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The Diagnostic Potential of the Combined Determination of Serum Monoamine Oxidase and N-Acetyl-β-*D*-Glucosaminidase for Fibroproliferative Liver Diseases

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Summary: The simultaneous determination of the catalytic activities in serum of monoamine oxidase (EC 1.4.3.4) and N-acetyl-β-*D*-glucosaminidase (EC 3.2.1.30) was performed in patients with various non-liver diseases, acute hepatitis and fibroproliferative liver disorders (cirrhosis and fibrosis) and the predictive values of the positive (both activities are pathologically elevated) and negative test results (normal activity of monoamine oxidase and/or N-acetyl-β-*D*-glucosaminidase) were estimated. It was found that the incidence of the positive result is extremely low (0.024, 5/207) in patients suffering from a great variety of non-liver and liver diseases (except cirrhosis) but rather great in liver cirrhotic subjects (0.44, 18/41). A fraction of only 0.07 of liver fibrotic patients had a positive test result. Based on these data the estimated predictive value of the positive result for liver cirrhosis at a prevalence of 0.03 is 0.47. This value increases strongly with higher prevalence of cirrhosis (population preselected for chronic liver diseases). The negative predictive value for cirrhosis and the positive value for fibrosis are low. Thus, the probability of the presence of cirrhosis in patients with suspected chronic liver diseases is great in cases of abnormally high activities of both monoamine oxidase *and* N-acetyl-β-*D*-glucosaminidase. Negative test results (normal catalytic activities of one or both enzymes), however, do not prove the absence of liver cirrhosis and/or liver fibrosis.

Die diagnostische Leistungsfähigkeit der kombinierten Bestimmung der Monoaminoxidase und N-Acetyl-β-D-Glucosaminidase im Serum für fibroproliferative Lebererkrankungen

Zusammenfassung: Die gleichzeitige Bestimmung der katalytischen Aktivitäten im Serum von Monoaminoxidase (EC 1.4.3.4) und N-Acetyl-β-*D*-Glucosaminidase (EC 3.2.1.30) wurde bei Patienten mit verschiedenen Nicht-Lebererkrankungen, akuter Hepatitis und fibroproliferativen Lebererkrankungen (Lebercirrhose und -fibrose) durchgeführt und die Vorhersagewerte des positiven (beide Enzymaktivitäten pathologisch erhöht) und negativen Testergebnisses (normale Aktivität der Monoaminoxidase und/oder N-Acetyl-β-*D*-Glucosaminidase) wurden geschätzt. Die Ergebnisse zeigen, daß die Häufigkeit des positiven Ergebnisses extrem niedrig (0,024, 5/207) ist bei Patienten mit sehr verschiedenen Nicht-Leber- und Lebererkrankungen (ausgenommen Lebercirrhose), aber groß ist bei lebercirrhotischen Patienten (0,44, 18/41). Ein Anteil von nur 0,07 der leberfibrotischen Patienten hat ein positives Testergebnis. Basierend auf diesen Werten beträgt der geschätzte Vorhersagewert des positiven Ergebnisses 0,47 für Lebercirrhosen, wenn deren Prävalenz mit 0,03 angenommen wird. Dieser Wert nimmt mit größerer Prävalenz der Lebercirrhose (für chronische Lebererkrankungen präselektionierte Population) stark zu. Der negative Vorhersagewert für Cirrhose und der positive Wert für Fibrosen sind niedrig. Somit ist die Wahrscheinlichkeit des Vorliegens einer Lebercirrhose bei

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Patienten mit vermuteten chronischen Lebererkrankungen im Falle pathologisch erhöhter Monoaminoxidase *und* N-Acetyl-β-*D*-Glucosaminidase-Aktivitäten hoch. Negative Testergebnisse (normale katalytische Aktivität eines der oder beider Enzyme) schließen jedoch eine Lebercirrhose und/oder Leberfibrose nicht aus.

