<u>26</u>]	Elevated KD & KLS	[<u>24</u> , <u>27</u>]
M-CSF	Mesenchymal cell types (endothelium, fibroblasts)	[<u>28</u>]	Elevated	[<u>29</u> , <u>30</u>]
Osteoprotegrin	Osteoblasts, endothelial and smooth muscle cells	[<u>31</u>]	Elevated	[<u>32</u>]
<i>Ccl1</i> (I-309)	Monocytes, T cells, endothelial cells; recruits Th2 T cells, monocytes, endothelial and smooth muscle cells	[<u>33-36</u>]	No data	NA
<i>Ccl2</i> (MCP-1)	Monocytes & stromal cells (epithelium, endothelium, fibroblasts, smooth muscle cells)	[<u>37</u>]	Elevated	[<u>38-40</u>]
<i>Ccl5</i> (Rantes)	Multiple	[<u>41</u>]	Elevated	[<u>42</u>]
<i>Cxcl10</i> (IP-10)	Multiple, regulated by interferons	[<u>43</u>]	Elevated	[<u>17</u> , <u>40]</u>
<i>Cxcl11</i> (I-TAC)	Endothelial, epithelial cells and leukocytes; recruitment of T cells and non-homeostatic recruitment of plasma cells	[<u>44</u> , <u>45</u>]	No data	NA

- **NA** = not applicable

Table 3- KLS Multiplex ELISA data

	hIL13	hMCP1	hIL6	hIL17	hTNFRII	hIP10	hRANTES	hITAC
<u>Sample</u> ID	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
#1	510.4	100200	54000	171.2	25700	125.8	15700	1935
#2	286	566.8	23	458.2	3120	70.8	34000	1050.5
#3	502.4	2510	40	190.8	5540	72.6	156600	1099.5
#4	163.6	358	11.8	107.4	2765	56.4	30300	59.2
#5	204	361	11	132	955	51.2	86200	17.8
#6	218.6	529.8	11	250.8	690	51	29700	21.8
#7	104.2	115.6	12.6	130.2	934.6	55	45600	104.6
#8	93.6	161.2	7.4	100	975.1	51.2	39700	10
	hOPG	hM-CSF	hTNFa	hIL10	hIL1ra	hI309	hIFNa	hIL1b
<u>Sample</u>	pg/ml	pg/ml	pg/ml	pg/ml				
<u>ID</u>		<u>P0/</u>	<u>P8/</u>	<u>pg/111</u>	pg/ml	pg/ml	pg/ml	pg/ml
<u>10</u> #1	27.8	62.4	60.4	<u>pg/111</u> 14.4	<u>pg/ml</u> 25050	<u>pg/ml</u> 48.4	<u>pg/ml</u> <0.8	<u>pg/ml</u> <0.4
	27.8 8.4							
#1		62.4	60.4	14.4	25050	48.4	<0.8	<0.4
#1 #2	8.4	62.4 88	60.4 <4.7	14.4 2.4	25050 361.6	48.4 4.8	<0.8 5.8	<0.4 <0.4
#1 #2 #3	8.4 9.4	62.4 88 58.4	60.4 <4.7 <4.7	14.4 2.4 1.4	25050 361.6 882.8	48.4 4.8 55.6	<0.8 5.8 46.2	<0.4 <0.4 <0.4
#1 #2 #3 #4	8.4 9.4 1.2	62.4 88 58.4 <15.6	60.4 <4.7 <4.7 <4.7	14.4 2.4 1.4 0.4	25050 361.6 882.8 38.8	48.4 4.8 55.6 4.8	<0.8 5.8 46.2 14.8	<0.4 <0.4 <0.4 <0.4
#1 #2 #3 #4 #5	8.4 9.4 1.2 0.8	62.4 88 58.4 <15.6 <15.6	60.4 <4.7 <4.7 <4.7 <4.7	14.4 2.4 1.4 0.4 0.8	25050 361.6 882.8 38.8 <15.6	48.4 4.8 55.6 4.8 <1.6	<0.8 5.8 46.2 14.8 <0.8	<0.4 <0.4 <0.4 <0.4 <0.4

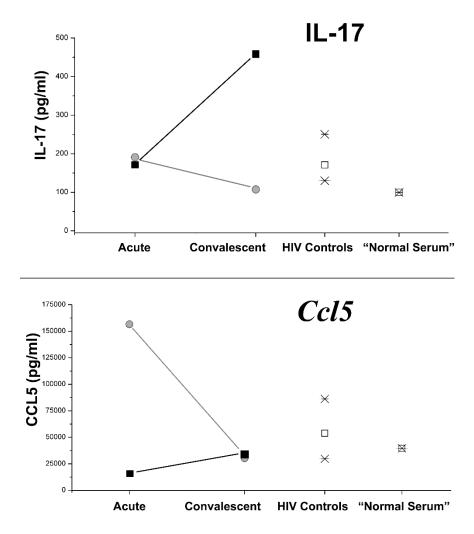


Figure 1. IL-17 and *Ccl5* did not differ between KLS patients and HIV controls. Patient 1 (severe KLS) black squares; Patient 2 (typical KLS) gray circles. The HIV control subjects' mean (open square) and range of analyte values are indicated in the third column. The level of analyte in a single HIV negative "normal serum" is shown as a square in the final column.

