Biological Markers for Alcohol Withdrawal Seizures: A Retrospective Analysis

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Key Words

Alcohol withdrawal • Seizures • Homocysteine • Carbohydrate-deficient transferrin • Prolactin • Detoxification • Dependence • Alcoholism

Abstract

Aims: Alcohol withdrawal seizures (AWS) are among the most important possible complications during the detoxification treatment of alcohol-dependent patients. Pharmacological therapy is often used during detoxification, but can cause dangerous side effects [Eur Addict Res 2010;16:179–184]. In separate studies several biological markers have been described as being associated with AWS risk. We investigated the role of homocysteine (HCT), carbohydrate-deficient transferrin (CDT) and prolactin (PRL) as biological markers for the risk of developing AWS. **Methods:** The present study included 189 alcohol-dependent patients of whom 51 had a history of AWS. We investigated the HCT, CDT and PRL levels of all patients and calculated sensitivity and specificity. Bayes' theorem was used to calculate positive (PPV) and negative (NPV) predictive values. **Results:** The highest combined sensitivity and specificity for %CDT was reached at a plasma cutoff value of 3.75%. The combination of HCT at a cutoff value of 23.9 $\mu\text{mol/l}$ and %CDT at a cutoff value of

3.75% showed the best predictive values (sensitivity 47.1%, specificity 88.4%, PPV 0.504, NPV 0.870). **Conclusion:** A combined assessment of HCT and CDT levels can be a useful method to identify patients at a higher risk of AWS, which may lead to a more individualized therapy.

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Introduction

Alcohol abuse and dependency represent a high risk for health [1] and an avoidable cost for society [2]. Therapeutic options, especially with regard to detoxification, play an important role. Alcohol withdrawal seizures (AWS) are among the most important possible complications of alcohol detoxification. To prevent AWS and other possible complications like delirium tremens several medication schemes are in use during detoxification. However, pharmacological therapy can be associated with side effects like hyponatremia, thrombopenia and allergic reactions [3]. Predictors for individual risks of withdrawal seizures during alcohol detoxification could open the way for an individualized pharmaceutical therapy or for a more intensive care of the detected patients.

The pathophysiological mechanisms of the development of AWS are not completely clarified. There is evidence that homocysteine (HCT) is elevated in nonabstinent alcoholics and decreases during alcohol withdrawal [4, 5]. It is shown that a higher HCT blood level is suitable to predict first-onset AWS [6] and is also correlated with previous withdrawal seizures [7]. In recent years different cutoff values for HCT plasma levels were specified. The most common fasting plasma level of HCT averages between 5 and 15 $\mu\text{mol/l}$. A HCT level between 4.9 and 11.7 $\mu\text{mol/l}$ seems to be an adequate interval in populations without a nutritional deficit [8], but the convenience of these cutoff levels is low for an assessment of AWS risk. In alcohol-dependent patients the HCT levels are highly elevated. Bleich et al. [9] showed that the highest combined sensitivity and specificity to predict the occurrence of first-onset AWS is calculated at a HCT plasma level of 23.9 $\mu\text{mol/l}$.

Carbohydrate-deficient transferrin (CDT) has been proposed as the most specific biomarker of chronic alcohol misuse [10] and probably reflects, at least partially, the severity of alcohol dependence. Hence, CDT was discussed as a possible biomarker to detect patients with higher risk of AWS. In an investigation to confirm %CDT as a predictive parameter for AWS, the best predictive validity was calculated at a cutoff value of 5.4%, but the authors pointed out that CDT alone is not suitable as a predictor for individual withdrawal seizure risk [11].

Regarding prolactin (PRL), there is clinical evidence that PRL levels increase following acute and chronic ethanol exposure [12–14] and decrease in abstinent patients [15, 16]. Also, as recent study results indicate, PRL serum levels seem to be associated with the severity of alcohol dependence and withdrawal symptoms [17]. PRL is supposed to be associated with the dopaminergic system. This supposable association would associate the tuberoinfundibular with the mesocorticolimbic dopamine system. Both systems are regulated by D_2 receptors. There seems to be an association between these receptors and the affective and cognitive functions [18], PRL secretion and the activity of the tuberoinfundibular dopamine neurons [19]. Dopamine inhibits the release of PRL in the hypothalamus [20]. Long-term alcohol consumption leads to a chronic stimulation of dopaminergic receptors [21, 22].

