Pediatric Rheumatology

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Towards a histological diagnosis of childhood small vessel CNS vasculitis

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Abstract

Background Primary small vessel CNS vasculitis (sv-cPACNS) is a challenging inflammatory brain disease in children. Brain biopsy is mandatory to confirm the diagnosis. This study aims to develop and validate a histological scoring tool for diagnosing small vessel CNS vasculitis.

Methods A standardized brain biopsy scoring instrument was developed and applied to consecutive full-thickness brain biopsies of pediatric cases and controls at a single center. Stains included immunohistochemistry and Hematoxylin & Eosin. Nine North American neuropathologists, blinded to patients' presentation, diagnosis, and therapy, scored de-identified biopsies independently.

Results A total of 31 brain biopsy specimens from children with sv-cPACNS, 11 with epilepsy, and 11 with non-vasculitic inflammatory brain disease controls were included. Angiocentric inflammation in the cortex or white matter increases the likelihood of sv-cPACNS, with odds ratios (ORs) of 3.231 (95CI: 0.914–11.420, $p=0.067$) and 3.923 (95CI: 1.13–13.6, $p=0.031$). Moderate to severe inflammation in these regions is associated with a higher probability of sv-cPACNS, with ORs of 5.56 (95CI: 1.02–29.47, $p=0.046$) in the cortex and 6.76 (95CI: 1.26–36.11, $p=0.025$) in white matter. CD3, CD4, CD8, and CD20 cells predominated the inflammatory infiltrate. Reactive endothelium was strongly associated with sv-cPACNS, with an OR of 8.93 ($p=0.001$). Features reported in adult sv-PACNS, including granulomas, necrosis, or fibrin deposits, were absent in all biopsies. The presence of leptomeningeal inflammation in isolation was non-diagnostic.

Conclusion Distinct histological features were identified in sv-cPACNS biopsies, including moderate to severe angiocentric inflammatory infiltrates in the cortex or white matter, consisting of CD3, CD4, CD8, and CD20 cells, alongside reactive endothelium with specificity of 95%. In the first study of its kind proposing histological criteria for evaluating brain biopsies, we aim to precisely characterize the type and severity of the inflammatory response in patients with sv-cPACNS; this can enable consolidation of this population to assess outcomes and treatment methodologies comprehensively.

Cynthia Hawkins and Susanne Benseler have contributed equally to the study and therefore co-senior authors.

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Keywords Sv-cPACNS, PACNS, cPACNS, Brain biopsy, Inflammatory brain disease

Introduction

Small-Vessel Childhood Primary Angiitis of the Central Nervous System (sv-cPACNS) is a severe, immune-mediated inflammatory disease affecting the central nervous system (CNS). It is a subtype of Childhood Primary Angiitis of the CNS (cPACNS), which is categorized based on the size of the blood vessels involved. sv-cPACNS specifically involves small blood vessels and is angiography-negative, distinguishing it from the angiography-positive large-to-medium vessel vasculitis (p-cPACNS) [1, 2]. It commonly presents with severe headache, seizures, and/or focal neurological deficits. Early recognition leads to early initiation of targeted therapy that can reverse the devastating neurological inflammatory attack. If left untreated, the disease can lead to severe neurological damage or even death [3–5].

The diagnostic tools for suspected sv-cPACNS have significant limitations; the clinical presentation is heterogeneous, the laboratory tests lack sensitivity and specificity, and neuroimaging characteristics are often widely overlapping with other inflammatory brain disorders. Magnetic Resonance Imaging (MRI) and Cerebrospinal fluid (CSF) analysis alone or in combination have not sufficiently demonstrated positive predictive value to establish the diagnosis and cannot differentiate sv-cPACNS from other mimics [6]. Computed Tomography Angiography (CTA) or Magnetic Resonance Angiography (MRA), though well studied in p-cPACNS, it often is normal in cases of sv-cPACNS [7]. Further, vessel wall magnetic resonance imaging that has shown to be valuable to evaluate cerebral vessels has not been vastly studied in cases of sv-cPACNS [8]. As none of the findings are specific for sv-cPACNS, brain biopsy has remained the only definitive diagnostic test, provided it is performed early, with high-quality samples, and from targeted regions [9, 10]. Brain biopsy is essential not only for diagnosing sv-cPACNS but also for excluding other conditions like infections and malignancies.

Uncertainty about what constitutes CNS vasculitis on brain biopsy remains [6]. Histopathology descriptions are driven from small case series that typically indicate only the prominent cell type in the inflammatory infiltrate. In sv-cPACNS, a vasocentric lymphocytic infiltrate is typically reported, but no standardized criteria or systematic approach for evaluating brain biopsies exist, complicating comparisons across patients and studies [11, 12]. Therefore, the aims of the study are to (1) develop an instrument for the standardized evaluation of elective brain biopsies performed for suspected inflammatory brain diseases in children, (2) determine histological characteristics of sv-cPACNS on brain biopsy compared to inflammatory and epilepsy controls and (3) propose histological criteria for the diagnosis of sv-cPACNS on biopsy in children.

Methods

A comprehensive, stepwise approach was undertaken to integrate systematic literature review findings and expert opinion to develop a comprehensive brain biopsy scoring tool through stages of "Item generation", "Item reduction" and "Tool development". The scoring tool was subsequently utilized in standardized evaluation of brain biopsies in cases of Sv-cPACNS.

Histopathological scoring tool development

Literature review, expert panel creation and item generation (Fig. 1)

An exhaustive literature review was conducted via PubMed/ MEDLINE based on the following key terms: "central nervous system vasculitis", "demyelinating autoimmune diseases", "multiple sclerosis", "neuromyelitis optica", "acute hemorrhagic leukoencephalitis", "transverse myelitis", "acute disseminated encephalomyelitis", "neurosarcoidosis", "Rasmussen encephalitis", "hemophagocytic lymphohistiocytosis", "Limbic encephalitis", "Autoimmune encephalitis", "anti NMDAR encephalitis", "brain biopsy", "histology" or "histocytochemistry" or "immunohistochemistry". The initial tool consisted of 56 variables derived from published literature in both adult and pediatric cohorts. This preliminary tool was distributed to a panel of nine expert neuropathologists across Canada for evaluation and feedback.

