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## No neurochemical evidence of neuronal injury or glial activation in children with Paediatric Acute-onset Neuropsychiatric Syndrome. An explorative pilot study

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### ABSTRACT

**Objective:** Paediatric Acute-onset Neuropsychiatric Syndrome (PANS) is characterised by an acute onset of obsessive compulsive disorder, combined with at least two other neuropsychiatric symptoms with acute onset. Diagnostic criteria also require that no specific medical aetiology is identified. Although there are no verified aetiological biomarkers, PANS is assumed to be a neuroinflammatory disorder with a possible autoimmune aetiology. Neurochemical markers such as neurofilament light (NfL, a neuronal injury marker) and glial fibrillary acidic protein (GFAP, an astrocytic activation marker) have not been published for this patient group.

**Method:** Blood samples from 17 children meeting diagnostic criteria for PANS, after assessment at a child neuropsychiatry clinic were analysed for serum concentrations of NfL and GFAP. Ten age-matched children without any neurological or psychiatric disorder served as a comparison group.

**Results:** No difference was found in mean NfL and mean GFAP serum concentrations between children with PANS and controls.

**Conclusion:** Neuronal injury and astrocyte activation do not seem to be a major event in PANS. The study group was small, and even if findings may be reassuring for parents and patients, they should be interpreted with caution and verified in larger cohorts and possibly with other markers in both serum and CSF.

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### KEYWORDS



Paediatric Acute-onset Neuropsychiatric Syndrome; PANS; glial fibrillary acidic protein; GFAP; neurofilament light; NfL

### Introduction

The acronym PANS (Paediatric Acute-onset Neuropsychiatric Syndrome) is clinically defined by an abrupt, dramatic onset of obsessive-compulsive disorder or severely restricted food intake combined with at least two out of seven other similarly severe symptoms with acute onset; anxiety, emotional lability and/or depression, irritability/aggression and/or severely oppositional behaviours, behavioural (developmental) regression, deterioration in school performance (related to attention-deficit/hyperactivity disorder/ADHD-like symptoms, memory deficits, cognitive changes), sensory or motor abnormalities and somatic

signs and symptoms, including sleep disturbances, enuresis, or urinary frequency. In addition, the research diagnostic criteria require that symptoms are not better explained by a known neurological or other medical disorder (Swedo et al. 2012).

The term PANS was a modification of a preceding diagnostic term, PANDAS (Paediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal infections), a similar clinical condition but implicating a temporal association with group A streptococcal infection and an ensuing autoimmune reaction (Swedo et al. 1998). By the term PANS aetiological associations were eliminated and hitherto no defined medical causes or reliable biomarkers have

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been identified, although infections and autoimmune reactions have been suggested (Frankovich et al. 2015). Infections, mostly upper respiratory, are commonly reported symptoms preceding the onset of PANS (Chang et al. 2015). In addition, autoimmune reactions, known to be associated with some neuropsychiatric disorders, have therefore also been assumed to have a connection with PANS (Chang et al. 2015). Gilbert et al. (2018) emphasised the need to define better the clinical manifestations, laboratory and neuroimaging findings, therapeutic responses, and clinical course in these disorders and that, to date, there is no significant evidence supporting any specific aetiologies for PANDAS and PANS.

Neurofilament light chain (NfL) protein, is a component of the neuron cytoskeleton and is released into the cerebrospinal fluid (CSF) and blood when neurons are injured, providing a neurodegenerative biomarker of neuronal damage (Khalil et al. 2018). An important aspect is that the NfL proteins indicate neuronal injury independent of causal pathways (Khalil et al. 2018). NfL levels in cerebrospinal fluid and in serum have been found to correlate significantly in paediatric patients with acquired demyelinating syndromes (Wong et al. 2019).

Glial fibrillary acidic protein (GFAP) is an astrocytic cytoskeletal protein, the expression and secretion of which is upregulated when astrocytes are activated (Yang and Wang 2015). In children with symptoms consistent with PANS, specific causes, such as N-methyl-D-aspartate receptor (NMDAR) encephalitis, the most common form of autoimmune encephalitis, needs to be considered (Wang et al. 2020). Several studies have demonstrated that not only neurons, but also astrocytes express functional NMDA receptors and that the pathogenic antibodies against the NMDAR, causing the anti-NMDAR encephalitis, affect not only the neuronal receptors, but also the NMDAR on astrocytes (Ismail and Faustmann 2020).

In the 1990s, GFAP was analysed in cerebrospinal fluid (CSF) of children and adolescents with autism, of whom 70% also had intellectual disability (ID) and 21% had epilepsy, and results were contrasted with findings in similarly aged cases without autism or other neuropsychiatric disorders. GFAP levels in children with autism were found to be at a level almost three times higher than in the group of children without autism. In one aetiological subgroup of children with autism, consisting of three children with a basal ganglia disorder, the highest GFAP levels were found (Ahlsén et al. 1993). Also a more recent study, using serum levels of GFAP, demonstrated elevated levels in

children with autism compared to controls (Wang et al. 2017).