Other investigations showed that PRL can act as a predictive value for the increased risk of seizures during alcohol withdrawal. However, a combined assessment of PRL and HCT did not lead to clear better predictive values compared to the predicting HCT alone [23, 24].

The aims of our study were to investigate (1) whether HCT, CDT and PRL may act as biological markers to appraise the individual risk of AWS, and (2) whether the combined assessment of different parameters may lead to better results.

Subjects and Methods

This study is part of the Franconian Alcoholism Research Studies, a large prospective research study [4]. Approval of the local ethical committee of the University of Erlangen-Nuremberg, in accordance with the ethical standards of the 1964 declaration of Helsinki, was obtained. Written informed consent was obtained from all patients. The same patient sample has been used in other previously published studies focusing on an association between PRL, HCT and previous AWS [7, 9, 23, 24]. All 189 (153 male, 36 female) alcohol-dependent patients fulfilled the criteria of alcohol dependency according to the International Classification of Diseases (ICD-10). The inclusion to the study occurred on the day of admission to the detoxification unit. All patients were treated with clomethiazole and carbamazepine during detoxification, hence none of the patients suffered from AWS during the study period. Therefore, we used the history of AWS to assess the risk of seizures as previously described [7, 23, 25]. Patients with concomitant psychiatric disorder, other substance abuse apart from alcohol and nicotine or common risk factors for hyperhomocysteinemia, like altered nutritional status or endocrinological problems, were not included in the study. Further exclusion criteria were neurological disorders like epilepsy, known risk factors for the development of withdrawal seizures, such as cerebral diseases or lesions and relevant neurological deficits, liver cirrhosis, and renal or pancreatic dysfunction. Patients did not receive any medication (i.e. antipsychotics, vitamin substitution supplements) that can affect the plasma level of HCT.

Blood samples were taken at admission before pharmacological detoxification was started. We analyzed HCT (Axis® Homocysteine EIA/Oslo, Norway, IBL-No. AX 513 01), CDT (%CDT turbidimetric immunoassay, reference value <2.6%, Axis-Shield®, Norway) and PRL (microparticle enzyme immunoassay, reference value: males <18 ng/ml, Abbott AxSYM, cat. # 7A62-22, Abbott Park, Ill., USA).

Statistical Analysis

All parameters followed a standard distribution according to the Kolmogorov-Smirnov test.

Group comparisons between patients with versus without a history of seizure were performed by *t* tests. PRL, HCT and %CDT serum levels were fed into logistic regression analysis (ENTER method) to determine if independent predictive properties existed. Only parameters showing a trend ($p < 0.1$) towards significant prediction in the logistic regression of all three parameters were used for further analysis.

A receiver operator characteristics (ROC) curve and the area under the curve (AUC) were used to measure the effectiveness of the different markers. The combination of markers was calculated after standardization to a mean of 0 and SD of 1 (*z*-transformed). The *z*-transformation was necessary to adapt the weighting of the

variables. AUCs were calculated and comparisons were made between markers and their combination, using DeLong's method. The standard method for AUC confidence intervals was applied. Comparisons of one biomarker or cluster between the female and male subgroup were made using the following statistic:

$$Z = (AUC_M - AUC_F) / \sqrt{SE_M^2 + SE_F^2},$$

where AUC_M/AUC_F = area under the curve for male/female subgroup and SE_M/SE_F = standard error of the AUC, which was compared with the standard normal distribution.

A cutoff value for %CDT was calculated as the value with the highest combined sensitivity and specificity (Youden's index). The previously published cutoff value for HCT fixed at 23.9 $\mu\text{mol/l}$ was used [9].

Sensitivity and specificity were calculated for all biomarkers using these cutoff values.

