Objective. The main objective was to develop a trajectory for von Willebrand factor (vWF) antigen in childhood primary CNS vasculitis (cPACNS) after treatment and compare this with disease activity and other inflammatory markers.

Methods. A single-centre cohort study of consecutive children diagnosed with cPACNS was performed. Demographic, clinical, laboratory, imaging and histological data were collected at diagnosis and during standardized clinic visits. Outcome measures included disease activity measured by physician global assessment and serial measures of vWF antigen. Analysis included descriptive statistics and parametric methods.

Results. The study cohort consisted of 39 children diagnosed with cPACNS: 25 with angiography-negative cPACNS and 14 with angiography-positive disease. Twenty-one patients were female, median age at diagnosis was 9.8 years, and median follow-up was 18 months. All patients presented with neurological deficits. Disease activity and neurological outcome improved significantly during follow-up. vWF antigen levels were increased at diagnosis in 65% of children with cPACNS and were decreased significantly after treatment. In contrast, levels of CRP and ESR fluctuated over time. Higher vWF antigen levels at diagnosis were associated with lower measures of disease activity at 12 months.

Conclusion. In our study, all children with cPACNS improved over time. Changes in CRP and ESR, other laboratory tests, and MRI did not consistently reflect altered disease activity. However, vWF antigen may help clinicians to identify changes in disease activity during follow-up and predict treatment response. Controlled studies are necessary to evaluate the sensitivity and specificity of vWF antigen as a biomarker of disease activity.

Key words: CNS, vasculitis, childhood, disease activity.

Introduction

CNS vasculitis is an inflammatory brain disease that often presents with severe neurological deficits and strokes in previously healthy children [1]. Inflammation of cerebral blood vessels in this disorder may develop with no known underlying cause, as in primary angiitis of the CNS (PACNS), or may be secondary to infection or systemic inflammatory disease [2]. The effects of childhood PACNS (cPACNS) can be devastating. A review of the published literature in 2001 found a mortality rate of 60%, and more than half of children who survived were left with moderate-to-severe long-term neurological deficits [3–5]. However, recent evidence suggests that survival and neurological outcome is improved if CNS vasculitis is diagnosed early and treated with aggressive immunosuppressive medications [6–10].

Making the diagnosis of cPACNS currently relies on invasive testing. Initial laboratory investigations, such as...
blood counts and inflammatory markers, are frequently normal [1, 6]. MRI of the brain shows T2 lesions in >90% of patients with CNS vasculitis, but these findings lack specificity to confirm the diagnosis [11]. Therefore angiography is used to identify CNS vasculitis involving medium to large cerebral vessels, and elective brain biopsies are needed to diagnose CNS vasculitis affecting small cerebral vessels [1, 6, 12–14].

Currently there is no gold standard measure of disease activity in cPACNS. Deciding whether a patient has active disease is a clinical judgment based on the presence of clinical features, elevated inflammatory markers and MRI findings. This can be extremely difficult given that the patient’s symptoms may be non-specific and that changes in inflammatory markers and neuroimaging may be subtle or absent. It is undesirable to repeat invasive testing, such as lumbar puncture or brain biopsy, on a regular basis to monitor disease activity. An ideal marker of disease activity should be sensitive, reliable, objective and measured by non-invasive and easily available testing.

Von Willebrand factor (vWF) antigen is a plasma protein that is synthesized primarily by megakaryocytes and endothelial cells and mediates platelet aggregation and adhesion. Release of vWF antigen is increased when vascular endothelium is damaged or inflamed. Measurement of serum levels is routinely available at most local laboratories. Elevated serum levels of vWF antigen have been identified in multiple systemic vasculitides, including Kawasaki disease, Henoch–Schönlein purpura and granulomatosis with polyangiitis (Wegener’s) [15–19]. Higher levels of vWF antigen levels were found in patients with active vasculitis compared with those in remission and healthy control subjects [20–22]. Therefore vWF antigen may serve as a surrogate marker of disease activity in CNS vasculitis. The objectives of this pilot study are as follows: (i) to describe the clinical, laboratory and imaging characteristics of our cohort of children with cPACNS at diagnosis and during follow-up; (ii) to determine disease activity longitudinally during follow-up; and (iii) to develop a trajectory for vWF antigen after treatment and to compare this with disease activity and inflammatory markers.

