Cerebral folate deficiency

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Cerebral folate deficiency (CFD) can be defined as any neurological syndrome associated with low cerebrospinal fluid (CSF) 5-methyltetrahydrofolate (5MTHF), the active folate metabolite, in the presence of normal folate metabolism outside the nervous system. CFD could result from either disturbed folate transport or from increased folate turnover within the central nervous system (CNS).

We report on a novel neurometabolic syndrome in 20 children, which we term ‘idiopathic CFD’. Typical features became manifest from the age of 4 months, starting with marked unrest, irritability, and sleep disturbances followed by psychomotor retardation, cerebellar ataxia, spastic paraplegia, and dyskinesia; epilepsy developed in about one third of the children. Most children showed deceleration of head growth from the age of 4 to 6 months. Visual disturbances began to develop around the age of 3 years and progressive sensorineural hearing loss started from the age of 6 years. Neuroimaging showed atrophy of frontotemporal regions and periventricular demyelination in seven children, slowly progressive supra- and infratentorial atrophy in three children, and normal findings in the remainder. Because active folate transport to the CNS occurs through receptor-mediated folate receptor protein 1 (FR1) endocytosis, DNA sequencing of the FR1 gene was performed and found to be normal. However, CSF protein analysis revealed a non-functional FR1 protein, suspected to result from either post-translational defects of FR1 protein N-glycosylation, the presence of folate antagonists with irreversible binding, or autoantibodies blocking the folate binding site of FR1. Oral treatment with 5-formyltetrahydrofolate (folinic acid) should be started in low doses at 0.5–1mg/kg/day, but in some patients higher daily doses of folinic acid at 2–3 mg/kg/day are required to normalize CSF 5MTHF values. This proposed treatment protocol resulted in a favourable clinical response in patients identified before the age of six years while partial recovery with poorer outcome was found beyond the age of 6 years. Careful clinical and EEG monitoring should be performed 1, 3, and 6 months after the beginning of treatment. After four to six months of folinic acid treatment, CSF analysis should be repeated in order to prevent over- or under-dosage of folinic acid.

Secondary forms of CFD have been recognized during chronic use of antifolate and anticonvulsant drugs and in various known conditions such as Rett syndrome, Aicardi-Goutières syndrome, 3-phosphoglycerate dehydrogenase deficiency, dihydropteridine reductase deficiency, aromatic amino acid decarboxylase deficiency, and Kearns-Sayre syndrome. The pathogenic link between these underlying specific disease entities and the observed secondary CFD has not been resolved.

Introduction

Since the 1940s, folate deficient states and inborn errors of folate metabolism and transport mechanisms have increasingly been recognized to play a role in macrocytic anaemia, atherosclerosis and thrombosis, neuropsychiatric disorders, and neural tube defects. Depletion of folate causes pernicious anaemia and demyelinating lesions of white matter around blood vessels in the brain and spinal cord. The histopathology of demyelinating lesions in the spinal cord shows vacuolization of myelin sheaths of the posterior and lateral columns, and often the anterior columns, which has been called ‘subacute combined degeneration’.

Several folate transport mechanisms from the extracellular compartment to the cell interior have been identified in humans. The most important of these are the reduced folate carrier 1 (RFC1) and the family of folate receptor proteins (FR) which possess different binding properties and are distributed at various sites (see Fig. 1a,b). Both transport systems also subserve the folate transport across the intestinal, placental, and blood-brain barriers. The CNS depends on normal folate homeostasis and intact transport mechanisms across the choroid plexus. For passage across the blood–CNS barrier, the predominant folate form in plasma, i.e. 5MTHF, is bound by the FR1, anchored to choroid epithelial cells and followed by endocytosis, storage, and subsequent delivery to the spinal fluid compartment where it will be transported into neuronal tissues.

A specific disorder of folate transport across the choroid

See end of paper for list of abbreviations.
plexus was described for the first time in an adult male with a slowly progressive neurological disease characterized by a cerebellar syndrome, distal spinal muscular atrophy, pyramidal tract dysfunction, and perceptive hearing loss. Biochemical investigations indicated low CSF folate despite normal folate levels in serum and red blood cells, suggesting a specific folate transport disorder across the choroid plexus. Further CSF analysis revealed an unusually low immunoreactive soluble

