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The Vascular Effects of Infection in Pediatric Stroke (VIPS) Study

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Abstract

Background—The most common cause of arterial ischemic stroke (AIS) in a previously healthy child is a large vessel cerebral arteriopathy. Varicella zoster virus is an established etiology, and recent data implicate a non-specific effect of additional common viral infections on cerebral vessels. The Vascular effects of Infection in Pediatric Stroke (VIPS) study is a multicenter cohort study that will test the hypotheses that (1) infection can lead to childhood AIS by causing vascular injury, and (2) the resultant arteriopathy, and inflammatory markers, predict recurrent stroke.

Methods—We are prospectively enrolling 480 children (aged 1 month through 18 years) with AIS and collecting (1) extensive infectious histories (through parental interview), (2) blood and serum samples (and CSF, when clinically obtained), and (3) clinically obtained but standardized brain and cerebrovascular imaging studies. Imaging studies are being centrally reviewed and adjudicated. Centralized laboratory assays will include serologies (acute and convalescent) and molecular assays for herpes viruses, and levels of inflammatory markers. Subjects are followed prospectively for recurrent ischemic events for the duration of the study (minimum of 1 year). We are banking biological specimens (including DNA) for future studies of specific infectious agents and mediators of inflammation relevant to thrombosis and vascular injury.

Analysis Plan—In a cross-sectional analysis, we will use logistic regression techniques to measure the association between markers of infection (from the clinical history and laboratory assays) and cerebral arteriopathy. In a prospective cohort analysis, we will use survival analysis techniques to determine whether cerebral arteriopathy and inflammatory markers predict recurrent stroke.

Conclusions—VIPS will shed light on the vascular effects of infection in childhood stroke. Because arteriopathy is likely the major predictor of recurrent stroke in children, a better

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understanding of the vascular injury pathway is critical for the development of rational strategies for secondary stroke prevention in children.

INTRODUCTION

Stroke is an increasingly recognized cause of childhood disability.¹ Population-based estimates of the annual incidence of childhood stroke range from 2.3 to 13 per 100,000 children.^{2–5} However, our current understanding of the pathogenesis of childhood AIS is limited. Etiological factors have largely been derived from hospital series and case reports, and include a wide range of disorders from congenital heart disease to blood cancers to rare inborn errors of metabolism.^{6–9} Sickle cell disease and congenital heart disease have been emphasized in this literature, and are likely strong risk factors for childhood AIS. However, stroke is rarely the initial presentation of these diseases, and thus they are unlikely to explain AIS in an otherwise healthy child. Instead, recent data suggest the importance of cerebral arteriopathies: as many as 64% of previously healthy children with a first AIS have an arteriopathy of a cervical or intracranial vessel.⁷ These arteriopathies appear to be important as a risk factor for recurrent stroke in children.^{10–12}

While childhood cerebral arteriopathies include established entities such as arterial dissection and moyamoya, most of these children have isolated unilateral focal stenosis of large intracranial arteries. This entity does not have features of spasm or dissection, and its occasional beaded and irregular appearance on angiogram have led to suggestions that it represents a focal large-vessel vasculitis.^{13, 14} Serial imaging often shows the lesions to be dynamic, indicating that this focal stenosis is acquired, as opposed to congenital.¹³ Although such imaging also suggests that this arteriopathy is rarely progressive after the initial 6 months—and hence this disorder has been called “transient cerebral arteriopathy (TCA)” — residual arterial lesions frequently persist beyond this time interval.^{10, 13}

Infection has been implicated as an etiology of this focal cerebral arteriopathy of childhood, and a focal vasculitis is the presumed underlying mechanism. Because of its known ability to invade vessel walls, possibly by direct spread from adjacent cranial nerves, varicella zoster virus (VZV) has received particular attention.¹⁵ There have been multiple case reports of a focal cerebral arteriopathy in children presenting with acute AIS within weeks to months of chicken pox (primary VZV infection) or, less commonly, herpes zoster (secondary VZV infection);^{16–25} varicella zoster IgG antibodies or DNA (by PCR) have been detected in the CSF in a small number of these cases.^{17, 18, 24} A VZV-antigen positive giant cell arteritis of the middle cerebral artery has been demonstrated pathologically in a fatal case of post-varicella stroke in a child (in addition to several fatal cases of post-varicella strokes in adults).^{26–29} A study of 70 childhood AIS cases (with or without vascular abnormalities) found that the prevalence of chicken pox in the year prior to the stroke (31%) was higher than the annual prevalence of chicken pox reported in the regional population (9%).³⁰ Lastly, in a case-control study that selected subjects with AIS *and an idiopathic focal arteriopathy*, and compared them to healthy controls, the association with varicella appeared strong: calculated odds ratio of 17.5 (95% CI, 2.8 to 117).³¹ The term “post-varicella arteriopathy” (PVA) has been coined for a focal cerebral arteriopathy in children with a history of varicella in the past 12 months.^{32, 33} Other herpes viruses have also been implicated in the pathogenesis of childhood cerebral arteriopathies, although only in case reports: herpes simplex virus type 1 (HSV-1)³⁴ and Epstein-Barr virus (EBV), the latter confirmed by positive EBV DNA in the CSF.³⁵ Enterovirus has been implicated in an 18 month old boy with focal stenosis of the proximal middle cerebral artery and enteroviral RNA in his CSF.³⁶

