

SUPPLEMENTAL MATERIAL

Herpesvirus Infection and Childhood Stroke

DETAILED METHODS:

Laboratory Assays: Serological assays for HSV1, HSV2, VZV, CMV, and EBV were performed using enzyme-linked immunosorbent assays (ELISA). For HSV, an IgM assay non-specific to subtype (1 versus 2) was performed first. We used the ELISA assay from Wampole Laboratories (Princeton, NJ) for the first batch, and after this was discontinued, the assay from Diamedix (Miami, FL) for the second batch. Kits were validated with an outside reference laboratory. The relative sensitivity of the assay used was 79% and the specificity 90%. If positive, then an IgG assay for the individual subtypes was performed (Focus Diagnostics, Cypress, CA). Assays against VZV, CMV, and EBV viral capsid antigen were performed following the manufacturer's instructions (Wampole Laboratories, Princeton, NJ). Assays were performed in batches and blinded to both case/control and clinical status. Assays for serologies were validated in the CALM laboratory and were concordant with gold-standard clinical reference lab results spanning the reportable range. The intra- and inter-assay reproducibility coefficients of variation were within the manufacturer specifications.

Data Analysis: The definitions for positive acute herpes serologies were established *a priori* according to the manufacturer's instructions and using the algorithms shown in Supplemental Tables 1 and 2. For all patients, an IgM above the manufacturer's threshold was considered positive for *acute* infection (i.e., positive acute serologies). For those with *paired samples*, serological evidence of acute infection required either (1) a fourfold or greater rise in IgG titers from acute to convalescent samples, (2) positive *acute* IgM titer, or (3) positive *convalescent* IgM titer. Because the HSV IgM titer was non-specific for subtype (1 versus 2), IgG titers for HSV 1 and HSV2 were used to make this distinction when possible.

Supplemental Table 1. Algorithm for defining positive acute herpes serologies with single (acute) blood samples

	Serologic definition
Acute HSV	HSV1/2 IgM \geq 1.1
Acute HSV1	HSV1/2 IgM \geq 1.1 AND HSV1 IgG \geq 1.1 AND HSV2 IgG < 1.1
Acute HSV2	HSV1/2 IgM \geq 1.1 AND HSV1 IgG < 1.1 AND HSV2 IgG \geq 1.1
Acute HSV 1/2 (indeterminate)	HSV1/2 IgM \geq 1.1 AND (HSV1 IgG < 1.1 AND HSV2 IgG < 1.1)
Acute CMV	CMV IgM \geq 1.1
Acute EBV	EBV VCA IgM \geq 1.1
Acute VZV	VZV IgM \geq 1.1

Supplemental Table 2. Algorithm for defining positive acute herpes serologies with paired (acute & convalescent) blood samples:

	Either	or	or
Acute HSV	Four-fold rise in HSV1 or HSV2 IgG titers (see below)	Acute HSV1/2 IgM \geq 1.1	Convalescent HSV1/2 IgM \geq 1.1
Acute HSV 1	Convalescent HSV1 IgG \geq 4 X Acute HSV1 IgG	Acute HSV1/2 IgM \geq 1.1 AND [Acute or Convalescent HSV1 IgG \geq 1.1]	Convalescent HSV1/2 IgM \geq 1.1 AND [Acute or Convalescent HSV1 IgG \geq 1.1]
Acute HSV2	Convalescent HSV2 IgG \geq 4 X Acute HSV2 IgG	Acute HSV1/2 IgM \geq 1.1 AND [Acute or Convalescent HSV2 IgG \geq 1.1]	Convalescent HSV1/2 IgM \geq 1.1 AND [Acute or Convalescent HSV2 IgG \geq 1.1]
Acute HSV 1/2 (indeterminate)		Acute HSV1/2 IgM \geq 1.1 AND [Acute and Convalescent HSV1 IgG <1.1] AND [Acute and Convalescent HSV2 IgG <1.1]	Convalescent HSV1/2 IgM \geq 1.1 AND [Acute and Convalescent HSV1 IgG <1.1] AND [Acute and Convalescent HSV2 IgG <1.1]
Acute CMV	Convalescent CMV IgG \geq 4 X Acute CMV IgG	Acute CMV IgM \geq 1.1	Convalescent CMV IgM \geq 1.1
Acute EBV	Convalescent EBV VCA IgG \geq 4 X Acute EBV VCA IgG	Acute EBV VCA IgM \geq 1.1	Convalescent EBV VCA IgM \geq 1.1
Acute VZV	Convalescent VZV IgG \geq 4 X Acute VZV IgG	Acute VZV IgM \geq 1.1	Convalescent VZV IgM \geq 1.1

