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ROC analysis of alcoholism markers – 100% specificity

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Abstract A combination of 4 so-called markers of alcoholism, i. e. methanol, aceton + 2-propanol, γ-glutamyltransferase and carbohydrate deficient transferrin, was investigated in 341 blood samples from alcoholics and non-alcoholics. From the history of alcohol consumption, four defined subgroups were formed: non-alcoholics divided into (A) 33 persons with no ethanol consumption during the past year and (B) 60 persons with daily consumption less then 40 g ethanol. Alcoholics were divided into (C) 177 persons with no ethanol at the time of admission/first blood sampling (withdrawal therapy) and (D) 71 persons with positive ethanol levels on admission/first blood sampling. All markers showed different extents of overlap between the collectives of alcoholics and non-alcoholics. By logistic regression, a formula was developed combining these markers with different mathematical weights. Thus an "Alc-Index" could be calculated for each individual. The ROC curve connecting all individual values gives an ideal form with 100% specificity and nearly 93% sensitivity. The threshold between the collectives of alcoholics and non-alcoholics was defined by the Alc-Index value 1.7. This was associated with no false positives among the non-alcoholics while nearly 93% of the alcoholics exceeded this index. The ROC-based calculation of the Alc-Index thus seems to be the most effective method for the diagnosis of alcoholism.

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Introduction

A conviction for driving under the influence of alcohol often leads to confiscation of the driving licence. If the person involved is a serial offender or if there is otherwise suspicion of severe alcohol abuse (i.e. alcoholism), the status of this person must be re-evaluated before the driving licence can be returned. Such judgement is often carried out by a psychological examination alone although this is medical disease and there exist clinical and biochemical features to diagnose this disorder. Recent research has shown that alcoholism can be more reliably assessed by measuring blood levels of so-called biochemical markers of alcoholism, e. g. certain plasma levels of methanol (MeOH), the sum of acetone and 2-propanol (A + 2P), γ-glutamyltransferase (γ-GT) and carbohydrate deficient transferrin (CDT) are linked to chronic alcohol abuse [1, 2]. In the past years, several efforts have been made to distinguish between non-alcoholics and persons with different types and histories of alcohol abuse by applying markers in isolation or in combination [3].

We have evaluated the diagnostic significance of the aforementioned "markers of alcoholism" either in isolation or in different combinations. This set of markers has been applied to four defined collectives.

Materials and methods

Subjects analysed

A total of 341 individuals were investigated and all were volunteers who gave informed consent (Table 1).

Non-alcoholics

This sample was subdivided into 2 groups of which 33 persons (group A) were permanent residents in a psychiatric ward of a hos-

Table 1 Description of the non-alcoholic and alcoholic collectives

Group	Non-alcoholics (n = 99)		Alcoholics (n = 248)	
	A Permanent resident of a hospital n = 33	B Volunteers n = 60	C Administered to withdrawal, not acutely intoxicated n = 177	D Administered to withdrawal, acutely intoxicated n = 71
Description of ethanol consumption habit	0 g/day	max. 40 g/day	200–300 g/day	200–300 g/day
Male/female	25/8	27/33	165/12	62/9
Age range/median (years)	32–74/60	17–75/26	23–80/42	23–68/43

pital. These individuals had been resident for at least 1 year with no access to alcohol. Their state of health and the history of taking drugs was confirmed by evaluation of the medical records. The main diseases of these permanent residents were schizophrenia, psychosis and mental retardation, sometimes accompanied with hypothyreosis, heart insufficiency, disorder of fat metabolism and diabetes. They were under treatment with neuroleptics, benzodiazepines, anticholinergics and anticonvulsives.

A total of 60 healthy volunteers (group B) declared an alcohol consumption of usually less than 40 g per day (27 males and 33 females). Their drinking behaviour, the state of health and the question of drug intake was confirmed by interviews. No diseases and no therapeutic drug intake could be confirmed.

Alcoholics

The group classified as alcoholics consisted of 248 individuals who were undergoing withdrawal therapy and were under permanent clinical observation. The definition was based on a previous history of alcoholism over a mean time range of 6–15 years, a quantity of daily alcohol consumption varying between 200 and 300 g and accordance with the criteria for ICD 10. A further condition to be included into this study was a period of 3 weeks without abstinence before admission to the hospital. In addition to the alcoholism some patients had liver diseases, gastritis, degenerative diseases of the locomotor system, and hypertension. In some patients the alcoholism was associated with the abuse of opioids (heroin, methadone and tramadol), benzodiazepines and clomethiazol.