The positive likelihood ratio (LR+) was calculated as the sensitivity/(1 - specificity). 95% CIs for sensitivity, specificity and positive predictive values (PPV) were calculated using Wilson's method. *p* values <0.05 (two-sided) were considered statistically significant. All calculations were made for male and female patients separately to study gender differences. To formally test for an interaction between gender and HCT/CDT or the seizure scores, multinomial logistic regression using a saturated model were calculated. Data were analyzed employing PASW™ for Windows 17.0 (SPSS Inc., Chicago, Ill, USA) and Analyse-it™ (Analyse-it™ Software, Leeds, UK).

Results

Table 1 shows the demographic characteristics of the study population.

189 patients were included in the study (153 male, 36 female). 51 had suffered AWS (44 male, 7 female) in the past. Patients with a history of AWS had significantly higher %CDT and HCT levels (CDT, seizure vs. no seizure: 6.71 ± 4.19 vs. 5.36 ± 3.31 , *t* test = -2.073, d.f. = 74, *p* = 0.042 and HCT, seizure vs. no seizure: 32.56 ± 26.20 vs. 19.54 ± 9.97 , *t* test = -3.460; d.f. = 55, *p* = 0.001) and showed a trend towards higher PRL levels (seizure vs. no seizure: 16.79 ± 11.46 vs. 13.09 ± 7.61 , *t* test = -1.869, d.f. = 50.961, *p* = 0.067).

There were no significant differences of age, daily alcohol intake (g/day), years of drinking, frequency of previous detoxifications and blood alcohol concentration (mg/dl) between seizure and nonseizure patients: age (43.67 ± 8.5 vs. 43.63 ± 9.3 , *t* = -0.025, *p* = 0.98), daily alcohol intake (275.3 ± 178.6 vs. 232.6 ± 188.1 , *t* = -1.438, *p* = 0.154), years of drinking (18.1 ± 9 vs. 18.3 ± 11 , *t* = 0.156, *p* = 0.876), frequency of previous detoxifications (14.4 ± 22 vs. 9.6 ± 14 , *t* = -1.422, *p* = 0.160), blood alcohol concentration (140.5 ± 144 vs. 127.8 ± 104 , *t* = -0.576, *p* = 0.566).

Table 1. Demographic characteristics of the study population

	History of AWS	Mean $\pm$ SD
Age	No Yes	43.63 ± 8.27 43.67 ± 8.45
Years of drinking	No Yes	18.34 ± 11.00 18.1 ± 9.03
Daily alcohol intake, g	No Yes	232.61 ± 188.07 275.30 ± 178.58
Previous alcohol withdrawal seizures	No Yes	0.00 ± 0.00 2.57 ± 2.84
Frequency of previous detoxifications	No Yes	9.57 ± 14.34 14.35 ± 22.34
Blood alcohol concentration, mg/dl	No Yes	127.79 ± 103.97 140.49 ± 144.18
CDT day 0	No Yes	5.36 ± 3.32 6.71 ± 4.19
HCT day 0	No Yes	19.53 ± 9.97 32.56 ± 26.20
Prolactin	No Yes	13.09 ± 7.61 16.79 ± 11.46

In the logistic regression model only HCT and CDT had a predictive property for a history of seizures ($R^2 = 0.178$; PRL: *B* = 0.028, *p* = 0.203; HCT: *B* = 0.041, *p* = 0.004; CDT: *B* = 0.088, *p* = 0.094). Therefore, only HCT and CDT were included in further analyses.

For %CDT the best cutoff value was calculated at 3.75%. For HCT the previously published cutoff value fixed at 23.9 $\mu\text{mol/l}$ was used [9]. At the cutoff value of 3.75%, %CDT showed a high sensitivity (76.5%), and a lower specificity (44.2%). HCT (cutoff value: 23.9 $\mu\text{mol/l}$) reached a low sensitivity (56.9%), but a higher specificity (78.3%). The combination of HCT and %CDT led to an increased specificity (88.4%), while sensitivity persisted at a low level (47.1%). The best predictive quality was calculated for the combined assessment of HCT and %CDT (PPV = 0.504, NPV = 0.870). NPV diversified between 83.9% and 91.7%. We found a higher sensitivity for the assessments 'CDT $\geq 3.75\%$ ', 'HCT $\geq 23 \mu\text{mol/l}$ ', 'HCT $\geq 23 \mu\text{mol/l}$ or %CDT $\geq 3.75\%$ ' and 'HCT $\geq 23 \mu\text{mol/l}$ and %CDT $\geq 3.75\%$ ' in men, while a lower specificity than women except for the assessment 'HCT $\geq 23 \mu\text{mol/l}$ '. Other relevant gender differences were not found (table 2). Multinomial logistic regression models did not show a significant interaction between each variable tested and gender (data not shown).

