Antiepileptic drugs interact with folate and vitamin B12 serum levels

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Abstract: OBJECTIVE: Antiepileptic drugs (AEDs) are important for the treatment of epilepsy, psychiatric diseases, and pain syndromes. Small studies have suggested that AED treatment reduces serum levels of folate and vitamin B12. METHODS: This prospective monocenter study aimed at testing the hypothesis that AED treatment is associated with folate and vitamin B12 serum levels in a large population. A total of 2730 AED-treated and 170 untreated patients with epilepsy and 200 healthy individuals were enrolled. RESULTS: Treatment with carbamazepine, gabapentin, oxcarbazepine, phenytoin, primidone, or valproate was associated with lower mean serum folate levels or with a higher frequency of folate levels below the reference range in comparison with the entire group of patients, untreated patients, or controls. Treatment with phenobarbital, pregabalin, primidone, or topiramate was associated with lower vitamin B12 levels compared with the entire group of patients. Vitamin B12 serum levels were higher in patients treated with valproate compared with the entire group of patients, untreated patients, and healthy controls. Folate or vitamin B12 levels below the reference range were associated with higher mean corpuscular volume (MCV) and higher homocysteine plasma levels. Vitamin substitution for 3 months in 141 patients with folate or vitamin B12 levels below the reference range yielded normal vitamin levels in 95% of the supplemented patients and reduced MCV and homocysteine plasma levels. INTERPRETATION: Treatment with most of the commonly used AEDs is associated with reduced folate or vitamin B12 serum levels and is a risk factor for hyperhomocysteinemia. Oral substitution is effective to restore vitamin, MCV, and homocysteine levels. Ann Neurol 2011;

Antiepileptic drugs interact with folate and vitamin B12 serum levels

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Authors' contributions

Michael Linnebank initiated the study, did the analyses and wrote the manuscript. Susanna Moskau and Alexander Semmler enrolled the patients and collected the results. Guido Widman collected the patient data. Birgit Stoffel-Wagner was responsible for the laboratory analyses. Michael Weller supervised the analyses and helped to write the manuscript. Christian E. Elger planned the study together with Michael Linnebank and supervised all steps of the study.

Ethics Committee

This study was approved by the local ethics committee.

Abstract

Objective

Antiepileptic drugs (AED) are important for the treatment of epilepsy, psychiatric diseases and pain syndromes. Small studies suggested that AED treatment reduces folate and vitamin B12 serum levels.

Methods

This prospective monocenter study aimed at testing the hypothesis that AED treatment is associated with folate and vitamin B12 serum levels in a large population. 2730 AED-treated and 170 untreated patients with epilepsy and 200 healthy individuals were enrolled.

Results

Treatment with carbamazepine, gabapentin, oxcarbazepine, phenytoin, primidone or valproate was associated with lower mean serum folate levels or with a higher frequency of folate levels below the reference range in comparison with all other patients, untreated patients or controls. Treatment with phenobarbital, pregabalin, primidone or topiramate was associated with lower vitamin B12 levels compared with the entire group of patients. Vitamin B12 serum levels were higher in patients treated with valproate compared with the entire group of patients, untreated patients and healthy controls. Folate or vitamin B12 levels below the reference range were associated with higher mean corpuscular volume (MCV) and higher homocysteine plasma levels. Vitamin substitution for three months in 141 patients with folate or vitamin B12 levels below the reference range yielded normal vitamin levels in 95% of the supplemented patients and reduced MCV and homocysteine plasma levels.

Interpretation

Treatment with most of the commonly used AED is associated with reduced folate or vitamin B12 serum levels and is a risk factor for hyperhomocysteinemia. Oral substitution is effective to restore vitamin, MCV and homocysteine levels.

Objective

Antiepileptic drugs (AED) are frequently used in the treatment of epilepsy, psychiatric diseases and pain syndromes. Their side effects include osteoporosis, atherosclerosis, fatigue, peripheral neuropathies cerebellar ataxia, neuropsychological impairment and teratogenesis including reduced cognitive functions in children of mothers treated with valproate.[1-9] Several studies have suggested that treatment with distinct AED like valproate, carbamazepine or phenytoin is associated with reduced mean serum levels of folate and vitamin B12 and that this may mediate AED side effects. As metabolism of homocysteine depends on folate and vitamin B12, hyperhomocysteinemia also was observed in associated with AED. However, the results of the published studies are conflicting concerning the association of the different AED with folate, vitamin B12 or homocysteine plasma levels. In addition, sample sizes were small, and data are lacking for most of the newer AED.[10-14] Thus, valid and detailed data on the association of the commonly used AED with folate and vitamin B12 serum levels are missing.

Suffering from epilepsy is unlikely to directly interfere with serum levels of folate or vitamin B12. Thus, patients with epilepsy are a suitable population to investigate the association of AED treatment with folate and vitamin B12 serum levels. This prospective monocenter cohort study tested the hypothesis that AED treatment is associated with folate and vitamin B12 serum levels in a population of 2730 AED-treated and 170 untreated patients with epilepsy.