Since not all subjects were acutely intoxicated on admission and/or blood samples were taken up to 2 days after admission, the alcoholics were further subdivided into group (C) with 177 individuals who were not intoxicated on admission and/or at the time of blood sampling and group (D) including 71 individuals who were under the influence of alcohol at the time of blood withdrawal with alcohol levels ranging from 0.10 to 3.9‰; with a median of 1.03‰.

Markers of alcoholism

Blood samples of the 341 individuals were centrifuged and serum was stored at –20 °C directly after sampling. This serum was used for the determination of the following markers:

Methanol (MeOH)

Methanol is produced endogenously and can therefore be detected in blood with a normal concentration range of 0.5–1.0 mg/l serum. Elevated concentrations of methanol are therefore a strong indication of recent alcohol consumption [4]. Determination was performed using the head-space GC method.

Sum of acetone + 2-propanol (A + 2P)

Caused by a pathological metabolism, often in combination with hypoglycaemia, the levels of acetone and 2-propanol are more frequently elevated among alcoholics when compared to non-alcoholics [5, 6, 7]. Acetone and 2-propanol, which are in a redox-balance, were determined using the GC head-space analysis. The levels measured were combined to give a sum value.

γ-Glutamyltransferase (γ-GT)

γ-GT is an indicator for liver disease used in clinical diagnostics. It is an indicator for the induction of liver enzymes, which can also be elevated due to alcohol abuse [8] or medications. The determination was carried out enzymatically using photometric detection [9].

Carbohydrate deficient transferrin (CDT)

Transferrin is a transport protein for iron, with a carbohydrate side chain [10, 11]. Under normal conditions healthy individuals possess tetrasialotransferrin which contains four terminal sialic acid residues. During chronic alcohol consumption the carbohydrate side chains produced have a reduced number of sialic acid residues leading to the formation of carbohydrate deficient transferrins (CDT). The number of sialic acid residues present on the deficient molecule can vary but the main form is the diasialotransferrin [12]. CDT determination was carried out by an ELISA technique after separation of different isoforms with micro-anionic exchange columns (ELISA CDTect, Pharmacia, Uppsala) [13]. The calibration was carried out in a range up to 200 U/l and values over 200 U/l were above the cut-off. For the determination of CDT it is important to use either fresh serum or to store serum at –20 °C immediately after centrifugation [14].

Receiver operating characteristic analysis

The receiver operating characteristic analysis (ROC analysis) [15] was performed to assess the ability of the markers to differentiate between alcoholics and non-alcoholics by computing sensitivity and specificity for each possible cut point of the associated diagnostic tests. The results were recorded in the so called ROC curve. This was done univariately for the isolated markers and multivariately for a combination of the markers. This combination was performed via logistic regression analysis. All calculations were carried out using SAS software [16].

Results and discussion

Individual markers

Methanol (MeOH)

The elimination of MeOH is strongly suppressed by ethanol concentrations above 0.2–0.5‰. Elevated levels

Table 2 Medians and standard deviations (SD) of the four markers associated to four groups

	Non-alcoholics				Alcoholics			
Group	A		B		C		D	
Ethanol consumption habit	0 g/day		max. 40 g/day		200–300 g/day not acutely intoxicated		200–300 g/day acutely intoxicated	
	Median (range)	S.D.	Median (range)	S.D.	Median (range)	S.D.	Median (range)	S.D.
Methanol (mg/l)	0.6 (0–2.7)	0.7	3.0 (0–10.3)	2.9	2.5 (0–43.5)	7.8	22.4 (0.5–63.3)	12.3
Acetone + 2-propanol (mg/l)	3.7 (1.0–51.3)	8.9	2.8 (0.1–8.0)	1.4	27.8 (2.1–1211)	163.8	25.9 (3.2–599)	81.4
γ-GT (U/l)	13 (6–53)	12	10 (5–47)	8	46 (6–2348)	232	40 (10–1855)	235
CDT (U/l)	12 (6–33)	5	22 (6–35)	7	43 (8–200*)	165	52 (10–200*)	152

* cut off value for CDT (exceeded by 5 individuals of group C and 4 individuals of group D)

of MeOH in blood return to the normal concentration range within several hours up to one day after abstinence depending on the preceding MeOH level [17, 18, 19, 20, 21, 22, 23].