Table 4- KLS/KD Acute Phase Analytes

Analyte (pg/ml)	<u>1 MS</u>	<u>s report</u>	Control	KD	Other	Other	Reference
(PD))	HIV cont Mean	rols HIV KLS P1, P2	Control	(KLS)	disease 1	disease 2	hererenee
IL-1beta	<0.4	<0.4,	310	1110	-	-	[<u>2]</u>
		<0.4					
IL-1ra	59	25050, 882	410	no data	7400 (urosepsis)	-	[<u>46]</u>
IL-6	12	54000, 40	<5	123	26 (enterovirus)	-	[8]
			1.5	164	6 (febrile illness)	-	[Z]
IL-10	1.9	14, 1.4	16	125	27 (febrile illness)	-	[<u>47]</u>
			4	122	34 (febrile illness)	-	[<u>10]</u>
IL-13	176	510, 502	35	no data	57 (RSV)	-	[<u>48]</u>
IL-17	171	171, 191	2	25	-	-	[<u>49]</u>
IFN-a [‡]	<0.8	<0.8, 46	-	-	-	-	-
IFN-g [§]	<10	27, <10	-	-	-	-	-
TNF-a	<5	60, <5	10 <3.4	24 8	- <3.4 (measles)	- 12 (anaphylaxis)	[<u>25]</u> [<u>50]</u>
sTNFRI	-	-	-	2750000	1550000 (encephalitis)	-	[<u>51</u>]
sTNFRII	860	25700, 5540	-	(>5000)	-	-	[27]
M-CSF*	<16	62, 58	-	-	-	-	-
Osteoprotegrin	2	28, 9	40	101	68 (JSLE)	80 (febrile illness)	[<u>32]</u>
<i>Ccl1</i> (I-309)	1.9	48, 56	-	-	-	-	-
<i>Ccl2</i> (MCP-1)	336	100200, 2510	-	443	83 (HSP)	328 (febrile illness)	[<u>40]</u>
			223 290	829 1320	-	-	[<u>52]</u> [<u>39]</u>
<i>Ccl5</i> (RANTES) [†]	53833	15600, 156600	-	-	-	-	-
<i>Cxcl10</i> (IP-10)	52	126, 73	-	538	59 (HSP)	417 (febrile illness)	[<u>40]</u>
			128	2469 3587	- 921 (febrile illness)	-	[<u>52]</u> [<u>17]</u>
<i>Cxcl11</i> (I-TAC)	48	1935, 1100	68	no data	254 (HCV cryoglobulinemia)	-	[<u>53]</u>

P1= patient 1; **P2** = patient 2

‡ conflicting data based on bioassays [18, 19]

§ published data in units/ml without conversion factor [21]

* published data in units/ml without conversion factor [73]

+ published data in arbitrary units mRNA in peripheral blood mononuclear cells (PBMC) [84]