Methods

This single-centre cohort study included consecutive children diagnosed with definite or suspected cPACNS at the Hospital for Sick Children (SickKids) in Toronto, Canada, between June 2001 and October 2010. Patients were considered to have definite cPACNS if they fulfilled the Calabrese criteria, including a newly acquired neurological deficit, angiographic and/or histological features of CNS vasculitis and no evidence of a systemic condition associated with these findings [23]. These patients were further grouped into angiography-positive cPACNS and angiography-negative cPACNS based on whether there was angiographic evidence of vessel inflammation. Children with suspected angiography-negative cPACNS presented with a newly acquired neurological deficit, clinical features consistent with cPACNS, normal cerebral angiography and no histological evidence of CNS vasculitis due to parental refusal of brain biopsy. All patients included in the study had levels of vWF antigen measured serially as part of their standardized clinical assessments. Patients were excluded if they were older than 18 years of age and if they had a primary disorder involving vWF antigen.

Ethics approval was obtained from the institutional review board (Research Ethics Board file number 100013043). All patients included in the study were enrolled in BrainWorks, an international collaborative study of outcomes in children with inflammatory CNS diseases, and informed consent was obtained for all associated studies, including the current study. The participation rate in BrainWorks at our institution is 100% for children with inflammatory CNS diseases.

Data collection at diagnosis

The initial work-up of a patient with a potential diagnosis of cPACNS includes the following components: (i) history and physical examination, including a general examination to look for systemic disease manifestations and a detailed neurological examination; (ii) laboratory investigations, such as complete blood cell count and differential, ESR and CRP; (iii) cerebrospinal fluid (CSF) analysis, including opening pressure, CSF protein level, CSF cell count and oligoclonal bands; (iv) bacterial and viral cultures, PCR and serological analysis of blood and CSF to assess for underlying infection following institutional protocol; (v) brain MRI; and (vi) conventional and/or MR angiography. Brain biopsy is performed when required to confirm a diagnosis of angiography-negative cPACNS.

Data collection during follow-up

All children with cPACNS were treated using institutional protocols [1, 21]. Clinical, laboratory, imaging and histological data were collected. At SickKids, patients with cPACNS are treated and monitored based on institutional protocols [1, 24]. Standardized clinic visits occur at 3-month intervals. Each visit involves monitoring for clinical features of disease, assessment of neurological outcome, measurement of laboratory parameters, and adjustment or confirmation of the treatment regimen. Routine laboratory investigations at each visit include inflammatory markers (ESR, CRP) and complete blood cell counts. Repeat brain MRI is also performed at regular intervals during follow-up.

Study outcomes

The primary study outcome was disease activity based on physician global assessment (PGA) using a 10-cm visual analogue scale. This was determined by the same treating physician (S.M.B.) at diagnosis and at the end of each standardized clinic visit before the measure of vWF antigen was available. As stated previously, there is no established measure of disease activity in cPACNS. However, PGA using a visual analogue scale has been validated as a sensitive measure of disease activity in other inflammatory diseases, including RA, SLE and ulcerative colitis [25–27]. Remission was defined as a PGA of 0 cm. The minimum clinically significant difference on a visual analogue scale is 1 cm [28]. Therefore a disease flare was defined as an
increase in PGA by at least 1 cm in the presence of recurrent symptoms, laboratory changes and/or MRI findings.