The levels of MeOH show group-related ranges and profiles of distribution (Table 2). In group A, they resemble the Gaussian type of distribution while in group B there is a bimodal distribution with a second peak in an elevated concentration range. In group C the distribution type is less defined and the range extends up to 45 mg/l. Group D shows an expressed block of cases in the range between 15 and 30 mg/l containing approximately 70%. In our opinion, group A reflects a true negative control. The second peak of group B would be explained by the maximum quantities of alcohol consumption allowed for the definition of this group; a male weighing 70 kg could then reach 0.7‰, so that some inhibition of methanol elimination would occur. The heterogeneous distribution in group C seems to reflect different stages of alcohol withdrawal while group D corresponds with the expectation, but two individuals with low values are difficult to explain. An elevated methanol concentration alone cannot be used to define alcoholism because recent continuous alcohol consumption can also cause elevated levels. At low concentrations, no discrimination can be made between alcoholics and non-alcoholics. However, concentrations above 10 mg/l seem to be indicative of pathological forms of alcohol consumption. The disadvantage of this marker is the rapid recovery in phases of abstention.

Sum of acetone and 2-propanol (A + 2P)

The distribution of this combination is much more heterogeneous (Table 2), e.g. non-alcoholics of group A showed a few values above 10 mg/l. These are associated with metabolic disorders, e.g. diabetes (two cases), fat metabolic disease (one case) and reduced food intake (one case). In group B non-alcoholic individuals generally showed lower concentrations, all in the range up to 10 mg/l. Group C and D alcoholic individuals showed a wide distribution with only a few cases below 10 mg/l and an pronounced peak between 10 and 30 mg/l.

In alcohol withdrawal, the symptoms observed showed positive correlation with acetone and 2-propanol levels, which normalise after 1 week of abstinence [24] But also a disturbance of the metabolic pathway due to diabetes, ketoacidosis or hunger can cause elevated levels which are not associated with alcohol consumption.

If disease-related outliers in group A are excluded then the upper threshold in non-alcoholics is around 10 mg/l.

γ-Glutamyltransferase (γ-GT)

In this study, the majority of the non-alcoholics showed γ-GT concentrations below 20 U/l while concentration profiles of groups C and D were very much in parallel (Table 2). In group A, elevated values were always associated with long term medication with neuroleptics or phenobarbital. Group B individuals had three observations above 30 U/l.

The γ-GT levels will increase only after long periods of alcohol abuse. Therefore short periods of alcohol abuse and/or relapses of alcoholics cannot be detected with this marker [25]. After complete abstinence from alcohol, the γ-GT levels return to normal within several weeks [26, 27]. The diagnostic use of γ-GT as a marker for alcoholism is also reduced in cases of liver disease, some medications or infections.

Although in groups C and D the history in the last days before sampling was different, the concentrations were very similar which underlines the usefulness of this as a "long-term memory" marker.

Carbohydrate deficient transferrin (CDT)

Similar to methanol, group A showed a Gaussian distribution with a clear peak at the interval of 10–20 U/l (Fig. 1, Table 2). The same type of distribution was also observed for non-alcoholics of group B where the peak was shifted to 30 U/l. This shift was obviously due to some alcohol consumption as stated.

Alcoholic groups C and D behaved more or less in parallel with around one-third of all values below 30 U/l. Again similar to γ-GT, the differences in the alcohol his-