Methods

<u>Patients</u>

This prospective study included serial in- and out-patients of the Department for Epileptology of the University Hospital Bonn, Germany, who suffered from epilepsy. Period of recruitment was January 1st to December 31st 2006. Patients with epilepsy not treated with any AED for at least three months were enrolled as disease controls. In Germany, there is no mandatory folate supplementation of basic food. The German *Robert-Koch-Institut* of the Ministry of Health recommends (2010) that all women at child bearing age, who do not prevent pregnancy, supplement 400 µg folate per day, albeit it states that this is not common practice and that only 9% of the German women have a sufficient uptake of naturally occurring folate or synthetic folic acid, respectively (www.rki.de). The guidelines of the German Society of Neurology recommend supplementation of 5mg folic acid per day for AED-treated women with epilepsy who plan to become pregnant. For our study, patients were not eligible if they supplemented any vitamin more than once a week, although we recommend such folic acid supplementation in respective cases. Otherwise unselected 100 female and 100 male blood donors served as second control group. For those healthy controls, no further data were available. The study was approved by the local ethics committee.

<u>Clinical protocol and laboratory analyses</u>

Folate, vitamin B12, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total red blood cells, mean corpuscular volume, hematocrit, leukocytes, platelets, calcium, potassium, sodium, ammoniac, gamma-glutamyl transferase, glutamic oxaloacetic transaminase, glutamate pyruvate transaminase, creatinine, fasting homocysteine plasma levels and triglycerides were determined in the clinical routine laboratory. Patients with serum folate or vitamin B12 serum levels below the reference range were advised to substitute the lacking vitamin. Substitution was per os, 5 mg folic acid or 900

µg vitamin B12, respectively. Substituted patients were invited to return after three months. Patients who did not follow the invitation to come to our clinic for the management of vitamin substitution received written advice for their family doctor.

Fasting plasma total homocysteine concentrations were measured by particle-enhanced immunonephelometry with a BN II system (Siemens Healthcare Diagnostics, Eschborn, Germany). The reference interval for normal was 4.9-15.0 µmol/l. Fasting serum vitamin B12 and folate concentrations were measured of a competitive chemiluminescent immunoassay with an Access™ immunoassay system (Beckman Coulter, Krefeld, Germany). Reference intervals were 3-17 ng/ml for folate and 180–920 pg/ml for vitamin B12.

Statistics

SPSS version 16 was used for statistical analysis (SPSS, Chicago, IL, USA). Pearson's Chi^2 tests, t-tests, univariate correlation analysis (Pearson) and univariate analysis of variance (ANOVA) were used for univariate analysis of numeric and metric data, respectively. Multiple linear regression analysis with a probability for inclusion of 0.05 and of 0.10 for exclusion was used to analyze factors of influence on folate and vitamin B12 levels.

Results

Study sample

2900 serial patients met the inclusion criteria. In patients who presented more than once to the clinic during the period of recruitment, the first visit was evaluated. 170 patients were not treated with AED, 958 patients were on AED monotherapy, and 1772 patients were treated with two or more AED. Intake of AED and personal data are summarized in Supplementary table 1. In this rather young study population, age did not correlate with serum folate or vitamin B12 levels (not shown). Women had higher serum folate levels (mean ± 1 SD: 6.34 ± 4.0 ng/ml) than men (5.49 ± 3.30; t = 6.4; p<0.001), whereas vitamin B12 serum levels showed no association with gender (not shown).

AED and folate levels

The 2730 patients treated with AED had significantly more often subnormal serum folate levels than the 170 untreated patients and the 200 healthy controls (Table 1). Multivariate analysis of AED intake (yes or no, all AED simultaneously, including patients taking more than one AED) together with age, gender and vitamin B12 levels as covariables revealed that intake of carbamazepine, gabapentin, oxcarbazepine, phenytoin or valproate was associated with lower mean folate levels compared with the entire group of patients (Table 2). The association of carbamazepine and valproate with lower folate levels was dose-dependent, whereas the association of gabapentin, oxcarbazepine and phenytoin was not. Although intake of primidone and topiramate was not associated with lower folate levels per se, the daily dose of these AED correlated negatively with folate levels.

Table 3 shows that the mean folate level in patients treated with AED monotherapies was 6.0±3.5 ng/ml, which did not differ from the healthy controls, but was lower than in untreated patients (6.6±3.7 ng/ml). The prevalence of subnormal folate levels was 16% and, thus, higher than in controls and untreated patients. Carbamazepine and phenytoin monotherapies were

associated with lower mean folate serum levels compared with untreated patients and healthy controls, and patients treated with carbamazepine, gabapentin, phenytoin or primidone monotherapy had serum folate levels below the reference range more often than untreated patients and controls. Correlation analysis of AED dose with folate levels did not reveal significant results for patients with monotherapy.