Introduction

Although there has been a substantial increase in recent years in our knowledge of the metabolism and structure of various connective tissue components, such as collagen (1–3), proteoglycans (4), and structural glycoproteins (2–5) in normal and fibrotic liver, the pathobiochemical mechanisms of intercellular excess deposition and molecular decomposition in chronically injured liver parenchyma are still not fully understood (3, 6, 7). The largely unknown pathogenesis of the fibrotic transition of the diseased liver, the chronicity of the fibrotic process and the ubiquitous distribution of the connective tissue components in the body might be the main reason why, despite intense efforts, the search for clinically reliable, organ- and disease-specific and sensitive biochemical parameters of fibrotic liver diseases (fibrosis and cirrhosis, respectively) has so far been rather unsuccessful. Among several parameters of collagen and proteoglycan/glycoprotein metabolism (4, 8, 9) the determinations of the activities in serum of monoamine oxidase (EC 1.4.3.4) (10–13) and N-acetyl-β-*D*-glucosaminidase (EC 3.2.1.30) (14–19) have been proposed as potential useful diagnostic tools. During the evaluation of their diagnostic validity (20, 21) it was found that the combined determination of both enzymes can be of help in substantiating liver cirrhosis.

Materials and Methods

Specimen collection

The activities of monoamine oxidase and N-acetyl-β-*D*-glucosaminidase were measured in sera of 114 patients with a great variety of non-liver diseases (including malignant and benign tumours, diseases of the gastro-intestinal tract and cardio-vascular system, diabetes mellitus, dermatological and neurological disorders and various, unspecified forms of non-liver diseases), in sera of 51 patients with non-fibrotic liver diseases (including cholecystitis, acute viral hepatitis, chronic active hepatitis, chronic persistent hepatitis) and 83 patients with fibrotic (fibrosis, cirrhosis) liver diseases. The subdivision of chronic and acute liver diseases is based on the results of clinical investigation, liver function tests and histologic examination of liver biopsy specimens, respectively, as reported elsewhere (20).

Determination of monoamine oxidase activity (EC 1.4.3.4) in serum

Enzyme catalytic activity was measured in serum either instantly after venous blood collection or, if not possible, in sera which were deep-frozen for 5 to 8 days to avoid loss of catalytic activity (22). The colorimetric procedure of *Ono* et al. (23) including modifications as described was applied (22). The analytical reliability of the test has been reported (22).

Determination of N-acetyl-β-D-glucosaminidase activity (EC 3.2.1.30) in serum

In principal, the procedure of *Findlay* et al. (24) using *p*-nitrophenyl-β-*D*-glucosaminide as substrate was followed. Some modifications incorporated into the test and analytical criteria have been described recently (21).

Statistical analyses

Estimates of the diagnostic criteria of the combined use of monoamine oxidase and N-acetyl-β-*D*-glucosaminidase, i.e. diagnostic sensitivity, specificity, positive and negative predictive values, are based on definitions (25) summarized in more detail previously (20, 21). A positive test result is defined as pathologically elevated catalytic concentrations of both monoamine oxidase (> 630 U/l) and N-acetyl-β-*D*-glucosaminidase (> 32 U/l). A negative result is given if the catalytic concentrations of monoamine oxidase (≤ 630 U/l) and/or N-acetyl-β-*D*-glucosaminidase (≤ 32 U/l) do not exceed the upper limit (+2s) of the reference range.

Results

Correlation of the catalytic concentrations of monoamine oxidase and N-acetyl-β-D-glucosaminidase in sera of patients with fibrotic liver diseases and non-liver disorders

The majority of patients (89/144) suffering from non-liver and nonfibrotic liver diseases had both normal monoamine oxidase (≤630 U/l) and N-acetyl-β-*D*-glucosaminidase (≤32 U/l) (fig. 1a). Pathologically elevated catalytic concentrations of N-acetyl-β-*D*-glucosaminidase (46/144) were far more frequent than abnormally high catalytic concentrations of monoamine oxidase (11/144). It is noteworthy that the fraction of patients having both elevated catalytic concentrations of monoamine oxidase and N-acetyl-β-*D*-glucosaminidase was found to be very small (0.014, 2/144). In all cases of patients afflicted with acute viral hepatitis catalytic concentrations of monoamine oxidase were within the normal range, but elevated catalytic concentrations of N-acetyl-β-*D*-glucosaminidase were noted in a fraction of 0.71 (15/21) (fig. 1b). Thus, in contrast to the latter enzyme, catalytic concentrations of monoamine oxidase in serum obviously are not influenced by the process of liver cell necrosis.