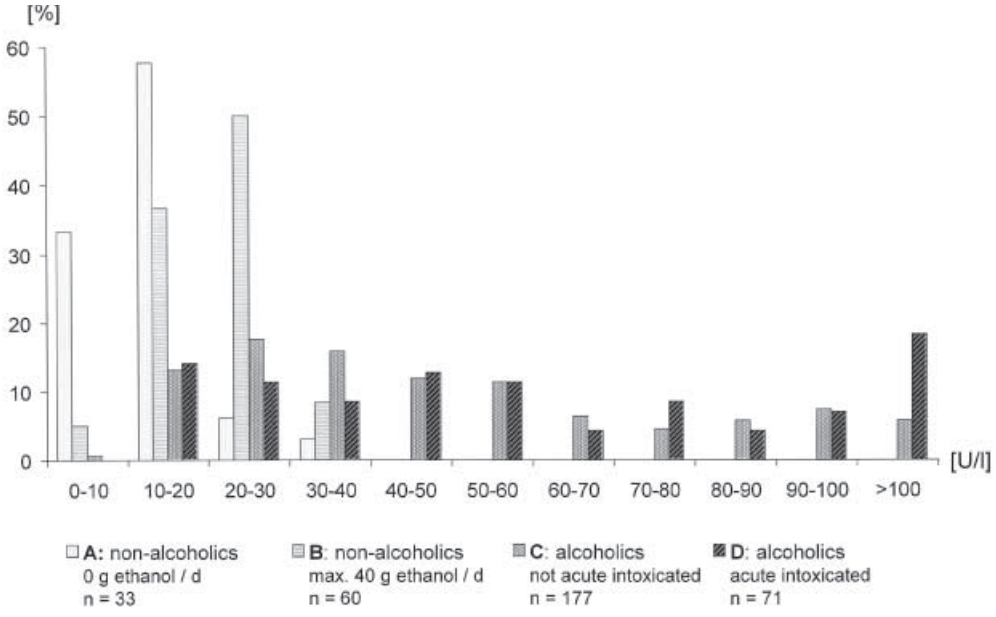

Fig. 1 Distribution of CDT activities (n = 341)

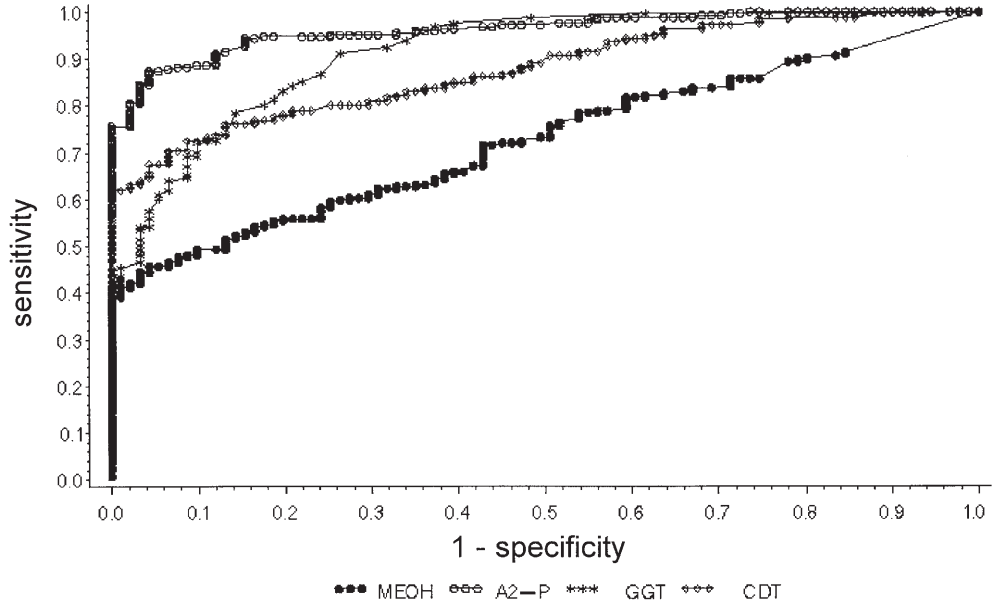

Fig. 2 Univariate ROC curves of the four markers (n = 339)

tory between both collectives apparently did not affect this marker.

A daily consumption of 60–80 g ethanol during a period of 7 days results in an increased CDT-level [28]. After abstinence the deficient forms will be slowly reduced and replaced by tetrasialotransferrin but the half-life is approximately 2 weeks. Disease-related increases of CDT are rare but exceptionally this can be due to liver disease (e.g. biliary cirrhosis and chronic active hepatitis) but there are also genetic variants of D-transferrin and an inborn error of glycoprotein metabolism [29]. CDT is now thought to be one of the most reliable markers of alcohol abuse [30].

Combination of markers

A method to increase the sensitivity in detecting alcoholics can be the combination of different markers. This combination was performed by a statistical evaluation applying two different statistical methods.