AED and vitamin B12 levels

The frequency of vitamin B12 levels below the reference range in the entire group of patients treated with AED did not significantly differ from untreated patients or controls (Table 1). However, treatment with phenobarbital, pregabalin, primidone or topiramate was associated with lower mean vitamin B12 levels compared with the entire group of patients (Table 2). The association of pregabalin and topiramate with lower mean vitamin B12 levels was dose-dependent. Although lamotrigine treatment was not associated with mean vitamin B12 serum levels or with the prevalence of vitamin B12 levels below the reference range, the daily dose of lamotrigine negatively correlated with mean vitamin B12 levels. Surprisingly, valproate treatment was dose-dependently associated with higher mean vitamin B12 levels in comparison to all other patients.

In the group of patients treated with AED monotherapies, mean vitamin B12 serum levels and the frequency of subnormal vitamin B12 serum levels did not differ from untreated patients or controls (Table 3). Concerning the different AED, only the mean vitamin B12 serum levels of patients treated with valproate monotherapy were higher than those of untreated patients or controls in a dose-dependent manner. Accordingly, the prevalence of subnormal vitamin B12 serum levels was significantly lower in patients treated with valproate monotherapy than in untreated patients and, for trend, in healthy individuals.

Subnormal folate or vitamin B12 levels: associated laboratory changes

We compared routine laboratory parameters of the patients with subnormal folate or vitamin B12 serum levels with those of patients with normal folate and vitamin B12 levels. Subnormal folate levels were associated with lower vitamin B12, lower red blood cells, higher mean corpuscular volume, higher mean corpuscular hemoglobin, higher lymphocyte counts, higher platelets, lower calcium, higher gamma-glutamyl transferase and higher homocysteine (Supplementary table 2). Subnormal vitamin B12 levels were associated with lower folate, lower red blood cells, higher mean corpuscular volume, higher mean corpuscular hemoglobin, lower mean corpuscular hemoglobin concentration, higher lymphocytes, higher platelets and higher homocysteine (Supplementary table 3). As expected from these data, intake of those AED associated with subnormal folate or vitamin B12 serum levels, generally was associated with higher homocysteine plasma levels. However, this effect depended on folate and vitamin B12 serum levels, which strongly negatively correlated with homocysteine plasma levels (the lower folate or vitamin B12, the higher homocysteine). Because of this expected dependency, separate analyses for the association of the AED with homocysteine plasma levels are not presented in detail. In brief, patients treated with carbamazepine (mean homocysteine plasma level ± 1 standard deviation: 16.8±6.1 µmol/L), oxcarbazepine (16.1±4.3), phenobarbital (17.5±5.6), phenytoin (19.4±8.5), primidone (16.6±3.6) or topiramate (18.9±7.5) had homocysteine plasma levels above the reference range, whereas untreated patients (11.9±3.0) and patients treated with clobazam (12.9±2.9), clonazepam (13.3±3.0), lamotrigine (13.5±3.9), levetiracetam (11.9±3.2) or valproate (13.5±4.6) had mean homocysteine plasma levels within the reference range. Results for pregabalin were borderline (15.0±6.4).

<u>Vitamin substitution</u>

Individuals with subnormal folate or vitamin B12 serum levels were asked to perform vitamin substitution under study conditions or to substitute vitamins with the help of their local neurologists if they decided not to return to our hospital because of large distances or other reasons. A total of 141 patients were substituted at our Department, 109 with folate (5 mg/day per os), 16 with vitamin B12 (900 µg/day per os), and 16 with both. Of these patients, 104 returned after a minimum of three months while still under substitution. Vitamin substitution restored normal vitamin levels in 95% of these patients, mean homocysteine plasma levels normalized from 16.6 ± 9.3 µmol/l to 11.6 ± 5.7 µmol/l, and MCV decreased from 91.2 ± 5.5 fl to 89.4 ± 5.9 fl (Supplementary tables 4 and 5). Some patients or their parents reported marked improvements of the cognitive performance or seizure frequency after vitamin substitution. However, in most of these patients also AED treatment was changed at the presentation in our Department. Thus, such speculative clinical effects of vitamin substitution could not be monitored validly in the present study.

Interpretation

In human metabolism, folate is, inter alia, a cofactor for purine- and thymidine synthesis, and vitamin B12 is a cofactor in the synthesis of succinyl-CoA from methylmalonyl-CoA. Jointly, they act in the remethylation of homocysteine to methionine, a precursor of S-adenosylmethionine, an ubiquitous methyl group donor. Deficiency of folate or vitamin B12 can lead to reduced blood cell production, chromosomal instability and disturbed DNA methylation, reduced synthesis of catecholamines and myelin as well as hyperhomocysteinemia. Thus, subnormal folate or vitamin B12 serum levels are associated with anemia, cognitive deficits, vascular disease, osteoporosis, cancer, psychiatric disease, spontaneous abortion and congenital malformations. Individuals with subnormal folate or vitamin B12 serum levels should be substituted, and women who could become pregnant

should prophylactically supplement folate.[15] Homocysteine is a neurotoxic excitatory amino acid acting at the N-methyl-D-aspartate (NMDA) receptor. Thus, reduced mean folate and elevated homocysteine levels associated with AED treatment might promote seizures and neuronal damage contributing to the brain atrophy observed in 20-50% of patients with epilepsy.[16] In addition, elevated homocysteine levels may underlie the increased risk of atherosclerosis in epilepsy patients.[9]