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**Figures 1:** (a) shows that after intestinal absorption across the intestinal barrier with the use of a reduced folate carrier (RFC), folates enter the circulation and can be transported into mesenchymal tissues, for example red blood cells (RBC) by the folate receptor protein 2 (FR2). After entry into the cell, folate monoglutamate (F) can be retained within cells after polyglutamylation by folylpoly-gamma-glutamate synthetase (FPGS), the major part of which will be tightly bound to proteins (F– stands for folylpolyglutamate). After passage across choroid epithelial cells using FR1 mediated endocytosis, folates will enter cerebrospinal fluid and enter neuronal cells by RFC, located at axons and dendrites. Within neurons, part of the folate pool will be catabolized by oxidation to dihydrofolates (DHF) and folic acid, which can be reconverted to reduced tetrahydrofolate by dihydrofolate reductase (DHFR). In vitro experiments indicate that part of the folate pool can be oxidized to form 6-formyltetrahydropterin and para-aminobenzoyl-glutamate. (b) shows three different folate transport mechanisms to and from the cell interior, i.e. FR1-mediated endocytosis, reduced folate carrier (RFC1), and adenosine triphosphate-dependent folate exporter. Folate monoglutamates can also enter mitochondria and will be converted to the folylpolyglutamate form. F– can be reconverted to its monoglutamate components by lysosomal gamma-glutamyl-hydrolase (GGH). ATP, adenosine triphosphate; ADP, adenosine diphosphate.

**Figure 2:** Active folate transport to the central nervous system occurs mainly at the choroid plexus epithelial cells, where folate receptor protein 1 (FR1), anchored at the plasma side, binds and incorporates 5-methyltetrahydrofolate through an endocytic process, after which conversion to folylpolyglutamate takes place by the action of folylpoly-gamma-glutamate synthetase (1). Stored folylpolyglutamate can be converted again to its monoglutamate form by gamma-glutamyl-hydrolase (2) after which the monoglutamate form of folate can leave the choroid plexus to the spinal fluid compartment. Part of the anchored FR1 will be cleaved from its glycosyl-phosphatidylinositol-peptide anchor and released to the spinal fluid as soluble FR1 (sFR1) without losing its physico-chemical properties. At the apical surface reduced folate carrier (RFC1) facilitates export of folate monoglutamate. Expression of FR1 is thought to correlate inversely with extracellular folate concentrations. F, folate monoglutamate; F–, folylpolyglutamate.
Figure 3: Structural formula of folic acid. This is composed of an unreduced 2-amino-4-hydroxypteridine molecule linked through methylene (C9-position) to p-aminobenzoylmonoglutamate. The R group, which is also shown, represents the carried one-carbon group at different oxidation states in the form of methyl, methenyl, or formyl, attached to the N-5 and/or N-10 positions. The metabolically active form tetrahydrofolate is reduced at 5,6,7,8 positions of the pteridine group.

Figure 4: Metabolic pathways showing the interconversion of different folate forms in the brain with major transport of 5-methyl-tetrahydrofolate (5MTHF) across the choroid plexus where in cerebral folate deficiency a non-functional folate receptor protein is thought to be responsible for reduced folate transport to the central nervous system. At the upper left the conversion of homocysteine to methionine is shown by the enzyme methionine synthase requiring 5MTHF and vitamin B12. Methionine is converted to the activated methyl-donor S-adenosylmethionine, necessary for methylation reactions. Other folate interconversions and metabolic pathways are also shown. SAH, S-adenosylhomocysteine; Ser, serine; Gly, glycine; THF, tetrahydrofolate; DHF, dihydrofolate; DHFR, dihydrofolate reductase; SHMT, serine hydroxymethyltransferase; MTHFR, methylenetetrahydrofolate reductase; SAM, S-adenosylmethionine; 5-formyl-THF is synonymous with folinic acid.
FR1 protein, which can be found in CSF after cleavage from its glycosyolphosphatidylinositol (GPI-anchor) at the apical membrane surface of choroid plexus epithelial cells (see Fig. 2). Moreover, the binding of radioactively labelled folate to FR proteins was unusually high in the study by Wevers et al. suggesting diminished expression and/or secretion of FR1 and impaired folate release from its binding site. However, the exact cause remains unclear.

This paper will focus on the typical clinical and biochemical findings as well as treatment guidelines for a novel neurometabolic syndrome associated with low 5MTHF in spinal fluid and the absence of any detectable folate disorder outside the nervous system. For this syndrome, which can be separated from the case of an adult male but where the exact pathogenic mechanism remains unknown, we propose the name idiopathic CFD. In addition we will outline the differential diagnosis for other systemic folate disorders and known disease entities where secondary CFD can be found.