Discriminant score

For discriminant analysis ,a randomised distribution of the 341 individuals into two collectives was carried out. The first collective included about two-thirds of the individuals from each of the four defined groups (non-alcoholics A+B and alcoholics C+D) and was used to define

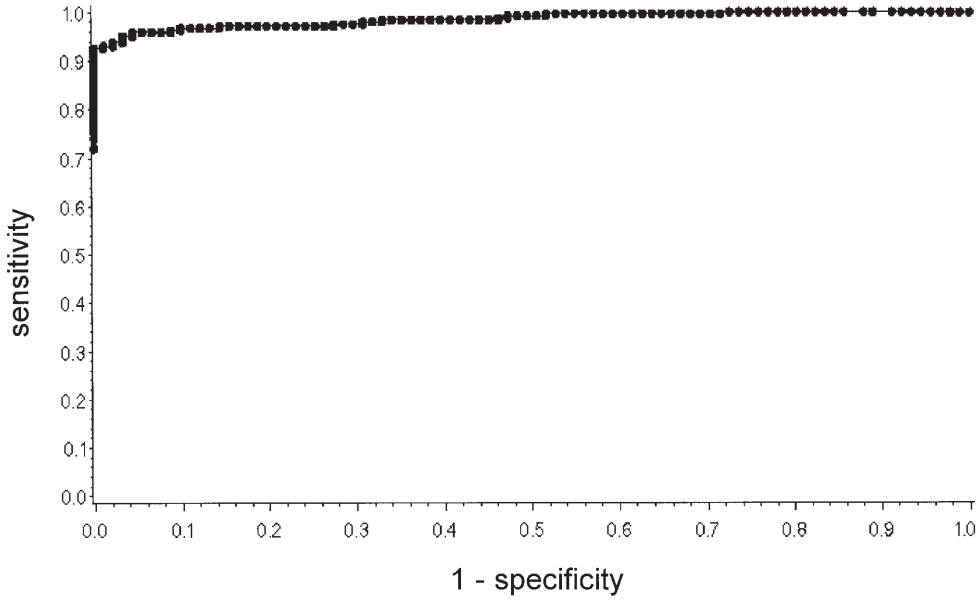

Fig. 3 Multivariate ROC curves of the four markers combined to an index by logistic regression analysis (n = 339)

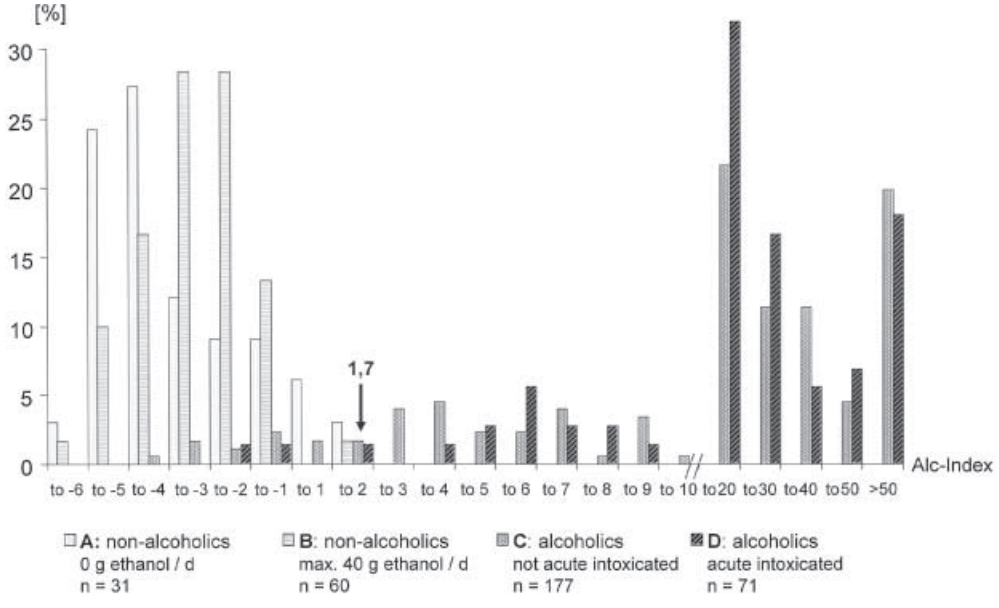

Fig. 4 Distribution of the Alc-indices (n = 339)

weighting factors. A discriminant score must be established to minimise within-group differences and to maximise between-group differences [31].