The present single-center study analyzed a large sample of 2730 unselected AED-treated patients with epilepsy (Supplementary table 1). Epilepsy patients treated with AED had more often folate serum levels below the reference range than untreated epilepsy patients. Although not a proof of causality, this at least argues for AED treatment and against epilepsy as the reason for subnormal vitamin levels (Table 1). Admittedly, as a potential source of bias, untreated patients will generally have less severe or more recently evolved disease than treated patients. Accordingly, Volpe et al. found that children with intractable epilepsy had significantly lower intakes of folate and vitamin B12 (among other nutrients) than healthy children.[20] Thus, it is possible that patients on polytherapy (more likely, intractable epilepsy) may differ from the controls in the present study (and the non-treated epilepsy patients as well) in terms of dietary vitamin intake. This could have confounded our results, although it may not explain the observed AED-specific effects on folate and vitamin B12 serum levels, and although most of the patients of our study were adults (Supplementary table 1). A study with AED-treated patients due to other indications than epilepsy could help to clarify that topic. As possible confounder concerning gender, women had higher folte-levels than men arguing that more female than male patients supplemented folic acid or cared for a folate-rich nutrition, although regular folic acid supplementation more than two days a week was an exclusion criterion.

Treatment with carbamazepine, gabapentin, oxcarbazepine, phenytoin, primidone or valproate was associated with lower mean serum folate levels or was associated with a higher frequency

of folate levels below the reference range compared with the entire group of patients or with untreated patients and controls (Tables 2 and 3). This association was dose-dependent for carbamazepine, primidone and valproate, suggesting that a reduction of these AED may restore vitamin levels. Similarly, intake of phenobarbital, pregabalin, primidone or topiramate was associated with lower vitamin B12 serum levels, and the association of pregabalin and topiramate was dose-dependent (Tables 2 and 3). As the daily dose of of lamotrigine negatively correlated with mean vitamin B12 levels, a minor association of lamotrigine with vitamin B12 levels cannot be excluded, although the intake of lamotrigine was not per se associated with folate or vitamin B12 levels.

Some of the associations were only observed when the entire sample was analyzed. In the subgroup of patients with AED monotherapy, only the associations of carbamazepine, gabapentin, phenytoin and primidone with low folate serum levels and the association of valproate with higher vitamin B12 serum levels were significant. Therefore, these associations may be the most relevant ones. Monotherapies with phenobarbital and other AED that were rarely used in our population were not included in this analysis. Thus, additional associations of these AED with folate and vitamin B12 levels cannot be excluded, as suggested by the analysis of all patients including those with polytherapy. The lack of association of lamotrigine, oxcarbazepine, pregabalin, and topiramate monotherapy with vitamin B12 levels may be due to the smaller sample size in the monotherapy subgroup analysis in combination with rather small effect sizes as indicated by the standardized regression coefficient Beta and the ANOVA test value F (Tables 1-3). Accordingly, these associations may be of minor relevance.

In summary, only the intake and daily doses of clobazam, clonazepam and levetiracetam were not at all associated with serum folate or vitamin B12 levels in our study population (Tables 2 and 3). This means that AED with different modes of action and interaction were associated with folate or vitamin B12 serum levels. Whereas the strong enzyme inductors carbamazepine

and phenytoin were associated with lower folate levels with a relatively high effect size, also the enzyme inhibitor valproate was associated with lower mean folate levels, albeit with a lower effect size (Table 2). Also gabapentin, which is presumably neither a hepatic enzyme inhibitor nor inductor, was associated with lower mean folate serum levels. Thus, mechanisms underlying associations of AED with lower folate serum levels may not be restricted to hepatic enzyme induction interfering with the folate metabolism. They remain speculative and might include interferences with diet, absorption, plasma binding, cellular metabolism and renal excretion.

In our study, serum levels of folate or vitamin B12 were associated with several laboratory changes (Supplementary table 2). We did not analyze associations of clinical parameters like seizure frequency in association with vitamin levels besides positive anecdotic reports, but the clinical relevance of low folate and vitamin B12 serum levels has been evidenced by numerous studies. Some of the typical AED side effects are possible symptoms of folate or vitamin B12 deficiency, such as fatigue, osteoporosis, atherosclerosis, polyneuropathy, neuropsychological impairment or teratogenesis, and hyperhomocysteinemia due to low folate or vitamin B12 serum levels may promote seizures.[1-7, 9, 17-18]

Treatment with carbamazepine, oxcarbazepine, phenobarbital, phenytoin, primidone or topiramate additionally was associated with higher homocysteine plasma levels, treatment with pregabalin revealed borderline results. However, these associations were not independent, but were explained by the influence of AED on folate and vitamin B12 serum levels as major determinants of homocysteine plasma levels. Our observations are in line with a smaller study of Belcastro et al.21, who observed hyperhomocysteinemia in association with carbamazepine, oxcarbazepine, phenobarbital and topiramate, but not in association with lamotrigine and levetiracetam. The association of some AED with hyperhomocysteinemia argues that the association of the same AED with folate and vitamin B12 levels is clinically

relevant. Interestingly, valproate, associated with lower folate but higher vitamin B12 levels, was associated with normal homocysteine levels.