	M	San	San	San	San	San	San	San	San	San	San			Ν	San	San	San	San	San	San	San	San	San	San	San			×	San	San	San	San	San	San	San	San	San	San	San	San	Sar	Sar	Sar	Sar	Ξe
3	Mean	nple 58	nple 56	nple 54	nple 52	nple 50	nple 48	nple 46	nple 44	Sample 42	nple 40		SD	lean	àmple 78	Sample 38	nple 36	nple 34	nple 32	nple 30	nple 28	nple 26	nple 24	nple 22	nple 20		SD	lean	Sample 72	nple 70	Sample 68	Sample 66	ample 64	Sample 62	nple 60	nple 18	nple 16	mple 14	nple 12	mple 10	mple 8	mple 6			Fest ID
		58	56	54	52	50	48	46	44	42	40				78	38	36	34	32	30	28	26	24	22	20				72	70	68	66	64	62	60	18	16	14	12	10	8	6	4	2	Sample ID
60	5.0	0.7	1.3	19.6	0.3	9.1	11.0	1.9	0.1	3.6	2.7		6.1	6.0	1.6	0.4	8.5	14.2	7.3	0.4	0.8	3.5	2.8	8.1	18.8		25.0	15.0	44.3	2.9	23.1	0.4	0.4	0.3	14.1	9.8	0.3	91.5	1.3	1.5	30.5	3.5	0.5	low sample volume	pa/ml
00	2.0	0.4	0.6	7.0	0.4	3.2	4.0	1.5	0.2	1.4	1.4		3.3	3.0	0.5	0.037	3.7	5.0	4.6	0.051	0.6	1.2	1.7	4.7	11.2		10.5	5.7	16.4	1.3	4.2	0.2	0.061	0.091	3.3	4.3	0.099	39.4	1.0	0.7			0.3	low sample vo	pg/ml
02	9.9	5.6	5.6	19.4	2.7	7.5	3.8	6.8	16.7	4.7	25.9		47.6	37.3	80.8	25.5	165.4	24.7	21.2	15.2	8.0	33.4	30.0	3.6	2.7		491.8	278.7	40.6	20.7	4.1	354.2	109.0	105.5	85.6	93.5	27.3	762.3	45.4	45.6	53.0	571.1	1862.9	blume low sample volume	na/m
1 31	26.5	14.9	6.4	6.6	6.5	11.6	157.0	3.3	23.2	11.2	24.0		186.7	87.6	109.7	11.3	39.3	15.9	31.4	25.0	33.7	16.0	14.0	644.3	22.7		845.4	371.2	109.6	484.9	257.1	108.7	134.7	191.4	3487.8	40.7	68.9	67.2	29.3	162.3				131.2	
367.5	842.0	1065.3	983.8	1011.9	474.1	725.2	495.3	1627.3	362.7	856.3	818.4		233.9	641.5	586.1	506.1	499.7	677.5	653.2	399.7	266.4	792.3	650.8	1071.4	953.6		2513.9	2125.5	1366.2	2189.8	1458.7	1624.0	1393.6	1360.7	3285.9	574.3	983.5	2878.9	752.0	804.6	1837.0	1201.8	11140.2	1156.1	na/m
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						\vdash				13.0		┝				14.6									_	_																		45.6	pa/m
										118.5						73.6									_				5 478.2															low sample volume	pa/ml
4 4	2.8	4.8	6.0	2.1	1.6	2.6	2.2	1.5	2.9	1.7	2.5		1.4	4.0	3.3	3.6	4.2	3.5	3.5	4.9	3.1	2.6	4.1	3.9	7.8		40.9	28.2	18.1	22.6	21.9	13.8	4.0	9.7	47.1	15.0	36.9	169.9	3.8	11.5	9.7	16.4			pa/ml
104.0	261.1	373.4	355.8	227.3	194.1	131.1	211.6	147.0	193.4	371.5	406.0		288.6	355.6	207.8	1208.3	331.2	260.4	230.3	364.8	317.3	215.7	198.4	244.5	333.3		174.4	347.0	389.7	235.1	250.0	250.7	114.3	437.1	433.2	259.7	328.8	738.4	119.0	312.3	486.7	229.2	621.5	low sample volume	pa/m
238.4	1504.4	1760.2	1331.6	1175.2	1137.3	1725.8	1772.3	1623.3	1358.3	1527.4	1632.4			ω		_					2861.1																_	5674.8	_	4128.9	_			4529.8	pa/m
-	1115.2			896.0	1059.9	1265.9	1302.8	1030.0	1190.9	882.9	1390.8		708.1	2267.1	2427.4	3575.8	2803.7	2062.2	1302.3	2310.5	2106.6	3158.1	2198.5	1485.9	1507.0		1829.8	4052.6	2631.4	3955.6	3419.6	8103.5	2940.9	3738.8	4789.1	2524.1	3585.6	6497.2	1919.8	2768.0	3107.1	4465.0	7552.4	2843.2	pa/ml
		3209s	3208s	3207s	3206s	3205s	3204s	3203s	3202s	3201s	3200s				2103s	2209s	2208s	2207s	2206s	2205s	2204s	2203s	2202s	2201s	2200s				1107s	1106s	1105s	1104s	1103s	1102s	1101s	1208s	1207s	1206s	1205s	1204s	1203s	1202s	1201s	1200s	
		Childrens Healthy Controls													IU Febrile Controls	Childrens Febrile controls													IU KD samples							Childrens KD samples									

AUSHON BIOSYSTEMS

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 Aushon Multiplex Immunoassay Analysis Report

 Johnson
 Species:
 Huma

 Yale University
 Matrix:
 Serun

 11/20/2015
 # of Samples:
 78

 7701
 Total Plex:
 12

Serum and Plasma 78 12

Human

Client/Study: Institute: Receive Date: SL Lot #:

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		1075.8	1337.2	143.7	29	682.3	685	0.144	814.3	697.4	10.8	2.0	4.9		Meen
	3209p	945.1	1943.3	1405	3.8	409.8	47.7	0.045	882.4	98,4	5.8	94	0,7	57	Sende 57
	3.208p	10842	1532.9	2563	7.1	847.8	38.8	82	1030.5	31.1	8.2	9	15	8	Sample 55
-	d/ 02.6	957.5	1251.7	127.4	21	966.7	22.0	0.031	1045.6	44.2	20.0	73	197	: 8	Sende 53
-	danze	10119	1033.7	1061	1.8	576,4	38,4	0.058	573.0	905	27	65	8	. 9	Sande 51
-	denze	11257	1324.6	118	29	478.1	420	8	723.5	642	2	30	82	: :	Sample 49
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-	92020	1162.4	1235.2	1132	2	8	434	: 8	428.2	7.5	18.6	12	: 2	n t	Sampe 43
	dt nz s	857.1	1116.0	1540	1.7	500.3	16,4	0.094	500.7	85,4	49	1.4	3.5	: 5	Semple 41
	dnoze	1404.3	9.08H	2013	23	505.1	100.2	8	753.5	1017	26.8	13	21	: 12	Sampie 39
	1000				:									;	
		743.1	1822.8	139.1	15	169.0	8.8	24	245.8	673	467	30	5.6		8
		2186.7	8.002	23.4	4.0	358.5	Let	=	648.8	123.9	8	2.9	6.0		Meen
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III Sakela Antal	denz z	33985/	877.3	8254	: 5	7.665	27	; e	577.8	792	27.5	2008	: 8	*	Sempe 37
	donz z	3002.9	7212.3	266.9	3.8	121	57.7	0.061	424.5	108.1	165.6	34	82	: 8	Semple 35
	2207p	18407	2699.0	1794	3.6	413.8	81	60	657.8	61.3	24.2	45	140	: ::	Semple 33
	2.206p	1167.5	1459.3	137.5	31	500.6	606	8	622.8	207.0	22.2	2	7.4	4	Sample 31
	2205p	20600	2023.2	198,4	63	438.3	49.3	0.027	5.655	116.2	20.2	0.052	02	8	Sample 29
Childrens Febrile Controls	2204p	2031.0	2460.8	1627	313	438.0	231.7	8	220.4	251.7	8	0.7	0.7	27	Semple 27
	2.203p	2853.5	3884.2	1531	24	425	233.2	82	1061.6	166.7	41.7	13	3.2	53	Semple 25
	2202p	21039	2465.6	1490	45	368.0	8.06	6	740.7	157.0	31.1	17	27	в	Sample 23
	2201p	1438.4	1672.8	2511	3.6	668	42.0	80	824.4	94.1	3.6	43	8.5	22	Sende 21
	2200p	14133	1784.4	198.4	7.9	507.6	45.8	02	976.1	96.0	27	10.1	169	19	Semple 19
		2010.8	2510.0	275.0	71.6	192.2	402.2	26.0	5097.7	347.1	1296.7	6.1	11.9		80
		4096.3	4970.7	367.9	34.6	280.7	354.7	7.4	2719.8	280.7		3.5	8.3		Meen
	1107p	3119.4	3975.4	9969	18.1	275.3	315.5	68	1028.9	84.7		low sample volume	low sample volume	п	Sample 71
	1106p	4602.5	5104.0	2789	20.8	211.5	182.5	02	2066.4	300.2		1.3	5.2	8	Sampie 69
	1105p	3137.9	208.9	surrion sydues not	low sample volume	low semple volume	61.8	0.046	797.8	low sample volume		low sample volume	low sample volume	67	Semple 67
IU KD cases	1104p	79475	8774.9	5042	14.4	145.8	177.8	8	1049.0	85,4		2	8	8	Sample 65
	1103p	38717	8002.4	1892	41	6101	44.2	9600	0.006	40.0		0.066	42	8	Semple 63
	1102p	38180	4027.5	5485	8.8	592	260.8	0.085	1143.8	87.4		690.0	0.6	ଣ	Sample 61
	1101p	4783.1	5551.9	469.7	48.8	943	1204.3	8	3290.2	129.3		35	162	59	Sands 59
	12080	2357.9	3621.8	1623	ŧ.	223.2	170.5	5	450.7	171.0		40	9.8	17	Sample 17
	12070	36478	3680.4	1526	39.2	614	101.8	5	996.2	13.2		2	2	5	Sample 15
_	12060	61983	B12.5	10738	239.6	519.4	1256.1	104.6	4657.4	214.7	- I	21.8	× I	13	Sample 13
-	12050	47073	0 0120	578 51 10	: 1	3400		0.00		401		: !	2	÷	Same 14
-	1010	799460	3020.7	5747	10.0	2,650	1 C20	3 5	70147	210	95	3	1	. م	Sources
Unidians KU samples	12020	4087.0	5015.7	845	16.2	100	119.2	0049	1047.3	212.0		20	34		Sampas
	1201p	86955	12365.6	5748	17.1	565	790.6	2	21362.0	9.6		1.0	40		Sample 3
	1200p	1582.0	2768.5	94.4	3.6	308.1		L .	676.1	82.0	28.8	1.0		-	Sample 1
			pg/ml			100 mi	m(6d	pg/ml	D0/m			μ(đđ	m(6d	Sample ID	Test ID
	Sample ID		NTNERI			hEotaxin	BIWI	NL13	NMOP1			NL4	NL1b		
					12		Total Plex:				7701		SL Lot #:		
					78		# of Samples:				11/20/2015		Receive Date:		
					Serum and Plasma		Matric				Yale University		Institute:		
					Human		Species:				Johnson		Client/Study:		
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Table 6- Plasma Data Set

