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Inflammatory Biomarkers in Childhood Arterial Ischemic Stroke: Correlates of Stroke Etiology and Recurrence

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Abstract

Background and Purpose—Among children with arterial ischemic stroke (AIS), those with arteriopathy have the highest recurrence risk. We hypothesized that arteriopathy progression is an inflammatory process, and that inflammatory biomarkers would predict recurrent AIS.

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[*]See Appendix

Methods—In an international study of childhood AIS, we selected cases classified into one of the three most common childhood AIS etiologies: definite arteriopathic (N=103), cardioembolic (N=55), or idiopathic (N=78). We measured serum concentrations of high sensitivity C-reactive protein (hsCRP), serum amyloid A (SAA), myeloperoxidase (MPO), and tumor necrosis factor alpha (TNF-α). We used linear regression to compare analyte concentrations across the subtypes, and Cox proportional hazards models to determine predictors of recurrent AIS.

Results—Median age at index stroke was 8.2 years (IQR 3.6, 14.3); serum samples were collected at median 5.5 days post-stroke (IQR 3, 10 days). In adjusted models (including age, infarct volume, and time to sample collection) with idiopathic as the reference, the cardioembolic (but not arteriopathic) group had higher concentrations of hsCRP and MPO, while both cardioembolic and arteriopathic groups had higher SAA. In the arteriopathic (but not cardioembolic) group, higher hsCRP and SAA predicted recurrent AIS. Children with progressive arteriopathies on follow-up imaging had higher recurrence rates, and a trend towards higher hsCRP and SAA, compared to children with stable or improved arteriopathies.

Conclusion—Among children with AIS, specific inflammatory biomarkers correlate with etiology and—in the arteriopathy group—risk of stroke recurrence. Interventions targeting inflammation should be considered for pediatric secondary stroke prevention trials.

INTRODUCTION

Although childhood arterial ischemic stroke (AIS) is a heterogeneous disorder, most cases fall into one of three broad etiologic categories: arteriopathic, cardioembolic, and idiopathic.[1] Presence of an arteriopathy (cervical or cerebral) confers an increased risk of recurrent AIS.[2–5] In the prospective, multicenter "Vascular effects of Infection in Pediatric Stroke" (VIPS) study, children with arteriopathic stroke had a 21% (95% CI, 14%–29%) chance of recurrence within 1 year, compared to 8% (95% CI 3%–18%) with cardioembolic and 5% (95% CI, 2%–12%) with idiopathic stroke.[2] Childhood arteriopathies are themselves heterogeneous and poorly understood,[6] yet mounting evidence suggests that infection and inflammation play a role in their pathogenesis. The VIPS study, and others, provide evidence that acute infection, such as the common cold or herpesviruses, act as triggers for childhood AIS.[7–10] Arterial wall imaging studies detecting enhancement in the wall of affected vessels in childhood arteriopathies may suggest an acute inflammatory process.[11, 12] Children whose arteriopathies progress after their index stroke have the highest risk of recurrent AIS.[13, 14] We hypothesized that arteriopathy progression is an inflammatory process, and that markers of inflammation would predict recurrent AIS in childhood. To explore this hypothesis, we measured serum levels of four soluble immune mediators in children with AIS enrolled in the VIPS study: hsCRP, SAA, MPO, and TNF-α. These four analytes were selected because of their published associations with adult stroke and vascular disease.[15, 16]

METHODS

Study subjects and sample collection

The VIPS study prospectively enrolled and centrally confirmed 355 children (29 days through 19 years) with AIS at 37 international sites from 1/2010–3/2014. Details of our methods for enrollment, case confirmation, data collection, classification of etiology, parental interview, sample collection, and ascertainment and confirmation of recurrent AIS are published.[2, 6–8, 17] In brief, a team of pediatric stroke neurologists and neuroradiologists confirmed cases after central review of imaging and clinical features. A single neuroradiologist (M.W.) estimated infarct volume using the ABC/2 method.[18] A central team similarly reviewed all clinically-obtained cerebrovascular imaging and clinical data to classify cases as "definite," "possible," or "no arteriopathy"; "no arteriopathy" cases were further classified into cardioembolic, other specific etiology, or idiopathic.[6] When arteriopathic cases had follow-up imaging, the team classified evolution as "stable", "improves or resolves", "progresses", or "progresses then improves or resolves". For analysis, these categories were dichotomized as "stable/improving/resolved" vs. "progression" (regardless of subsequent improvement). The study protocol included a minimum follow-up of 1 year. Recurrent AIS, defined as "a new acute infarction in an arterial territory with corresponding new or worsening clinical signs and symptoms," was centrally confirmed.[2]