To our knowledge it has not been systematically analysed whether children suffering from PANS would have detectable signs of neuronal/axonal or astrocyte cell damage. Since NfL and GFAP can now be analysed in serum, sampling in children is facilitated. Moreover, for both these biomarkers, serum concentrations have been shown to reflect the levels in the CSF (Kuhle et al. 2016; Novakova et al. 2017; Högel et al. 2020). Since this is the first time these data are analysed in a group of children with and without a symptom combination referred to as PANS, there are no other reference data among children.

As mentioned above, it has been assumed that neuroinflammation or autoimmune mechanisms may be involved in PANS, but reliable biomarkers have been lacking. The aim of this study is to examine if signs of neuronal injury or glial cell activation could be found by analysing the neurochemical markers NfL and GFAP in serum in serum of children meeting criteria for PANS.

## Methods

### Study participants

Our initial cohort of children with PANS consisted of 23 children, referred between 2015 and 2017 and assessed at the Child Neuropsychiatry Centre (CNC)/Gillberg Neuropsychiatry Centre in Gothenburg. During the study period, CNC was the referral centre for children with suspected PANS in Gothenburg and the southwestern region of Sweden and these 23 children were deemed to meet clinical criteria for PANS. Data from the clinical assessments of these children have been reported previously (Johnson et al. 2019).

For the present study 11 of the 23 children (7 boys), mean age 13 years, gave consent to have blood test taken for analysis of NfL and GFAP. In addition, 6 children (3 boys), mean age 9.6 years, later included in the cohort, were able to participate in blood sampling. Thus, 17 children could be analysed for serum NfL and GFAP.

The time from onset of PANS symptoms to the time point of blood sampling varied between 1 month and 5 years. At the time of assessment, however, all children had moderate to severe impairments that markedly influenced their school attendance and parental and family situation.

Four of the 17 patients had autism, no child in the study group had diagnosed intellectual disability. In addition to OCD and at least two other

**Table 1.** Serum concentrations of neurofilament light and glial fibrillary acidic protein.

Study groups	PANS group <i>n</i> = 17 (7 girls, 41,2%) <sup>a</sup>	Control group <i>n</i> = 10 (5 girls, 50%)	
Mean age	11.3 years	9.4 years	
Age range	7–16 years <sup>b</sup>	5–15 years <sup>c</sup>	
	Mean (SD)	Mean (SD)	<i>p</i> -Value
Neurofilament light	4.7 pg/ml (2.2)	4.6 pg/ml (1.7)	<i>p</i> = .910
Glial fibrillary Acid Protein	103 pg/ml (73.12)	112 pg/ml (64.37)	<i>p</i> = .730

PANS: Paediatric acute-onset neuropsychiatric syndrome.

<sup>a</sup>( $\chi^2 = 0.20$ ,  $p = .656$ ), <sup>b</sup>M (SD):11.8 (3.0), <sup>c</sup>M (SD): 9.6 (3.4). *T*-test for age range difference between groups:  $t = -1.78(25)$ ,  $p = .087$ .

neuropsychiatric symptoms, four children had psychotic symptoms (delusions, hallucinations).

The comparison group included 10 children (5 boys, 5 girls), mean age 9.4 years, who did not have any neurological or psychiatric disorders. Their blood samples were taken in connection with investigation for non-neurological/non-psychiatric disorders, mainly allergic diseases, confirmed in their paediatric records, at a paediatric outpatient clinic in Gothenburg.

### Serum analyses

NfL and GFAP were measured using ultrasensitive Single molecule array (Simoa) technology, using commercially available kits, according to instructions from the manufacturer (Quanterix, Billerica, MA). The analyses were performed in one round of experiments, using one batch of reagents by board-certified laboratory technicians who were blinded to clinical data. For a quality control (QC) sample with an NfL concentration of 15.0 pg/mL, the repeatability was 7.2%. For a QC sample with an NfL concentration of 47.3 pg/mL, the repeatability was 1.7%. For a QC sample with a GFAP concentration of 131 pg/mL, the repeatability was 6.1%. For a QC sample with a GFAP concentration of 365 pg/mL, the repeatability was 4.3%.

The study was approved by the local ethical review board. All parents and participants provided informed consent after receiving oral and written information of the study and gave consent both to research and permission to publish.

### Statistical analysis

We conducted two-tailed *t*-tests in order to test mean differences between the PANS group and the comparison group. Since sample sizes were small, we cross-checked the *t*-test results with the non-parametric Mann-Whitney *U* test, which yielded very similar *p*-values. We calculated Spearman correlations between the time point of PANS onset and the time point of blood sampling.