Table 7. Demographic Cl	naracteristics	s of Study Subjects	5
Demographics	Kawasaki	Febrile Control	Afebrile Control
	(N= 16)	(N=11)	(N=10)
Age (years)	3.2 (1.6)	3.2 (2.3)	4.2 (1.6)
Gender (M)	8 (50%)	1 (9%)	5 (45%)
Race			
Caucasian	9 (56%)	10 (91%)	10 (100%)
Black or African-	5 (31%)	1 (9%)	0 (0%)
American			
Asian	2 (13%)	0 (0%)	0 (0%)
Ethnicity			
Hispanic or Latino	0 (0%)	0 (0%)	2 (20%)
Non-Hispanic or Latino	16 (100%)	11 (100%)	8 (80%)

Data reported as mean (SD) or n (%).

Subject	1	Fever	s and Kawasaki Dise Physical Exam	WBC/Plt	Alb	CRP	ESR	ECHO	IVIG	Final Diagnosis	KD
Subject	Site	(days)	Findings [‡]	$(k/\mu L)$	(g/dL)	(mg/dL)	(mm/h)	Abnl [†]	Response	Filai Diagnosis	Algorithm Result
	1				Kawas	aki Disease	Group				
1101	IU	7	R/Con/Mu/Ext	13.6/432	3.0	34.4	N/A	Y	Y	KD	+
1102	IU	8	R/Con/Mu/Ext/LN	9.9/347	3.1	14.4	79	N	Y	KD	+
1103	IU	5	R/Mu/Ext/LN	20.8/247	4.2	13.1	73	Ν	Y	KD	+
1104	IU	5	R/Con/Mu/Ext	23.7/250	2.4	29	62	Y	Y	KD	+
1105	IU	8	R/Con/Mu	10.9/386	3.6	1.6	34	Ν	Y	Incomplete KD	+
1106	IU	9	R/Con/Mu/Ext/LN	12.1/385	3.6	2.7	35	N	Y	KD	+
1107	IU	9	R/Con/Mu/Ext	15.6/501	3.2	8.3	83	N	Y	KD	+
1200	MN	7	R/Con/Mu/LN	15.5/1068	2.2	8.38	114	Y-CA	Y	KD	+
1201	MN	6	R/Con/Mu/Ext	20.9/365	2.4	23.40	66	N	Y	KD	+
1202	MN		R/Con/Mu/Ext		2.5			Ν		IVIG-resistant KD or	+
		9		10.0/263		17.30	42		Ν	other	
1203	MN	5	R/Con/Mu/LN	18.0/361	3.1	3.26	74	N	Y	KD	+
1204	MN	5	R/Con/Mu/Ext	12.8/401	2.8	12.90	49	Ν	Y	KD	+
1205	MN	6	R/Con/Mu/Ext/LN	10.3/276	2.6	15.60	86	Ν	Y	KD	+
1206	MN	5	R/Con/Mu/Ext	8.6/289	2.8	18.40	70	N	Y	KD	+
1207	MN	5	R/Con/Mu/Ext	8.9424	3.5	12.90	38	N	Y	KD	+
1208	MN	8	R/Con/Mu/Ext/LN	22.1/725	2.8	18.60	93	Ν	Y	KD	+
					Febr	ile Illness G	roup				
2101	IU	2	None	9.7/215	N/A	12.6	34	N/A	N/A	Osteomyelitis, MSSA bacteremia	-
2200	MN	1	None	9.8/390	N/A	0.96	N/A	N/A	N/A	UTI	_
2201	MN	1	Mu	10.0/334	3.9	0.51	N/A	N/A	N/A	Influenza	_
2202	MN	5	Con	11.7/230	N/A	2.19	N/A	N/A	N/A	RSV Bronchiolitis	_
2203	MN	4	Mu	19.5/313	N/A	27.50	N/A	N/A	N/A	Pyelonephritis	-
2204	MN	3	Mu	19.2/367	N/A	2.54	N/A	N/A	N/A	Strep Pharyngitis	_
2205	MN	4	Mu	9.7/253	N/A	N/A	N/A	N/A	N/A	UTI	-
2206	MN	2	LN	8.6/426	N/A	3.83	N/A	N/A	N/A	Pharyngeal Abscess	-
2207	MN	1	None	22.4/277	N/A	N/A	N/A	N/A	N/A	Pneumonia	-
2208	MN	2	LN	26.8/427	2.7	12.70	N/A	N/A	N/A	Lymphadenitis	-
2209	MN	2	None	16.7/432	N/A	N/A	N/A	N/A	N/A	UTI	-