Secondary outcomes included serial measures of vWF antigen and neurological outcome. vWF antigen levels were collected prospectively at diagnosis and at standardized follow-up visits. Measurement of vWF antigen levels is routinely available at our institution, and the assay has been validated with healthy controls for age and sex. Levels above 1.40 IU/ml are considered abnormal. Neurological outcome was determined by the Pediatric Stroke Outcome Measure (PSOM), which is a Likert-scaled neurological examination measure of five domains: cognitive/behavioural, language expression, language comprehension and right or left sensory/motor deficits. Complete recovery is defined as a PSOM summary score of 0, and a moderate to severe deficit is defined as a PSOM summary score of at least 1 in any domain [29].

Statistical analysis

Characteristics of the study cohort were assessed using descriptive statistics. Relationships between vWF antigen, disease activity, ESR and CRP were assessed using Pearson correlations. Variables found to be positively skewed were described with median and range, and then log transformed before entry into parametric analysis. Trajectories of disease activity, neurological outcome, inflammatory markers and vWF antigen over time were determined using linear mixed-effects models. Relationships between vWF antigen and regressors were also explored using simple linear regression. The validity of all models was assessed using fit diagnostic of residuals. Analyses were performed using R statistical software (http://www.R-project.org) and SAS statistical software for Windows version 9.2 (SAS Institute, Cary, NC, USA).

Results

Patient cohort

The study cohort consisted of 39 children who were diagnosed with cPACNS between June 2001 and October 2010 and had repeated measures of vWF antigen. Twenty-one patients (54%) were female, and the median age at diagnosis was 9.8 years (range 3.3–17.8 years). The median duration of follow-up was 18 months (range 3–78 months). The cohort was composed of 25 patients (64%) with angiography-negative cPACNS (20 biopsy-confirmed, 5 suspected) and 14 (36%) patients with angiography-positive cPACNS. There were more females in the group of children with angiography-negative cPACNS, which is consistent with the larger cohort of patients with this disease at SickKids [30]. The demographic data are summarized in Table 1.

At diagnosis

All 39 patients presented with neurological deficits. At presentation, 67% of children complained of headaches, 59% had seizures, 33% experienced a decreased level of consciousness and 23% had fever. Cognitive dysfunction was noted in 54%, and behaviour and/or mood abnormalities were present in 28%. Motor deficits were common, as 54% of the children presented with hemiparesis and 13% had ataxia. Vision problems, including optic neuritis or papilloedema, occurred in 26% and speech defects were found in 28%.

The results of the initial investigations are presented in Table 2. Leucocytosis was present in 50% of patients, anaemia was detected in 26% and thrombocytosis was found in 34%. Elevated levels of vWF antigen, ESR and CRP were identified at diagnosis in 65, 67 and 23% of children tested, respectively. At diagnosis, high inflammatory markers were correlated with high levels of vWF antigen ($r = 0.43$, $P = 0.053$ for ESR; $r = 0.5$, $P = 0.005$ for CRP). However, using vWF antigen as an adjunct to the traditional inflammatory markers led to the identification of an abnormality in 81% of patients, or five additional patients.

Lumbar puncture was performed in 30 of the study patients. Opening pressure was measured in 14 patients and was elevated in 43%. CSF leucocytosis was identified in 69% of patients (range 0–131 × 10^6/l), and elevated CSF protein levels >0.40 g/l were found in 50% of patients (range 0.10–1.75 g/l). Abnormal findings on brain MRI were identified in 95% of children (Fig. 1A, Table 2). Two patients with normal brain MRI presented with characteristic features of cPACNS, including seizures, cognitive dysfunction, behaviour changes and headaches, and this prompted further work-up to confirm a diagnosis of angiography-negative cPACNS. Abnormal findings involved white matter in 79% of patients and grey

