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Cerebral folate deficiency: A neurometabolic syndrome?☆,☆☆

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ABSTRACT

Background: Cerebral folate deficiency (CFD) is increasingly recognized in various neurological conditions, raising the question of whether it might represent a clear-cut clinical syndrome.
Methods: Retrospective analysis of patients with low cerebral spinal fluid (CSF) 5-methyltetrahydrofolate (5MTHF) values was performed.
Results: 58 pediatric patients with low (-2nd to -3rd standard deviation) and 45 patients with very low 5MTHF values (<3rd standard deviation) were identified, including 22 patients with defined underlying neurological conditions. The leading symptoms were mental retardation ($n=84$), motor retardation ($n=75$), epilepsy ($n=53$), ataxia ($n=44$) and pyramidal tract signs ($n=37$). There was no relationship between 5MTHF levels and the severity of clinical disease, the duration of clinical disease, distinct neurological symptoms and antiepileptic drug treatment, respectively. Genetical analysis for mutations in the folate receptor 1 gene proved normal in all 16 children studied.
Conclusions: For the majority of patients CFD is not a clear-cut neurometabolic syndrome but the common result of different genetic, metabolic or unknown processes. Nevertheless, CFD may represent a treatable disease-modifying factor which should therefore be addressed in prospective studies.

1. Introduction

During recent years cerebral folate deficiency (CFD), characterized by low cerebrospinal fluid (CSF) folates, has emerged to an important differential diagnosis in various neurological disorders. CFD may be associated with different underlying conditions such as 5,10-methylene-tetrahydrofolate reductase, 3-phosphoglycerate dehydrogenase, dihydrofolate reductase (DHFR) or dihydropteridine reductase deficiency, hereditary folate malabsorption, Rett syndrome, Aicardi–Goutières syndrome or mitochondrial disorders and has also been linked to autistic features [1–7]. One explanation for the high clinical impact of folate deficiency might be the fact that folate is a cofactor in processes contributing to preserve the genome, to regulate gene expression, to amino acid metabolism, myelin formation and neurotransmitter synthesis. Therefore, screening for low CSF 5-methyltetrahydrofolate (5MTHF) levels has been recommended for various neurological disorders of unknown origin [8].

Based on case series a distinct clinical phenotype of CFD has been proposed, characterized by normal development until 4–6 months of age and evolving agitation, insomnia, psychomotor retardation, deceleration of head growth, ataxia, hypotonia, spasticity, dyskinesia and epilepsy [9]. The underlying etiology causing CFD is not understood. In some cases CFD was shown being related to blocking auto-antibodies to folate receptors [10]. Recently, three patients with impaired uptake of folate to the CNS based on a mutation in the folate receptor 1 (*FOLR1*) gene coding for folate receptor alpha, leading to progressive movement disturbance, psychomotor decline, white matter disease and epilepsy after an initial period of normal neuromotor development have been reported [11,12]. This contrasts to patients with DHFR deficiency who present with neurologic disease combined with systemic MTHF deficiency including megaloblastic anemia [13,14] and patients with mutations in the proton-coupled

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folate transporter (PCFT) in whom systemic folate deficiency is also present [15].

Whereas delineation of a clinical phenotype facilitates diagnosis, focusing on few clinical characteristics might exclude patients with uncommon symptoms from effective treatment.

Since 1999 CFD diagnostics has been routinely performed in a large series of patients treated at RWTH Aachen University Hospital, showing otherwise unexplained severe neurological diseases. Some of these patients have been published previously in order to illustrate specific aspects of CFD [1,2,10]. Data on a larger series of randomly selected patients, in contrast, have not yet been reported.

2. Patients and methods

Clinical charts of patients studied for CFD between 1999 and 2007 at our hospital by analyzing CSF 5MTHF levels have been analyzed retrospectively. CSF 5MTHF values had been determined by means of high-pressure liquid chromatography using electrochemical detection as described previously [16]. The main diagnoses, the biochemical findings and major clinical symptoms were recorded. Among others, this included symptoms considered typical for CFD, such as psychomotor retardation, microcephaly, ataxia, spasticity, dyskinesia, dystonia and epilepsy. The symptoms were compared with CSF 5MTHF concentrations, including only CSF samples obtained prior to any folinic acid supplementation. Patients were divided into two groups showing CSF 5MTHF values between age-related -2nd to -3rd standard deviations (low 5MTHF; moderate CFD) and values age-related<-3rd standard deviations (very low 5MTHF; severe CFD) (Table 1). Data were compared using the Wilcoxon rank-sum test for independent samples.

From randomly selected patients genetical analysis of the FOLR1 gene has been performed as reported previously [12]. The analysis was performed in line with the German law on gene diagnostics.