[‡]Rash (R), non-exudate conjunctivitis (Con), mucous membrane changes (Mu), erythema and/or painful swelling of the extremities (Ext), cervical lymphadenopathy (LN), coronary artery aneurysms (CA).

[†] Considered abnormal based on American Heart Association criteria [1]. Subject 1101 had an LAD z-score of +2.69 with marker perivascular brightness around the coronaries. Subject 1104 had an LAD z-score of +3.19 with perivascular edema present. Subject 1200 had coronary artery aneurysms (Y-CA) with an LAD z-score of +9.42 and an RCA z-score of +8.56.

Alb, Albumin; CRP, C-Reactive Protein; ECHO, Echocardiogram; ESR, Erythrocyte Sedimentation Rate; IU, Indiana University; IVIG, Intravenous Immunoglobulin; JIA, Juvenile Idiopathic Arthritis; KD, Kawasaki Disease; MN, Children's Minnesota; MSSA, Methicillin-Susceptible *Staphylococcus aureus*; N/A, Not Available; Plt, Platelet Count; RSV, Respiratory Syncytial Virus; UTI, Urinary Tract Infection; WBC, White Blood Cell Count.

1. Newburger JW, Takahashi M, Gerber MA, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. Circulation **2004**; 110(17): 2747-71.

Table 9.	Diagnosis of Afebrile Control Subjects	
Subject	Final Diagnosis	KD Algorithm Result
3200	Dog Bite, Facial Laceration	-
3201	Supracondylar Humerus Fracture	-
3202	Femur Fracture	-
3203	Supracondylar Humerus Fracture	-
3204	Ulna Fracture, Radius Fracture	-
3205	Tonsillar Laceration, Oral Injury	-
3206	Ulna Fracture, Radius Fracture	-
3207	Supracondylar Humerus Fracture	-
3208	Closed Head Injury, Concussion	-
3209	Closed Head Injury, Motor Vehicle Collision	-

