Selected Topics in Clinical Enzymology

Editors
D. M. Goldberg · M. Werner

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Editors-In-Chief
David M. Goldberg · Mario Werner

Associate Editors
Erich Kaiser · Hans-Jörg Gibitz

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David M. Goldberg, M.D., Ph.D.
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CORRELATIONS AND FACTOR ANALYSIS OF ENZYMES IN SERUM OF PATIENTS WITH HEPATIC METASTASES

Heiko Assel, Jörg Fedderke, Ellen Schmidt and Stefan Voges

Department of Gastroenterology and Hepatology, Centre for Internal Medicine and Dermatology, Medical School, Karl-Wiechert-Allee 9, D 3000 Hannover 61, Federal German Republic

Abstract

Determination of correlations and factor-analysis make it possible to summarize and interpret parameters of clinical chemistry. These methods were used to obtain knowledge about enzyme changes in serum of patients with liver metastases.

In a retrospective study we investigated 653 enzyme patterns of 508 patients. Clinico-chemical parameters were correlated. We were able to extract four factors representing liver cell damage, cholestasis, extent of tumor growth in the liver, and disturbance of liver function. The results are in good accordance with existing pathophysiological concepts.

Introduction

The diagnosis of hepatic metastases is important as involvement of the liver determines therapy and prognosis. In most cases, clinico-chemical parameters already indicate the existence of metastases. Enzyme patterns in serum typically show liver cell damage, cholestasis, and disturbances of liver cell function. Determination of correlations and factor analysis provide knowledge about the behavior of enzymes in malignant liver disease.

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Materials and Methods

In a retrospective study, 653 enzyme patterns of 508 patients with liver metastases were investigated. The determinations of enzyme activities in serum were made by Test-Combinations of Boehringer. The patient data were evaluated via punched cards by means of the SPSS-Programme (1). Correlations were calculated by the formula of Pearson.

Factor analysis

Many laboratory findings show correlations which are caused by fundamental similarities which can be extracted by factor analysis. We calculated factor loadings from correlation coefficients using orthogonal factor analysis with Varimax-rotation (2,3,4).

The following parameters were investigated: aspartate aminotransferase, EC 2.6.1.2 (GOT); alanine aminotransferase, EC 2.6.1.1 (GPT); glutamate dehydrogenase, EC 1.4.1.3 (GLDH); lactate dehydrogenase, EC 1.1.1.27 (LDH); alkaline phosphatase, EC 3.1.3.1 (ALP); γ-glutamyl-transferase, EC 2.3.2.2 (GGT); cholinesterase, EC 3.1.1.8 (CHE); bilirubin; Quick prothrombin test; and the liver weight at biopsy. Data were correlated only when determined within the last month of life.

Results

GGT was pathological in 88% of all cases, and was increased more than 10-fold in one-third. ALP was elevated in 79% of the patients; only in 5% was activity higher than 10 times above normal. Hyperbilirubinaemia occurred in 40% of the cases. GOT, with 63.5%, showed pathological values more frequently than GPT with 55%. The behavior of LDH was

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similar to that of GOT. An increase of GLDH was observed in 51.5% of the patients. CHE was decreased in 62% of the patients, but increased in 3%.

Determination of correlations led to highly significant coefficients of more than 0.5 between the following:

GOT - GPT	GPT - GLDH
ALP - GGT	ALP - bilirubin

Liver weight had the highest correlation to GOT (0.484) and GGT (0.483). These, too, were highly significant. Between CHE and the Quick test, the coefficient was only 0.3470.

TABLE 1. Varimax Rotated Factor Matrix for Ten Parameters in 508 Patients with Liver Metastases

	Factor 1	Factor 2	Factor 3	Factor 4
GOT	0.517	0.314	0.404	0.345
GPT	0.971	0.153	0.171	-0.001
GLDH	0.613	0.239	0.004	0.105
LDH	0.088	0.123	0.362	0.122
ALP	0.208	0.779	0.048	0.240
GGT	0.221	0.817	0.335	-0.044
CHE	0.060	-0.023	-0.177	-0.557
Bilirubin	0.334	0.420	-0.099	0.476
Quick test	-0.124	-0.081	-0.041	-0.606
Liver weight	0.232	0.166	0.802	0.071

By factor analysis, four factors were extracted: GOT, GPT and GLDH have the highest vector weightings for Factor 1 which represents liver cell damage. GGT, ALP and bilirubin prevail among vector weightings for Factor 2 which can be interpreted as an indicator for cholestasis. Because liver weight contributes much more than the other parameters to Factor 3, this