Epidemiologic data from the International Pediatric Stroke Study (IPSS), an international registry of childhood ischemic stroke cases enrolled at 30 centers, also suggest that common childhood infections in general may play a role in the development of the arteriopathy seen in children with AIS. Among 508 children enrolled with AIS, a recent upper respiratory infection (URI) was noted in 9.1% of those with a cerebral arteriopathy, compared to 4.5% of those without an arteriopathy (OR 2.1, $p=0.048$).³⁷ This association with URI was stronger for children with idiopathic arterial stenosis versus those with other arteriopathy (e.g., arterial dissection, moyamoya) or no arteriopathy: OR 2.8 (95% CI, 1.3–6.1; $p=0.003$).

Cerebral arteriopathies in children are of particular clinical significance in that they appear to confer an increased risk for recurrent stroke. In a Californian study, the overall 5-year cumulative recurrence rate was 19% (95% CI, 12–30%) after an index stroke in a child (>28 days to 18 years of age).¹⁰ While there were no recurrences among children with normal vascular imaging, children with a stenosing vascular abnormality had a 5-year cumulative recurrence rate of 66% (95% CI, 43–87%; $p<0.0001$ for the comparison). Similarly, in a German study, children whose strokes were classified as “vascular” in etiology had a four-fold odds of suffering a recurrence compared to children with idiopathic strokes.³⁴ A British group, on the other hand, did not find an association between arterial stenosis and recurrence, except in the setting of moyamoya, which is a progressive disease.¹² However, they found that a persistent leukocytosis independently predicted recurrence (OR 1.2; 95% CI, 1.04–1.42), suggesting that chronic infection or inflammation may increase risk of recurrence.

To improve our understanding of the role of infection in the development of cerebral arteriopathies in children, we designed the Vascular effects of Infection in Pediatric Stroke (VIPS) study to test the overall hypotheses that infection can lead to AIS by causing vascular injury, and the resultant arteriopathy predisposes children to recurrent stroke. The specific aims are (1) to determine the association between infection and the arteriopathy observed in children with AIS, and establish the temporal nature of this relationship; and (2) to prospectively determine if arteriopathy and inflammatory markers predict stroke recurrence. A better understanding of the vascular injury pathway is critical for our long-term goal of developing therapeutic interventions for secondary stroke prevention in children.

METHODS

Overall Study Design

For Aim 1, we are utilizing a cross-sectional study design to determine the prevalence of infection and arteriopathy in children with AIS. The cases are children aged greater than 28 days through 18 years with an acute diagnosis of AIS, enrolled within 2 weeks of the stroke ictus. The study methods include chart review, parental interview, centralized imaging review, and collection of blood samples with centralized assays for biomarkers of infection. Testing for herpes viruses (VZV, HSV 1 and 2, EBV, and cytomegalovirus [CMV]) will be performed in all cases. Additional biological samples (serum, plasma, DNA, and urine; and CSF, if obtained for clinical purposes) will be aliquoted and banked to create a repository for future studies, which will further explore the associations between infection, inflammation and arteriopathy. For Aim 2, we are performing a prospective cohort study utilizing the childhood AIS cases identified for Aim 1. We are following these subjects prospectively for the duration of the study period, but a minimum of 1 year.

Setting

VIPS has built upon the existing infrastructure of the IPSS. The Hospital for Sick Children (University of Toronto), which directs the IPSS, serves as the VIPS Network Coordinating Center and Data Core, expanding current IPSS databases and web-based data entry systems to accommodate VIPS-specific clinical, radiographic and laboratory assay data. UCSF serves as the VIPS Study Coordinating Center, Imaging Core, and Biostatistics Core. The Center for Advanced Laboratory Medicine (CALM) at Columbia University serves as the VIPS Laboratory Core.