Expert panel consensus meeting, tool development and item reduction (Fig. 1)

Following the initial round of literature reviews, a consensus meeting was held in Ottawa, Canada at the Children's Hospital of Eastern Ontario. The expert panel discussions led to the truncation and revision of items, modification of the tool design to enhance scoring efficiency and feasibility, and the development of biopsy and feature-specific definitions. It was recommended that each case and control slide deck include a full set of standardized stains, such as Hematoxylin & Eosin (H&E) and immunohistochemistry (IHC) (Fig. 2). Suggested antibody panels included markers like anti-CD3, anti-CD4, anti-CD8, anti-CD20, and anti-CD68, which were chosen to identify various immune cell subsets. Severity definitions were also clarified: mild inflammation was described as scattered lymphocytes, moderate as more extensive but not vessel-obliterating inflammation, and severe as inflammation leading to vessel wall obliteration

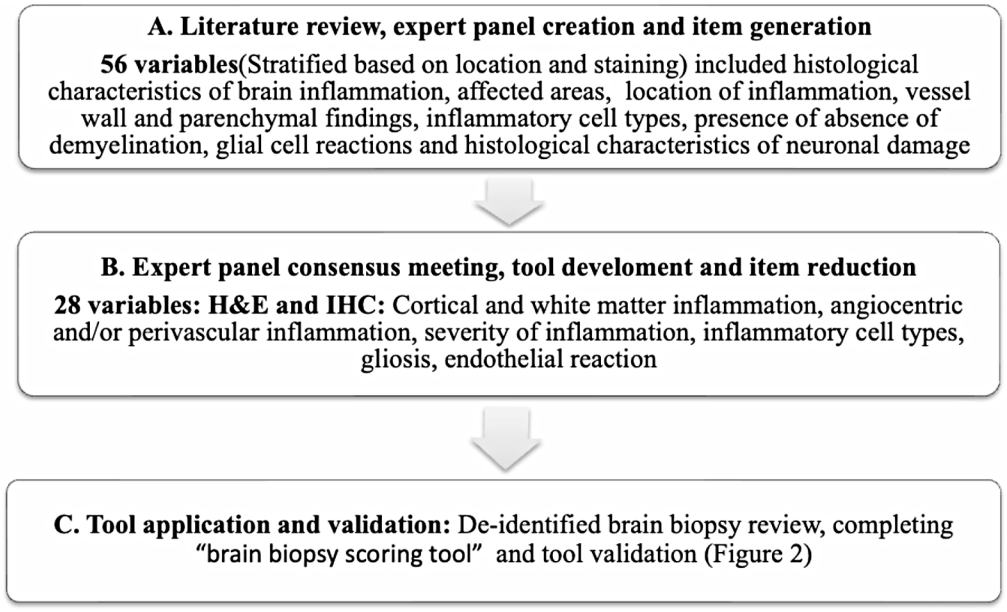

Fig. 1 Histopathological brain biopsy scoring tool development for small vessel childhood primary angiitis of CNS (sv-cPACNS)

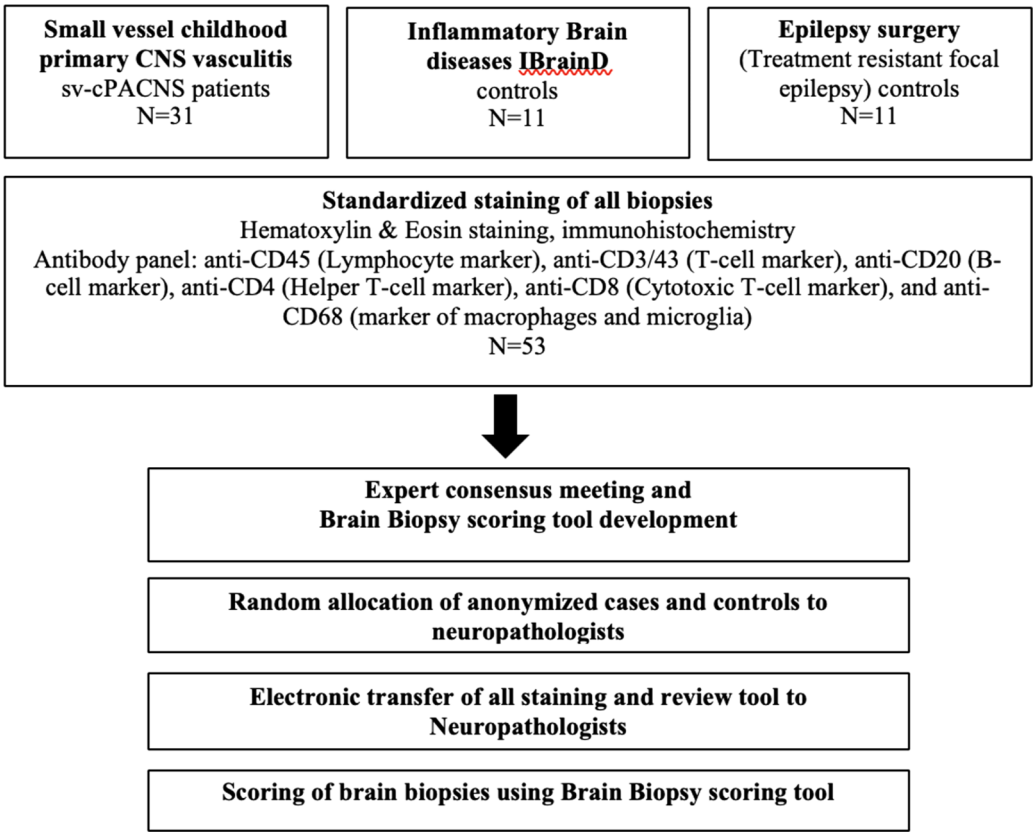

Fig. 2 Histopathological Brain biopsy scoring application on cases of small vessel childhood primary angiitis of CNS (sv-cPACNS) and inflammatory brain diseases (IBrainD) and epilepsy controls

(Brain Biopsy Scoring Tool- Supplementary material) (See results section, tool development). Following the consensus meeting, adjustments were made and agreed upon by the panel members. Once finalized, the tool was locked to ensure consistency in evaluations across cases.