The generalised squared distance function calculated for these collectives was applied to the second collective which included about one-third (a total number of 106) of each of the four groups. The group identification was made for all four groups. No sample was identified as false positive, but out of the total number of 106 individuals, 13 alcoholics (12.3%) remained unidentified.

Receiver operating characteristic analysis (ROC analysis)

ROC analysis determines the relationship between sensitivity (percentage of correct positive results) and specificity (percentage of correct negative results). For each value the correlation of sensitivity and specificity was calculated and all values observed plotted in the form of so-called ROC curves. This was first performed univariately, as shown in Fig. 2. The plot shows the rate of false positives (i.e. 1–specificity) on the X-axis and of correct positives on the Y-axis. Two individuals from group A who were known diabetics were excluded from the ROC analysis. Within the ROC curves of the individual markers, A + 2P showed the best correlation between sensitivity and specificity, followed by γ-GT, CDT and MeOH (Fig. 2).

If the 4 markers are combined, the resulting multivariate ROC curve shows a more ideal line (Fig. 3). To plot this curve, a formula was developed by logistical regression, where [MeOH] and [A + 2P] are the concentrations

Table 3 Minima, maxima and medians of the Alc-indices in the four groups

	Non-alcoholics		Alcoholics	
Group Ethanol consumption habit	A 0 g/day	B max. 40 g/day	C 200–300 g/day not acutely intoxicated	D 200–300 g/day acutely intoxicated
Minima	–5.41	–5.05	–3.53	–1.77
Maxima	16.05	1.65	519.58	244.43
Median	–3.13	–2.38	18.03	18.27

in mg/l and [γ-GT] and [CDT] are the activities in U/l; factors related to each marker reflect the different statistical weights relative to specificity and sensitivity.

$$\text{Alc-Index} = 0.1121 \times [\text{MeOH}] + 0.4082 \times [\text{A} + 2\text{P}] + 0.0907 \times [\gamma\text{-GT}] + 0.1254 \times [\text{CDT}] - 7.7736$$

By using this approach a more ideal separation between alcoholics and non-alcoholics was achieved. By choosing an appropriate threshold point it is possible to obtain either maximum sensitivity or specificity.

Our aim was to obtain a high sensitivity and simultaneously no false positives (i.e. 100% specificity). The efficiency of this approach can be controlled when group-specific values are considered. All individuals of groups A and B showed Alc-Indices of less then 1.7 (Fig. 4). If the threshold value to distinguish alcoholics from non-alcoholics is defined as 1.7, all individuals of groups A and B would be classified as non-alcoholics. This value provides 100% specificity as there is no false positive identification. Applying the Alc-Index of 1.7 to groups C and D, 229 out of 248 alcoholics were diagnosed accordingly, i.e. a sensitivity of 92.7%. Among the alcoholics, who were not under the influence of alcohol when admitted, 16 out of 177 could not be identified as alcoholics. Among 71 of the alcoholics who were acutely influenced by alcohol only 3 could not be verified by this approach.

Conclusion

The application of single markers is disadvantageous because there always exist overlaps between the collectives of alcoholics and non-alcoholics. The single markers can also be influenced by recent ingestion of alcohol, by e.g. metabolic disorders or hunger (acetone and 2-propanol), infections and medications (γ-GT) and liver disease (γ-GT and CDT). Therefore one parameter alone should not be regarded as reliable even if the value is observed in "a safe" area of concentration.

By combining these four markers to form an index called the Alc-Index, the specificity and sensitivity of the test system was considerably improved (Fig. 4, Table 3). The Alc-Index represents one threshold instead of four separate thresholds for each single marker. It also avoids false conclusions drawn from pathology values of one marker in isolation. The advantage of this combined approach is that non-alcoholics are not at risk of being misdiagnosed as alcoholics. On the other hand it is extremely effective because only a few alcoholics will remain undetected.

Although we have included into our evaluation individuals with diseases, disorders and taking medications, we would strictly warn against the use of these markers in isolation. Although they have the highest evidential value, the diagnostic procedure should always include medical investigations to be complemented in doubtful cases by a psychological examination. Especially the risk of false positives would then seem to be avoided. Even if a metabolic disorder from another origin exists, this would not lead to false conclusions because a combination of markers is always evaluated.

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