VIPS has 34 sites as of January 1, 2011: 17 U.S., 7 Canadian, and one site each in Argentina, Australia, Chile, China, Germany, Hong Kong, Malaysia, Philippines, Serbia, and the United Kingdom (see Appendix). All sites have met the following site selection criteria: basic laboratory capabilities necessary for processing biological specimens; access to a refrigerated centrifuge, minus 70 degree freezer, and dry ice (an exception was made for a small number of centers, which will be allowed to send samples on cold packs); an MRI scanner with a minimum magnet strength of 1.5 Tesla; ability to perform a minimum neuroimaging protocol, including brain MRI and brain vascular imaging, as part of their *routine clinical care*; MRI slice thickness of 5 mm or less; and ability to provide DICOM-formatted data burned onto a CD or DVD. Our decision to use only clinically obtained imaging was both financial and ethical in that it circumvents the potential risks of sedation in children who would require anesthesia for their research scans.

Inclusion and Exclusion Criteria

Potential cases are identified primarily through the clinical practice of the IPSS co-investigators who are considered the pediatric stroke experts at their respective institutions, and are thereby made aware of acute pediatric stroke cases. In addition, co-investigators advertise the study to their colleagues in child neurology, hematology, and pediatric critical care. Inclusion criteria are: (1) acute AIS (using the clinical and radiographic criteria defined below) within 2 weeks of enrollment and (2) age greater than 28 days and less than 19 years at the time of the stroke ictus (3) parental consent. Cases will be excluded if (1) there is no parent or guardian available for interview, or (2) the minimum neuroimaging protocol, defined below, was not performed. Because we are interested in the association between infection and arteriopathy in all cases, even among children otherwise predisposed to stroke, we include children with known stroke risk factors, such as sickle cell disease, congenital heart disease, and moyamoya.

Cases are initially confirmed by the local co-investigator using clinical and radiographic diagnostic criteria for stroke: (1) clinical: a focal neurological deficit of acute onset or a seizure; (2) radiographic: a CT or MRI showing a focal brain infarct conforming to an established arterial territory in a location and of a maturity consistent with the neurological signs and symptoms. They are then subjected to a centralized review and case confirmation process, described below.

Medical Records Abstraction

Data regarding the index AIS are obtained through review of medical records by the local co-investigator, and abstracted onto the Case Report Forms (CRFs). These data include neurologic presentation of the stroke; past medical history, with particular attention to perceived risk factors for childhood stroke;⁶⁻⁹ and etiologic work-up (diagnostic studies performed to identify etiology of the stroke). The CRFs include particular detail regarding serious acute infections: bacteremia/sepsis, meningitis, encephalitis.

Parent/Surrogate Interview

The site investigator or project coordinator performs a standardized interview of the parents/guardians of each enrolled subject as soon as possible and at most within 1 week of enrollment (maximum of 3 weeks after stroke ictus). Data obtained from the interview includes presence or absence of recent infection, recent febrile infection, or prior chicken pox, type of infection (e.g., upper respiratory versus urinary tract infection), and infectious symptoms (such as cough, vomiting, diarrhea, etc.). In addition, we ask the date of the most recent episode of each specific type of infection in that child's life. As a measure of infectious burden, we ask parents to estimate the number of infections, duration of infections, and missed school/preschool/daycare due to infection in the 6-month interval preceding stroke. Additional interview questions pertain to gender, age, self-reported ethnicity, indicators of socioeconomic status (most advanced level of parental education and household income), vaccination history, and family history of stroke at a young age. Questions also include "distracter variables" in order to assess for potential recall bias. For example, we ask whether the subjects have a remote history of urinary tract infections, abnormal head shape, and umbilical hernias in the neonatal period.

Definitions/Diagnostic Criteria for Infection

Recent infection (the primary exposure variable) is defined as parental recall of any infection within 4 weeks preceding the stroke ictus date, exclusive of CNS infections (meningitis/encephalitis), bacteremia/sepsis, or chicken pox (infections with distinct mechanisms for causing AIS that will therefore be considered separately). This includes any one of the following subtypes (further defined below): URI, pneumonia, acute otitis media, sinusitis, and acute gastroenteritis. Because the association between infection and stroke in adult studies was strongest for febrile infections,^{38, 39} and because infectious symptoms (such as cough and nasal discharge) can mimic allergic symptoms and are relatively ubiquitous in young children, we are also measuring recent febrile infection, defined as parental report of a temperature greater than 38° C (100.4°F) that was attributed to an infectious etiology within 4 weeks preceding the stroke ictus. URI is defined as respiratory symptoms (cough, rhinorrhea, sore throat) attributed by the parents to an infectious etiology. Similarly, acute gastroenteritis will be defined as two of three symptoms of vomiting, diarrhea, or fever, attributed by the parents to an infectious etiology. Acute otitis media, pneumonia, and sinusitis will be defined as parental report of a physician diagnosis of those illnesses.