Vitamin substitution yielded normal vitamin serum levels and decreased homocysteine plasma levels and MCV in individuals with subnormal folate or vitamin B12 serum levels within approximately three months of substitution (Supplementary table 4). In Germany, where the present study was conducted, folate fortification of flour or food is not mandatory, although an increasing amount of food is voluntarily fortified. In countries with mandatory folate fortification of flour, like USA or Canada, the relevance of AED treatment for folate serum levels may be different from countries without fortification.

In conclusion, the present epidemiological data shows that treatment with any of the commonly used AED other than levetiracetam and benzodiazepines, but in particular with carbamazepine, gabapentin, phenytoin and primidone, is associated with lower mean serum levels of folate or vitamin B12 or, accordingly, with a higher frequency of folate or vitamin B12 serum levels below the reference range. Since such conditions are associated with clinically adverse conditions, AED treatment requests regular controls of folate and vitamin B12 serum levels. Because the method of determination and the reference ranges of folate or vitamin B12 have remained controversial,[19] prophylactic supplementation may be considered as an alternative to regular measurements and selective substitution, especially in women at child-bearing age. For restoration of vitamin levels below the reference range, substitution of 5 mg folate or 900 µg vitamin B12 per os for three months were effective here (Supplementary Table 5). As 5 mg folate is the daily dose recommended by the German Society of Neurology for AED-treated women with epilepsy (2010), a strict implementation of this recommendation is likely to prevent folate deficiency referring to our data. However, lower doses like 1 mg/d folate and 15 µg/d vitamin B12 per os are eventually more suited for continuation and for prophylactic substitution. This has to be defined in prospective studies. The dose-dependency of the association of intake of some AED with folate and vitamin B12

serum levels suggests that reductions of AED dose, if possible, may also reduce side effects mediated by reduced folate or vitamin B12 levels. Studies are needed to further proof causality of AED treatment with associated laboratory changes like increased MCV or homocysteine plasma levels mediated by reduced folate and vitamin B12 levels, and studies are needed to examine the full clinical implications of these potential AED side effects.

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Table 1: Folate and vitamin B12 serum levels in the study population

	Folate				Vitamin B12			
	Mean serum levels (ng/ml) ± SD	Frequency of subnormal serum levels	Versus untreated patients	Versus healthy controls	Mean serum levels (pg/ml) ± SD	Frequency of subnormal serum levels	Versus untreated patients	Versus healthy controls
AED-treated patients (n=2730)	5.8±3.7	0.17	13.7; <0.001	16.3; <0.001	381±184	0.06	0.17; 0.685	0.19; 0.661
Untreated patients (n=170)	6.6±3.7	0.06		0.01; 0.950	366±187	0.07		0.71; 0.400
Healthy controls (n=200)	6.3±3.7	0.06	0.85; 0.357		366±160	0.05	0.71; 0.400	

The frequencies of subnormal serum folate or vitamin B12 levels in AED-treated patients, untreated patients and healthy controls were compared by Pearson's Chi^2 analysis (Chi^2; p). SD = standard deviation. For SI units, folate (ng/ml) must be multiplied with 2.27 (=nmol/l), vitamin B12 (pg/ml) with 0.74 (=pmol/l).

Table 2: AED and vitamins in all patients: linear regression analysis

AED (yes/no)	Folate			Vitamin B12		
	Mean serum levels (ng/ml) ± SD	AED intake and serum levels (Beta; p)[1]	AED dose and serum levels (Beta; p)[2]	Mean serum levels (pg/ml) ± SD	AED intake and serum levels (Beta; p)[1]	AED dose and serum levels (Beta; p)[2]
All patients (n=2900)	5.9±3.7			380±184		
Clobazam (n=229)	5.6±4.0	-0.018; 0.312	0.001; 0.998	382±181	0.023; 0.193	-0.047; 0.480
Clonazepam (n=44)	6.3±5.3	0.010; 0.582	0.292; 0.069	405±270	0.010; 0.572	-0.037; 0.819
Carbamazepine (n=721)	4.9±3.1	-0.160; <0.001	-0.094; 0.014	342±146	-0.030; 0.124	0.021; 0.570
Gabapentin (n=128)	5.9±3.7	-0.038; 0.033	0.081; 0.366	359±148	-0.004; 0.821	-0.-022; 0.803
Lamotrigine (n=1001)	6.3±3.9	0.034; 0.076	-0.051; 0.091	378±175	0.001; 0.946	-0.069; 0.023
Levetiracetame (n=1062)	5.8±3.7	-0.012; 0.493	-0.018; 0.564	376±185	0.017; 0.336	-0.047; 0.180
Oxcarbazepine (n=390)	5.8±3.6	-0.039; 0.042	-0.090; 0.079	365±180	0.001; 0.997	-0.063; 0.222
Phenobarbital (n=193)	5.3±3.7	-0.030; 0.091	-0.080; 0.273	358±156	-0.045; 0.010	-0.054; 0.463
Phenytoin (n=157)	4.5±2.8	-0.099; <0.001	-0.118; 0.132	373±202	0.023; 0.199	0.140; 0.085
Pregabalin (n=231)	5.3±3.8	-0.028; 0.118	-0.061; 0.366	344±186	-0.040; 0.025	-0.153; 0.025