---

**Table 1** Demographic characteristics at diagnosis for children with PACNS

<table>
<thead>
<tr>
<th></th>
<th>All cPACNS</th>
<th>Angiography-negative cPACNS</th>
<th>Angiography-positive cPACNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients, n (%)</td>
<td>39 (100)</td>
<td>25 (64)</td>
<td>14 (36)</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>21 (54)</td>
<td>20 (80)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Median age at diagnosis, years</td>
<td>9.79</td>
<td>9.46</td>
<td>11.2</td>
</tr>
<tr>
<td>Minimum, maximum age, years</td>
<td>3.3, 17.8</td>
<td>5.5, 17.8</td>
<td>3.3, 16.5</td>
</tr>
<tr>
<td>Median duration of follow-up, months</td>
<td>18</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>Minimum, maximum follow-up, months</td>
<td>3, 78</td>
<td>3, 78</td>
<td>3, 71</td>
</tr>
</tbody>
</table>
matter in 38%. Overall, MRI changes were unilateral in 47% of children and unifocal in 30%.

Fourteen patients (36%) had evidence of vasculitis on conventional and/or MR angiograms. By definition, 100% of the 14 patients with angiography-positive cPACNS had vasculitis on angiogram. None of the 25 patients with angiography-negative cPACNS had a positive angiogram. Brain biopsies were performed in 20 patients in the study, and all biopsies showed histological evidence of vasculitis involving the small cerebral vessels (Fig. 1B). Five patients with suspected angiography-negative cPACNS did not have a brain biopsy, mostly due to parental refusal; however, all testing for secondary causes of CNS vasculitis and anti-neuronal antibodies was negative. Statistical analyses were repeated with these five patients removed, and the results did not change.

All children had active disease based on PGA and an abnormal PSOM summary score at diagnosis. The median PGA of disease activity at diagnosis was 6.3 (range 1.3–9.5) for all patients. The median PSOM summary score at diagnosis was 2.0 (range 1.0–10) for all children in the cohort. Children with elevated vWF antigen levels did not differ in disease subtype, disease severity, clinical presentation, neuroimaging findings, inflammatory markers or other laboratory measures from those who had normal values.

During follow-up
All children with cPACNS were treated based on institutional protocols. High-dose prednisone was started in 38 children and was tapered over 12 months in 32 patients (25 angiography negative, 7 angiography positive).
and over 2–6 months in 6 children with angiography-positive disease. Twenty children with angiography-negative cPACNS and seven children with progressive angiography-positive cPACNS were treated with 6 months of i.v. CYC before starting maintenance therapy with AZA or MMF. Three children with angiography-negative cPACNS were started directly on maintenance medications. All 14 patients with angiography-positive cPACNS were given acetylsalicylic acid long term, while nine received unfractionated and/or low-molecular-weight heparin for up to 2 months.

Neurological symptoms resolved such that 69% children were asymptomatic by 24 months of follow-up. Leucocytosis, anaemia and abnormal platelet counts improved quickly and were abnormal in only 10% of children at 1 year of follow-up. MRI brain lesions either improved or remained stable in 76% of children after treatment. New MRI lesions were identified in 22% of children—they developed during disease flares in five patients and without flare in three patients.

Levels of vWF antigen decreased significantly over time in all patients (intercept β = −0.17, s.e. = 0.03, \( P < 0.001 \)). Median levels of vWF antigen, ESR and CRP were highest at diagnosis (Table 3). However, no significant correlation was found for ESR and CRP with vWF antigen levels during follow-up. Levels of ESR appeared to fluctuate more after treatment than vWF antigen (Fig. 2B).

Disease activity was elevated at diagnosis and decreased significantly over time in all patients (intercept \( \beta = −1.9, \) s.e. = 0.12, \( P < 0.001 \)). This is depicted visually in Fig. 2A. The pattern of progressive decline in vWF antigen levels followed the continual decrease in disease activity more closely than ESR and CRP (visual inspection, Fig. 2B). An inverse trend was identified between elevation in inflammatory markers at diagnosis and disease activity after treatment. In a post-hoc linear regression analysis including all patients with complete data at diagnosis and at 1 year of follow-up, we found that lower disease activity at 12 months was associated with an elevated baseline vWF antigen (\( \beta = −2.8, \) s.e. = 1.2, \( P = 0.038 \)) when adjusted for baseline levels of PGA (\( \beta = −0.3, \) s.e. = 0.3, \( P = 0.287 \)), ESR (\( \beta = −0.3, \) s.e. = 0.4, \( P = 0.371 \)) and CRP (\( \beta = 0.2, \) s.e. = 0.3, \( P = 0.592 \)).