We define CNS (central nervous system) infections as meningitis or encephalitis within 4 weeks of the stroke ictus/index date. These diagnoses will require supportive documentation in the medical records, in addition to parental report. A diagnosis of bacteremia/sepsis will be based on positive blood culture results. Criteria for the diagnosis of meningitis/encephalitis will include a physician diagnosis *and* a cerebrospinal fluid profile supporting the diagnosis (elevated leukocyte count and protein). Prior chicken pox will be defined by parental report alone because this has been shown to be specific for the diagnosis, and because a physician diagnosis often is not made as children with a suspected diagnosis are discouraged from coming to clinic (to prevent spread to other children).⁴⁰

The time interval we selected for the definition of recent infection is arbitrary, based only on prior literature using a similar cut-off.^{38, 41} However, the strength of the association between infection and stroke may vary by the temporal proximity of the infection. Therefore, in addition to defining recent infection, recent febrile infection, and the infectious subtypes as dichotomous variables (yes/no) within defined time-frames, we will ask the parents to estimate the date of onset of the most recent infection. This will allow us to evaluate time-dependent associations over a range of possible times.

We define infectious burden based on parental report in three ways: (1) the estimated total number of infectious illnesses in the past 6 months, (2) the estimated total number of days of infectious illness in the past 6 months (a reflection of both frequency and duration of illness), and (3) the estimated total number of days of missed school/preschool/daycare due to infectious illness. A fourth measure of infectious burden will be the sum of positive testing for herpes viruses: positive herpes serologies or positive PCR, suggestive of either acute or remote infection. This will be a categorical variable ranging from 0 (all herpes virus testing negative) to 5 (evidence of either acute or remote infection with all five herpes viruses tested for in this study).

Neuroimaging Review

The purpose of centralized imaging review is (1) to confirm the index AIS diagnosis, as described above, (2) to describe and categorize vascular findings, and (3) to confirm recurrent AIS. The imaging studies subjected to review include (1) baseline brain MRI studies, (2) baseline vascular imaging studies (MRA, CTA, and/or conventional angiogram), (3) all follow-up vascular imaging studies in children with abnormal baseline vascular imaging, and (4) follow-up brain MRI (or CT) studies performed for suspected recurrent stroke. At most IPSS centers, follow-up vascular imaging is routinely performed on all children with abnormalities on baseline vascular imaging; it will be used to aid in the classification of the arteriopathies (as their evolution can help distinguish different subtypes), and to assess their natural history. Follow-up brain MRI imaging is routinely performed on all children with clinical symptoms of recurrent stroke, and will be used for the confirmation of recurrences for Specific Aim 2.

The minimum neuroimaging protocol for subject inclusion in the study will consist of the following MRI sequences: axial T2 images, axial diffusion weighted images (DWI), axial or coronal FLAIR images, and time of flight magnetic resonance angiography (MRA) of the brain. MRA of the neck and axial or coronal gradient echo (GRE) images of the brain are strongly recommended. Conventional angiography and CT angiography (CTA) will be accepted in lieu of MRA, although all VIPS sites have indicated that MRA is their first-line vascular imaging study.

Centralized radiology review is performed by two study neuroradiologists (M.W. and C.L.S.), with adjudication by a third (A.J.B.). Disputes are resolved by discussion and consensus among the three radiologists. The radiologists abstract data regarding approximate infarct size, location, and acuity, and any associated hemorrhage. In their vascular interpretation, because diagnostic criteria for vascular abnormalities in children are not firmly established, they will primarily classify the vascular imaging as normal or abnormal, and then completely describe vascular findings. They will also categorize them based on prevalence using the definitions for arteriopathy described below. We will reassess these definitions after the first 50 sets of imaging studies are reviewed. If we feel that a useful vascular classification system can be developed, we will create specific definitions for the categories, and then re-review the vascular imaging with independent classification by the two primary reviewers, and adjudication by the third. We will then calculate inter-observer reliability.

Definitions/Diagnostic Criteria for Arteriopathy

We define arteriopathy as any stenosing abnormality of a cervical or intracranial artery, which is further subcategorized into small/medium versus large vessel disease.¹⁰ Because complete vessel occlusion may represent thrombus rather than an underlying vascular abnormality, isolated occlusion will be classified separately. Because the label “TCA” can, by definition, only be applied to subjects with follow-up vascular imaging (demonstrating