Primidone (n=103)	5.4±4.4	-0.024; 0.174	-0.311; 0.002	354±158	-0.035; 0.047	-0.006; 0.950
Topiramate (n=351)	5.6±4.2	-0.012; 0.492	-0.113; 0.033	341±168	-0.069; <0.001	-0.155; 0.004
Valproate (n=645)	6.3±3.6	-0.045; 0.026	-0.101; 0.011	496±316	0.324; <0.001	0.084; 0.037

Only AED that were taken by at least 1% (n=29) of the patients were included. The overall significance for folate was $F=12.35$; $p<0.001$ and $F=28.69$; $p<0.001$ for vitamin B12.

[1] The standardized coefficient Beta and p are given for linear regression analysis. All AED were analyzed simultaneously with age and gender as additional covariables.

[2] Linear regression: The standardized coefficient Beta and p are given for AED dose with age and gender as covariables in addition to folate, when vitamin B12 was the independent variable and vice versa.

Table 3: AED and vitamins in patients with monotherapy: linear regression analysis

| | Folate | | | | | | | Vitamin B12 | | | | | | |
| | Mean serum levels[2] | | | | Frequency of subnormal serum levels[3] | | | Mean serum levels[2] | | | | Frequency of subnormal serum levels[3] | | |
AED	(ng/ml) ± SD	Versus no AED	Versus controls	AED dose		Versus no AED	Versus controls	(pg/µl) ± SD	Versus no AED	Versus controls	AED dose		Versus no AED	Versus controls
All patients (n=958[1])	6.0±3.5	4.06; 0.044	1.07; 0.302		0.16	6.55; 0.011	16.3; <0.001	389±190	2.36; 0.125	1.09; 0.297		0.06	0.237; 0.627	0.247; 0.380
Carbamazepine (n=215)	5.1±3.0	18.6; <0.001	12.83; <0.001	-0.099; 0.144	0.24	22.3; <0.001	17.9; <0.001	339±143	3.28; 0.071	2.08; 0.150	-0.060; 0.381	0.09	0.64; 0.455	0.450; 0.535
Gabapentin (n=26)	6.0±3.6	0.557; 0.456	0.134; 0.715	0.325; 0.122	0.16	4.57; 0.039	3.94; 0.047	355±193	0.103; 0.749	0.044; 0.834	-0.151; 0.273	0.04	0.28; 0.897	0.066; 0.797
Lamotrigine (n=293)	7.0±4.0	0.953; 0.329	3.55; 0.060	0.089; 0.448	0.05	0.12; 0.832	0.23; 0.881	347±148	1.84; 0.176	1.07; 0.301	-0.004; 0.973	0.08	0.16; 0.851	1.254; 0.263
Levetiracetame	6.4±3.8	0.115;	0.063;	0.043;	0.10	1.44;	1.95;	404±235	2.15;	1.93;	0.044;	0.04	0.366;	0.030;

(n=67)		0.735	0.801	0.731		0.266	0.162		0.143	0.166	0.736		0.762	0.863
Oxcarbazepine (n=96)	6.1±3.3	1.08; 0.300	0.143; 0.706	-0.084; 0.419	0.11	2.53; 0.155	3.35; 0.095	379±187	0.377; 0.540	0.430; 0.513	0.055; 0.593	0.07	0.053; 0.805	0.629; 0.293
Phenytoin (n=15)	4.0±1.6	6.92; 0.009	5.42; 0.021	0.160; 0.599	0.27	8.36; 0.018	9.633; 0.013	412±338	0.944; 0.332	0.808; 0.370	0.232; 0.385	0.07	0.00; 0.988	0.080; 0.778
Primidone (n=10)	5.4±5.3	1.036; 0.310	0.61; 0.436	-0.431; 0.208	0.40	15.1; 0.004	17.1; 0.003	292±145	2.07; 0.151	1.43; 0.233	0.295; 0.128	0.20	2.52; 0.159	3.98; 0.104
Topiramate (n=26)	6.4±4.7	0.075; 0.785	0.010; 0.921	-0.073; 0.689	0.16	3.27; 0.089	3.94; 0.070	304±84	3.69; 0.056	2.51; 0.115	0.233; 0.214	0.12	0.962; 0.399	2.00; 0.164
Valproate (n=176)	6.5±3.6	0.025; 0.876	0.422; 0.516	-0.102; 0.179	0.08	0.531; 0.529	0.909; 0.408	487±197	43.4; <0.001	36.0; <0.001	0.153; 0.044	0.02	5.12; 0.029	3.01; 0.096
No AED (n=170)	6.6±3.7		0.627, 0.429		0.06		0.035; 0.885	364±186	0.023; 0.880			0.06		0.406; 0.653
Healthy controls (n=200)	6.3±3.7	0.627, 0.429			0.06	0.035; 0.885		366±160		0.023; 0.880		0.05	0.406; 0.653	

[1]Since monotherapies with patient numbers <10 were not analyzed (n=34 patients), the sum of the patient numbers shown in the table is 924.