Tool application and validation (Fig. 2)

Cases and controls were meticulously de-identified and assigned unique study IDs to maintain confidentiality. These cases were then randomly distributed between two neuropathologists, who independently evaluated them without knowledge of case-control status, or the evaluations performed by the other reviewer, ensuring an unbiased assessment. Regarding specimen processing, pre-existing slides were utilized in the study, specifically chosen from a standard selection of stained and archived materials. In general H&E stains and IHC stains specific to inflammatory cell markers (e.g., CD3, CD20) were included to provide a comprehensive view of inflammatory and cellular characteristics. Slides were scanned, and images were distributed on DVDs for review. Data were entered into the finalized tool called "brain biopsy scoring tool" that was developed based on final expert consensus panel (Fig. 1). All study data were managed using the secure, web-based REDCap system, which ensured standardized data capture and analysis across multiple sites [15]. Reviewers had access only to their assigned cases and were blinded to other evaluations.

Patients and controls

Patients and controls were identified from a single-center prospective cohort of children under 18 years of age at Hospital for Sick Children between 1998 and 2014. Cases consisted of small vessel vasculitis patients diagnosed based on Calabrese criteria [6] of newly acquired neurologic deficits with no evidence of underlying systemic illness and negative angiography findings enrolled in the Brainworks CNS vasculitis registry; Controls were selected from two pools: (a) epilepsy patients who underwent surgery (b) patients with various inflammatory brain disorder that underwent brain biopsy to avoid diagnostic odyssey such as N-methyl-D-aspartate receptor (NMDAR), Rasmussen's encephalitis, secondary Hemophagocytic lymphohistiocytosis (HLH), Febrile infection-related epilepsy syndrome (FIRES), Tuberculosis infection and Acute necrotizing encephalopathy of childhood. Only those whose brain biopsy was completed and available for cases and controls for review on-site at SickKids were included in the study.

Table 1 Demographics and clinical characteristics of children with sv-cPACNS, epilepsy and inflammatory brain diseases

	sv-cPACNS Cases *N* = 31	Epilepsy controls *N* = 11	Inflammatory controls *N* = 11
Median age at diagnosis in years (range)	11 (4–18)	3 (0.1–17)	6 (1–18)
Sex (% females)	21 (67%)	5 (45%)	5 (45%)
Median time from presentation to brain biopsy in days (range)	70 (24–258)	529 (316–1460)	78 (21–312)
Patients treated with steroids prior to biopsy for at least 4 weeks (%)	18 (58%)	1 (9%)	4 (36%)
Patients treated with Cyclophosphamide prior to brain biopsy (%)	0%	0%	0%
Lesional brain biopsy (%)	23 (74%)	8 (72%)	7 (63%)

Statistical analysis

Descriptive summary statistics were performed, including means with SD, medians with IQR for continuous variables, and percentages for categorical variables. Univariate analyses, such as the student's t-test, Chi-square, or Fisher's exact test, were used to compare variables between groups. Multivariable logistic models were employed to investigate associations between histological variables and sv-cPACNS diagnosis. Variable reduction was conducted based on hypothesis-driven univariate analysis before multinomial logistic regression. Odds ratios (OR) from logistic regression measured the association strength between histological characteristics and diagnostic categories, using the control group (Epilepsy cohort) as reference. Agreement between pathologists was evaluated using the Kappa statistic. Multiple correspondence analysis (CA) assessed multidimensional relationships between histological variables and diagnoses. All analyses were performed using SAS software, V.9.4 (SAS Institute, Cary, NC, USA).

Results

The study included 31 patients clinically diagnosed with sv-cPACNS who had brain biopsies available at the time of diagnosis. Additionally, 11 epilepsy patient controls and 11 non-vasculitic inflammatory brain disease patient controls were part of the study cohort. Females made up 67% of the sv-cPACNS cases, with a mean age of 11 ± 3 years at diagnosis. Steroid therapy was initiated within four weeks of obtaining a brain biopsy in 58% of these cases, and none received cyclophosphamide therapy before the biopsy. There was a wide variability in term of timing of the brain biopsy with mean of 70 days from the onset of presentation to the timing of the biopsy. Among the sv-cPACNS cases, 23 biopsies (74%) were considered lesional (Table 1).

Tool development

Neuropathologist agreement was achieved on quality parameters, histological features, and mandatory stains for evaluation of brain histopathology. It was determined that adequate specimen sampling requires all three layers

of brain tissue and, ideally, should be obtained from the targeted lesion. A total of 56 histological features were identified, separated by anatomical location and inflammatory subsets. Variables were modified and reduced following the consensus meeting as discussed in item reduction section, with total of 28 variables selected for analysis. The **Brain Biopsy Scoring Tool**, detailed in the supplementary material, aimed to design a structured comprehensive framework for the histopathological assessment of brain tissue inflammation, gliosis, and related findings across the leptomeninges, cortex, and white matter. Key components include:

- **Inflammation Localization**: Classified as angiocentric or parenchymal, further distinguished by vessel size (large with smooth muscle, small without smooth muscle) and specific vessel location (intramural, perivascular, or unsure).
- **Severity of Inflammation**: Graded as mild (scattered lymphocytes), moderate, or severe (vessel wall obliteration).
- **Gliosis**: Subtyped into astrogliosis or microglial activation/microgliosis, with localization categorized as perivascular or diffuse.
- **Additional Findings**: Includes perineuronal changes, myelin loss, reactive endothelium, infarction, and neuronophagia.
- **Immunostaining Markers**: Incorporates CD45, CD3, CD4, CD8, CD20, and CD68 to evaluate inflammatory cell types across brain regions.