1 Supplement references

- 2 1. Dinarello, C.A., *Biology of interleukin 1*. FASEB J, 1988. **2**(2): p. 108-15.
- Maury, C.P., E. Salo, and P. Pelkonen, *Circulating interleukin-1 beta in patients with Kawasaki disease*. N
 Engl J Med, 1988. **319**(25): p. 1670-1.
- Arend, W.P., et al., *Interleukin-1 receptor antagonist: role in biology*. Annu Rev Immunol, 1998. 16: p. 27 55.
- 7 4. Gabay, C., Interleukin-6 and chronic inflammation. Arthritis Res Ther, 2006. 8 Suppl 2: p. S3.
- 8 5. Gattorno, M., et al., Synovial fluid T cell clones from oligoarticular juvenile arthritis patients display a
- 9 prevalent Th1/Th0-type pattern of cytokine secretion irrespective of immunophenotype. Clin Exp Immunol,
 10 1997. 109(1): p. 4-11.
- Ueno, Y., et al., *The acute phase nature of interleukin 6: studies in Kawasaki disease and other febrile illnesses*. Clin Exp Immunol, 1989. **76**(3): p. 337-42.
- Gupta, M., et al., *Cytokine modulation with immune gamma-globulin in peripheral blood of normal children and its implications in Kawasaki disease treatment.* J Clin Immunol, 2001. 21(3): p. 193-9.
- 15 8. Kim, D.S., Serum interleukin-6 in Kawasaki disease. Yonsei Med J, 1992. **33**(2): p. 183-8.
- 16 9. Moore, K.W., et al., *Interleukin-10*. Annu Rev Immunol, 1993. **11**: p. 165-90.
- 17 10. Kim, D.S., et al., *Increased serum interleukin-10 level in Kawasaki disease*. Yonsei Med J, 1996. **37**(2): p.
 18 125-30.
- Okada, Y., et al., *Effect of corticosteroids in addition to intravenous gamma globulin therapy on serum cytokine levels in the acute phase of Kawasaki disease in children.* J Pediatr, 2003. **143**(3): p. 363-7.
- Minty, A., et al., *Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses.* Nature, 1993. 362(6417): p. 248-50.
- Jaffe, J.S., et al., *Human lung mast cell activation leads to IL-13 mRNA expression and protein release.* Am J
 Respir Cell Mol Biol, 1996. **15**(4): p. 473-81.
- Schmid-Grendelmeier, P., et al., *Eosinophils express functional IL-13 in eosinophilic inflammatory diseases.* J Immunol, 2002. **169**(2): p. 1021-7.
- Peck, A. and E.D. Mellins, *Plasticity of T-cell phenotype and function: the T helper type 17 example.*Immunology. **129**(2): p. 147-53.
- Jia, S., et al., *The T helper type 17/regulatory T cell imbalance in patients with acute Kawasaki disease.* Clin
 Exp Immunol. **162**(1): p. 131-7.
- 31 17. Ko, T.M., et al., *CXCL10/IP-10 is a biomarker and mediator for Kawasaki disease*. Circ Res, 2015. **116**(5): p.
 876-83.

- Rowley, A.H., et al., Serum interferon concentrations and retroviral serology in Kawasaki syndrome. Pediatr
 Infect Dis J, 1988. 7(9): p. 663-6.
- 35 19. Ogle, J.W., et al., *Absence of interferon in sera of patients with Kawasaki syndrome*. Pediatr Infect Dis J,
 36 1991. 10(1): p. 25-9.
- Mosmann, T.R. and R.L. Coffman, *TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties.* Annu Rev Immunol, 1989. **7**: p. 145-73.
- Matsubara, T., S. Furukawa, and K. Yabuta, *Serum levels of tumor necrosis factor, interleukin 2 receptor, and interferon-gamma in Kawasaki disease involved coronary-artery lesions.* Clin Immunol Immunopathol,
 1990. 56(1): p. 29-36.
- 42 22. Matsubara, T., et al., *Decreased interferon-gamma (IFN-gamma)-producing T cells in patients with acute*43 *Kawasaki disease.* Clin Exp Immunol, 1999. **116**(3): p. 554-7.
- 44 23. Darrah, P.A., et al., *Multifunctional TH1 cells define a correlate of vaccine-mediated protection against*45 *Leishmania major.* Nat Med, 2007. **13**(7): p. 843-50.
- 46 24. Furukawa, S., et al., Serum levels of p60 soluble tumor necrosis factor receptor during acute Kawasaki
 47 disease. J Pediatr, 1994. 124(5 Pt 1): p. 721-5.
- 48 25. Ahn, S.Y., et al., *Tumor necrosis factor-alpha levels and promoter polymorphism in patients with Kawasaki*49 *disease in Korea*. Yonsei Med J, 2003. 44(6): p. 1021-6.
- 50 26. Diez-Ruiz, A., et al., Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. Eur J
 51 Haematol, 1995. 54(1): p. 1-8.
- 52 27. Blanchard, J.N., et al., *Recurrent Kawasaki disease-like syndrome in a patient with acquired* 53 *immunodeficiency syndrome*. Clin Infect Dis, 2003. **36**(1): p. 105-11.
- Sweet, M.J. and D.A. Hume, *CSF-1 as a regulator of macrophage activation and immune responses.* Arch
 Immunol Ther Exp (Warsz), 2003. 51(3): p. 169-77.
- Igarashi, H., et al., *High serum levels of M-CSF and G-CSF in Kawasaki disease*. Br J Haematol, 1999. 105(3):
 p. 613-5.
- 58 30. Oana, S., M. Terai, and Y. Kohno, *Serum M-CSF levels in Kawasaki disease*. Br J Haematol, 1999. **107**(2): p.
 59 462-3.
- 60 31. Hofbauer, L.C., et al., *Effects of immunosuppressants on receptor activator of NF-kappaB ligand and*
- 61 osteoprotegerin production by human osteoblastic and coronary artery smooth muscle cells. Biochem
 62 Biophys Res Commun, 2001. 280(1): p. 334-9.
- Simonini, G., et al., Osteoprotegerin serum levels in Kawasaki disease: an additional potential marker in
 predicting children with coronary artery involvement. J Rheumatol, 2005. 32(11): p. 2233-8.