In the group of liver fibrosis the proportion of patients having pathologically increased catalytic concentrations of N-acetyl-β-*D*-glucosaminidase (0.50, 21/42) was found to be quite larger than that with abnormal catalytic concentrations of monoamine oxidase (0.17, 7/42) (fig. 1c). Even among liver fibrotic

subjects the incidence of abnormally elevated catalytic concentrations of both enzymes was very low (0.07, 3/42). In contrast to the patients with non-liver diseases, non-fibrotic liver disorders and liver fibrosis, a quite large fraction (0.44, 18/41) of subjects suffering from liver cirrhosis had both pathologically elevated catalytic concentrations of monoamine oxidase and N-acetyl-β-*D*-glucosaminidase (fig. 1d). Abnormally high catalytic concentrations of monoamine oxidase (0.56, 23/41) occured less frequently than elevated catalytic concentrations of N-acetyl-β-*D*-glucosaminidase (0.71, 29/41). Normal catalytic concentrations of both enzymes were found in only 7/41 patients with liver cirrhosis. Compared with data shown in figure 1c the transition of liver fibrosis to cirrhosis is accompanied by a considerable increase in the fraction of patients having both abnormal catalytic concentrations of monoamine oxidase and N-acetyl-β-*D*-glucosaminidase, which is mainly due to an increase in the proportion of pathologically elevated catalytic concentrations of monoamine oxidase among liver cirrhotic subjects. In patients suffering from a great variety of non-liver and liver diseases (except liver cirrhosis) the fraction with pathologically elevated catalytic concentrations of both enzymes is very small (0.024, 5/207), but this fraction is nearly 20 times greater (0.44) in the population of cirrhotics.