H&E and Luxol Fast Blue (LFB) staining were used to assess the location and severity of inflammatory infiltrates, myelination, endothelial cell activation, and the extent and severity of gliosis. IHC staining was subsequently used to characterize the inflammatory cells.

Histological characteristics (Tool validation)

Inflammation was observed in all three brain layers in sv-cPACNS cases, with leptomeningeal inflammation present in 11/31 (35%) sv-cPACNS cases and 7/22 (31%) controls ($p=0.38$). Cortical or white matter inflammation was evident in 18/31 (58%) sv-cPACNS cases and 6/22 (27%) controls. Angiocentric inflammation capturing both intramural and perivascular inflammation, was observed in 15/31 (48%) sv- cPACNS cases compared with 5/22 (22%) controls. Intramural inflammation was noted in 16 (30%) of all biopsies, out of which 11/16 (68%) were reported in sv-cPACNS cases. Intramural infiltration showed sensitivity of 22% and specificity of 86%. Cortical astrogliosis and microglial activation was reported in 11/31 (35%) of cases (Table 1). Moderate to severe cortical or white matter vessel inflammation was reported in 11/31 (35%) of sv-cPACNS cases (Table 2) (Fig. 3). The inflammatory infiltrate was found to be T-cell mediated (CD3+82%, CD4+75%, CD8+80%) with 60% of sv-cPACNS biopsies also showing evidence of B cells infiltration. Immunohistochemistry straining identified moderate to severe CD3 (46%), CD4 (46%) and CD8 (26%) lymphocytic subtypes in cases (Table 2). Reactive endothelium was observed in 21/31 (67%) of cases compared to 4/22 (18%) of the controls (Chi-square 0.004). Features typically reported in adult sv-PACNS including granulomas, necrosis, or fibrin deposits were absent in all biopsies.

Table 3 presents the results of a multinomial logistic regression analysis, examining how the location of angiocentric inflammation and the severity of moderate to severe inflammation affect the likelihood of sv-cPACNS. Cortical or white matter angiocentric inflammation showed an increased likelihood of sv-cPACNS, with odds ratios (OR) of 3.231 (95% CI: 0.914–11.420, $p=0.067$) and 3.923 (95% CI: 1.13–13.6, $p=0.031$), respectively. Moderate to severe inflammation raised the probability of sv-cPACNS, with ORs of 5.56 (95% CI: 1.02–29.47, $p=0.046$) in the cortex and 6.76 (95% CI: 1.26–36.11, $p=0.025$) in WM. Within the inflammatory infiltrate subtypes, moderate to severe CD3 inflammation was associated with the highest increase in odds of sv-cPACNS (OR: 7.8, 95% CI: 1.47–41.21, $p=0.015$). This was followed by increased odds of moderate to severe inflammation by CD8 (OR: 5.77, 95% CI: 1.12–29.85, $p=0.036$), CD4 (OR: 3.63, 95% CI: 0.78–16.75, $p=0.09$), and CD20 (OR: 4.2, 95% CI: 0.43–42.64, $p=0.21$). Angiocentric localization of CD8 and CD20 inflammatory infiltrates suggested the clearest association with (Table 3). Reactive endothelium was strongly associated with a higher likelihood of sv-cPACNS, with an odds ratio of 8.93 (95% CI: 2.38–33.55, $p=0.001$).

Correspondence analysis was successful in representing the contingency table for a two-dimension solution (see *e*Figure 1) as determined by trace analysis for dependencies ($\chi 2=69.64$, df=34, $p=0.0003$). The quality of representation for each row and column is provided as contributions to the total χ^2 statistic and Pearson's residuals (see *e*Table S1 and *e*Table S2). CA revealed distinct clustering of variables around the three different diagnoses based on the two possible dimensions of severity of infiltrate (largest explained inertia, 72%) and location of inflammation (smaller explained inertia, 28%) (Fig. 4). Histological features distinguishing sv-cPACNS from controls in CA included severity of inflammatory infiltrate consisting of CD3, CD4 and CD8 lymphocytic subtypes (Contribution to chi square was 2.15,1.22, 1.22 respectively). This indicates that sv-cPACNS cases were differentiated from controls primarily by the severity of inflammation rather than its location. As was also noted in the multinomial logistic regression, CD3 inflammation

Table 2 Histological characteristics in brain biopsies of children with SVcPACNS, epilepsy and inflammatory controls