Laboratory methods

Blood samples were collected locally as soon as possible after enrollment, up to 21 days post-stroke. They were centrifuged at 3000 rpm for 10 minutes, with serum samples immediately separated, aliquoted and stored in 1.2 mL cryovials at −70°C. Samples were then shipped on dry ice to the Center for Advanced Laboratory Medicine (CALM) at Columbia University, and were run in batches by technicians blind to clinical status. HsCRP and SAA concentrations were measured using a clinically-validated BNII nephelometer (Siemens Dade Behring, Deerfield, IL). TNF-α (Invitrogen, Camarillo, CA) and MPO (R&D Systems Inc., Minneapolis, MN) concentrations were measured using enzyme-linked immunosorbent assays following the manufacturer's instructions. Assay performance was within the manufacturer's specifications.

Data analysis

Analysis focused on cases that could be classified with a high degree of certainty into one of the three most common etiologic groups: arteriopathic, cardioembolic, or idiopathic (Figure 1). From the overall VIPS cohort of 355 children, we excluded those with "possible" arteriopathy (likely a mixture of etiologies),[6] or other specific etiologies not falling into the three major categories of interest. We also excluded cases with major infections (sepsis, meningitis/encephalitis, endocarditis) which would impact serum concentrations of inflammatory biomarkers. We compared analyte concentration levels in the remaining children. Kruskal-Wallis tests were used to make unadjusted comparisons of each analyte individually across the three etiologic groups; linear regression models examined the associations between individual analytes and stroke etiology while adjusting for potential confounders (age, sex, infarct volume, time from stroke to blood sampling, seizures at

presentation, and clinical infection in the week preceding stroke). For regression analyses, analyte concentration levels were used as outcomes and log-transformed to reduce the skewness of residuals; our primary predictor was etiology group with idiopathic as a reference.

To assess variables related to risk of recurrent AIS, we used survival analysis techniques as previously described; the outcome was defined as the time from index AIS to first recurrent AIS and cases were censored at death or loss to follow-up.[2] To determine whether analyte concentrations correlated with recurrent AIS, we created Cox proportional hazards models. Each analyte was analyzed individually to determine its potential association with recurrence. Analyte concentrations were log-base 2 transformed to yield relative hazards associated with a doubling of concentration. We adjusted for stroke etiology, as well as those variables included in the linear regression models above. To investigate potential interactions by stroke etiology, we included an interaction term in our Cox models, and performed analyses stratified by subtype. Only the arteriopathic and cardioembolic subgroups were assessed in these analyses because of the paucity of outcomes (recurrence) in the idiopathic group. Among the subgroup of children with arteriopathic stroke and follow-up vascular imaging, we analyzed arteriopathy progression as a dichotomous predictor of recurrent AIS; to maintain consistency across models, we adjusted for the potential confounders described above.

Our α-level was set at 0.05. All analyses were conducted using Stata v12 (Stata Corp., College Station, TX).

RESULTS

The present analysis included 236 children with AIS whose etiology was classified into one of three major groups (Figure 1): idiopathic (n=78), arteriopathic (n=103), or cardioembolic (n=55). Median age at stroke ictus was 8.2 years overall (interquartile range [IQR], 3.6, 14.3), and was higher in the idiopathic group (11.8) than the arteriopathic (7.5) or cardioembolic (5.1) groups (p=0.07); 134 (57%) were boys. The median time from stroke ictus to collection of the serum sample was 5.5 days (IQR 3, 10 days); we included four samples collected beyond the 21 day window (Figure 2). Concentrations of hsCRP and SAA correlated with infarct size, but MPO and TNF-α did not (Supplemental Figure I).

