INTRODUCTION

Early detection of alcoholism is vitally important as one-third of patients suffering from mental disorders have health problems caused by alcohol abuse (Jaff and Anthony, 2005). Alcohol dependence diagnostics can be carried out by means of history-taking, questionnaires and biochemical tests (Heather, 2001). The metabolism of ethanol is rather rapid in comparison to that of many other drugs; in fact, it is complete. Its products are indistinguishable from the metabolic products of food-stuffs and body energy sources. Therefore, it is essential to use markers indicating the consequences of alcohol abuse (Thom, 2001).

Laboratory pathology findings can generally not be detected with one disease only, e.g. alcohol addiction. However, no marker is known to be sufficiently specific and sensitive to diagnose chronic excessive alcohol intake (Javors et al., 2003). Carbohydrate-deficient transferrin (CDT) offers the best combination. Not as reliable but widely used in clinical practice are gama-glutamyltransferase (GGT), aspartate-aminotransferase (AST), alanine-aminotransferase (ALT) and erythrocyte mean cell volume (MCV) were assessed three times in 238 alcoholics admitted to hospital: on admission, after 24 h and after 7 days. Results: All the values were significantly higher than those in healthy persons. The fastest activity decrease was seen in GLDH. The kinetics of GLDH and AST were more applicable than GGT kinetics after a week, but GLDH kinetics were most reliable. GLDH was the most specific laboratory marker with almost 90% specificity. The sensitivity of combination MCV and GLDH kinetics after 1 week of abstinence was pathognomonic by 97.2%. This decision tree gave us a model with 84.5% accuracy. Conclusions: GLDH is an equally accurate marker of alcoholism in comparison to others, if its significantly faster decrease is taken into consideration. We strongly believe that watching changes in the activity of laboratory markers of alcoholism is an effective yet overlooked aid.

METHODS

Our research included those inpatient alcohol addicts who met the diagnostic criteria for alcohol addiction according to ICD-10 (1993) and DSM-IV (1994). The control group was selected from general practice healthy patients and blood donors. They

ASSESSMENT AND DETECTION

Glutamate Dehydrogenase as a Marker of Alcohol Dependence

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Abstract — Aims: The aim of this study was to examine glutamate dehydrogenase (GLDH) in the diagnostic combinations as a result of new findings. Methods: GLDH, gama-glutamyltransferase (GGT), aspartate-aminotransferase (AST), alanine-aminotransferase (ALT) and erythrocyte mean cell volume (MCV) were assessed three times in 238 alcoholics admitted to hospital: on admission, after 24 h and after 7 days. Results: All the values were significantly higher than those in healthy persons. The fastest activity decrease was seen in GLDH. The kinetics of GLDH and AST were more applicable than GGT kinetics after a week, but GLDH kinetics were most reliable. GLDH was the most specific laboratory marker with almost 90% specificity. The sensitivity of combination MCV and GLDH kinetics after 1 week of abstinence was pathognomonic by 97.2%. This decision tree gave us a model with 84.5% accuracy. Conclusions: GLDH is an equally accurate marker of alcoholism in comparison to others, if its significantly faster decrease is taken into consideration. We strongly believe that watching changes in the activity of laboratory markers of alcoholism is an effective yet overlooked aid.
were both male and female, aged 18–65. The excluding criteria were acute right heart failure, toxic circulatory failure, obstructive jaundice and severe respiratory insufficiency, acute viral hepatitis, severe acute intoxication (mushrooms), hypovolemic shock, liver metastases, hepatomas, abscesses, primary biliary cirrhosis and sclerosing cholangitis.

From among the control subjects, a group of ‘laboratory-healthy’ persons whose MCV, AST, ALT and GGT values were within the reference range were selected.

A blood sample was taken from every subject three times: on admission to hospital, after 24 h and again after 7 days. We took blood samples from the healthy control group only once as we did not expect any further changes and recurring blood tests would therefore be considered ethically disputable.

The serum activities of GLDH, AST, ALT and GGT were assessed in all tests for both groups. The MCV value, however, was defined only in the first test because of its rather slow return to normal after the cessation of drinking. The slower decrease kinetics of its value and high expenses were the reasons that CDT was assessed only once and merely for the alcoholics.

The number of days passed between the last consumption of alcohol and the first blood test was stated by means of history taking and clinical evaluation.