[2]The mean serum folate or vitamin B12 levels of patients treated with AED monotherapy were compared with those of the patients who were not treated with AED or with controls, respectively, by ANOVA (F; p). "AED dose": Linear regression analysis (Beta; p) with AED doses, age, gender and the serum level of the vitamin other than the independent variable as covariables and folate or vitamin B12 serum levels as independent variable.

[3]The frequency of subnormal folate or vitamin B12 serum levels in patients treated with the respective AED in monotherapy was compared with the frequency in untreated patients ("no AED") or controls by Pearson's Chi^2 test ($Chi^{2;}$ p).

Supplementary table 1: Patients and treatment

AED	All patients				Patients with monotherapy			
	n (ratio)	Daily dose (mg) ± SD (range)	Age (years) ± SD (range)	Female gender	n (ratio)	Daily dose (mg) ± SD (range)	Age (years) ± SD (range)	Female gender
All patients	2900		38.2±14.7 (3-85)	0.50	958 (1.00)		36.8±15.7 (3-85)	0.53
Clobazam	229 (0.08)	15.2±9.3 (3-60)	38.7±13.2 (8-80)	0.55	5 (<0.01)	16.0±9.2 (5-50)	44.6±13.4 (31-67)	0.80
Clonazepam	44 (0.02)	2.1±1.7 (1-8)	43.7±15.0 (15-78)	0.68	3 (<0.01)	1.3±0.8 (0.5-2)	45.7±26.6 (15-63)	0.67
Carbamazepine	721 (0.25)	1067±463 (100-2800)	41.1±13.4 (9-80)	0.48	215 (0.22)	934±443 (100-2700)	14.0±13.4 (9-75)	0.51
Diazepam	26 (<0.01)	11.5±8.1 (3-30)	39.6±8.9 (25-54)	0.42	1 (<0.01)	10	43	0.00
Ethosuximid	21	783±331	28.8±13.0	0.48	1	775	7	1.00

	(<0.01)	(250-1500)	(7-56)		(<0.01)			
Felbamat	16 (<0.01)	2481±1027 (800-3600)	32.1±11.4 (16-54)	0.25	1 (<0.01)	3600	54	0.00
Gabapentin	128 (0.04)	2049±1016 (800-3600)	45.6±16.0 (15-83)	0.51	26 (0.03)	1827±883 (600-4800)	52.7±15.7	0.50
Lamotrigine	1001 (0.35)	385±239 (12.5-1500)	37.3±13.9 (3-85)	0.51	293 (0.31)	300±163 (12.5-850)	34.7±15.6	0.59
Levetiracetam	1062 (0.37)	2539±997 (25-7000)	39.5±13.8 (12-78)	0.48	67 (0.07)	2183±837 (500-4000)	40.8±15.8 (12-74)	0.51
Lorazepam	12 (<0.01)	2.0±1.1 (1-5)	35.3±13.8 (13-56)	0.42	0			
Mesuximid	3 (<0.01)	650±433 (150-900)	23.7+7.6 (17-32)	0.33	0			
Oxcarbazepine	390 (0.13)	1401±620 (150-3600)	35.9±14.3 (4-78)	0.48	96 (0.10)	1079±541 (150-3000)	35.7±15.7 (8-78)	0.50
Phenobarbital	193	123±73	41.3±12.3	0.44	7	193±84	43.0±4.9	0.86

	(0.07)	(15-450)	(13-72)		(<0.01)	(100-300)	(36-49)	
Phenytoin	157 (0.05)	300±108 (50-600)	41.4±14.2 (13-79)	0.40	15 (0.02)	318±103 (100-500)	48.0±16.4 (24-79)	0.33
Potassium-bromide	4 (<0.01)	1806±1117 (850-3400)	28.5±9.5 (18-38)	0.75	0			
Pregabalin	231 (0.08)	328±154 (50-900)	38.8±12.5 (14-73)	0.44	3 (<0.01)	200±87 (150-300)	52.0±18.0 (35-71)	1.00
Primidone	103 (0.04)	579±316 (60-1750)	42.7±14.1 (16-76)	0.49	10 (0.01)	656±276 (250-1125)	43.9±17.9 (23-73)	0.70
Sultiam	24 (<0.01)	243±167 (50-600)	18.5±11.5 (5-46)	0.58	13 (0.01)	215±424 (50-450)	12.7±4.9 (6-23)	0.62
Tiagabin	11 (<0.01)	59±81 (15-300)	40.7±10.3 (22-55)	0.36	0			
Topiramate	351 (0.12)	299±178 (20-1000)	35.7±13.0 (4-76)	0.51	26 (0.03)	213±164 (25-800)	36.9±15.5 (12-76)	0.65
Valproate	645	1545±780	36.0±14.2	0.44	176	1290±631	32.5±14.8	0.45