- 65 33. Miller, M.D., et al., *A novel polypeptide secreted by activated human T lymphocytes.* J Immunol, 1989.
 66 143(9): p. 2907-16.
- 67 34. Miller, M.D., et al., Sequence and chromosomal location of the I-309 gene. Relationship to genes encoding
 68 a family of inflammatory cytokines. J Immunol, 1990. 145(8): p. 2737-44.
- Singoni, A., et al., *The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells.* J
 Immunol, 1998. 161(2): p. 547-51.
- 36. Haque, N.S., et al., *Chemokine receptor-8 (CCR8) mediates human vascular smooth muscle cell chemotaxis and metalloproteinase-2 secretion.* Blood, 2004. **103**(4): p. 1296-304.
- 73 37. Deshmane, S.L., et al., *Monocyte chemoattractant protein-1 (MCP-1): an overview*. J Interferon Cytokine
 74 Res, 2009. 29(6): p. 313-26.
- 75 38. Terai, M., et al., Dramatic decrease of circulating levels of monocyte chemoattractant protein-1 in
 76 Kawasaki disease after gamma globulin treatment. J Leukoc Biol, 1999. 65(5): p. 566-72.
- Asano, T. and S. Ogawa, *Expression of monocyte chemoattractant protein-1 in Kawasaki disease: the anti- inflammatory effect of gamma globulin therapy*. Scand J Immunol, 2000. **51**(1): p. 98-103.
- Chung, H.S., et al., *Production of chemokines in Kawasaki disease, Henoch-Schonlein purpura and acute febrile illness.* J Korean Med Sci, 2004. **19**(6): p. 800-4.
- 41. Olson, T.S. and K. Ley, *Chemokines and chemokine receptors in leukocyte trafficking*. Am J Physiol Regul
 Integr Comp Physiol, 2002. 283(1): p. R7-28.
- Wong, M., E.D. Silverman, and E.N. Fish, *Evidence for RANTES, monocyte chemotactic protein-1, and macrophage inflammatory protein-1 beta expression in Kawasaki disease.* J Rheumatol, 1997. 24(6): p.
 1179-85.
- 43. Liu, M., et al., *CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications.*87 Cytokine Growth Factor Rev. 22(3): p. 121-30.
- 44. Cole, K.E., et al., Interferon-inducible T cell alpha chemoattractant (I-TAC): a novel non-ELR CXC chemokine
 with potent activity on activated T cells through selective high affinity binding to CXCR3. J Exp Med, 1998.
 187(12): p. 2009-21.
- 91 45. Johansson, C., et al., *Differential expression of chemokine receptors on human IgA+ and IgG+ B cells*. Clin
 92 Exp Immunol, 2005. 141(2): p. 279-87.
- 93 46. Olszyna, D.P., et al., *Levels of inhibitors of tumor necrosis factor alpha and interleukin 1beta in urine and*94 *sera of patients with urosepsis.* Infect Immun, 1998. 66(8): p. 3527-34.
- 95 47. Noh, G.W., et al., *Effects of intravenous immunoglobulin on plasma interleukin-10 levels in Kawasaki*96 *disease*. Immunol Lett, 1998. 62(1): p. 19-24.

- 97 48. Chung, H.L., et al., *Age-related difference in immune responses to respiratory syncytial virus infection in*98 *young children.* Pediatr Allergy Immunol, 2007. **18**(2): p. 94-9.
- 99 49. Sohn, M.H., et al., *Circulating interleukin 17 is increased in the acute stage of Kawasaki disease*. Scand J
 100 Rheumatol, 2003. **32**(6): p. 364-6.
- 101 50. Furukawa, S., et al., *Kawasaki disease differs from anaphylactoid purpura and measles with regard to* 102 *tumour necrosis factor-alpha and interleukin 6 in serum.* Eur J Pediatr, 1992. **151**(1): p. 44-7.
- 103 51. Korematsu, S., et al., *The characterization of cerebrospinal fluid and serum cytokines in patients with*104 *Kawasaki disease*. Pediatr Infect Dis J, 2007. **26**(8): p. 750-3.
- Shikishima, Y., T. Saeki, and N. Matsuura, *Chemokines in Kawasaki disease: measurement of CCL2, CCL22 and CXCL10.* Asian Pac J Allergy Immunol, 2003. 21(3): p. 139-43.
- Antonelli, A., et al., *High serum levels of CXCL11 in mixed cryoglobulinemia are associated with increased circulating levels of interferon-gamma*. J Rheumatol. **38**(9): p. 1947-52.

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