Folate metabolism and transport

Folates are a family of structurally related compounds. The basic structure consists of a 2-aminopyrimidine molecule linked through a methylene carbon to para-aminobenzoylmoono- or polyglutamate (Fig. 3). Only the reduced folate forms can function as the active cofactors in cellular metabolism. These carry one-carbon units at three different oxidation levels in the form of methyl, methenyl, or formyl groups, attached to the N-5 and/or N-10 position of tetrahydrofolate, which can be interconverted enzymatically.

The physiological function of the various reduced folate forms subserves the de novo synthesis of purines and thymidine, methylation of DNA, the conversion of homocysteine to methionine, and the formation of the active methyl-group donor S-adenosylmethionine (SAM), which is used for the transfer of methyl groups. 5MTHF is one of the physiological active folate forms and is the precursor of the activated methyl-group donor SAM (see Fig. 4). SAM is used in more than 100 chemical reactions among which are the methylation of DNA, fatty acids, phospholipids, polysacharides, and proteins. For example, the methylation of arginine at position 107 within myelin-basic protein is necessary to maintain stability of CNS myelin. In addition, the universal methyl-group donor SAM acts in the catabolism of biogenic monoamines.

An adequate folate pool and metabolism is not only necessary for DNA synthesis and replication, cell division, growth and survival, but also for normal embryonic growth, as well as development and maturation of the nervous system. Both genetic and non-genetic predisposing factors affecting folate metabolism and transfer across the placental barrier have been identified as having a role in the origin of neural tube defects.

Cellular folate uptake across the plasma membrane is accomplished by three different transport mechanisms, i.e. folate-receptor mediated endocytosis by FR1 and FR2, the RFC1, and an adenosine triphosphate-dependent folate exporter system. FR1 is mainly distributed at epithelial cells, such as choroid plexus, lung, thyroid, and renal tubular cells, while FR2 is mainly located within mesenchymal derived cells, such as red blood cells. Both are high-affinity proteins which function at the physiological nanomolar range of extracellular folate concentrations. RFC1 is ubiquitously distributed and represents a low-affinity folate transporting system with bidirectional transport across cellular membranes. The adenosine triphosphate-dependent folate exporter regulates intracellular folate monoglutamate concentrations.

Intact transport mechanisms across the intestinal, placental, and blood-brain barriers are crucial to achieve adequate folate stores within organs and the CNS (see Figs 1a, 1b, 2). For passage across the blood–CSF barrier, plasma 5MTHF is bound by the FR1, anchored to choroid epithelial cell membranes by a GPI moiety. This is followed by receptor-mediated endocytosis, storage, and subsequent export. The choroid plexus is the main site of active folate transport to the CNS, as the FR1 proteins possess a high affinity for folate in the nanomolar range which represents the physiological concentration in plasma. Folate transport by FR1 across the choroid plexus is an active transport leading to a two-fold higher concentration

**Figures 5:** (a) shows number of patients diagnosed and treated with folinic acid before age of 6 years (indicated by open bars) who have a favourable prognosis and sometimes recover dramatically. This contrasts with children diagnosed at age of 6 years or above (indicated by black bars) who show partial and delayed recovery. (b) summarizes age at onset of main clinical features.

in CSF compared with plasma. The FR1 is mainly localized at the basolateral surface of choroid epithelial cell membranes, facing the plasma-side, where it functions through receptor-mediated endocytosis. After endocytosis and release of folate from its receptor into the cytosol, part of the endocytotic membrane-attached FR1 will recycle to the basolateral choroid cell surface. Another part of the intracellular FR1 pool reaches the apical surface where it is cleaved from its GPI-peptide anchored to the membrane and released into CSF without losing its binding characteristics. For normal expression of FR1 on the cell membrane surface and proper functioning of this folate receptor mediated endocytosis, many cooperating genetic and non-genetic factors have been identified as important determinants, such as intracellular homocystein concentration, the extent of N-glycosylation of FR1, and the sphyngolipid and cholesterol content in membranes. At the apical surface of choroid epithelial cells RFC1 facilitates folate export. RFC1 is also located at vessel membranes of the blood-brain barrier, where its contribution to the main bulk of folate transport is of minor importance because it has low folate affinity and will only bind folate concentrations within the micromolar range.

Clinical features of idiopathic CFD
Twenty children (5 females, 15 males; 19 of Caucasian and one of Tamil origin) presented with a uniform history and clinical phenotype (Fig. 5b). Two children from unrelated families originated from a first-cousin marriage. Pregnancy, birth, and neonatal periods were normal except for two children born preterm.