GLDH, AST, ALT, GGT and CDT were defined in serum, but MCV in blood. According to the recommendations of the International Federation of Clinical Chemistry (IFCC), AST, ALT and GGT were ascertained by means of reference procedures at 37 °C (Committee on Reference Systems for Enzymes, 2002a, 2002b), whereas CDT with an immunochemical method by means of Bio-Rad%CDT kit. MCV was determined with a blood test on Abbott Cell-DYN 610 and Mellet Schloesing Laboratories MS4 equipment. We defined the catalytic GLDH activity with new Deutsche Gesellschaft fuer Klinische Chemie (DGKC) (1992)method and a Dialab kit.

Statistical analysis was carried out by using SPSS 12.0.1 Windows analysis software. Frequencies, arithmetic mean, median, variance, standard deviation and standard error of the arithmetic mean, highest and lowest values, specificity and sensitivity, t-test, non-parametric Wilcoxon W, Mann–Whitney U in Komolgorov–Smirnov Z tests, Spearman’s rho, Friedman’s, Cochran’s tests and ROC (receiver-operating characteristic) curve were used. Reference values were determined by using the non-parametric method (90%).

As an additional aid to diagnostics, we also applied the system of intelligent data analysis, a decision tree model, which could perform millions of combinations from the entire database, including all or just one part of the data (Lenič et al., 2003).

The study was approved by the Slovenian Committee on Medical Ethics on 6 January 2004, no. 50/01/04.

### RESULTS

#### General data

The control group consisted of 245 healthy persons [199 men (81.2%) and 46 women (18.8%)] and 238 alcohol dependants [199 men (83.6%) and 39 women (16.4%)]. There were no statistically significant differences between the groups, \( P (c^2 \text{test}) = 0.631 \). The mean (SD) age of healthy persons was 44.9 (±11.9), and that of alcohol-dependent ones was 44.3 (±8.8) years. There were no statistically significant differences between groups, \( P (t\text{-test}) = 0.520 \).

The period since last consumption of alcohol in healthy subjects [6.92 (±7.77) days] was significantly longer than that of alcohol-dependent ones [3.3 (±4.81) days] with Mann–Whitney \( U = 2378.0 \), Wilcoxon W = 29,639, \( Z = -4.635 \), \( P < 0.0005 \).

Among healthy subjects, there were 149 (60.8%) teetotallers, 142 (57.96%) of them being physically and laboratory healthy.

#### Markers activity in healthy subjects and alcoholics

The description of CDT, MCV, AST, ALT, GGT and GLDH serum activity in both groups is shown in Table 1.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Status</th>
<th>( n )</th>
<th>CDT (%)</th>
<th>MCV (μL)</th>
<th>AST (μkat/L)</th>
<th>ALT (μkat/L)</th>
<th>GGT (μkat/L)</th>
<th>GLDH (nkat/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>Reference level</td>
<td>≤2.5</td>
<td>81–94</td>
<td>≤0.58</td>
<td>≤0.74</td>
<td>≤0.92</td>
<td>≤124.0</td>
<td></td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>107</td>
<td>–</td>
<td>92.71 ± 4.29</td>
<td>0.42 ± 0.18</td>
<td>0.52 ± 0.43</td>
<td>0.73 ± 1.25</td>
<td>38.99 ± 38.63</td>
<td></td>
</tr>
<tr>
<td>Alcoholics</td>
<td>199</td>
<td>3.49 ± 1.07</td>
<td>99.97 ± 5.00</td>
<td>1.28 ± 1.17</td>
<td>1.16 ± 1.07</td>
<td>5.68 ± 7.94</td>
<td>351.53 ± 439.00</td>
<td></td>
</tr>
<tr>
<td>Reference level</td>
<td>≤2.5</td>
<td>–</td>
<td>81–94</td>
<td>≤0.52</td>
<td>≤0.56</td>
<td>≤0.63</td>
<td>≤64.5</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>Healthy subjects</td>
<td>34</td>
<td>91.14 ± 4.25</td>
<td>0.32 ± 0.16</td>
<td>0.29 ± 0.34</td>
<td>0.28 ± 0.18</td>
<td>19.85 ± 20.49</td>
<td></td>
</tr>
<tr>
<td>Alcoholics</td>
<td>39</td>
<td>2.7 ± 1.01</td>
<td>98.97 ± 4.85</td>
<td>0.96 ± 1.07</td>
<td>0.73 ± 0.91</td>
<td>5.53 ± 5.22</td>
<td>296.08 ± 535.16</td>
<td></td>
</tr>
</tbody>
</table>

Reference levels: CDT (Bio-Rad%CDT kit); MCV (Osredkar, 2005); AST, ALT, GGT (Committee on Reference Systems for Enzymes, 2002a, 2002b); GLDH (Kravos and Malešič, 2008).