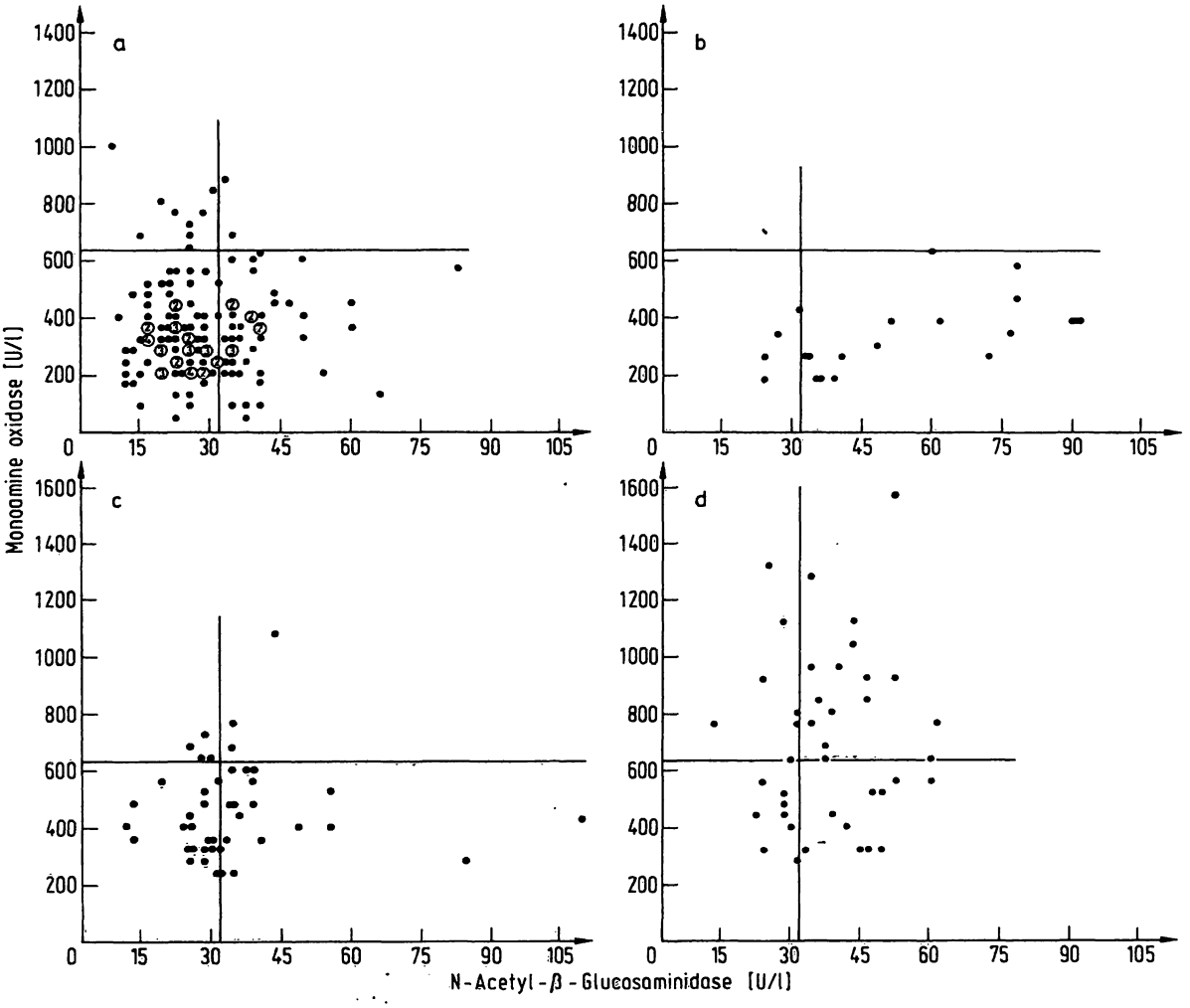

Fig. 1. Correlation of the catalytic concentrations of monoamine oxidase and N-acetyl-β-*D*-glucosaminidase in sera of patients with miscellaneous non-liver and non-fibrotic liver diseases (a, n = 144, r = 0.022), acute viral hepatitis (b, n = 21, r = 0.519), liver fibrosis (c, n = 42, r = −0.031), and liver cirrhosis (d, n = 41, r = 0.048).
Numbered circles in a indicate the cases with identical catalytic concentrations of both enzymes. The diagrams are subdivided by lines marking the upper "normal" limit (+2s value) of catalytic concentrations of both enzyme activities.

Predictive values of the combined determination of monoamine oxidase and N-acetyl-β-D-glucosaminidase for liver fibrosis and cirrhosis

The results described above suggest that the simultaneous determination of monoamine oxidase and N-acetyl-β-*D*-glucosaminidase might provide an aid in the diagnosis of liver cirrhosis. Therefore predictive values of the positive (pathologically elevated activities of both enzymes) and negative test result (normal activities of one or both enzymes) were estimated with respect to a reference population includ-

ing non-liver and liver diseases (except fibrosis and cirrhosis). As anticipated from the data in figure 1d and documented in figures 2 and 3, predictive values of the positive result are much better than those of the negative test result. Based on a prevalence of chronic liver diseases (including cirrhosis and fibrosis) of 0.03 in West-Germany (26), the positive predictive value is estimated to be 0.34 (sensitivity 0.20, specificity 0.988) for fibrosis and cirrhosis (fig. 2). For liver cirrhosis alone (fig. 3) the respective predictive values is 0.47 (sensitivity 0.341, specificity 0.988). Clearly, the predictive values improve strongly among patients selected for chronic liver disorders (figs. 2, 3). Thus, at a prevalence of cirrhosis of only 0.20 the probability of the presence of liver cirrhosis in the case of a positive test result is greater than 0.85 (fig. 3).