Inflammatory markers and stroke etiology

In our unadjusted analyses, there were significant differences in all analyte concentrations across all three etiologic groups (Table 1). In the adjusted models, compared to children with idiopathic stroke, children with cardioembolic stroke had higher concentrations of three analytes (hsCRP, SAA, and MPO), while those with arteriopathic stroke had higher levels of only one analyte, SAA. (Adjustment for seizures and infection in the preceding week had minimal impact on the models; data not shown).

Inflammatory markers and recurrent AIS

The 236 children were followed for a median of 23 months (range 7 days to 60 months) following index stroke. During follow-up, 31 children had a recurrent AIS: 24 (23%) of 103

in the arteriopathic group, 5 (9%) of 55 in the cardioembolic group, and 2 (3%) of 78 in the idiopathic group. Higher concentrations of two analytes—hsCRP and SAA—were associated with an increased hazard of recurrent AIS in children with arteriopathic, but not cardioembolic stroke (Table 2; test for interaction, p=0.87 for hsCRP and p=0.31 for SAA). The effect size was similar for both analytes: doubling of their concentrations increased the hazard of recurrence after arteriopathic AIS by 16% (Table 2). Cumulative recurrence risk after arteriopathic AIS, stratified by analyte concentration above versus below the median, is shown in Figure 3 (A&B). The two markers were highly collinear (r=0.82, p<0.0001), so could not be included in the same Cox model.

Arteriopathy progression and recurrent AIS

Among the 103 children with arteriopathic stroke, 62 (60%) had centrally-reviewed follow-up vascular imaging (Supplemental Table I). The median time from index stroke to final vascular imaging included in these analyses was 5 months (IQR 1, 12 months). The arteriopathy was classified as progressive in 30 (48%) and nonprogressive (stable or improved) in 32 (52%). In an analysis of these 62 children, arteriopathy progression increased the hazard of recurrent AIS three-fold (adjusted HR 3.1, 95% CI 1.1, 8.7, p=0.036). Among children with progressive arteriopathy, the 1-year cumulative risk of recurrence was 46% (95% CI 25, 84%) compared to 25 % (95% CI, 12, 52%) among those with nonprogressive arteriopathy (Figure 3C).

Inflammatory markers and arteriopathy progression

The median hsCRP concentration was 1.62 mg/L (IQR 0.46, 7.28) in the progressive group (N=28), compared to 0.87 mg/L (IQR 0.16, 4.13) in the nonprogressive group (N=31; unadjusted p=0.20, Wilcoxon rank-sum test). The median SAA concentration was 14.2 mg/L (IQR 1.7, 59) in the progressive group (N=29), compared to 3.9 mg/L (IQR 1.4, 13.3) in the nonprogressive group (N=29; unadjusted p=0.067, Wilcoxon rank-sum test). Adjusted linear regression models did not find significant associations between arteriopathy progression and log-transformed concentrations of hsCRP (p=0.283) and SAA (p=0.089).

DISCUSSION

In a large, international study of childhood AIS, serum concentrations of three of four measured inflammatory biomarkers differed by stroke etiology, even after adjusting for potential confounders. Two of these—the acute phase reactants CRP and SAA—predicted risk of recurrent AIS among children with arteriopathic stroke, the subgroup at highest risk for recurrence. Children with progressive arteriopathies had the highest recurrence risk, and a trend towards higher hsCRP and SAA concentrations. These findings have important implications for the development of new strategies for secondary stroke prevention in childhood.

Two prior studies of childhood AIS, the Swiss Neuropediatric Stroke Registry Study Group (N=12 cases, N=7 controls) and a single-center US study (N=50 cases), measured serum levels of inflammatory biomarkers; both found elevated hsCRP, while other markers, including TNFα, were not significantly different.[19, 20] Elevations in these serum markers

measured post-stroke could reflect, in part, downstream effects of the infarct itself related to tissue destruction and breakdown of the blood brain barrier. Because we could collect only post-stroke serum samples, we adjusted our analyses for infarct size, seizures, and timing of the serum sample relative to the stroke. Although residual confounders may exist, several pieces of evidence indicate that these markers also reflect *upstream* mechanisms underlying the stroke pathogenesis. First, adult studies similarly found that serum levels of soluble immune mediators (TNFα, IL-6, and IL-1β) correlate with stroke etiology.[21, 22] Second, we observed large variations in biomarker concentrations even amongst children with very low volume infarcts that should have had minimal systemic effects (Supplemental Figure I). Third, the observed associations between biomarkers and recurrence risk in the arteriopathic group suggest that inflammation may be contributing to the pathogenesis of subsequent strokes.