### Results

Mean serum NfL was 4.7 pg/ml (SD 2.2) and 4.6 pg/ml (SD 1.7) in children with PANS and in the comparison group, respectively, *t*-test:  $M_{Diff} = 0.09$ , 95% CI [−1.59,1.77],  $t(25)=0.11$ ,  $p = .910$ .

Mean serum GFAP was 103 pg/ml (SD 73.12) in the PANS group and 112 pg/ml (SD 64.37) in the comparison group, respectively, *t*-test:  $M_{Diff} = -9.75$ , 95% CI [−67.28, 47.78],  $t(25) = -0.35$ ,  $p = .730$ . Mann-Whitney *U* test gave similar results,  $p = .941$  (NfL) and  $p = .570$  (GFAP).

Time between onset of PANS symptoms and the time point of blood sampling did not relate to levels of either NfL  $r = -.02$   $p = .929$  or GFAP  $r = -.08$   $p = .769$ .

Mean serum GFAP of the four children with autism in the PANS group was 127.1 pg/ml (SD 121.3) and 95.8 pg/ml (SD 56.6) in the children with PANS without autism. No further calculations were performed due to very small numbers (Table 1).

### Discussion

This study was based on 17 children referred to a child and adolescent neuropsychiatric clinic, at the time of the study the main referral centre for children with suspected PANS in Gothenburg and the southwestern part of Sweden. All included children met the research criteria for PANS<sup>1</sup> and had had an abrupt onset of OCD and at least two associated, severe neuropsychiatric symptoms. Co-occurring psychotic symptoms (delusions, hallucinations) were reported in 23.5% of the children in the PANS group, which is in line with other reports (Frankovich et al. 2015; Murphy et al. 2015). A basal ganglia involvement is presumed in PANDAS and there are studies implicating immune-mediated basal ganglia and striatal involvement also in PANS aetiology and in the pathogenesis of psychotic disorders (Silverman et al. 2019). However, as discussed by Gilbert et al. (2018) studies of PANDAS and PANS are inconclusive regarding pathophysiology, diagnostic markers and treatment. The need for cross-institutional collaboration and well-conducted

investigations of underlying biological mechanisms in children with PANDAS/PANS has also been emphasised, given the severe nature of the symptoms and considerable effects on family and child functioning (Wilbur et al. 2019).

In the present study, we could not demonstrate increased levels of NfL and GFAP in serum of children with PANS, compared to a control group of children without symptoms of any neurological or psychiatric disorder. Levels of NfL and GFAP did not correlate with the elapsed time between onset of PANS and blood tests. In summary, our study did not reveal any signs of neuronal/axonal or astrocyte damage in children with PANS.

The four children with autism in our PANS group had GFAP concentrations that did not differ significantly from GFAP levels in the children with PANS without autism. However, the small study groups with only four children diagnosed with autism do not allow any conclusion to be drawn. These results differ from previous studies, in which elevated levels of GFAP were found in CSF (Ahlsén et al. 1993) and serum (Wang et al. 2017) in children with autism compared to controls. However, none of the children with autism and PANS in our study had intellectual disability, while this was present in 70% of the children with autism reported in the 1990s (Ahlsén et al. 1993).

Clear limitations of this study are the small study groups and the large differences in time periods between onset of PANS symptoms and blood sampling. However, all children had moderate to severe symptoms and impairment at blood sampling. Regarding the symptom trajectory over time it is also difficult to distinguish if symptom exacerbations are related to intercurrent infections, immunologic factors, or a spontaneous symptom course of PANS.

Strengths of the study are that all patients were clinically well defined and assessed by the same paediatricians, and that it was possible to use analyzes of serum, since previous research has demonstrated correlation between these two biomarkers in cerebrospinal fluid and serum.

Thus, although our study groups of patients with PANS and comparison children were small, findings may be reassuring for parents and patients.

## Conclusions

Neurofilament light (NfL, a neuronal injury marker) and glial fibrillary acidic protein (GFAP, an astrocytic activation marker) were analysed in serum of 17 children meeting criteria for PANS and concentrations

were compared with 10 age-matched controls. Mean NfL and mean GFAP concentrations did not differ between children with PANS and controls and thus no neurochemical evidence of neuronal injury or glial activation was found in children with PANS. These findings should be interpreted with caution and verified in larger cohorts.

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None.

## Statement of interest

HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg, all unrelated to the work presented in this paper.

KB has served as a consultant or at advisory boards for Abcam, Axon, Biogen, Lilly, MagQu, Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg, all unrelated to the work presented in this paper.

MJ has received research grants from Shire and been engaged as a speaker or consultant by Eli Lilly, Shire, Ginsana, PCM Scientific, Evolan, and New Nordic, all unrelated to the work presented in this paper.

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