##### Changes of markers’ activities in the course of time

It was discovered that mean AST, ALT, GGT and GLDH serum activities diminished significantly \( P < 0.001 \) after the resumption of abstinence. See Fig. 3 for results. Only the data of those alcoholics whose blood had been taken in all three tests are included \( (n = 191) \).

Spearman’s non-parametric rho showed a statistically significant correlation between GGT and GLDH activity in all three tests; high correlation ranged from 0.363 to 0.869 \( (P < 0.01) \).

##### Markers’ specificity and sensitivity

GLDH reached the highest specificity in healthy subjects, whereas AST was slightly lower. Both enzyme activities are similar, most probably because of the same release mechanism.
from the mitochondria. The value of high MCV sensitivity is diminished by its low specificity, but GLDH specificity is comparable to that of other markers. Gradually, the level of GLDH, AST and ALT in alcoholics decreased statistically significantly (Table 2).

The sensitivity of serum GLDH kinetics was highest in the first 24 h after cessation of drinking; it was still rising after a week’s period, yet slightly more slowly than that of AST (Table 3).

**Table 2. Markers’ specificity and sensitivity in healthy subjects and alcoholics.**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Specitivity in healthy subjects</th>
<th>Sensitivity first test alcoholics</th>
<th>Sensitivity second test alcoholics</th>
<th>Sensitivity third test alcoholics</th>
<th>Cochran Q (for sensitivity)</th>
<th>df (for sensitivity)</th>
<th>P (for sensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDT</td>
<td>71.7%</td>
<td>61.8%</td>
<td>34.9%</td>
<td>74.193</td>
<td>2</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>86.1%</td>
<td>50.0%</td>
<td>40.1%</td>
<td>20.868</td>
<td>2</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>84.9%</td>
<td>75.9%</td>
<td>4.133</td>
<td>2.127</td>
<td>2</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>GGT</td>
<td>84.9%</td>
<td>72.9%</td>
<td>72.9%</td>
<td>2.0005</td>
<td>2</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>GLDH</td>
<td>89.8%</td>
<td>65.5%</td>
<td>34.4%</td>
<td>57.855</td>
<td>2</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. GLDH and AST sensitivity kinetics in alcoholics.**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Sensitivity I–II</th>
<th>Sensitivity I–III</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLDH</td>
<td>76.29%</td>
<td>81.25%</td>
</tr>
<tr>
<td>AST</td>
<td>68.10%</td>
<td>83.33%</td>
</tr>
</tbody>
</table>

Denotations I, II and III indicate first, second and third blood test.

**Marker combinations and decision tree induction method**

Various marker combinations used for laboratory diagnostics of alcohol dependence were assessed by including those 180 alcoholics (out of 238) whose data were available from all three tests. Pathological values of serum CDT, GGT, AST, MCV, GLDH and the decrease of GLDH activity between first and second and also first and third blood tests were taken into consideration; a positive difference between two blood tests (or an activity decrease) was estimated to be as pathological. In combinations with pathology tests, at least one out of two markers was determined to be diagnostically useful. Sensitivity by more than 95% was assumed as pathognomonic. For data, see Table 4.

A decision tree was built on the basis of learning set (n = 483), randomly divided into training set (second/third, n = 322) and testing set (first/third, n = 161). Every person was described with three markers: GLDH, MCV and GGT, without inclusion of AST and ALT, which were also available.

The decision tree proved to be very effective in diagnosing alcohol dependence, with a multi-method approach accurately
Fig. 3. Changes of markers’ activities with regard to time (Friedmann’s and Wilcoxon signed ranks non-parametric tests). AST (μkat/L) (far left), ALT (μkat/L) (middle left), GGT (μkat/L) (middle right), GLDH (nkat/L) (far right) ($P < 0.001$) where first box = first measurement, second box = second measurement, third box = third measurement. The distribution of activities is demonstrated with “box-and-whiskers plot” (central line indicates median, boxes first and third indicate the third part; handles first and ninth indicate the tenth part.