Systemic inflammation could play a complex role in AIS pathogenesis. Circulating immune mediators can activate the coagulation system, promoting thrombosis, and can injure arterial and cardiac endothelium.[23] The different patterns of immune activation we observed across our three etiologic subgroups suggest that inflammation may play different roles in different childhood stroke etiologies. We speculate that systemic inflammation interacts with other pediatric stroke risk factors, like congenital heart disease and trauma. In a child with a structurally abnormal heart, inflammation might trigger intracardiac thrombus formation and cardioembolic stroke. Inflammation might make the cervical arteries more vulnerable to dissection; exposure to trauma after arteries have been "primed" by inflammation could trigger arteriopathic stroke.

The link between inflammation, arteriopathy and stroke recurrence in childhood has previously been postulated, but never directly studied.[12, 20] Arterial wall imaging studies suggest that some childhood arteriopathies may be inflammatory in nature.[11, 12] Other studies have reported a correlation between arteriopathy progression and increased risk of recurrence.[13, 14] We confirmed that children with progressive arteriopathy have the highest risk of recurrent AIS: almost half had a recurrence within one year, most within the first 30 days, compared to 12% of the VIPS cohort overall.[2] We demonstrated for the first time that higher levels of two inflammatory biomarkers (hsCRP and SAA) correlate with higher recurrence risk after arteriopathic stroke, particularly in the first 60 days (Figure 3, A&B). We observed a trend towards a correlation between those markers and arteriopathy progression (but were likely underpowered because only a subset of cases had follow-up vascular imaging). Although our observational data can demonstrate only correlation, and not causation, we hypothesize that arteriopathy progression results from on-going inflammation of the affected arteries. This hypothesis could be tested in a randomized controlled trial of anti-inflammatory therapy for secondary stroke prevention in childhood. Such a trial design, however, would need to consider the role of infection and other factors in pathogenesis, and the concern for immunosuppression in the setting of acute infection.

The four specific immune mediators we measured provide a window into a complex immune response. SAA concentrations were elevated in both our arteriopathic and cardioembolic cases, and correlated with recurrence in the arteriopathic group. SAA is not only a biomarker, but a participant in the innate immune response. It promotes adhesion, migration,

and infiltration of lymphocytes and monocytes; regulates production of cytokines by inflammatory cells; and increases generation of extracellular matrix metalloproteinases.[24, 25] Through these effects, SAA plausibly participates in damage to arterial or cardiac endothelium that may be relevant to childhood stroke. SAA is elevated in adults with both Takayasu arteritis and giant cell arteritis, and correlates with disease activity of inflammatory arteriopathies.[26, 27]

HsCRP was significantly elevated only in our cardioembolic cases, although there was a trend towards higher levels in arteriopathic cases. Levels also correlated with recurrence risk in the arteriopathic group. CRP is an acute phase reactant used clinically as a non-specific marker of inflammation; it predicts a broad variety of cardiovascular and non-cardiovascular causes of morbidity and mortality, including adult AIS, prognosis after stroke, and atherosclerosis.[28–30] Mounting evidence indicates that CRP, like SAA, is not only a biomarker but a participant in the innate immune response.[31, 32] The aforementioned Swiss study reported higher median hsCRP levels in their 12 cases of childhood AIS (median 5.9 μg/ml, range 0.13, 98) than seven age-matched control children (median 0.12 μg/ml; range 0.003, 4.1; p=0.007).[19] The single-center U.S. study of childhood AIS reported similar median CRP concentrations for cardioembolic (N=11) and arteriopathic (N=26) subtypes, compared to our study, but was underpowered to detect a significant difference.[20] Regardless, the non-specificity of CRP may limit its utility in distinguishing among mechanisms of stroke subtype, while it may still be useful as a marker of recurrent stroke risk among children with arteriopathic stroke.