non-progression after six months), we chose to use the more inclusive label that can be applied to baseline imaging: focal cerebral arteriopathy of childhood (FCA).³⁷ This is defined as a unilateral focal stenosis of one or multiple adjacent segments of the large vessels of the circle of Willis (the vessels typically involved in TCA, such as the distal internal carotid or proximal middle or anterior cerebral arteries), in the absence of diagnostic features of arterial dissection or moyamoya. Within the category of FCA, we also define the subgroup of TCA: features of FCA on baseline imaging that show the typical evolution of TCA on follow-up imaging (e.g., “banding” or “beading” of the vessel at baseline progressing to smooth stenosis).^{13, 32} Arterial dissection will be defined by a focal irregular stenosis of a large vessel in the presence of a double lumen, intimal flap, or pseudoaneurysm.^{11, 32, 42} Moyamoya will be defined as bilateral stenosis or occlusion of the distal internal carotid or proximal middle cerebral or anterior cerebral arteries, and abnormal collateral vascular networks in the vicinity of the stenosis/occlusion (the so-called moyamoya vessels).⁴³ This designation will include both the primary (idiopathic) form and secondary disease (e.g., in the setting of sickle cell disease or genetic syndromes such as Down syndrome). Diffuse cerebral vasculitis will be defined as multifocal areas of segmental narrowing and dilatation (beading) involving small or medium vessels.^{44, 45}

We recognize that the FCA category, as currently defined, may be heterogeneous, and could include cases of arterial dissection without diagnostic imaging features, early (unilateral) moyamoya, and large vessel vasculitis. However, this category represents both the most prevalent group of described vascular abnormalities in children with AIS, and the least well understood. Our strategy is to err towards over-inclusivity in this category, selecting out only the well-defined arteriopathies. We can then use the data obtained in this study to better understand this group, and devise a rational scheme for further sub-categorization.

Ascertainment of recurrences

We anticipate that, in the event of a recurrence, most children will be readmitted to same hospital where they were initially enrolled, and the local investigator (as the resident childhood stroke expert) will be notified of their admission on a clinical basis. Although a referral bias could exist if first stroke cases are referred to a tertiary center (VIPS enrolling site), but then re-present to their initial hospital only, we suspect this is unlikely; recurrent strokes in children are typically a source of great concern, and are probably more likely to result in a re-referral to the regional tertiary center. Regardless, the site investigator or project coordinator will also perform phone follow-up every 4 months, and at the completion of the study follow-up period, to inquire about recurrent strokes. In addition, investigators will perform quarterly reviews of the cases’ medical records to identify any readmissions, urgent care visits, or follow-up head imaging suggestive of a recurrence.

Confirmation of recurrences

Recurrences will be initially confirmed by the local co-investigator using the same clinical and radiographic criteria used for the index stroke, with the added criterion of radiographic evidence of new infarction. They will then be subjected to a centralized adjudication process. Similar to the adjudication process for the initial case confirmation, the imaging and clinical history will be independently reviewed by a study neuroradiologist (M.W.) and pediatric stroke neurologist (H.J.F.), with adjudication of disagreements by a third investigator (A.J.B.). TIAs will be excluded from the definition of recurrence out of concern for observer bias: the decision as to whether or not focal symptoms represent a TIA may be affected by treating physician knowledge of the case’s exposure status (presence or absence of arteriopathy). Silent infarctions (seen on imaging, but with no clinical correlation) will be noted but not included as an outcome due to incomplete ascertainment (follow-up brain imaging will not be performed in all subjects).

Biologic Sample Collection/Handling/Storage

We are collecting approximately 16 mL of blood at the time of enrollment (10 mL for serum and 6 for plasma in EDTA), or a maximum of 3 mL/kg in a 6 week period; exact volumes vary slightly by individual site IRB requirements. If needed, blood may be drawn on separate successive days to comply with site specific IRB approved algorithms for pediatric phlebotomy. Convalescent serum and plasma samples are collected 7–28 days after the initial sample collection. We anticipate that convalescent samples will not be available for 10% of participants. In addition, we will collect 10 ML of urine from willing participants and 5 mL of research CSF when lumbar puncture is performed for clinical indications. Blood samples are centrifuged, aliquoted, and frozen at -70 degrees C at the enrolling site. Samples are shipped up to twice yearly on dry ice to CALM for storage and batched analysis. Samples will be analyzed for herpes virus serologies and PCR, and inflammatory markers. The remaining samples, including buffy coat specimens to be used for potential future genetic testing, will be stored and curated in a repository at CALM.