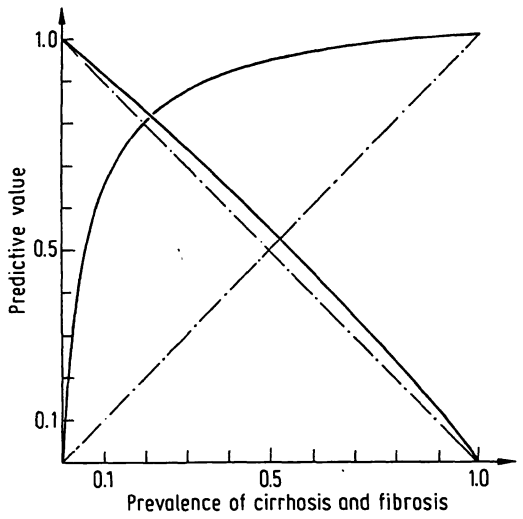

Fig. 2. Predictive values of the positive (both monoamine oxidase and N-acetyl-β-*D*-glucosaminidase are pathologically elevated) and negative test result (one or both enzymes are within the reference range) as a function of disease prevalence (liver cirrhosis and fibrosis). The reference population consists of patients with various non-liver and non-fibrotic liver diseases including cases with acute hepatitis. —·—·— indicates predictive values of a hypothetical test, in which the decision between positive and negative result is made by coin tossing (further explanation in results).

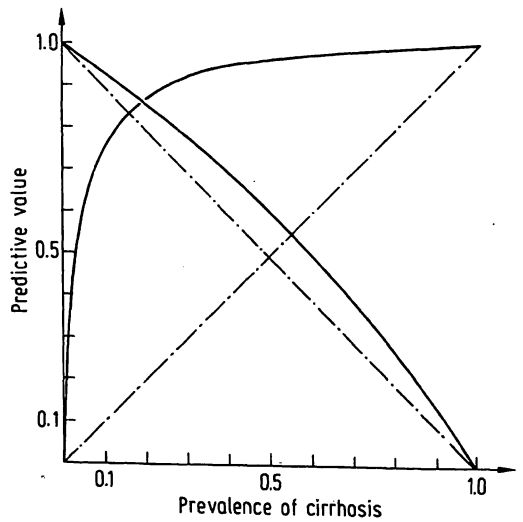

Fig. 3. Predictive values of the positive and negative test result, defined as in fig. 2, as a function of disease prevalence (liver cirrhosis). Reference population and definition of a hypothetical test (—·—·—) are described in fig. 2.

In relation to the positive predictive values of a hypothetical test, in which the decision between positive and negative result is made by coin tossing (the worst of all tests which do not give systematically wrong results, dotted lines in figs. 2 and 3), the maximal gain of information obtainable by the determination of the catalytic concentration of monoamine oxidase and N-acetyl-β-*D*-glucosaminidase, i.e. maximum difference between the curve and dotted line in direction of the y-axis (fig. 3), is about 67 per cent points for liver cirrhosis.

Discussion

The data provided in this study clearly document that, in comparison with N-acetyl-β-*D*-glucosaminidase, monoamine oxidase has a greater diagnostic specificity for liver cirrhosis. However, the former enzyme proves to have a better diagnostic sensitivity than the latter. The simultaneous determination of both enzymes in patients with suspected chronic liver diseases is suggested as a diagnostic aid for substantiating the presence of liver cirrhosis. The predictive value for this disease reached by the positive test result (0.47 at a prevalence of disease of 0.03) is higher than that obtained by the single determination of monoamine oxidase (0.30) (20) and far greater than that of N-acetyl-β-*D*-glucosaminidase (0.07) (21). Since the predictive values increase strongly with higher prevalence of cirrhotic subjects (fig. 3) the validity of a positive test result for cirrhosis becomes quite great in a population selected for hepatological disorders. However, the fact that the predictive values of negative test results are low should not be ignored, i.e. normal catalytic concentrations of monoamine oxidase and/or N-acetyl-β-*D*-glucosaminidase do not prove the absence of liver cirrhosis. Furthermore, the combined determination of both enzymes does not help in the diagnosis of liver fibrosis, due chiefly to the low incidence of pathologically elevated monoamine oxidase in this category of diseases. In conclusion, the probability of the presence of liver cirrhosis is high if the catalytic concentrations of both monoamine oxidase and N-acetyl-β-*D*-glucosaminidase are pathologically elevated in patients with suspected chronic liver diseases (population preselected for cirrhosis). The combined determination of both enzymes is of no use for the early detection of fibroproliferative liver diseases.

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