Variables	sv-cPACNS Cases *N* = 31	Epilepsy controls *N* = 11	Inflammatory controls *N* = 11	Chi-square (*p*-value)
Leptomeningeal inflammation (*N* = 53)*	11 (35%)	5 (45%)	2 (18%)	0.38
Cortical inflammation (*N* = 53)	13 (42%)	9 (82%)	5 (56%)	0.23
• absent	15 (48%)	2 (18%)	3 (33%)	
• angiocentric	3(10%)	0	1 (11%)	
• parenchymal				
Cortical vessel Inflammation localization (*N* = 51)	7 (24%)	0	3 (33%)	0.22
• intramural	9 (31%)	2 (18%)	2 (18%)	
• perivascular				
Cortical vessel inflammation Severity (*N* = 50)#	7 (23%)	2 (18%)	4 (36%)	0.05
• mild	9 (29%)	0	0	
• moderate	2 (6%)	0	2 (18%)	
• severe				
Cortical gliosis (*N* = 53)	14 (46%)	6 (64%)	6 (55%)	0.47
• absent	11 (35%)	2 (18%)	5 (45%)	
• astrogliosis	6 (19%)	2 (18%)	0	
• microglial activation/microgliosis				
Reactive endothelium (*N* = 52)	20 (64%)	0 (0%)	4 (36%)	0.004
White matter inflammation (*N* = 52)	13 (42%)	9 (82%)	8(73%)	0.11
• absent	15 (48%)	2 (18%)	3 (27%)	
• angiocentric	3 (10%)	0	0	
• parenchymal				
White matter vessel Inflammation localization (*N* = 53)	4 (15%)	0	2 (18%)	0.27
• intramural	9 (34%)	2 (20%)	1 (9%)	
• perivascular				
White matter vessel inflammation severity (*N* = 47)	7 (22%)	2 (20%)	1 (10%)	0.06
• mild	9 (30%)	0	0	
• moderate	2 (6%)	0	2 (18%)	
• severe				
White matter gliosis (*N* = 52)	16 (51%)	9 (81%)	8 (72%)	0.41
• absent	10 (32%)	1 (9%)	2 (18%)	
• astrogliosis	5 (16%)	1 (9%)	1 (9%)	
• microglial activation/ microgliosis				
CD3 positive cells (*N* = 48)	23 (82%)	4 (40%)	7 (63%)	--
CD3 Inflammation Localization (*N* = 53)	19 (68%)	3 (30%)	6 (60%)	0.05
• angiocentric	4 (14%)	1 (10%)	0 (0%)	
• parenchymal				
Severity of inflammation, CD3 (*N* = 48)	10 (35%)	4(40%)	4(40%)	< 0.001
• mild	13 (46%)	0 (0%)	0 (0%)	
• moderate	0 (0%)	0 (0%)	3 (30%)	
• severe				
CD4 positive cells (*N* = 48)	21 (75%)	4 (40%)	7 (63%)	--
CD4 Inflammation Localization (*N* = 48)	18 (64%)	4 (44%)	6 (54%)	0.49
• angiocentric	3(10%)	0 (%)	1 (9%)	
• parenchymal				
Severity of inflammation, CD4 (*N* = 48)	8 (29%)	4(44%)	4 (36%)	0.008
• mild	12 (43%)	0 (0%)	0 (0%)	
• moderate	1 (3%)	0 (0%)	3 (27%)	
• severe				
CD8 positive cells (*N* = 53)	25 (80%)	3 (27%)	6 (54%)	--
CD8 Inflammation Localization (*N* = 48)	20 (65%)	3 (27%)	20 (65%)	0.02
• angiocentric	5 (16%)	0 (0%)	0 (0%)	
• parenchymal				
Severity of inflammation, CD8 (*N* = 53)	17 (56%)	3 (27%)	3 (27%)	0.001
• mild	7 (22%)	0 (0%)	0 (%)	
• moderate	1 (3%)	0 (0%)	3 (27%)	
• severe				

Table 2 (continued)

Variables	sv-cPACNS Cases *N* = 31	Epilepsy controls *N* = 11	Inflammatory controls *N* = 11	Chi-square (*p*-value)
CD20 positive cells (*N* = 53)	18 (60%)	1 (12%)	4 (36%)	--
Severity of inflammation, CD20 (*N* = 46)	13 (43%)	2 (22%)	2 (20%)	0.416
• mild	4 (13%)	0 (0%)	2 (20%)	
• moderate	1 (3%)	0 (0%)	0 (0%)	
• severe				
CD68 positive cells (*N* = 49)	25 (86%)	6 (60%)	9/11(81%)	---
Severity of inflammation, CD68	8 (28%	3(30%)	6 (54%)	0.268
• mild	12(41%)	3(30%)	2 (18%)	
• moderate	5(17%)	0 (0%)	1(9%)	
• severe				

* Missing data is not shown

Severity was described by the consensus group as follows: mild was described as having scattered lymphocytes, severe described as having vessel wall obliteration, and moderate described as being in between mild and severe