After the first report of five children with CFD, the additional 15 patients with typical features have been selected for further study at Aachen University Hospital. Patients only entered the study after exclusion of known disorders and after parental informed consent. Their mean age at the time of diagnosis was 6.4 years (range 2 to 17y).

Normal development during the first 4 months was followed by deceleration of head growth from the age of 4 to 6 months and the onset of marked unrest, irritability, and sleep disturbances in 18 of the 20 patients. From 6 months of age, acquisition of neurodevelopmental milestones was delayed and even came to a standstill, leading to generalized hypotonia with poor postural control and signs of cerebellar ataxia on attempted grasping by the end of the first year. Upper limb movements became further complicated due to superimposed dyskinesias with ballistic movements and hand choreoathetosis. In addition to marked psychomotor delay, neurological examination from the age of 1.5 to 2 years showed progressive ataxia and dyskinesias with appearance of pyramidal signs in the lower limbs. Later an ascending paraparesis with pyramidal deficits leading to spastic tetraplegia developed in older patients.

Apart from severe cognitive delay, seven of the 20 children had additional autistic features, fulfilling the criteria of the Autistic Diagnostic Observation Schedule. Recurrent myoclonic-astatic seizures, absences and generalized tonic-clonic seizures have been noted among seven out of 20 children of whom six children required anticonvulsant drugs. Visual disturbances manifested as ocular strabismus around the age of 3 years at which time visual evoked potentials after flash stimuli began to show prolonged latencies followed by reduction and extinction of P2–wave amplitudes. If patients had a delayed diagnosis and treatment was not started until the age of 6 years, the central visual disturbance was followed by progressive visual loss and appearance of optic atrophy. Two patients diagnosed and treated at the age of 10 and 15 years also suffered from progressive sensorineural hearing loss which had started.

Figure 6: (a, b) MRI (T2-weighted) of 21-month-old male patient with cerebral folate deficiency showing normal myelin signal of callosal structures compared with decreased myelin signals for white matter and (c) loss of myelin signals around occipital horns.
Biochemical findings in idiopathic CFD

Neurodegenerative and inborn errors of metabolism as well as chromosomal disorders and MECP2 gene alterations were ruled out by extensive investigations. Normal values have been found for serum homocysteine, folic acid, vitamin B<sub>12</sub>, red blood cell folate concentration and leucocyte methylenetetrahydrofolate reductase activity.

CSF analysis showed reduced 5MTHF concentration in all patients compared with normal controls while a later CSF analysis in five patients showed a further decrease of 5MTHF (Fig. 7a). In nine of 20 patients CSF analysis showed a reduced concentration of 5-hydroxy-indoleacetic acid (5HIAA) in the presence of normal homovanillic acid (HVA) concentrations (except for a low HVA concentration in the oldest patient). CSF pterin analysis showed isolated reduction of neopterin concentration in six of 20 patients, while CSF biopterin was slightly reduced in two of 20 patients. In these latter patients, inborn errors of tetrahydrobiopterin synthesis and dihydropteridine reductase deficiency were excluded by appropriate tests. There is, as yet, no explanation for the observed folate deficiency and diminished pterin metabolites in spinal fluid.

After treatment with folinic acid, 5MTHF concentrations, pterins, 5HIAA and HVA normalized except for two patients whose 5HIAA levels remained low. The corrected 5MTHF values increased to a mean value of 73.1 nmol/l with a range of 45.4–120 nmol/l, which showed no statistical difference compared with 99 normal controls (mean value 82.01 nmol/l, SD 31; range 44 to 181 nmol/l). Reference values of the control group have been collected and published by our own laboratory. Various studies in animals and observations in humans confirm that folate depletion in the CNS reduces the turnover of the serotonergic and dopaminergic pathways.

Part of the membrane-anchored FR1 will be released into the spinal fluid compartment and can be isolated from CSF for further study. In four children with idiopathic CFD further CSF studies demonstrated a normal or slightly elevated concentration of immunoactive soluble FR1 (patients’ values 0.16–0.56 nmol/l; normal range 0.14–0.38 nmol/l), but studies with radioactively labelled folate showed severely decreased folate binding affinity (patients’ values 0.02–0.05 nmol/l; normal range 0.3–0.45 nmol/l). These CSF soluble FR1 protein studies are different from the findings of the patient described by Wevers et al. Sequencing of the encoding exons and the intron-exon boundaries of the FR1 and FR2 gene was completely normal. Therefore, it is very unlikely that idiopathic CFD is caused by a defect of the encoding exons of the FR genes but is rather due to a non-functional FR1 protein which explains the reduced folate transport to the CNS. The exact underlying cause remains unknown.