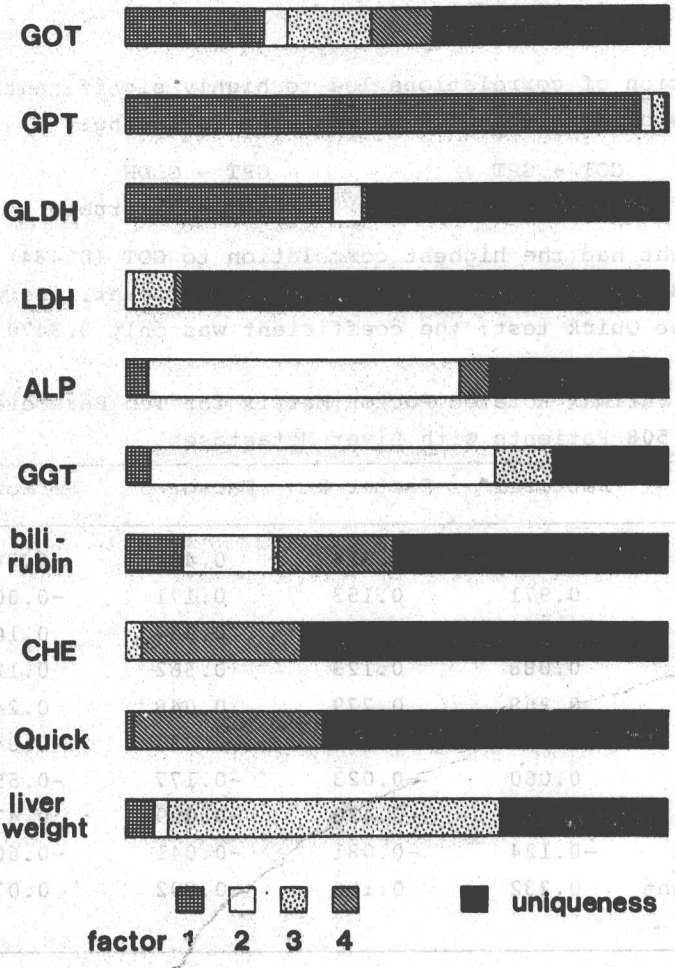

Fig. 1. Variance splitting of ten parameters among four factors described in text, and uniqueness attributable to each parameter.

could be a measure of the extent of hepatic metastases. The Quick test, CHE and bilirubin have the highest weightings for Factor 4 which indicates liver cell function.

If vector weightings are squared, the percentage of variance of every parameter is obtained due to the Factor concerned. Variance splitting of the investigated parameters is shown in Figure 1. For example, 26.7% of the variance of GOT can be referred to Factor 1, 4.5% to Factor 2, 16.3% to Factor 3 and 11.9% to Factor 4.

The variance that cannot be explained by the extracted Factors is called uniqueness. GPT has the lowest uniqueness, and LDH the highest. It is evident that liver cell damage is very important for the variance of GPT, whereas the variance of GOT is influenced also by Factor 3 (extent of hepatic metastasis), Factor 4 (liver cell function), and unknown factors (uniqueness).

It is interesting that LDH does not appear with any variance in Factor 1. The variance of bilirubin is divided between Factor 2 (cholestasis) and Factor 4 (liver cell function).

Discussion

Enzyme patterns in serum of patients with hepatic metastases typically show a combination of liver cell damage, cholestasis and disturbances of liver cell function (5,6). Our results show significant correlations between parameters of liver cell damage (GOT, GPT, GLDH) as well as between parameters of cholestasis (ALP, GGT, bilirubin). It seems to be typical for liver metastases that LDH does not correlate with GOT, GPT and GLDH. This is confirmed by factor analysis because it does not show any variance of LDH due to liver cell

damage. Remarkably, 83.9% of the variance of LDH cannot be explained by the extracted Factors (uniqueness); on the other hand, the uniqueness of GPT is only 0.4%. Altogether we can refer 55.04% of the variance of all investigated parameters to the four extracted Factors. Among the variance that can be explained, Factor 2 (cholestasis) has a relative importance of 29.3%, Factor 1 (liver cell damage) 28.3%, Factor 4 (liver function) 22.0%, and Factor 3 (extent of hepatic metastasis) 20.4%.

Kubale et al (7) applied factor analysis in acute viral hepatitis and found a relative importance of 52.9% for liver cell damage, 21.78% for cholestasis, and 16.35% for liver function. The results we have obtained are in good accordance with existing pathophysiological concepts.