MPO was elevated in our cardiac cases, and did not appear important in our arteriopathic cases. MPO levels correlate with active coronary artery disease (CAD), predicting risk of myocardial infarction.[16] Atherosclerosis and CAD are unlikely to be contributors to stroke risk in children, but other childhood cardiac disorders are associated with stroke risk and could explain some of the association with MPO. TNFα, which has been associated with cardioembolic stroke in adults,[21, 22] did not correlate with either stroke etiology or recurrence in our pediatric study, nor was it elevated in the cases of childhood AIS in the Swiss study.[19] TNFα is a less stable marker in stored specimens, however, and associations may thus be limited by measurement error.

In addition to the limitation of having only post-stroke serum samples available for analysis, our study has a number of other limitations. Although VIPS is the largest-ever prospective study of childhood AIS, some of our analyses may have been underpowered to detect actual differences between groups. Because we had only clinically obtained imaging, our analyses of arteriopathy progression were likely biased as children are more likely to get follow-up imaging if they have a recurrent stroke. Hence, our estimates of stroke recurrence among children with arteriopathy progression and non-progression were likely overestimates. For feasibility of enrollment, our blood samples were collected alongside clinical phlebotomy over a three-week window after the stroke; late samples may have been less likely to reflect pre-stroke inflammatory processes. Published pediatric normative values for most of our analytes are not available, and we did not have serum samples from healthy control children. The VIPS study collected blood samples only from trauma controls (for antibody titers), and the trauma would likely have affected the inflammatory biomarker concentrations. Lastly,

we measured only four biomarkers; since our study began, more immune mediators have been linked to stroke and vascular injury.[23] The renewal of the VIPS study proposes to address many of these limitations by collecting serum samples in a shorter time window (72 hours post-stroke), using multiplex technology to analyze a large number of inflammatory markers, and collecting serum samples from well children undergoing elective procedures.

CONCLUSIONS

Different inflammatory responses may underlie the heterogeneity in childhood stroke pathogenesis and recurrent stroke risk. Because children with progressive arteriopathies have the highest risk of recurrent AIS, a better understanding of the inflammatory processes underlying their arteriopathies will guide the development of secondary stroke prevention strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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APPENDIX