Assays for Herpes Viruses

Herpes viruses are the group of pathogens that has been most frequently implicated in the pathogenesis of vascular injury. As discussed above, VZV, HSV, and EBV have been implicated in reports of arteriopathy in children; VZV, CMV and HSV have also been implicated in the pathogenesis of atherosclerosis in adults.⁴⁶ We will perform serological assays for VZV, HSV, CMV, and EBV at CALM; the specific testing we will perform for each virus is shown in Table 1, below. Serologies will be performed using an automated immunochemiluminescent assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA). This is a solid-phase, two-step enzyme-labeled immunoassay very similar to a standard ELISA assay. It utilizes beads coated with viral antigen, and a chemiluminescent substrate for signal. Assays on this system for serologies for HSV 1, HSV 2, and CMV are standardized and FDA-approved. The system requires 20 microliters of serum for each of the tests. The assays have been validated in the CALM laboratory. On the acute samples, we will perform IgG and IgM serologies and plasma PCR (except for CMV, as the significance of a positive plasma CMV PCR is less certain). On convalescent samples, we will repeat IgM and IgG serologies. In addition, we will study all available CSF samples with viral PCR and intrathecal antibodies for HSV and VZV. For the cross-sectional analysis, the presence or absence of acute herpes virus infection and remote herpes virus infection (used for our definition of infectious burden) will be determined through independent review of all available data by two pediatric infectious disease specialists (C.G. and a clinical infectious disease fellow), with adjudication of disputes by a third investigator (M.S.E.). Data subject to review will include clinical history (including history of varicella vaccination or clinical infection), timing of the sample collections, and acute and convalescent serology results.

Assays for Inflammatory Marker

The rationale for our choice of inflammatory markers is shown in Table 2; these markers have been shown to predict recurrent stroke and cardiovascular events in adults, and we will determine whether a similar association exists for childhood AIS. We will measure C-reactive protein (hsCRP), serum amyloid A (SAA), myeloperoxidase (MPO), and lipoprotein-associated phospholipase A2 (Lp-PLA2) on all samples, as well as tissue necrosis factor alpha (TNF- α), on that majority of samples that are frozen and shipped on dry ice. Assays for hsCRP and SAA will be performed utilizing the Dade-Behring BN-II nephelometer (Dade-Behring, Deerfield, IL) The nephelometric method to measure hsCRP has become recognized as the national standard assay for this marker. Assays for MPO, TNF alpha, and Lp-PLA2 will be performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Biosource International, Camarillo, CA and diaDexus,

Inc., South San Francisco, CA). All assays will be performed by a research technician blinded to subject outcome status.

STATISTICAL ANALYSIS PLAN

Sample Size

We chose our sample size of 480 subjects based on power calculations for the primary association of interest, that between recent infection (within 4 weeks of the stroke ictus) and FCA. In a retrospective population-based cohort study childhood stroke based in Northern California,⁴⁷ the proportion of children with AIS who had an urgent care visit for an infectious illness in the 4 weeks preceding the stroke was 27% (95% CI 18–37%; unpublished preliminary data). The proportion of children with an idiopathic stenosis was 27% (16–41%) in the Californian cohort,¹⁰ and 23% (95% CI 19–27%) in the IPSS registry.³⁷ The point estimate for the odds ratio (OR) for the association between infection (variously defined) and stenosing arteriopathy ranged was 2.3 in the Californian cohort, and 2.4 in the IPSS registry.³⁷ Assuming a 25% prevalence of recent infection, and a 20% prevalence of idiopathic stenosis in the unexposed group, we would need 480 cases to have 80% power to detect an OR of 2.0 (alpha set at 0.05).

Aim 1

In a cross-sectional analysis, we will measure the association between the primary predictor (recent infection, as ascertained from the parental interview) and the outcome (FCA) using logistic regression; odds ratios and exact confidence intervals will be calculated. We will first perform a univariate analysis. We will assess potential confounders (such as age, gender and ethnicity) by determining whether they are associated with the predictor and outcome. Then, to determine whether recent infection is an independent risk factor for FCA, multivariate logistic regression models will be constructed, and will include potential confounders. Potential effect modifiers (such as race/ethnicity and geographic location) will be assessed through stratification and tests for interaction adding cross-product variables to the model. Secondary predictors (recent febrile infection, recent herpes virus infection, infectious burden) will be analyzed in a similar fashion. For categorical variables, we will use chi-square tests to compare proportions, or Fisher's exact, when necessary. For continuous variables, we will employ Student's *t*-tests. We will create additional logistic regression models to determine which of these variables are independent predictors of FCA. Because our primary analysis will include all cases, the reference group (children without FCA) will be heterogeneous, including children with no arteriopathy, and children with other forms of arteriopathy (such as dissection and moyamoya). As a secondary analysis, we will repeat the above analyses, excluding children with other forms of arteriopathy. In addition, because of literature suggesting an association between recent infection and arterial dissection in adults,^{48, 49} we will analyze the association between recent infection and other arteriopathy sub-types, including dissection.

We will perform additional analyses to assess potential recall bias in our primary analysis by comparing (1) the proportion of positive responses to “distracter variables” and (2) the proportion of reported VZV infection validated by biologic assay, accounting for prior varicella vaccination, in children with and without FCA.