Supplemental Table 3. Characteristics of VIPS childhood AIS cases with versus without convalescent blood samples

Characteristic	Single Samples N=139		Paired Samples N=187		P-value
	n	(%)	n	(%)	
Demographics					
Age in years, median (quartiles)	7.7	(3.1, 14.0)	7.6	(3.1, 14.5)	0.81
Male gender	79	(56.8)	107	(57.2)	0.94
Race/ethnicity					0.38
White, non-Hispanic	92	(66.2)	120	(64.2)	
Black, non-Hispanic	11	(7.9)	26	(13.9)	
East Asian	3	(2.2)	4	(2.1)	
Indian/South Asian	12	(8.6)	12	(6.4)	
Middle Eastern	1	(0.7)	2	(1.1)	
First Nations/Aboriginal	0	(0.0)	3	(1.6)	
Pacific Islander	1	(0.7)	0	(0.0)	
Mixed or other	18	(12.9)	16	(8.6)	
Unknown	1	(0.7)	4	(2.1)	
Ethnicity					0.007
Non-Hispanic	124	(89.2)	140	(74.9)	
Hispanic	9	(6.5)	36	(19.3)	
Mixed or other	6	(4.3)	11	(5.9)	
Country					0.0001
USA	75	(54.0)	130	(69.5)	
Canada	33	(23.7)	23	(12.3)	
Australia	8	(5.8)	5	(2.7)	
Philippines	8	(5.8)	8	(4.3)	
Chile	2	(1.4)	12	(6.4)	
United Kingdom	9	(6.5)	1	(0.5)	
France	4	(2.9)	2	(1.1)	
Serbia	0	(0.0)	5	(2.7)	
China	0	(0.0)	1	(0.5)	
Socioeconomic Status					
Residence					0.05
Urban	56	(40.3)	53	(28.3)	
Suburban	51	(36.7)	91	(48.7)	
Rural	32	(23.0)	43	(23.0)	
Household income (in US dollars)					0.10
<\$10,000	14	(10.1)	39	(20.9)	
\$10,000-30,000	22	(15.8)	37	(19.8)	
\$31,000-50,000	18	(12.9)	23	(12.3)	
\$50,000-100,000	42	(30.2)	44	(23.5)	
>100,000	27	(19.4)	33	(17.6)	
Missing	16	(11.5)	11	(5.9)	
Maternal education, highest level					0.06
Less than high school	9	(6.5)	32	(17.1)	

High school graduate	36 (25.9)	42 (22.5)	
Some college education	44 (31.7)	53 (28.3)	
Bachelor's degree, or equivalent	31 (22.3)	29 (15.5)	
Some graduate education	3 (2.2)	5 (2.7)	
Graduate degree	10 (7.2)	19 (10.2)	
Missing	6 (4.3)	7 (3.7)	
Number of routine visits to an MD office, past 24 months, median (quartiles)	2 (1, 4)	2 (2, 4.5)	0.56
Stroke Subtype			0.46
Definite arteriopathy	49 (35.3)	70 (37.4)	
Arterial dissection	11 (7.9)	14 (7.5)	
Transient cerebral arteriopathy	7 (5.0)	16 (8.6)	
Primary moyamoya	7 (5.0)	8 (4.3)	
Secondary moyamoya	6 (4.3)	10 (5.3)	
Secondary vasculitis	4 (2.9)	10 (5.3)	
Genetic arteriopathy	3 (2.2)	0 (0.0)	
PHACES	2 (1.4)	0 (0.0)	
Fibromuscular dysplasia	1 (0.7)	1 (0.5)	
Iatrogenic	0 (0.0)	1 (0.5)	
Primary vasculitis	0 (0.0)	0 (0.0)	
Not further classified	8 (5.8)	10 (5.3)	
Possible arteriopathy	16 (11.5)	14 (7.5)	
No arteriopathy	74 (53.2)	103 (55.1)	
Cardioembolic	25 (18.0)	46 (24.6)	
Spontaneous	22 (15.8)	39 (20.9)	
Iatrogenic	3 (2.2)	7 (3.7)	
Presumed embolic with hypercoaguable condition	2 (1.4)	6 (3.2)	
Other etiology	7 (5.0)	10 (5.3)	
Idiopathic (no known risk factors)	40 (28.8)	41 (21.9)	