Table 2. Measures of the effectiveness of HCT (cutoff value 23.9 $\mu\text{mol/l}$), CDT (cutoff value 3.75%) and the combination of both parameters

	Sensitivity (CI)	Specificity (CI)	PPV	NPV	LR(+)	PPV (0.03)	NPV (0.03)
HCT $\geq 23 \mu\text{mol/l}$							
All	56.9 (42.2–70.7)	78.3 (70.4–84.8)	39.5	87.9	2.62	7.5	98.3
Female	42.9 (9.9–81.6)	75.9 (56.5–89.7)	30.7	84.2	1.78	5.2	97.7
Male	59.1 (43.2–73.7)	78.9 (70.0–86.1)	41.2	88.5	2.80	8.0	98.4
CDT $\geq 3.75\%$							
All	76.5 (62.5–87.2)	44.2 (35.8–52.9)	25.5	88.3	1.37	4.1	98.4
Female	57.1 (18.4–90.1)	62.1 (42.3–79.3)	27.4	85.3	1.51	4.5	97.9
Male	79.5 (64.7–90.2)	39.4 (30.2–49.3)	24.7	88.5	1.31	3.9	98.4
HCT $\geq 23 \mu\text{mol/l}$ or CDT $\geq 3.75\%$							
All	86.3 (76.6–95.9)	34.1 (26.0–42.1)	24.7	90.0	1.31	3.9	98.9
Female	71.4 (29.0–96.3)	44.8 (26.4–64.3)	24.5	86.3	1.29	3.8	98.1
Male	88.6 (75.4–96.2)	31.2 (22.7–40.8)	24.4	91.7	1.29	3.8	98.9
HCT $\geq 23 \mu\text{mol/l}$ and CDT $\geq 3.75\%$							
All	47.1 (32.9–61.5)	88.4 (81.9–93.2)	50.4	87.0	4.06	11.2	98.2
Female	28.6 (3.7–71.0)	93.1 (77.2–99.2)	50.9	83.9	4.14	11.4	97.7
Male	50.0 (34.6–65.4)	87.2 (79.4–92.8)	49.3	87.5	3.89	10.8	98.3

PPV (0.03) = PPV assessed by using 3% prevalence for alcohol withdrawal seizures, NPV (0.03) = NPV assessed by using 3% prevalence for alcohol withdrawal seizures.

Table 3. ROC curve analysis

		AUC	SE	CI
CDT	All	0.61	0.044	0.52–0.70
	Female	0.68	0.107	0.48–0.89
	Male	0.59	0.050	0.49–0.69
HCT	All	0.66	0.051	0.56–0.76
	Female	0.60	0.153	0.30–0.90
	Male	0.66	0.056	0.59–0.79
HCT+CDT	All	0.69	0.045	0.60–0.78
	Female	0.73	0.126	0.48–0.98
	Male	0.69	0.049	0.59–0.79

As we used the prevalence of AWS observed in a more general population of alcohol-dependent adults (3% according to Le Strat et al. [26]), the estimated PPV were very low, whereas the NPV diversified between 97.7 and 98.9% (table 2).

ROC curves showed a significant dominance for the combined assessment of HCT and %CDT (AUC = 0.69) versus %CDT alone (AUC = 0.61, $p = 0.03$). There was also a difference between HCT and %CDT combined (AUC = 0.69) versus HCT alone (AUC = 0.66), although this was not significant ($p = 0.40$) (tables 3, 4).