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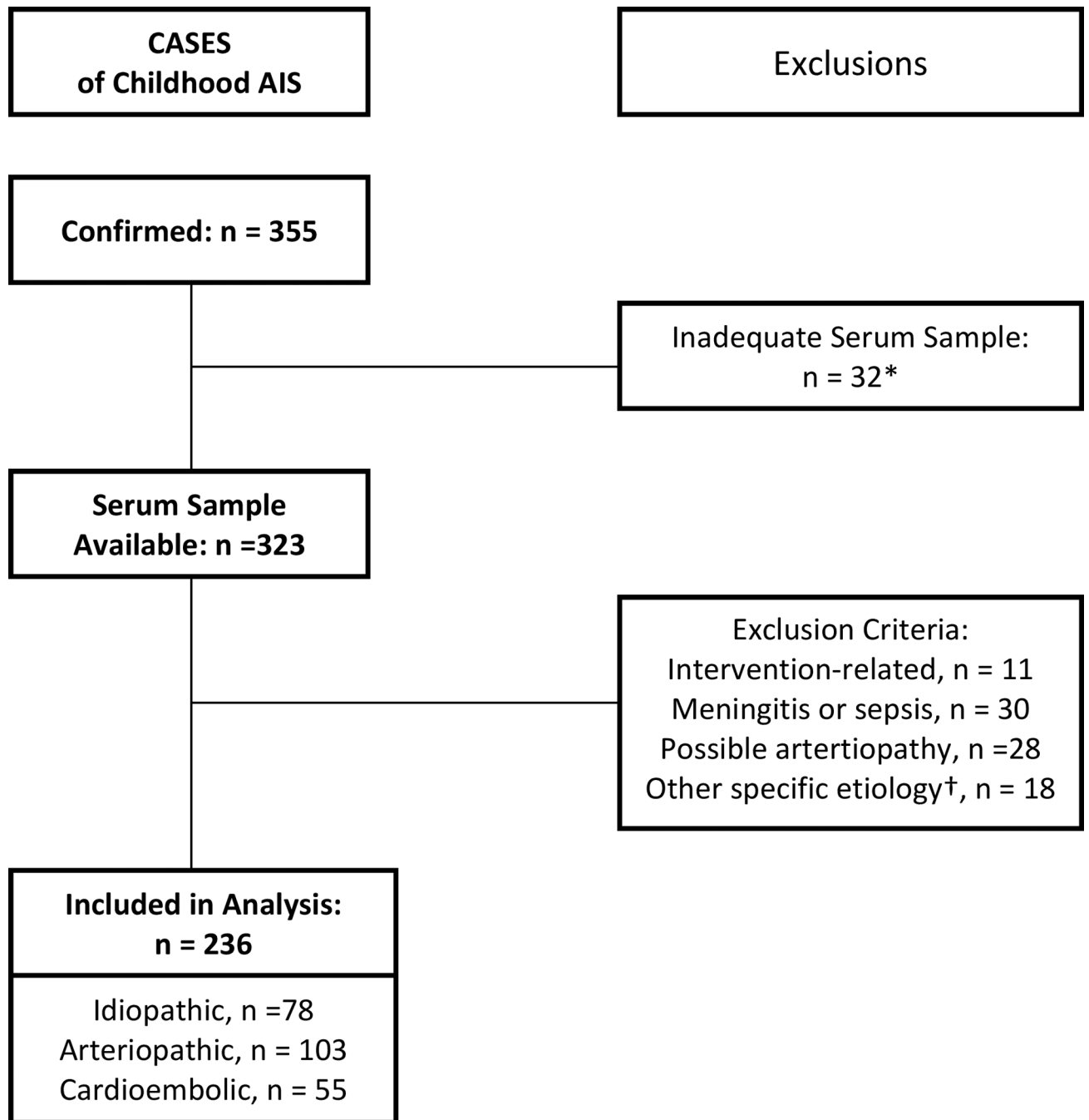
Figure 1.
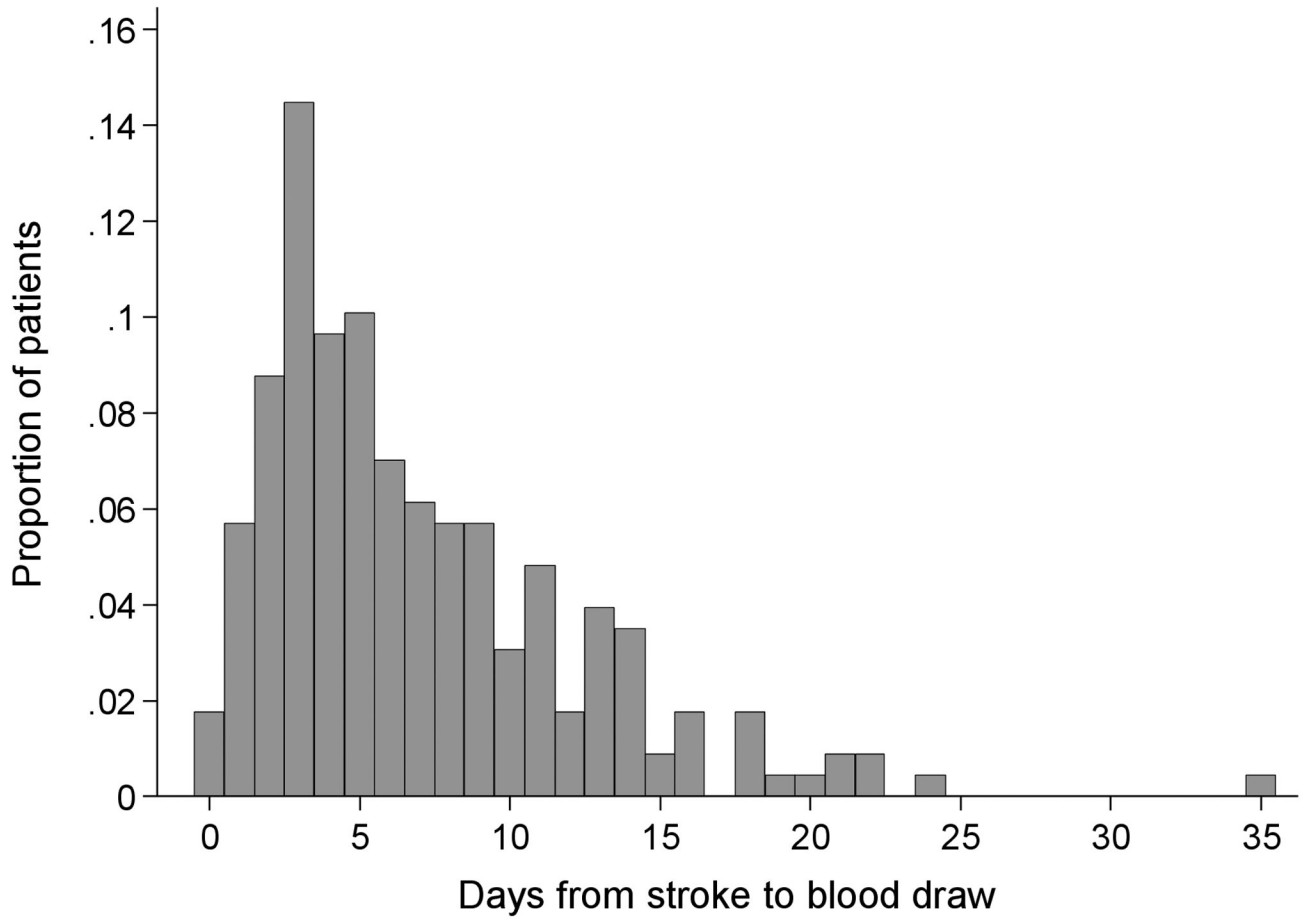