Supplemental Table 4. Evidence of past herpesvirus infection (positive IgG and negative IgM antibody titers) in childhood AIS cases* versus stroke-free trauma controls enrolled in USA, Canada, and Chile only (N=389)

Herpes Virus	Cases N=275		Controls N=114		OR†	95% CI	P-value
	n	(%)	n	(%)			
HSV1 or 2	49	(17.8)	23	(20.2)	0.90	(0.52, 1.59)	0.70
VZV	162	(58.9)	67	(58.8)	1.05	(0.67, 1.64)	0.84
CMV	77	(28.0)	41	(36.0)	0.71	(0.45, 1.14)	0.15
EBV	148	(53.8)	57	(50.0)	1.26	(0.81, 1.98)	0.31
Any herpes virus	220	(80.0)	96	(84.2)	0.84	(0.45, 1.50)	0.56

*Analysis based on acute blood samples only to allow case/control comparison

† Odds ratio (OR) adjusted for age

Supplemental Table 5. Clinical and stroke characteristics among cases of childhood AIS stratified by serologic evidence of acute herpesvirus exposure (gold standard algorithm using paired samples, N=187)

	Acute herpesvirus exposure				p-value
	Positive (N=85)		Negative (N=102)		
	n	(%)	n	(%)	
Demographics					
Age in years, median (quartiles)	9.6	(4.4, 14.8)	7.0	(1.6, 13.9)	0.07
Male gender	40	(47.1)	67	(65.7)	0.01
Race					0.24*
White	49	(57.6)	71	(69.6)	
Black	12	(14.1)	14	(13.7)	
Indian/South Asian	2	(2.4)	2	(2.0)	
East Asian	8	(9.4)	4	(3.9)	
First Nations/Aboriginal	1	(1.2)	1	(1.0)	
Middle Eastern	2	(2.4)	1	(1.0)	
Pacific Islander	0	(0.0)	0	(0.0)	
Mixed or other	4	(4.7)	0	(0.0)	
Unknown	7	(8.2)	9	(8.8)	
Ethnicity					0.56*
Non-Hispanic	64	(75.3)	76	(74.5)	
Hispanic	14	(16.5)	22	(21.6)	
Mixed or other	7	(8.2)	4	(3.9)	
Country					0.009*
USA	51	(60.0)	79	(77.5)	
Canada	11	(12.9)	12	(11.8)	
Australia	4	(4.7)	1	(1.0)	
Philippines	7	(8.2)	1	(1.0)	
Chile	4	(4.7)	8	(7.8)	
United Kingdom	0	(0.0)	1	(1.0)	
France	2	(2.4)	0	(0.0)	
Serbia	4	(4.7)	1	(1.0)	
China	1	(1.2)	0	(0.0)	
Parental report of infection					
Any clinical infection in the...					
Prior week	15	(17.6)	20	(19.6)	0.85
Prior month	36	(42.4)	42	(41.2)	0.76
Prior 6 months	49	(57.6)	54	(52.9)	0.57
Clinical infection in prior week					
Type of illness					
Cold/upper respiratory infection	4	(4.7)	9	(8.8)	0.39*
Stomach flu/gastroenteritis	2	(2.4)	3	(2.9)	1.00*
Ear infection/otitis media	4	(4.7)	2	(2.0)	0.41*
Pneumonia	2	(2.4)	2	(2.0)	1.00*