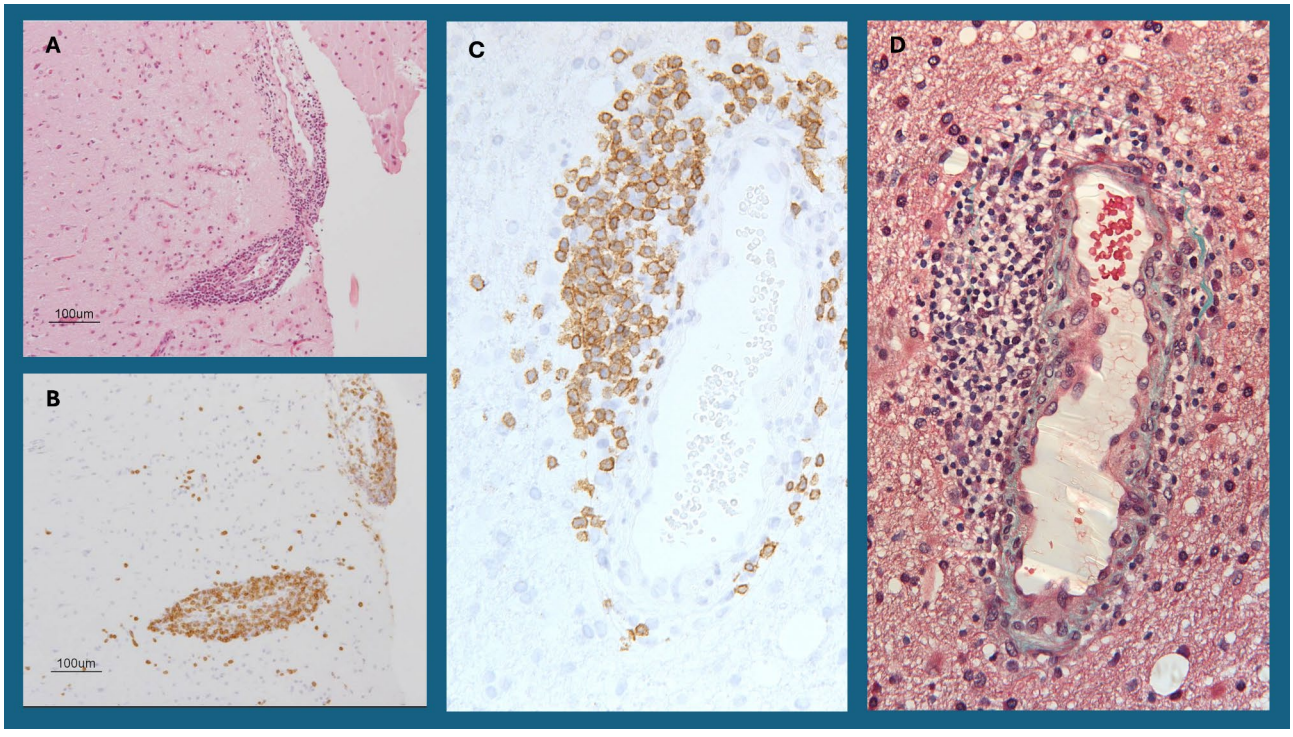

Fig. 3 Histology features observed in patients with sv-cPACNS. **A**: Moderate to severe angiocentric cortical inflammatory infiltrate, **B**: Perivascular white matter CD8 inflammatory infiltrate of moderate to severe severity. **C**: CD3 immunohistochemistry highlights the T-cells. **D**: Masson Trichome-stained section of brain showing a blood vessel with reactive endothelium and perivascular lympho-histiocytic infiltrates

severity had the highest contribution followed by equal contribution by CD4 and CD8 infiltrates.

Moderate to severe angiocentric inflammatory infiltrates in cortex or white matter consisting of CD3, CD4 or CD8 together with reactive endothelium showed sensitivity of 20% and specificity of 95% with negative predictive value of 0.4 and positive predictive value of 0.8. Inter-rater variability in determining the final diagnosis of sv-cPACNS was reflected in a Kappa score of 0.29–0.36.

Discussion

Small vessel (sv-)cPACNS is a rare and progressive immune-mediated inflammatory disease with variable clinical presentation, including headaches and fatigue, seizures, psychiatric symptoms, neurological deficits, and others. We report on largest study to date that aimed to develop a comprehensive evaluative tool for standardized review of brain biopsies in children with sv-cPACNS. Distinct histological characteristics that were found to set apart sv-cPACNS from controls included moderate to severe cortical or white matter angiocentric

Table 3 Multinomial logistic regression analysis of inflammation's location and severity in distinguishing sv-cPANS cases from controls

Variables	B coefficient	Wald Chi-Square	Odds Ratio (OR) 95% Confidence interval (CI)	*P*-value
Cortical inflammation location, angiocentric (*N* = 51)*	1.17	0.644	3.231 (0.914–11.420)	0.067
Cortical inflammation severity- moderate to severe (*N* = 52)	0.841	3.96	5.56 (1.026–29.478)	0.046
White matter inflammation location, angiocentric (*N* = 53)	0.634	4.6429	3.923 (1.132–13.602)	0.031
White matter inflammation severity- moderate to severe (*N* = 52)	0.8545	5.006	6.767 (1.268–36.118)	0.025
CD3 inflammation severity- moderate to severe(*N* = 48)	0.84	5.849	**7.8** (1.476–41.213)	0.015
CD3 inflammation location, angiocentric (*N* = 48)	0.94	0.60	2.6 (0.79–8.44)	0.11
CD4 inflammation severity- moderate to severe(*N* = 51)	1.29	2.74	3.64 (0.789–16.755)	0.09
CD4 inflammation location, angiocentric (*N* = 48)	0.59	0.97	1.80 (0.56–5.79)	0.32
CD8 inflammation severity- moderate to severe(*N* = 53)	1.75	4.38	**5.77** (1.12–29.85)	0.036
CD8 inflammation location, angiocentric (*N* = 53)	0.59	5.27	3.89 (1.221–12.428)	0.021
CD20 inflammation severity- moderate to severe (*N* = 49)	1.45	1.52	4.27 (0.43–42.64)	0.216
CD20 inflammation location, angiocentric (*N* = 48)	1.36	4.45	3.90 (1.102 13.802)	0.03
Reactive Endothelium (*N* = 42)	0.6756	10.49	8.93 (2.38–33.55)	0.001