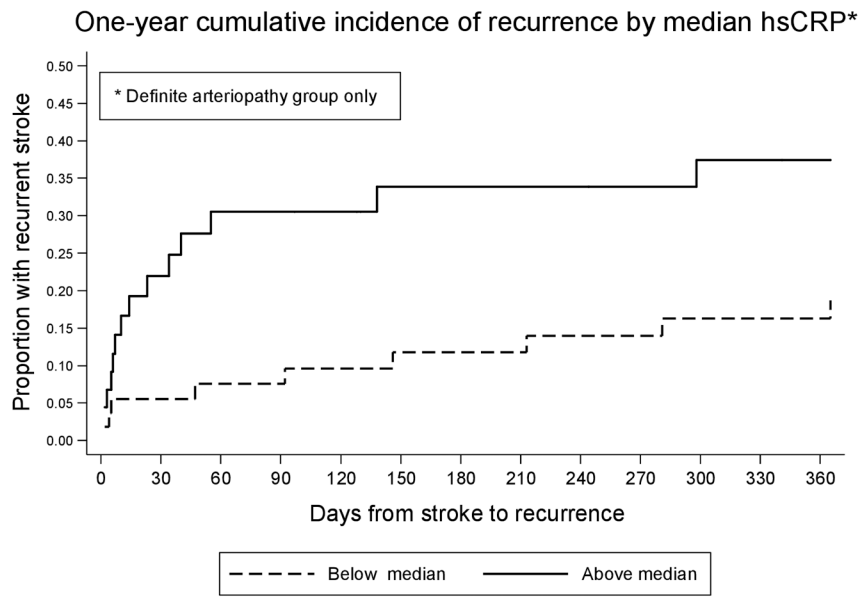Flow diagram showing the 236 VIPS cases included in the current analysis.