We acknowledge that the cut-off of 4 weeks in our definition of recent infection is somewhat arbitrary, derived from prior literature on infection and stroke in adults. Hence, we will explore the relationship between time since most recent infection (to the stroke ictus) and probability of FCA. Because this relationship is unlikely to be linear, we will use regression splines.⁵⁰ This technique fits a regression line that is continuous at specified cut points, and linear in the intervals between those cut points. This allows the predictor variable

(time) to be treated as continuous, despite the nonlinear relationship. We will also assess for seasonal variation by determining whether stroke in cases with FCA cluster in different seasons than those without.

Aim 2 Analysis

The associations between the primary predictor (arteriopathy) and the outcome (time from initial stroke to recurrent ischemic stroke) will be assessed with survival analyses. Cases will be censored at the date of death or their last contact (telephone or in-person) prior to the end of the study, whichever comes first. For univariate analyses, Kaplan-Meier curves will be constructed and log rank tests will be performed to test the significance of the difference between dichotomous predictors. Univariate Cox proportional hazards models will be used to determine hazard ratios as a measure of relative risk. To determine whether arteriopathy is an independent risk factor for recurrent AIS, multivariate Cox proportional hazards models will be constructed, and will include potential confounders such as gender, ethnicity, and markers of socioeconomic status. To determine whether the association between arteriopathy and recurrence varies by arteriopathy subtype (arterial dissection, moyamoya, FCA) we will perform stratified survival analyses. To determine whether a history of recent infection (prior to the index stroke) modifies the association, we will perform stratified analyses and also enter a cross-product variable into the Cox proportional hazards models to test for interaction. We will perform additional analyses to determine if levels of inflammatory markers predict recurrence. As these levels will be continuous, we will convert them to categorical variables by dividing them into quartiles. We will then employ the same survival analysis techniques to determine whether a higher level predicts recurrence. We will create multivariate Cox proportional hazards models to adjust for potential confounders, including infarct size, stroke severity, and concurrent post-stroke hospital-acquired infections (although uncommon in children).

DISCUSSION

We describe the design of the first prospective multi-center study to assess the relationship between childhood infections and the cerebral arteriopathy commonly observed in previously healthy children presenting with a first AIS. As a secondary aim, the VIPS study will prospectively establish the rate of recurrent AIS in children, and determine whether inflammatory markers and abnormalities on vascular imaging predict recurrence. Studies of childhood AIS have long been challenged by the relative rarity of this disease, making it difficult for any single center or small collaborative effort to achieve a powerful sample size. The VIPS study overcomes that hurdle by utilizing and building upon the existing infrastructure of the IPSS, an international effort that has enrolled more than 2,500 neonatal and childhood stroke patients since 2003.

Our primary predictor—recent infection—will be ascertained by parental interview. The major limitations of this approach are recall bias and inability to detect sub-clinical infections. However, we will manage recall bias by (1) minimizing such bias by performing interviews within a discrete interval from the stroke ictus, and (2) measuring residual recall bias through distractor variables. In addition, we will secondarily measure infection through biologic assays for herpes viruses, the class of viruses most implicated as mediators of vascular injury. This measure will be both resistant to recall bias, and sensitive to subclinical infections.

Our primary outcome—focal cerebral arteriopathy—will be ascertained through centralized review of vascular imaging studies. Our study takes advantage of clinically obtained brain and vascular imaging. This approach decreases the cost of the study, and circumvents ethical issues around subjecting children to the risk of anesthesia to perform imaging studies for

research purposes. However, a clear disadvantage is the variability of the imaging studies that will be obtained in VIPS. As we designed the minimum imaging protocol, we tried to maximize the standardization of the imaging studies across centers without excluding smaller institutions through overly rigorous criteria which would decrease the generalizability of our results.

Our primary outcome is also limited by the lack of a validated classification system for childhood cerebral arteriopathies. For this reason, our approach will be to thoroughly describe the vascular imaging abnormalities, and then attempt to develop and validate a classification system during the course of the study. However, our study will be limited by the capabilities of commonly employed imaging techniques. MRA, although the imaging study of choice in children because it is non-invasive and does not expose patients to radiation, is imperfect for characterizing arteriopathies; for example, compared to conventional angiography, MRA detected only half of the arterial dissections in one pediatric study.⁵¹ Hence, the arteriopathy that we call FCA in this study will likely still represent a variety of etiologies, including a focal arteritis, intracranial dissection, and even early moyamoya. Ultimately, better imaging techniques—such as arterial wall imaging for intramural inflammation or blood products⁵²—will be needed to better define the arteriopathies observed in children.