Flu/influenza	0	(0.0)	1	(1.0)	1.00*
Chicken pox	0	(0.0)	0	(0.0)	n/a
Roseola	0	(0.0)	0	(0.0)	n/a
Taken to a doctor for the illness	12	(14.1)	18	(17.6)	0.51
Treated with antibiotics for the illness	11	(12.9)	12	(11.8)	0.81
Admitted to the hospital for the illness	6	(7.1)	13	(12.7)	0.2
Missed school for the illness	8	(9.4)	6	(5.9)	0.36
Associated signs/symptoms					
Cough	7	(8.2)	10	(9.8)	0.71
Fever (temperature >101 F or 38.5 C)	9	(10.6)	11	(10.8)	0.97
Runny nose	4	(4.7)	7	(6.9)	0.76*
Vomiting	3	(3.5)	10	(9.8)	0.35*
Sore throat	3	(3.5)	4	(3.9)	1.00*
Diarrhea	2	(2.4)	2	(2.0)	1.00*
Ear pain	2	(2.4)	1	(1.0)	0.59*
Rash	0	(0.0)	2	(2.0)	0.50*
Ulcers in mouth	0	(0.0)	1	(1.0)	1.00*
Urinary frequency	0	(0.0)	0	(0.0)	n/a
Pain with urination	0	(0.0)	0	(0.0)	n/a
Ulcers on hands/feet	0	(0.0)	0	(0.0)	n/a
Total infectious illnesses, median (range)	1	(0, 2)	1	(0, 2)	0.99
Total days of illness, median (range)	3	(0, 10)	4	(1, 10)	0.93
Number of physician visits for illness, median (range)	0.5	(0, 1)	1	(0, 1)	0.77
Total school/daycare days missed for illness, median (range)	0	(0, 2)	0	(0, 3)	0.68
Prior varicella vaccination (ever)	49	(57.6)	67	(65.7)	0.34
Stroke Subtype					0.76
Definite arteriopathy	36	(42.4)	34	(33.3)	
Arterial dissection	9	(10.6)	5	(4.9)	
Transient cerebral arteriopathy	9	(10.6)	7	(6.9)	
Primary moyamoya	2	(2.4)	6	(5.9)	
Secondary moyamoya	6	(7.1)	4	(3.9)	
Secondary vasculitis	6	(7.1)	4	(3.9)	
Genetic arteriopathy	0	(0.0)	0	(0.0)	
PHACES	0	(0.0)	0	(0.0)	
Fibromuscular dysplasia	0	(0.0)	1	(1.0)	
Iatrogenic	0	(0.0)	1	(1.0)	
Primary vasculitis	0	(0.0)	0	(0.0)	
Not further classified	4	(4.7)	6	(5.9)	
Possible arteriopathy	5	(5.9)	9	(8.8)	
No arteriopathy	44	(51.8)	59	(57.8)	
Cardioembolic	19	(22.4)	27	(26.5)	
Spontaneous	17	(20.0)	22	(21.6)	
Iatrogenic	2	(2.4)	5	(4.9)	
Presumed embolic with prothrombotic	2	(2.4)	4	(3.9)	

condition					
Other etiology	4†	(4.7)	6‡	(5.9)	
Idiopathic (no known risk factors)	19	(22.4)	22	(21.6)	
Isolated patent foramen ovale (PFO)	4	(4.7)	1	(1.0)	
Migraine	1	(1.2)	1	(1.0)	
No PFO or migraine	14	(16.5)	20	(19.6)	
Stroke presentation					
Focal signs					
Hemiparesis	69	(81.2)	79	(77.5)	0.87
Dysarthria	25	(29.4)	29	(28.4)	0.17
Aphasia	22	(25.9)	17	(16.7)	0.09
Ataxia	22	(25.9)	18	(17.6)	0.39
Visual field deficit	8	(9.4)	14	(13.7)	0.59
Non-focal signs					
Headache	37	(43.5)	28	(27.5)	0.06
Decreased level of consciousness	31	(36.5)	23	(22.5)	0.10
Nausea/vomiting	20	(23.5)	22	(21.6)	0.72
Seizures at presentation	20	(23.5)	32	(31.4)	0.42
Vertigo	9	(10.6)	14	(13.7)	0.59
Diplopia	4	(4.7)	6	(5.9)	0.97*
Papilledema	0	(0.0)	1	(1.0)	0.78*
Infarct Characteristics at Baseline					
Location					
Vascular distribution of infarction					
Superficial MCA	49	(57.6)	58	(56.9)	0.91
Lenticulostriate	32	(37.6)	36	(35.3)	0.74
PCA	13	(15.3)	17	(16.7)	0.80
PICA	12	(14.1)	7	(6.9)	0.10
ACA	3	(3.5)	12	(11.8)	0.06*
SCA	8	(9.4)	5	(4.9)	0.23
Anterior choroidal	6	(7.1)	9	(8.8)	0.66
Basilar	8	(9.4)	8	(7.8)	0.70
AICA	0	(0.0)	2	(2.0)	0.50*
Other	3	(3.5)	3	(2.9)	1.00*
Infarct side					0.95
Left	26	(30.6)	32	(31.4)	
Right	36	(42.4)	43	(42.2)	
Bilateral	23	(27.1)	25	(24.5)	
Volume of largest infarct, cm ³ , median (quartiles)	14.9	(3.7, 66.6)	16.8	(2.7, 76.5)	0.91

† sickle cell (n=1), meningitis (n=1), genetic syndrome (n=2)

‡ sickle cell (n=2), head trauma (n=1), meningitis (n=1), cancer (n=1), genetic syndrome (n=1)

*Fisher's exact (all others were chi-square, except Wilcoxon rank sum for continuous variables)