Figure 2.
Histogram demonstrating time in days from stroke ictus to serum sample collection (n=236).

A.

One-year cumulative incidence of recurrence by median hsCRP*

* Definite arteriopathy group only

B.

One-year cumulative incidence of recurrence by median SAA*

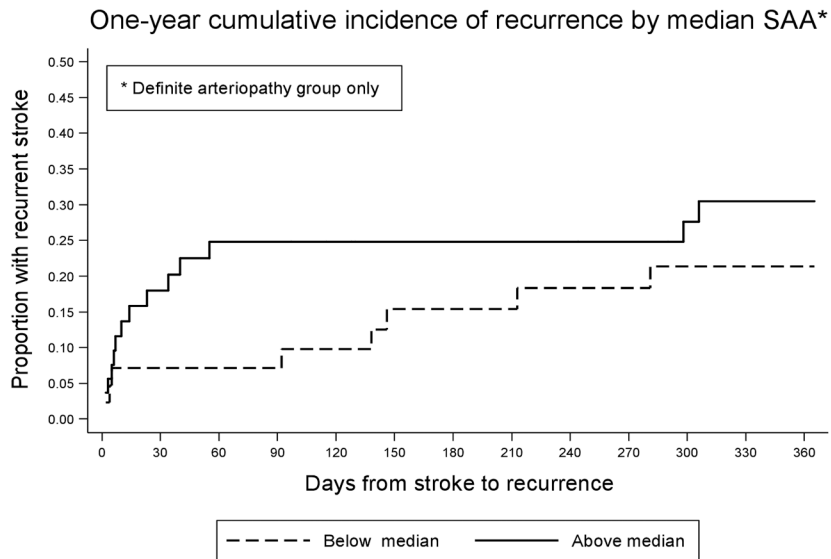* Definite arteriopathy group only

Figure 3.
Nelson-Aalen plots demonstrating cumulative incidence of recurrent arterial ischemic stroke (AIS) after index arteriopathic AIS. Panels A and B show recurrence for children with definite arteriopathy stratified by hsCRP (panel A; N=98) and SAA (panel B; N=97) concentrations that are above versus below the median concentration. Panel C shows recurrence among 62 children with definite arteriopathy and follow-up vascular imaging, stratified by arteriopathy progression.

Table 1

Levels of inflammatory markers in children with idiopathic, arteriopathic, and cardioembolic arterial ischemic stroke

Marker	Idiopathic			Arteriopathic				Cardioembolic				
	N	median	(IQR)	N	median	(IQR)	Adjusted p-value*	N	median	(IQR)	Adjusted p-value*	Unadjusted p-value†
hsCRP (mg/L)	78	0.48	(0.18, 2.5)	100	1.1	(0.28, 6.1)	0.071	54	4.6	(1.5, 14)	<0.0001	0.0001
SAA (mg/L)	77	4.1	(1.3, 8.4)	97	6.9	(1.8, 33)	0.042	52	7.7	(2.3, 32)	0.049	0.007
MPO (ng/mL)	78	156	(87, 342)	103	158	(73, 258)	0.21	55	327	(154, 532)	0.004	0.0001
TNFα (pg/mL)	78	2.6	(1.0, 4.1)	103	2.4	(0.47, 3.4)	0.53	55	3.3	(1.6, 4.5)	0.09	0.03

* from linear regression models using log-transformed outcomes and adjusted for age, gender, infarct volume, time from stroke to blood sample, seizure, and clinical infection in preceding week; reference is idiopathic

† Kruskal-Wallis tests across all 3 groups

Table 2

Adjusted hazard ratios* (HR) for inflammatory markers as a predictor of recurrent arterial ischemic stroke in children with arterial ischemic stroke.

Marker[†]	Overall (29 recurrences)				Arteriopathic (24 recurrences)				Cardioembolic (5 recurrences)			
	N	HR	(95% CI)	Adjusted p-value*	N	HR	(95% CI)	Adjusted p-value*	N	HR	(95% CI)	Adjusted p-value*
hsCRP (mg/L)	151	1.13	(0.99, 1.28)	0.06	98	1.16	(1.01, 1.32)	**0.034**	53	0.93	(0.63, 1.43)	0.73
SAA (mg/L)	147	1.11	(0.96, 1.28)	0.15	97	1.16	(1.001, 1.35)	**0.048**	52	0.49	(0.20, 1.22)	0.12
MPO (ng/mL)	155	1.08	(0.81, 1.43)	0.58	101	1.13	(0.81, 1.56)	0.47	54	0.92	(0.45, 1.90)	0.83
TNFα (pg/mL)	155	1.21	(0.96, 1.95)	0.12	101	1.12	(0.90, 1.39)	0.3	53	1.44	(0.56, 3.74)	0.45

*
From Cox proportional hazards models adjusted for age, gender, infarct volume, time to blood sample, seizure, and clinical infection in preceding week. "Overall" models include, and are adjusted for, cardioembolic and arteriopathic stroke subtype; idiopathic strokes were excluded due to the extremely low rate of recurrence in this group. Hazard ratios are interpreted as the relative hazard of recurrence risk associated with a doubling of the marker.

[†]
All marker concentrations were converted to log base 2 for analysis.