Six children developed flares during follow-up. Two disease flares did not produce any changes in laboratory testing, three disease flares were associated with increases in at least one inflammatory marker and vWF antigen, and the final disease flare was accompanied only by an abnormal elevation of vWF antigen.

Neurological outcome improved during follow-up in all children with cPACNS. There was a significant decrease in PSOM summary scores over time (\( P < 0.001 \)). The median PSOM summary score at 12 months was 0.8 and at 24 months was 0.5 (Table 3). PSOM scores were ≤0.5, which indicates a good neurological outcome with no impact on daily functioning, in 52% of children at 12 months and 65% at 24 months. Persisting neurological deficits were related to the development of dystonia secondary to stroke in children with angiography-positive disease and to ongoing mild cognitive dysfunction in those with angiography-negative cPACNS.

**Discussion**

Monitoring disease activity in children with cPACNS is extremely challenging. It is often difficult to determine whether non-specific symptoms, such as fatigue and headaches, are occurring due to disease flare or whether they are caused by more common conditions in childhood. Similarly, a child may continue to have seizures because they have persistent CNS inflammation or because of previous injury due to now inactive cPACNS. Lumbar puncture and brain biopsy are likely to yield abnormal results in the setting of active cPACNS, but are too invasive to repeat longitudinally to monitor disease activity. New MRI findings were identified in five children during disease flares, but also developed in three children without other signs of active cPACNS. Therefore, there are limitations in using neuroimaging to follow disease activity.

Identifying laboratory markers of increased disease activity for cPACNS would be ideal because laboratory measures are non-invasive and easier to measure serially. In our study, disease activity as measured by PGA and neurological outcome as evaluated by PSOM summary scores improved significantly during follow-up. Leucocytosis, anaemia and abnormal platelet counts were not prominent at diagnosis, resolved rapidly with

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diagnosis</th>
<th>6 months</th>
<th>12 months</th>
<th>18 months</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients at follow-up</td>
<td>39</td>
<td>27</td>
<td>26</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Median level of ESR, mm/h (range)</td>
<td>22.5 (1–120)</td>
<td>4 (1–72)</td>
<td>1 (1–27)</td>
<td>4 (1–22)</td>
<td>2.5 (1–21)</td>
</tr>
<tr>
<td>Median level of CRP, mg/l (range)</td>
<td>1.9 (0–100)</td>
<td>0.9 (0–32)</td>
<td>0.9 (0–28)</td>
<td>0.4 (0–7.1)</td>
<td>0 (0–3)</td>
</tr>
<tr>
<td>Median level of vWF antigen, IU/ml (range)</td>
<td>1.59 (0.83–3.86)</td>
<td>1.35 (0.75–3.64)</td>
<td>1.15 (0.58–1.6)</td>
<td>1.07 (0.42–1.51)</td>
<td>1.09 (0.58–1.4)</td>
</tr>
<tr>
<td>Median disease activity by PGA (range)</td>
<td>6.3 (1.3–9.5)</td>
<td>2.0 (0.0–6.0)</td>
<td>1.1 (0.0–6.4)</td>
<td>1.1 (0.0–7.6)</td>
<td>0.3 (0.0–8.0)</td>
</tr>
<tr>
<td>Median Pediatric Stroke PGA Outcome Measure summary score (range)</td>
<td>2.0 (1.0–10.0)</td>
<td>1.0 (0.0–10.0)</td>
<td>0.8 (0.0–7.0)</td>
<td>1.0 (0.0–3.0)</td>
<td>0.5 (0.0–4.0)</td>
</tr>
</tbody>
</table>
treatment and were not associated with disease activity. The highest levels of vWF, ESR and CRP were identified at diagnosis, which is in keeping with previous studies of systemic vasculitides [18–21]. vWF antigen levels decreased significantly after treatment and followed the pattern of decrease in disease activity more closely than ESR and CRP.