Table 4. Marker combinations

<table>
<thead>
<tr>
<th>Marker combination</th>
<th>No. with two pathological markers</th>
<th>No. with one pathological marker</th>
<th>No. without pathological marker</th>
<th>Combination sensitivity in percentage</th>
</tr>
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<tbody>
<tr>
<td>MCV, GLDH I-II</td>
<td>114</td>
<td>52</td>
<td>4</td>
<td>97.78</td>
</tr>
<tr>
<td>MCV, AST I-III</td>
<td>130</td>
<td>45</td>
<td>5</td>
<td>97.22</td>
</tr>
<tr>
<td>MCV, GLDH I-III</td>
<td>126</td>
<td>49</td>
<td>5</td>
<td>97.22</td>
</tr>
<tr>
<td>MCV, AST I-II</td>
<td>107</td>
<td>64</td>
<td>9</td>
<td>95.00</td>
</tr>
<tr>
<td>GGT, AST I-III</td>
<td>118</td>
<td>52</td>
<td>9</td>
<td>94.44</td>
</tr>
<tr>
<td>GGT, GLDH I-III</td>
<td>114</td>
<td>56</td>
<td>10</td>
<td>94.44</td>
</tr>
<tr>
<td>CDT, MCV</td>
<td>112</td>
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classified at 84.5%. More precisely: the sensitivity on the training set for alcohol dependants was 79.3% and the specificity for non-alcohol dependants 90.5%.

MCV was determined to be the leading parameter. Some precaution is needed in MCV evaluation because of its significantly low specificity. Caution in differential diagnostics of elevated MCV values is needed before final decisions about its cause. For the following course, see Fig. 4.

**DISCUSSION**

The mean age of 44.9 years for alcohol dependants was anticipated because of a relatively long period, at least 10 to 20 years, necessary for the development of disease. More than a quarter of alcohol dependants were admitted into the hospital under the influence of alcohol; they were evidently unable to give up drinking alone.

It was ascertained that GLDH, AST, ALT, GGT activities and the MCV value of alcoholics were significantly higher than those in healthy persons. The GLDH activities are high in comparison to those in the literature because the patient group consists of alcohol dependants. They were selected because we

wanted to include only clinically verified alcohol-dependent persons in order to exclude eventual heavy drinkers.

Increase in GLDH activity is particularly important. Only toxic effects that cause necrosis could release enzymes from the hepatocyte rough endoplasmic reticulum and mitochondria from where GLDH originates. Low alcohol consumption cannot cause such damage.
A similar decline was found in the activities of all markers (GLDH, AST, ALT and GGT) after 24 h, as well as after 7 days. The speed of restoring to reference levels was most rapid and explicit in GLDH but slightly less so in AST, ALT and GGT. A 24-h interval of alcohol abstention is sufficient for a reliable evaluation of the fall in GLDH activity, or even more when alcohol dependants had not drunk alcohol for 3–7 days.

We strongly believe that watching changes in the activity of laboratory markers of alcoholism is an effective yet overlooked aid in confirming the cessation of drinking. The kind of damage, half-life time and cell enzyme localization determine their changes in the plasma. After 24 h, it was found that normalization of GLDH activity was faster than that of AST; however, after 7 days, the normalization of AST activity proved to be slightly quicker. It can be concluded that serum mitochondrial enzyme activities return to normal sooner than the cytosolic ones (ALT), but GLDH normalizes even faster than AST. The difference most probably results from cytosolic AST, which is released even with minor cell damage. Furthermore, the fact that piridoxal phosphate was used for the determination of AST activity, which is not a standard practise in laboratories worldwide, should also be taken into consideration.

The kinetics of GLDH and AST is more applicable than GGT kinetics after a week’s cessation of drinking. Nevertheless, the GLDH kinetics is more reliable than that of AST as it is solely derived from the liver, in contrast to AST, which is not.

GLDH had the best specificity of all liver enzymes. AST had almost the same specificity, but it may originate from other tissues, which reduces its diagnostic value. Our results show that GGT specificity is comparable to others and considerably lower to GLDH.

We discovered 65.5% sensitivity in alcohol-dependent persons, rising to 72.2% in those who drank alcohol within 48 h prior to blood test (Kravos and Malešič, 2008). GLDH had the highest specificity of all nonspecific markers and with its comparable sensitivity to other liver enzymes is a fair aid in the screening and confirming of alcohol dependence.

Comparing accuracy as Stamm (Stamm et al., 1984a, 1984b) did, by summing up specificity and sensitivity, an almost identical accuracy between GGT and GLDH was discovered (GGT/GLDH = 162.2:162; by 200 as highest): an important new finding.