3. Results

103 patients with CFD were identified, 64 boys (mean 6 ± 5 years, range 0–20 years) and 39 girls (mean 5 ± 4 years, range 1–17 years). 58 showed low CSF 5MTHF levels (-3rd std. dev. to -2nd std. dev.) and 45 very low levels (below the -3rd std. dev.). 15 of the 45 children with very low levels showed CSF 5MTHF values lower than 10 nmol/l. In all patients laboratory and clinical signs of systemic folate deficiency were absent, ruling out severe enteral folate malabsorption, for example caused by PCFT mutations [15].

Whereas 22 patients showed distinct underlying clinical diagnoses (Table 2), no clear diagnosis was made in most cases so that the data will be further analyzed with regard to the distinct clinical findings (Table 3).

Hypothesizing that symptoms strongly linked to CFD should be associated with severe CFD, the ratio "number of patients with very low/low CSF 5MTHF" was calculated for distinct clinical features. However, none of the symptoms displayed in Table 3 was associated with a preponderance of severe CFD.

According to the literature, psychomotor retardation, microcephaly, ataxia, spasticity, dyskinesia, dystonia and epilepsy may be typical symptoms of CFD. Hypothesizing, that patients with severe CFD might show more symptoms than patients with moderate CFD we compared the number of symptoms present in the distinct patients with the severity of CFD. However, no relationship was found (Fig. 1).

A long standing CFD might also be related with more severe clinical disease. Although we cannot rule out that CFD may develop rapidly, we assumed that CFD should have been present for a longer period of time among older children. Therefore, we studied whether children above 5 years of age showing or not showing a distinct clinical feature differed with regard to their CSF 5MTHF values. However, no significant differences were found with regard to any of the clinical features studied (Table 3). In contrast, older children with white matter changes on MRI even tended to show higher CSF 5MTHF levels than children with normal white matter findings ($p = 0.0742$) (Fig. 2).

Different drugs, especially antiepileptics, have been discussed to impair cerebral 5MTHF uptake. 35 of the 103 patients had received antiepileptics, including valproic acid in 32 cases. However, patients with or without antiepileptic treatment did not differ with regard to their CSF 5MTHF findings.

A subgroup of 16 children has also been studied for a mutation in the *FOLR1* gene. Six of them showed low CSF 5MTHF, ten showed very low CSF 5MTHF levels. No mutations were detected in any of these theses children.

Table 1
Age-dependent classification of CSF 5MTHF levels.

Age [years]	Normal range [nmol/l]	Low CSF 5MTHF[a] [nmol/l]	Very low CSF 5MTHF[b] [nmol/l]
0–1.99	64–182	34.5–63	<34.5
2–4.99	63–111	27–62	<27
≥5	41–117	22–40	<22

[a] -3rd std. dev.<5MTHF<-2nd std. dev.
[b] 5MTHF<-3rd std. dev.

Table 2
Clinical and CSF 5MTHF findings of 22 patients with clear-cut neurological diagnoses.

Main diagnosis	Low CSF 5MTHF[a]	Very low CSF 5MTHF[b]	Total
Rett syndrome	4	2	6
Autism	3	1	4
Mitochondrial disorders	1	1	2
Joubert syndrome	1	0	1
Friedreich ataxia	0	1	1
Aicardi–Goutières syndrome	0	1	1
GTP cyclohydrolase I deficiency	1	0	1
Oculomotoric apraxia	1	0	1
3-Phosphoglycerate dehydrogenase deficiency	1	0	1
Pontocerebellar hypoplasia	0	1	1
Arthrogryposis	0	1	1
Schizophrenia	0	1	1
Myoadenylate deaminase deficiency	0	1	1

[a] -3rd std. dev.<5MTHF<-2nd std. dev.
[b] 5MTHF<-3rd std. dev.

Table 3
Prevalence of distinct clinical findings among patients with low CSF folates.

	N total	Low CSF 5MTHF[a]	Very low CSF 5MTHF[b]	Ratio very low/low CSF 5MTHF
Patient history				
Preterm infancy	10	5	5	1
Neonatal cerebral hemorrhage	4	2	2	1
Connatal infection	3	2	1	0.5
Neuroimaging				
CNS malformation	32	19	13	0.7
White matter changes	16	9	7	0.8
Periventricular leukomalacia	5	4	1	0.25
Clinical findings				
Mental retardation	84	50	34	0.7
Speech disorder	82	48	34	0.7
Motor retardation	75	50	25	0.5

[a] -3rd std. dev.<5MTHF<-2nd std. dev.
[b] 5MTHF<-3rd std.