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Please address correspondence to:

Dr. H. Assel, Abteilung für Gastroenterologie und Hepatologie, Zentrum für Innere Medizin und Dermatologie, Medizinische Hochschule Hannover, Karl-Wiechert-Allee 9, D3000 Hannover 61, Federal German Republic

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SPECIFIC ISOZYME PROFILES OF ADENOSINE DEAMINASE IN COLORECTAL ADENOCARCINOMA

Joop ten Kate, Juul T. Wijnen, Elly Herbschleb-Voogt, P. Meera Khan

Department of Human Genetics, Sylvius Laboratories, University of Leiden, Wassenaarseweg 72, 2333 Al, Leiden, The Netherlands

Gerrit Griffioen

Department of Gastroenterology and Hepatology, University Medical Center, Rijnsburgerweg 10, 2333 AA, Leiden, The Netherlands

and Fré T. Bosman

Department of Pathology, Faculty of Medicine, University of Leiden, Wassenaarseweg 62, 2333 Al, Leiden, The Netherlands

Abstract

Two broad groups of adenosine deaminase (ADA) isozymes are known to occur in man. The smaller of about 35K is called ADA-S. The ADA-S combines with another gene product called adenosine deaminase complexing protein (ADCP) to form several species of large ADA, or ADA-L of 300K and higher.

The activity and isozyme profiles of ADA in tumors and adjacent normal mucosa were investigated in nine patients with colorectal adenocarcinomas. The ADA was quantified by RIA and activity was measured by spectrophotometry. The isozymes of ADA were separated by Sephadex G-200 superfine column chromatography, gel electrophoresis, and IEF.

The tumors exhibited an overall increase of ADA. Their relative amounts of ADA-L appear to be proportional to the degree of differentiation of the tumors. The results of

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this preliminary study indicate that starch gel electrophoresis can replace column chromatography as a screening procedure for the identification of colorectal adenocarcinoma by specific changes in the ADA isozyme profiles.

Introduction

The enzyme adenosine deaminase (ADA; E.C. No. 3.5.4.4) is known to occur as isozymes (1). The so-called red cell ADA, a monomer in its functional form, has a molecular weight of 35K and is determined by a gene on chromosome 20 in man. This small molecular weight form of ADA is called ADA-S. The large ADA, or ADA-L, is a product of combination of ADA-S with the adenosine deaminase complexing protein (ADCP) which is coded for by a gene on chromosome 2 in man (2). The proportion of ADA-L to ADA-S is a reflection of the amount of ADCP, capable of binding to ADA-S, present in the tissue.

Nishihara et al (3) observed that free ADCP, usually present in considerable amounts in normal lung tissue, was absent in an extract of lung cancer tissue. Even though the normal human kidney is a major source of ADCP, Trotta and Balis (4) reported that ADA-L was absent in the tissue of clear-cell carcinoma of kidney. In the same report they presented evidence for the occurrence of tumor-specific variants of ADA in several specimens of colorectal adenocarcinoma. The overall ADA activity in the tumors was found to be significantly more than that observed in the normal adjacent tissue. They found that greater than 80% of the enzyme in the adjacent normal tissue was ADA-L in all the 12 patients investigated. On the other hand, in the tumor tissue the ADA-L was found to be considerably decreased, and in several cases they observed the appearance of a neo-isozyme with a molecular weight of 70K which we call the ADA-intermediate (ADA-I).

Here we present the preliminary results of our investigations on adenosine deaminase in the tumors and adjacent normal tissues of nine thoroughly investigated and well-documented cases of colorectal adenocarcinoma.

Materials and Methods

Collection of the specimens

The colonic tumor and the normal-looking adjacent tissue for enzyme studies were secured from surgical material and transported on ice to the Laboratory of Pathology immediately after resection. The tissues were cleaned by repeated gentle washes in 0.9% NaCl solution. The tumors were scraped gently but firmly to allow collection of specimens rich in tumor cells. Similarly the normal tissue was scraped to collect mucosal cells. These specimens were stored at -80°C (Forma-biofreezer, Ohio, USA) for five to 11 months. Carcinomas were classified and graded according to Morson (5) and Dukes (6).

Preparation of the tissue homogenates

Just before column chromatography each specimen was homogenized with a Potter-type Teflon glass homogenizer in a buffer at pH 7.0 containing 0.05 mol/l Imidazole-HCl, 0.1 mol/l NaCl, 15% sucrose, and 0.02% sodium azide.

The homogenate was centrifuged at 11,700 g for 60 min in a Sorvall Superspeed RC2B (Newton, USA) with an SS34 rotor. The supernatant was used for column chromatography, spectrophotometric assay, protein estimation, radioimmunoassay (RIA), gel electrophoresis and isoelectric focusing (IEF).

Column chromatography

The ADA-isozymes in the supernatant were separated on a Sephadex G-200 Superfine (Pharmacia, Uppsala, Sweden) column (95 x 1.6 cm or 82 x 1.6 cm) which was equilibrated with the same buffer as used for homogenization, but without sucrose.