	(0.22)	(40-5400)	(4-80)		(0.18)	(40-4500)	(7-80)
Vigabatrin	17 (<0.01)	1897±1067 (500-4000)	35.3±14.3 (8-55)	0.53	0		
Zonesamid	5 (<0.01)	215±122 (75-400)	30.2±15.9 (18-55)	0.40	0		
No AED	170 (0.06)		39.2±15.8 (16-83)	0.53			
Controls	200			0.50			

The daily dose is defined as the total intake of the respective AED per day. "Female gender" shows the ratio of women.

Supplementary table 2: Association of subnormal folate serum levels with further laboratory abnormalities

	Subnormal folate levels (n=478)	Normal folate levels (n=2422)	ANOVA[1]
Vitamin B12 (pg/ml)	325±176	390±182	51.2; <0.001
Red blood cells (*10^6/µl)	4.61±0.48	4.66±0.45	4.56; 0.033
Mean corpuscular volume (fl)	91.1±5.6	89.9±5.1	19.8; <0.001
Mean corpuscular hemoglobin (pg)	31.0±2.2	30.6±2.0	17.3; <0.001
Lymphocytes (*10^3/µl)	6.62±2.54	6.32±1.92	7.42; 0.006
Platelets (*10^3/µl)	267±73	256±68	10.6; 0.001
Calcium (mmol/l)	2.26±0.11	2.31±0.11	14.2; <0.001
Gamma-glutamyl transferase (U/l)	85.9±87.3	57.6±66.0	38.2; <0.001
Homocysteine (µmol/l)	17.7±10.3	12.3±5.2	19.9; <0.001

[1]F; p

Supplementary table 3: Association of subnormal vitamin B12 serum levels with further laboratory abnormalities

	Subnormal vitamin B12 levels (n=170)	Normal vitamin B12 levels (n=2730)	ANOVA[1]
Folate (ng/ml)	4.32±3.0	6.00±3.7	34.6; 0.001
Red blood cells (*10^6/µl)	4.55±0.46	4.65±0.46	7.78; 0.005
Mean corpuscular volume (fl)	91.6±6.0	90.0±5.2	14.2; 0.001
Mean corpuscular hemoglobin (pg)	31.1±2.3	30.6±2.0	6.67; 0.010
Mean corpuscular h. c. (g/dl)[2]	33.8±1.1	34.1±1.1	6.91; 0.009
Lymphocytes (*10^3/µl)	6.71±2.4	6.35±2.0	4.15; 0.042
Platelets (*10^3/µl)	279±71	257±69	16.2; 0.001
Homocysteine (µmol/l)	17.8±11.9	14.3±7.3	5.63; <0.001

[1]F; p

[2] Mean corpuscular hemoglobin concentration.

Supplementary table 4: Vitamin substitution

	Substituted due to subnormal levels	Follow-up after ≥3 months	Subnormal levels after ≥3 months
folate	n=125	n=94	n=5[3]
vitamin B12	n=32	n=20	n=0
total	n=141[1]	n=104[2]	n=5

[1]16 patients were substituted with both folate and vitamin B12.

[2]At follow-up, 10 patients were substituted with both folate and vitamin B12.

[3]After 3 months of substitution, 60% of patients treated with folate had folate serum levels higher than the top of the measurement range, i.e. 25 ng/ml. The 5 patients who presented with persistingly low levels all had serum folate levels below 2 ng/ml, thus, compliance seemed questionable.

Supplementary table 5: Laboratory changes after vitamin substitution

Parameter	Before substitution	After substitution[4]	t-test (t;p)
Folate (ng/ml)[1]	2.22±0.49	20.2±8.1	21.5; <0.001
Vitamin B12 (pg/ml)[2]	154±15.7	434±213	6.25; <0.001
Homocysteine (µmol/l)[3]	16.6±9.3	11.6±5.7	7.52; <0.001
Mean corpuscular volume (fl)[3]	91.2±5.5	89.4±5.9	3.70; <0.001

Mean absolute levels ± SD of laboratory parameters in patients with subnormal folate (n=125) or subnormal vitamin B12 plasma levels (n=32) before and after substitution as compared with the t-test.

After substitution, there were no significant changes on the parameters hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total red blood cells, hematocrit, leukocyte counts, platelet counts, calcium, potassium, sodium, ammoniac, gamma-glutamyl transferase, glutamic oxaloacetic transaminase, glutamate pyruvate transaminase, creatinine, and triglycerides.

[1]Patients substituted with folate only (n=125).

[2]Patients substituted with vitamin B12 only (n=32).

[3]All patients substituted with folate or vitamin B12 or both (n=141).

[4]After substitution, 60% of the patients had folate serum levels higher than the top of the measurement range, i.e., 25 ng/ml. Due to this technical limitation, the effective folate serum levels after substitution were supposedly higher than shown in this table.