Table 4. Comparison of ROC curve analysis

Contrast	Difference	SE	CI	p
All patients				
CDT vs. HCT	–0.05	0.068	–0.18 to 0.09	0.5007
CDT vs. HCT+CDT	–0.08	0.038	–0.16 to –0.01	0.0302
HCT vs. HCT+CDT	–0.04	0.044	–0.12 to 0.05	0.4002
Female				
HCT vs. CDT	–0.08	0.198	–0.47 to 0.30	0.6716
CDT vs. HCT+CDT	–0.04	0.112	–0.26 to 0.18	0.6931
HCT vs. HCT+CDT	–0.13	0.137	–0.40 to 0.14	0.3493
Male				
HCT vs. CDT	0.07	0.075	–0.07 to 0.22	0.3351
CDT vs. HCT+CDT	–0.10	0.040	–0.18 to –0.02	0.0121
HCT vs. HCT+CDT	–0.03	0.048	–0.12 to 0.06	0.5471

Discussion

The aim of the study was to analyze possible predictive qualities of HCT, CDT and PRL regarding AWS in order to optimize the assessment of the individual risk of AWS in a retrospective analysis.

For %CDT we found the best cutoff value at 3.75% (sensitivity 76.5%, specificity 44.2%). This result is not in concordance with the result of a previous study that cal-

culated a best %CDT cutoff value at 5.4% (sensitivity 39%, specificity 88%) for alcohol-related seizures [11]. The authors of this investigation assert that %CDT only is unsuitable for use as a predictive marker for AWS. PRL showed no relevant predictive qualities and was therefore omitted in the further statistical analysis.

The correlation between HCT levels and the risk of first-onset alcohol withdrawal seizures has been described in several investigations [6, 7, 9, 27]. How HCT induces AWS is not exactly revealed. Hyperhomocysteinemia leads to an upregulation of the NMDA receptor system. Ethanol acts as an antagonist of the NMDA receptors. One current hypothesis for the genesis of AWS is that during detoxification amino acids like glutamate and HCT lead to overstimulation of the NMDA receptors, but on the other hand the antagonistic effect of ethanol decreases [5, 28]. Investigations showed decreasing plasma HCT levels during alcohol detoxification to normal levels several days after alcohol abstinence was achieved [5–7, 29]. The convulsant action of HCT is also shown in experimental animal studies. For example, HCT infusions generated tonic-clonic seizures in rats [30]. In other investigations it is shown that NMDA and non-NMDA antagonists have a protective effect against HCT-induced seizures [31, 32].

In our study best values were found for the combined assessment of HCT at a cutoff value of 23.9 $\mu\text{mol/l}$ and %CDT at a cutoff value of 3.75% (sensitivity 47.1%, specificity 88.4%). In that case the PPV is 50.4%, so for those patients the risk of suffering an AWS is fairly high. Using the prevalence for AWS of a more general population leads to higher NPV. It is of note that our patient population was formed by severe alcohol-dependent patients, but the prevalence of 3% according to Le Strat et al. [26] was assessed in a general alcohol-dependent population.

Therefore, the informative value of this result seems to be limited.

These results may be unsatisfactory for clinical use to predict AWS because of the high possibility of false-positive and false-negative findings. Identifying patients with a very low risk of AWS can represent a high importance in order to avoid subjecting these patients to unnecessary pharmaceutical therapy which can cause dangerous side effects. In this context the NPV attains more weight. Patients with a plasma HCT level below 23.9 $\mu\text{mol/l}$ and %CDT level below 3.75% have a low risk of developing an AWS (9.2%). Particularly in patients above these cutoff values, a prophylactic medication with an antiepileptic drug should be taken into account.

In addition to HCT and %CDT, it can be useful to include other markers for a better significance of appraising the risk of AWS. We made a retrospective analysis. Therefore, the interpretation of our results is limited and investigations of further biological markers of AWS, using a prospective design, are necessary in order to decrease type II error. In many studies ethyl glucuronide was discussed as a new marker of alcoholism, but a recent investigation showed no correlation between ethyl glucuronide and a history of seizures [33]. Recent reports assessed the predictive value of thrombocytes and HCT. Berggren et al. [34] showed a correlation between thrombocytopenia and the development of delirium tremens and seizures in alcohol withdrawal patients.

In conclusion, in spite of its retrospective nature, our investigation may support the hypothesis that assessing certain biomarkers in alcohol-dependent patients can be valuable in appraising the individual risk of AWS and in allowing individual therapy strategies. Further studies are necessary to increase the interpretability of our results.

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