For molecular weight estimation, we used dextran blue (MW 2,000K, Pharmacia), aldolase (MW 158K, Boehringer, Mannheim, W. Germany), bovine serum albumin (MW 67K, Sigma, St. Louis, USA) and chymotrypsinogen-A (MW 25.7K, Sigma). An Ultrorac II (LKB 2070, Sweden) was used for collecting the fractions.

Enzyme assay by spectrophotometry

The ADA activity was measured using a spectrophotometer (model 250) attached to an automatic recorder, model 6050 (Gilford, Ohio, USA) with the temperature maintained at 37°C using a thermostatic waterbath (Tamson, Holland). For this assay (7), xanthine oxidase (0.5 U/mg protein) from Koch Light and nucleoside phosphorylase (25 U/mg protein) and adenosine from Boehringer were used.

Radioimmunoassay (RIA)

Pure ADA was obtained from human erythrocytes by combining the procedures of Schrader and Stacy (8) and Rossi et al (9) (Herbschleb-Voogt et al, in preparation). It was labeled essentially following Chechik et al (10), with the exception that the separation of the free iodine from the labeled protein was performed on a Biogel P60 (Biorad) column. The lactoperoxidase (82 U/mg protein) was obtained from Sigma, and hydrogen peroxide (30%) from Baker. Radioactivity was measured with an auto-gamma scintillation spectrometer 5120 (Packard, Illinois, USA).

The anti-ADA antiserum was raised in a rabbit. The RIA assay was essentially according to Daddona et al (11) (Herbschleb-Voogt et al, in preparation).

Protein estimation

The procedure of Lowry et al (12) was used with the Gilford spectrophotometer. The K-,Na-Tartrate, $CuSO_4$ and Na_2CO_3 were obtained from Baker, and Folin and Ciocalteau phenol reagent from BDH.

Starch gel electrophoresis

The operating conditions for electrophoresis and the gel staining method were as described by Spencer et al (13), except for the sample application. Slots of 8 x 1 mm were made in the gel, in which 50 µl of the sample was applied; 25 µl of the homegenate was mixed with 25 µl of Sephadex G-200 suspension in saccharose (BDH) (prepared by mixing water, saccharose, Sephadex G-200 in the ratio 100:40:5, v/w/w, respectively). The LKB, Bromma 2117 multiphor and the LKB 2103 Biochrom power supply for electrophoresis and the Lauda Ultra Kryomat TK 30D (W. Germany) for cooling were used. The electrostarch was from Otto Hillwer (Wisconsin, USA), agar from Difco (Detroit, USA) and phenazine methosulfate (PMS) and bimethyl-thiazolyl-diphenyl tetrazolium bromide (MTT) were from Sigma.

Cellogel electrophoresis

This was performed as described by van Someren et al (14) using Cellogel from Chemetron (Milan, Italy).

Isoelectrofocusing (IEF)

One mm thick gels were cast using a gel solution containing

TABLE 1. Specific Activities (SA, IU/mg protein x 10^3) and Absolute Specific Activities (ASA, IU/ng ADA x 10^3 as Measured by Radioimmunoassay) in the Tumors (T) and Adjacent Normal Mucosa (N) in Surgical Specimens from the Patients Described in Table 2

Case	Type	SA	Total ADA Index[a]	ng ADA/[b] mg protein	ASA
PQ	N	9.58		40.86	0.297
	T	17.61	183	70.53	0.250
PT	N	7.3		n.t.[c]	n.t.
	T	8.9	122	n.t.	n.t.
PS	N	8.02		24.40	0.329
	T	11.10	139	22.72	0.489
PR	N	7.09		17.65	0.345
	T	19.20	315	92.57	0.207
PAB	N	8.60		33.34	0.386
	T	11.72	136	51.39	0.251
PU	N	5.65		18.31	0.324
	T	7.25	84	17.39	0.318
PW	N	6.05		13.65	0.443
	T	10.77	177	75.95	0.142
PP	N	4.44		12.69	0.350
	T	11.09	252	48.07	0.231
PAC	N	6.52		12.75	0.511
	T	9.98	154	32.13	0.311
Mean ± SEM	N	7.0 ± 0.5		21.7 ± 3.7	0.373 ± 0.025
Mean ± SEM	T	11.8 ± 1.3		51.3 ± 9.5	0.275 ± 0.036
Student t-test		$P \leq 0.002$		$P \leq 0.01$	$P \leq 0.05$

a See the text; total ADA index = T/N x 100

b Measured by RIA

c n.t. = not tested

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