However, despite these limitations, the VIPS study will provide useful evidence regarding the role of infection in the development of cerebral arteriopathies in children, and rates and predictors of recurrent stroke. This information will be critical for the development of secondary stroke prevention strategies in children. In addition, VIPS will provide a valuable library of clinical information and imaging, with a corresponding repository of DNA and other biological samples, which will allow the testing of additional hypotheses to further our understanding of childhood stroke.

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Appendix: VIPS Sites (as of 1/1/2011)

Current enrolling sites

U.S.: Children's Hospital of Buffalo (Buffalo, NY), Children's Hospital of Philadelphia (Philadelphia, PA), Children's Hospital of Wisconsin (Milwaukee, WI), Children's National Medical Center (Washington, DC), Johns Hopkins University (Baltimore, MD), Maimonides Infants and Children's Hospital (Brooklyn, NY), Nationwide Children's Hospital (Columbus, OH), The Cleveland Clinic (Cleveland, OH), University of Colorado- Denver Children's Hospital (Aurora, CO), University of Texas Southwestern - Children's Medical Center (Dallas, TX), University of Utah- Primary Children's Medical Center (Salt Lake City, UT)

Canada: Alberta Children's Hospital (Calgary, AB), Children's Hospital of Eastern Ontario (Ottawa, ON), Hospital for Sick Children (Toronto, ON), Mc Master University Children's Hospital (Hamilton, ON), University of Alberta-Stollery Children's Hospital (Edmonton, AB), Winnipeg Children's Hospital (Winnipeg, MB)

Non-North American: Argentina (Hospital Italiano de Buenos Aires), Chile (Pontifica Universidad Catolica de Chile), China (Chinese PLA General Hospital), Germany (Munster University Paediatric Hospital), Hong Kong (Queen Mary Hospital), Philippines (University of the Philippines-Philippine General Hospital), Serbia (Mother and Child Healthcare Institute)

Sites obtaining IRB approval

US: Boston Children's Hospital (Boston, MA), Loma Linda University School of Medicine (Loma Linda, CA), Rainbow Babies and Children's Hospital (Cleveland, OH), St. Louis Children's Hospital (St. Louis, MO), University of Alabama Medical Center (Birmingham, AL), Vanderbilt University (Nashville, TN)

Canada: British Columbia Children's Hospital (Vancouver, BC)

Non-North American: Australia (Royal Children's Hospital Melbourne), Malaysia (Hospital Kuala Lumpur), United Kingdom (University College of London Institute of Child Health–Bristol Royal Hospital for Children)

Table 1

Testing for herpes viruses in the VIPS study

Virus	Serum/Plasma testing		CSF (on subset)	Further testing if evidence of acute infection
	Acute	Convalescent		
HSV1 HSV2	IgM, IgG	IgM IgG	PCR, intrathecal antibody	do type-specific antibody if evidence of acute infection found
VZV	IgM, IgG Qualitative PCR	IgM IgG	PCR, intrathecal antibody	
CMV	IgM IgG	IgM IgG	PCR	
EBV	VCA IgM, EBNA, VCA IgG Quantitative PCR		PCR	

Table 2

Choice of inflammatory markers for the VIPS study

Tests	Rationale
HsCRP	Acute phase reactant produced in the liver on stimulation by IL-6; plays an important role in the innate immune response.
	Predictive of myocardial infarction (MI) ⁵³⁻⁵⁷ and stroke ⁵⁶⁻⁶⁰ in several adult populations.
	Predictive of recurrent ischemic events in patients with TIA and stroke. ^{61, 62}
	Predictive of mortality after stroke in the NOMAS population (see preliminary data). ⁶³
	Elevated in 74% of children with a focal cerebral arteriopathy. ¹⁴
	Stable, standardized, and commercially available nephelometric assay.
Serum amyloid A	Predictive of cardiovascular events in adult women in the Women's Health Study (WHS); ⁵⁴ predictive of recurrent cardiac events after MI. ⁶⁴
	May facilitate LDL oxidative modification; ⁶⁵ acute phase protein with similar temporal distribution to CRP. ⁶⁶
MPO	Predictive of stroke and MI among patients with peripheral arterial disease. ⁶⁷
	Predictive of stroke among patients with Fabry disease. ⁶⁸
	MPO polymorphisms are associated with the extent of brain damage and functional outcome after stroke. ⁶⁹
	Predictive of MI in healthy populations ^{70, 71} and prognosis after MI. ⁷²⁻⁷⁴
TNF alpha	Predictive of recurrent MI or cardiovascular death after MI. ⁷⁵
Lp-PLA2	Predictive of stroke in several adult populations. ^{76, 77}
	Predictive of recurrent stroke and other vascular events in the NOMAS population (see prelim data). ⁶³