The rationale for exploring the potential use of vWF antigen as a marker for disease activity in cPACNS is that it is released by endothelial cells after vascular injury. Therefore, elevated levels of vWF antigen may reflect vascular inflammation and damage specifically. In contrast, ESR and CRP are acute-phase reactants that may increase in response to multiple possible stimuli in children, such as common viral illnesses. This is the likely explanation for the observed increases in ESR during follow-up that were not associated with increased disease activity in our study. At diagnosis of vasculitis, acute-phase reactants may be elevated due to the systemic inflammatory response, whereas increases in vWF antigen levels likely result from the underlying vascular process.

Interestingly, post-hoc analysis showed children in our study with higher levels of vWF antigen at diagnosis of cPACNS were more likely to have lower measures of
disease activity after 12 months of follow-up. One potential explanation for this finding is that patients with significantly increased inflammatory markers at diagnosis may have a more intense underlying inflammation that is highly responsive to anti-inflammatory treatment. This may help clinicians to identify a subgroup of patients who will respond to therapy more rapidly. Higher inflammatory markers have also been found to predict better treatment response in recent studies of GCA and idiopathic rapidly progressive glomerulonephritis [31–33]. This finding may be unique to more localized vasculitic processes because earlier research on systemic vasculitides, such as Kawasaki disease, have demonstrated an association between higher inflammatory markers and poorer outcome or treatment response [34–36].

vWF antigen may provide additional useful information for the clinician treating a child with cPACNS. In our study, elevated ESR and vWF antigen levels were identified at diagnosis in >60% of patients and increased CRP was found in 23%. With the addition of vWF antigen to the traditional inflammatory markers, an abnormality was documented in >81% of children at presentation. Also, the pattern of vWF antigen followed the decrease in disease activity during follow-up more closely than the classic inflammatory markers. vWF antigen levels declined progressively and slowly, whereas increases in CRP rapidly resolved after treatment and ESR fluctuated randomly during follow-up. The addition of vWF antigen also helped to identify one extra case of disease flare compared with ESR and CRP alone.

There were several limitations to our study. The study cohort was relatively small in size, which is not unexpected given that cPACNS is a rare disease. However, the small data set makes the search for a biomarker of disease activity particularly challenging. Laboratory markers and clinical measures were collected serially in a non-biased fashion in an attempt to address this challenge. In particular, the PGA of disease activity was assigned by the treating physician at the end of the clinic visit, and the laboratory markers, particularly vWF antigen, would not have been available at that time. The specificity of vWF antigen as a biomarker could not be assessed in the study due to the absence of a control group.

In summary, children with cPACNS presented with devastating neurological deficits and responded to treatment with significant improvements in disease activity and neurological function. Changes in the traditional inflammatory markers (CRP and ESR), other laboratory tests and neuroimaging findings did not consistently reflect altered disease activity. However, the addition of vWF antigen may help clinicians to better identify changes in disease activity during follow-up. Higher vWF antigen levels and inflammatory markers at diagnosis were associated with lower disease activity at 12 months. Prospective controlled studies are necessary to evaluate the sensitivity and specificity of this non-invasive test as a potential biomarker of disease activity in cPACNS. National and international collaboration in future studies would be helpful, given the rarity of this condition.

Rheumatology key messages

- VWF levels may reflect disease activity in children with primary CNS vasculitis.
- Decreasing vWF antigen levels mirror diminishing disease activity in childhood primary CNS vasculitis.
- CRP, ESR and neuroimaging changes do not consistently reflect disease activity in primary CNS vasculitis.

Acknowledgements

T.C. was supported through a studentship, in part, by the Ontario Student Opportunity Trust Fund—Hospital for Sick Children Foundation Student Scholarship Program.

Disclosure statement: The authors have declared no conflicts of interest.

References