Possible mechanisms are acquired defects of FR1 protein N-glycosylation, which in vitro is known to affect folate receptor expression and binding. However, this is unlikely because there was no indication for a generalized protein N-glycosylation defect. A second putative mechanism is the appearance of folate antagonists or development of an autoimmune disease with autoantibodies directed against the FR1 folate-binding sites.

Diagnosis and differential diagnosis

Systemic depletion of the pool of metabolically active reduced folates should be considered and has been encountered in children with malnutrition, intestinal malabsorption due to celiac disease, during the use of antifolate agents like methotrexate and, rarely, during chronic use of anticonvulsant drugs.

Figure 7: (a) shows 5-methyltetrahydrofolate concentrations in cerebrospinal fluid (CSF) from patients with the described idiopathic cerebral folate deficiency (CFD) syndrome (n=20) with the drop of 5MTHF concentrations among five patients. (b) shows only the CSF data with diminished values among 11 out of 26 investigated patients with Rett syndrome recruited from five centres in Europe and Israel. Grey shaded area represents normal range of aged-matched controls.
A rare form of hereditary folate malabsorption with macrocytic anaemia and low systemic folate status, together with severe neurological deficits and intracranial calcifications, is suspected to be caused by an intestinal RFC1 defect.\textsuperscript{1,2,6} The amino acids serine, glycine, and histidine act as the most important one-carbon donors to replenish the pool of unsubstituted tetrahydrofolates. Therefore, hereditary metabolic conditions associated with lowered levels of these amino acids can be expected to cause secondary reduction in the 5MTHF concentrations and, thus, the SAM-mediated methylation reactions. A de novo disorder of serumine synthesis due to 3-phosphoglycerate dehydrogenase deficiency leads to a recognizable clinical entity with microcephaly, intractable seizures, feeding disturbances, and severe neurological disability. The presence of low serine in the brain will consequently reduce the substrate for the enzyme serine hydroxy-methyltransferase in this disorder which will reduce the production of 5,10-methylenetetrahydrofolate, the precursor for 5MTHF and SAM.\textsuperscript{27}

Autosomal recessively transmitted forms of methylenetetrahydrofolate reductase deficiency (MTHFR) with reduced enzyme activity should always be considered because they can lead to low 5MTHF levels associated with homocysteine accumulation manifesting with a variable phenotype from mild to severe neurological disabilities. Therefore, leucocyte MTHFR activity was measured in all our patients.\textsuperscript{28,29}

Known rare inborn errors of metabolism associated with secondary CFD include dihydropyridine reductase and aromatic L-amino acid decarboxylase deficiency. Diagnosis depends on a high index of clinical suspicion and typical findings from CSF amino acid levels, biogenic monoamine, and pterin metabolites.\textsuperscript{30–32}

Two recent reports have described secondary CFD in a number of females with Rett syndrome and patients with variant or classical Aicardi-Goutières syndrome.\textsuperscript{33,34} Our preliminary findings on Rett syndrome, in an extended multicentre study from five European Centres and Israel, have now shown lowered 5MTHF to be present in CSF among 11 out of a total of 26 (25 females and one male) investigated patients with Rett syndrome. Data for these 11 patients have also been included in Figure 7b. Among the 10 females and one male with Rett syndrome and low CSF 5MTHF, \textit{MECP2} mutations or deletions were present in six, while in five patients the \textit{MECP2} gene was normal. The very low level of 5MTHF in a mutation-negative male with Rett-like features and four mutation-negative females with classical Rett syndrome brings up a new issue of whether CNS folate deficiency contributes to the phenotype of Rett syndrome. Analysis of the FR1 isolated from CSF of the first four reported females with Rett Syndrome and with CFD indicated similar unexplained findings of a non-functional FR1 protein in the presence of normal FR1 and FR2 genetic analysis. Further CSF studies of children with Rett syndrome have also been started in North America to detect the incidence of reduced folate transport to the brain, which may also carry implications for therapy. Care should be taken to exclude those patients with Rett syndrome receiving multivitamin preparations or treated with folate plus betaine, or in whom folate plus betaine had been stopped for less than three months before the CSF sampling, because folates can be stored and tightly bound to proteins for a long time (equal to or longer than 100 days).\textsuperscript{33} This long residence time may represent an important bias in determining the exact prevalence of CFD among the population with Rett syndrome. While the results of treatment with folinic acid in four patients with Rett syndrome showed partial improvement and prevented further regression, there was no dramatic recovery of lost functions.\textsuperscript{35}