*Number of observations used

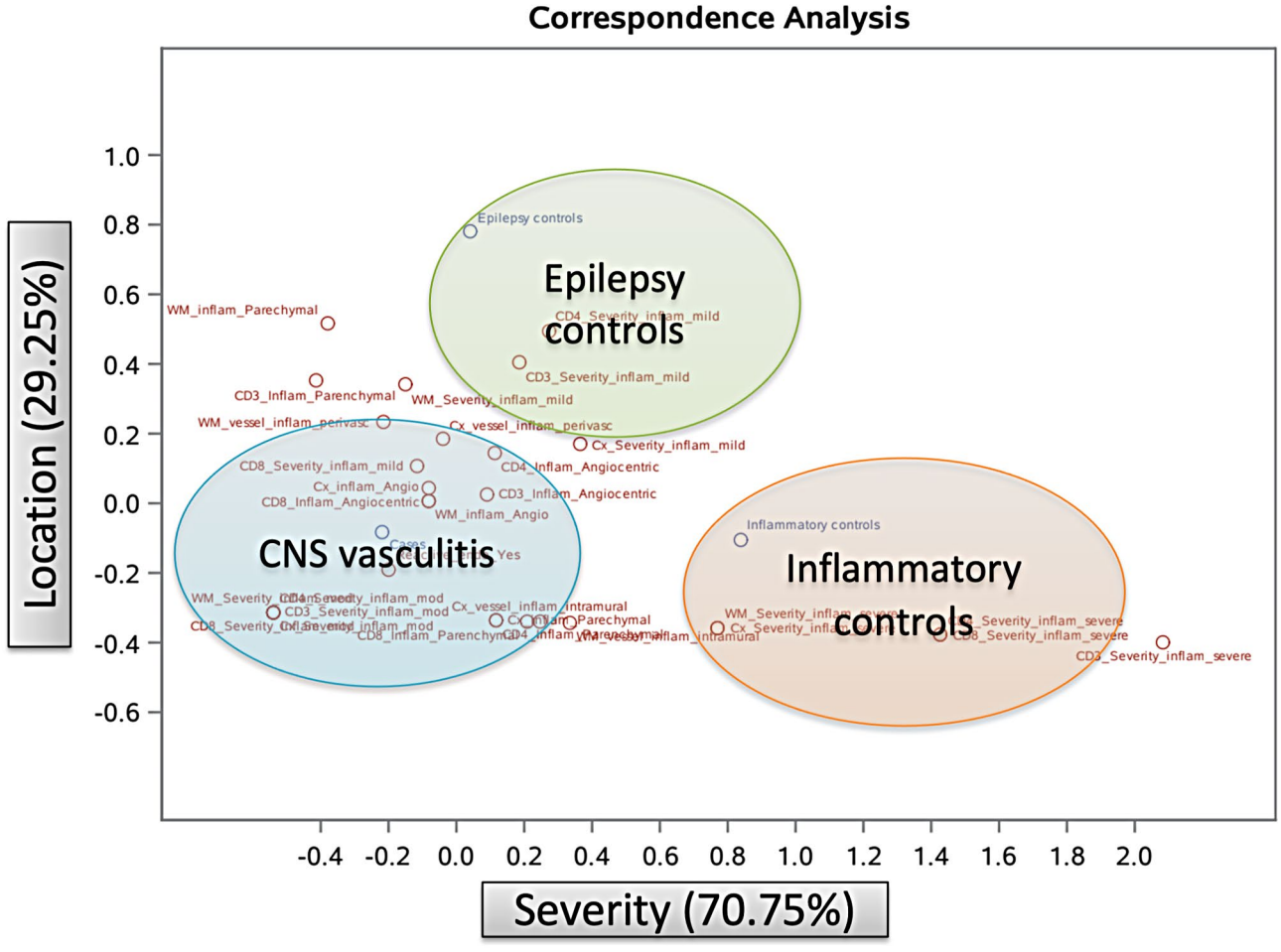

Fig. 4 Correspondence analysis of histological characteristics of brain biopsies in children with sv-cPACNS, epilepsy and inflammatory brain diseases. legends: Distance observed between points indicates level of association in a two-dimensional space representing the total variance of the factor scores (inertia). This method was able to separate the cases and controls based on location of the infiltrate and severity of infiltrate. Cases and controls are separated in the X-axis (Severity of inflammation). Epilepsy and inflammatory controls were not separated in the X-axis instead they were separated by the Y-axis (Location of inflammation)

inflammatory infiltrates in presence of reactive endothelium. The inflammatory infiltrate was found to be primarily T-cell mediated (CD3+82%, CD8+80%, CD4+75%, CD20 60%). Constellation of the following features: (a) moderate to severe angiocentric inflammatory infiltrates in cortex or white matter that (b) consisted of CD3 and CD4 or CD8 cell subtypes alongside (c) reactive endothelium showed sensitivity of 20% and specificity of 95% with negative predictive value of 0.4 and positive predictive value of 0.8.

Our results advise against biopsy of leptomeninges only, as leptomeningeal inflammation was observed in equal proportion in both cases and controls, and thus not a distinguishing histological feature of sv-cPACNS. Our data shows that among the inflammatory infiltrate subtypes, moderate to severe CD3 inflammation showed the highest increase in odds of sv-cPACNS diagnosis. Results suggest that while location of the inflammation is important, distinguishing perivascular from intramural inflammation was not found be statistically significant. Intramural inflammation in particular was found to be specific but not sensitive in patients without an alternative diagnosis. Further to this point, our data (through correspondence analysis) confirms that sv-cPACNS cases were distinguished from controls (epilepsy and inflammatory controls) more strongly on basis of severity of inflammation.

A consensus among a panel of nine experienced neuropathologists was reached regarding the following recommendations: (1) Employing H&E staining to ascertain the location and severity of inflammatory infiltrate; (2) Utilizing standardized immunohistochemistry (IHC) with anti-CD45, anti-CD3/43, anti-CD20, anti-CD4, anti-CD8, and anti-CD68 to characterize inflammation; (3) Collecting lesional biopsy samples from the leptomeninges, cortex, and white matter, with consideration of non-lesional biopsy (e.g., right frontal region) in cases where the lesion site is inaccessible or visibility is lacking; (4) Conducting biopsies before or within 7 days of initiating immunosuppression to uphold biopsy quality and enhance diagnostic probability.