The ROC curve, combining specificity and Sensitivity of AST had equal and of ALT lesser importance to other markers in men; in women, it was the opposite. GGT activity in women, with its high area under the curve, was distinct from other markers in diagnostic accuracy. It could be concluded that GLDH is an equally accurate marker of alcohol dependence as, for instance, GGT or CDT; if the significantly faster decrease in its activity since last alcohol consumption in alcohol dependants is taken into consideration and not overlooked.

With almost 90% specificity, GLDH was found to be the most specific laboratory marker, but the combination of GLDH and AST reached the highest specificity, with just a little more than 82%. Combinations containing MCV and activities of liver enzymes (or containing markers from two different organs) reached the highest sensitivities. A combination MCV and CDT had an even higher sensitivity, but it was not comparable because we could not measure CDT in healthy subjects. MCV could be an important unspecific marker of alcoholism as it is present in all best combinations.

Marker combinations play an important role in the confirmation of alcohol abuse. Insufficient attention has been devoted to the kinetics of decreasing enzyme activities so far, with perhaps partial exception in Weill’s (Weill et al. 1989) study.

When combining two markers, by taking kinetics into consideration, the best results are reached with combinations of MCV (which has slow decrease kinetics) and GLDH kinetics. The sensitivity of combination MCV and GLDH kinetics after 1 week of abstinence was pathognomonic by 97.2%. Combinations of MCV value and GLDH kinetics at 24 h or 7 days could be considered pathognomonic.

A combination of MCV and AST kinetics at 7 days had, in 72.2% of alcoholics, both markers pathologic in comparison to 70% at MCV and GLDH kinetics at 7 days, but a second combination could be regarded as more reliable because of lesser AST specificity and the fact that it could originate not solely from the liver.

The decision tree offers a qualitatively different access to the diagnostic evaluation of laboratory findings and is different from common practice. It is designed to set up its own new borders and criteria on the basis of all data from healthy subjects and alcohol dependants, that is, in contrast to generally accepted or established reference values. Among a myriad of possibilities, we have chosen the decision tree with MCV, GGT and GLDH markers because of their simplicity, easy examination and their moderate cost. We estimate that we could exclude eventual co-morbid diseases or drug influences with three-marker diagnostics. This decision tree gave us a model with 84.5% accuracy. We discovered excellent specificity at 90% and very high sensitivity at almost 80%. We feel confident about the model’s reliability and simple application, because of its three-marker basis. The value of MCV is the leading step (branch) in the tree, which is very important. MCV rarely plays an important role in the confirmation of alcohol dependence, as it is primarily increased in many haematological diseases, which must also be taken into consideration in the valuation of our decision tree for alcohol dependants, as it was discovered that MCV had a relatively low specificity. The high accuracy of our classification model provides an opportunity to apply it as a helpful method in finding and diagnosing alcohol dependence in everyday practice, with our exclusion criteria and differential diagnostic cautions taken into consideration.

We strongly believe that monitoring changes in the activity of laboratory markers of alcoholism is an effective yet overlooked aid. After all, owing to fewer possibilities for laboratory control in the cessation of drinking since, for instance GGT needs at least 5 days and CDT 14 days for first drop in activity, but on the grounds of alcohol intoxication, it is impossible to evaluate addiction. From a time point of view, GLDH is an ideal marker of alcohol dependency, because its activity starts to decrease immediately after the last drink and activity decline lasts long enough.

We strongly believe that the application value of determining GLDH activity in serum is in combination with other markers. Direct biochemical supervision over the maintenance of abstinence from last drink could be performed by the normalization of the activity of particular markers: from the 1st to 2nd day by determining alcohol in blood, from the 1st to 7th day by GLDH, from the 5th to 10th day by GGT, from the 7th to 28th day by CDT and up to 3 months by MCV. Pathological values return to reference values if the alcohol-dependent person abstains.
GGT and CDT are still regarded as the most useful parameters in the control of abstinence, while GLDH as a marker of breaks in abstinence still has to be considered. Tests for GGT, AST, ALT, MCV and GLDH are simple, effective and significantly cheaper than CDT, as well as some newer and still not accepted as standard.

Therefore, we can consider it as an important additional marker of alcohol dependence. GLDH would be helpful, above all, in distinguishing drunken alcohol addicts from drunken regular drinkers, traffic participants and in forensic cases. For confirmation of alcohol addiction, the kinetics of serum GLDH and AST activities after cessation of drinking are particularly important.

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The ICD-10 Classification of Mental and Behavioural Disorders. (1993) Geneva: WHO.