Because these preliminary data now indicate that CFD appears not to be a consistent feature in Rett syndrome, important further questions concerning the pathogenesis of secondary CFD in a selected number of patients with Rett syndrome versus the group of patients with Rett syndrome with normal CSF findings are raised. The exact link between the \textit{MECP2} phenotype and its influence on folate transfer processes at the choroid plexus remains to be established.

Diagnosis of idiopathic CFD can be established based upon the salient clinical features associated with a reduced CSF folate concentration and after exclusion of disturbed folate and vitamin B\textsubscript{12} homeostasis and metabolism outside the nervous system. The conditions leading to secondary CFD should be considered and excluded, as indicated above.

\textbf{Guidelines for treatment} 

After finding low 5MTHF values in CSF, all patients were treated and followed up for a period of at least one year. Folinic acid was the preferred folate substance as it represents a stable form of a metabolically active reduced folate compound in contrast to folic acid, which is an oxidized and metabolically inactive folate form. Paradoxically, treatment with folic acid may further enhance CNS depletion of the metabolically active pool of reduced folates because folic acid has a higher FR1 binding affinity compared with 5MTHF and consequently competes at the FR1 receptor with the transport and storage of reduced folate species.\textsuperscript{1} After informed parental consent, treatment consisted of oral administration with 0.5–1mg/kg body weight calcium folinic acid. Because side effects of gastrointestinal upset and an increased risk of seizures can be associated with calcium folinic acid, careful clinical and electroencephalogram (EEG) follow-up studies have been performed after 1, 3, and 6 months. Hunter et al.\textsuperscript{36} have previously described in a study on healthy volunteers that daily administration of 15mg folic acid to healthy volunteers for more than one month caused mental changes, sleep disturbances, and irritability. Some became hyperactive, whereas others became depressed and confused and had increasing difficulties with concentration.

In addition, Hommes et al.\textsuperscript{37} reported that intravenous folate at extremely high doses (45 to 120mg) in rats caused generalized seizures. Therefore, based on these earlier observations, extreme caution should be taken during the treatment of children with CFD to avoid overdosage and neurotoxicity with folinic acid. Two children manifested transient tics and agitation during folinic acid treatment, but all other children showed excellent tolerance with no adverse clinical or EEG side effects.

Although the group of youngest children, diagnosed and treated before the age of 6 years, showed a favourable and sometimes dramatic response with marked neurological recovery and cessation of seizures, the group of older children beyond the age of 6 years tended to show a more delayed response with incomplete neurological recovery (Fig 5a). However, treatment with folinic acid was able to prevent further deterioration. In children who did not show any clinical
effect or a doubtful effect after treatment during an initial period of 4 to 6 months at a folinic acid dose of 0.5–1mg/kg/day, a lumbar puncture was repeated in order to monitor the change of CSF 5MTHF levels. The reason for repeating the spinal fluid analysis after 4 to 6 months was to detect whether 5MTHF levels in CSF had normalized, remained low, or had become too high with the risk of clinical toxicity. CSF sampling to monitor CSF 5MTHF during treatment is also necessary because the natural course, as shown previously, indicated a further drop of 5MTHF among children with idiopathic CFD. Based on the initial CSF levels together with the monitored levels during treatment, dose readjustments for folinic acid have been calculated. During long-term treatment, CSF sampling has been repeated among selected children who showed insufficient recovery or possible signs of folate toxicity. Abrupt increases of the folinic acid dose by more than 0.5mg/kg/day over a period of less than one month have been avoided for safety reasons.

These guidelines for treatment with folinic acid have been derived from our own experience and the available literature and developed by the International Cerebral Folate Deficiency Group.

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References


**List of abbreviations**

- CFD: Cerebral folate deficiency
- FR: Folate receptor proteins
- FR1: Folate receptor protein 1
- GPI: Glycosylphosphatidylinositol
- HVA: Homovanillic acid
- MTHFR: Methylene-tetrahydrofolate reductase
- RFC1: Reduced folate carrier 1
- SAM: S-adenosylmethionine
- 5HIAA: 5-hydroxy-indoleacetic acid
- 5MTHF: 5-methyltetrahydrofolate

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