In adult patients with PACNS, diagnostic yield of brain biopsies range between 36 and 72% [13]. Results of a meta-analysis showed that brain biopsy indeed had the highest diagnostic yield (74%) when the indication was to evaluate for suspected PACNS [14]. Features reported in adult patients with sv-PACNS including granulomas, necrosis or fibrin deposits [15] were absent in all pediatric biopsies. Adult patients with predominantly lymphocytic infiltrates have the propensity toward a better prognosis [16] that can be indicative of either earlier disease detection or milder disease process. This subset of patients could potentially be compared with their pediatric counterparts for better delineation of disease trajectory. Differences between adult and pediatric sv-PACNS may result from distinct inflammatory pathways, genetic and environmental factors, and developmental variations, affecting inflammation's manifestation and progression in CNS vasculitis [17]. Of importance is that the vast majority of sv-cPACNS brain biopsies in children show lymphocytic, non-granulomatous lesions [18–20]. Our results elaborate on that of previous publication reporting angiocentric lymphocytic inflammatory infiltrate involving leptomeninges, cortex, and subcortical white matter, with swollen, reactive endothelia in biopsy specimen of patients with sv-cPACNS [9]. We further report on importance of severity of inflammation and lymphocytic inflammatory subtypes by providing a standardized scoring tool for comprehensive review of brain biopsies. A retrospective single-center study of brain biopsies in 66 children revealed a diagnostic yield of 69%, amongst which the most frequently diagnosed disease was CNS vasculitis [18]. New survey however, comparing differences in diagnostic and therapeutic approaches between Europe and North America, found that higher proportion of North American respondents would perform a brain biopsy to secure diagnosis (70.59 vs. 45.95%) in patients with sv-cPACNS [21].

The strength of this study is the largest number of blinded cases and controls, which provides a reliable perspective on the histological features of sv-cPACNS than the previous smaller series. Another strength of the study is inclusion of neuropathologists from across the country with varied experiences to eliminate reporting bias. Inclusion of subset of controls (Both inflammatory and epilepsy controls) allowed for identification and internal verification of constellation of histological findings associated with diagnosis of sv-cPACNS. Amongst limitations of the study is possibility of referral bias, given that cases were all selected from one centre. With studies of this kind, small sample size and small number of control subjects can skew the results and can impact the generalizability of findings. The small sample size also contributed to the wide confidence intervals observed in the multinomial logistic regression results, reflecting increased uncertainty and potential for error. The timing of the biopsy and use of steroids can confound specificity of the reported features. Also of note is that sparsity was observed. The study's low Kappa scores in determining the final diagnosis highlight the impact of rare diagnoses like sv-cPACNS on inter-rater agreement, as the Kappa statistic is sensitive to prevalence of a disease. In this context, low Kappa values do not signify poor overall agreement but emphasize the need for standardized histological criteria and a diagnostic atlas. Such resources could improve diagnostic consistency by reducing variability in interpretation and enhancing inter-rater reliability through clearer guidelines and training. To ensure

reliability and applicability, the proposed diagnostic tool and its identified histological features require further validation in an external patient cohort. Moreover, this research allows for the future opportunity to distribute the corresponding biopsy slides via an easily accessible web-based online atlas, which will be open to all physicians caring for children with primary small vessel CNS vasculitis. The slides can be categorized based on their distinct features and can be accompanied by pertinent details regarding the duration of symptoms and response to treatment. Myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) should be considered an important differential diagnosis for sv-PACNS vasculitis and screened for in all patients. Although a combination of clinical evaluation, imaging, laboratory tests can help distinguish sv-PACNS from MOGAD, potential overlaps may exist. While the primary challenge in assessing inflammatory conditions lies in discerning inflammation triggered directly by the initial injury from inflammation provoked by the body's response to the damage, the current proposed histological criteria for evaluating brain biopsies allow for a more detailed description of the type and severity of the inflammatory response. Future association of the results with neuroimaging findings in particular will be of value.

Conclusion

In conclusion, given that diagnosis of sv-cPACNS cannot be established in the absence of histologic demonstration of CNS angiitis, this study provides basis for standardized reporting of histological features of small vessel vasculitis through a systematic evaluative tool. We proposed characteristic histological features in support of the diagnosis of sv-cPACNS to include (a) moderate to severe cortical or white matter angiocentric inflammatory infiltrates composed of CD3 and CD4 or CD8 lymphocytic subtypes in presence of (b) reactive endothelium. The histologic criteria have specificity of 95%. This approach not only facilitates the detection of patients with sv-cPACNS but also provides an opportunity to combine study populations across different centers to evaluate outcomes and treatment approaches more effectively. This approach not only facilitates the detection of patients with sv-cPACNS but also provides an opportunity to combine study populations across different centers to evaluate outcomes and treatment approaches more effectively. It is crucial to note that patients without histologic confirmation of CNS angiitis should not be included in case reports, case series, or reviews of sv-cPACNS.

Abbreviations

cPACNS	Childhood primary angiitis of the central nervous system
Sv-cPACNS	Small vessel vasculitis childhood primary angiitis of the central nervous system
p-cPACNS	Angiography positive childhood primary angiitis of the central nervous
CSF	Cerebrospinal fluid
CT	Angiography Computed Tomography-based Angiography
MR	Angiography Magnetic Resonance Angiography
H&E	Hematoxylin & Eosin
IHC	Immunohistochemistry
CD	Cluster of Differentiation
NMDAR	N-methyl-D-aspartate receptor
HLH	Hemophagocytic lymphohistiocytosis
FIRES	Febrile infection-related epilepsy syndrome
MOGAD	Myelin oligodendrocyte glycoprotein antibody-associated disease

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

Study Design: SB, CH, MNN, and AD designed the study, including conceptualization and methodology. Data Collection and Processing: SS and AD were responsible for data collection and cleaning. PNT and MNN conducted data analysis and labeling. Generation, Analysis, and Communication of Results: SB, CH, MNN, and AD contributed to writing the original draft. JM, HBS, BE, CD, PWS, JK, DM, and HVV reviewed and edited the manuscript. Manuscript Composition and Correspondence: SB, CH, MNN, and AD wrote the manuscript. All authors read and approved the final version of the manuscript. MNN submitted the manuscript.

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Data availability

Datasets generated and/or analyzed during the current study are available on. reasonable request.

Declarations

Standard protocol approvals, registrations, and patient consents

The Declaration of Helsinki was followed when conducting the study. Ethics approval was obtained (REB#1000014279).

Consent for publication

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Conflict of interest

The authors do not declare any conflicts of interests.

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