

# Serum $\beta$ -N-Acetyl Hexosaminidase ( $\beta$ -NAH) as a Discriminant between Malignant and Benign Extrahepatic Biliary Obstruction: Comparison with Carcinoembryonic Antigen (CEA)\*

EITAN SCAPA,†‡§ PETER THOMAS,†‡ MATTHEW S. LOEWENSTEIN||†‡ and NORMAN ZAMCHECK†‡¶\*\*

†Mallory Gastrointestinal Research Laboratory, Boston City Hospital, ‡Department of Medicine, Harvard Medical School, ¶Department of Pathology, Boston University School of Medicine, Boston, MA and ||Mount Auburn Hospital, Cambridge, MA, U.S.A.

**Abstract**—Fifty-one patients (16 with malignant extrahepatic biliary obstruction, ten with benign extrahepatic biliary obstruction, eight with alcoholic liver disease, five with viral hepatitis and 12 with liver metastases) and 19 adult healthy controls were studied with determinations of  $\beta$ -N-acetyl hexosaminidase (a lysosomal enzyme which is cleared from the circulation by the Kupffer cells), carcinoembryonic antigen (CEA), serum bilirubin, alkaline-phosphatase and aspartate aminotransferase (AST). Both CEA and  $\beta$ -NAH were elevated in each disease group. Elevated  $\beta$ -NAH levels distinguished between benign and malignant extrahepatic biliary obstruction better than CEA levels.  $\beta$ -NAH levels for the malignant and the benign groups were  $47.6 \pm 14.7$  U/l and  $23.0 \pm 4.7$  U/l (mean  $\pm$  S.D.) respectively. The groups differed significantly ( $P < 0.001$ ). Plasma CEA levels for both groups were  $18.7 \pm 38.9$  and  $7.2 \pm 3.3$  ng/ml (mean  $\pm$  S.D.) respectively.  $\beta$ -NAH levels for the 19 normal controls were  $15.8 \pm 3.5$  U/l (mean  $\pm$  S.D.).  $\beta$ -NAH also was significantly elevated in patients with hepatic metastases ( $36.9 \pm 20.1$  U/l). In 25 cancer patients with metastases other than in the liver  $\beta$ -NAH levels ( $18.3 \pm 5.2$ ) were not significantly elevated over the control group. It has potential value as a marker for non-CEA-producing liver metastases.

## INTRODUCTION

MANY laboratories have reported elevations of plasma carcinoembryonic antigen (CEA) in patients with benign liver disease [1-4]. CEA is removed from the circulation of experimental animals by the liver via a two-stage mechanism, initially by uptake through a specific receptor site on the surface of the Kupffer cell [5, 6] followed by

transfer to the hepatocyte where lysosomal degradation occurs [6]. Others [7] have shown that a human lysosomal enzyme,  $\beta$ -N-acetyl hexosaminidase ( $\beta$ -NAH), is also removed from the circulation of animals by Kupffer cells, but via a different receptor — one that recognizes mannose and N-acetyl glucosamine. Diminished ability of Kupffer cells to phagocytose denatured albumin has been reported in approximately 50% of patients with benign and malignant biliary obstruction [8] and elevated CEA levels are found in 50% of patients with benign biliary obstruction [4]. Like CEA,  $\beta$ -NAH is known to be elevated in the serum of patients with cirrhosis and intra- and extrahepatic cholestasis [9], in chronic alcoholics with acute ethanol intoxication [10] and in fulminant hepatitis [11].

We measured CEA and  $\beta$ -NAH levels in

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§Current address: Gastrointestinal Unit, "Assaf Harofeh" Medical Center, Tel-Aviv University School of Medicine, Zariffin, 70300, Israel.

\*\*To whom requests for reprints should be addressed at: Gastrointestinal Research Laboratory, Mallory Institute of Pathology, Boston City Hospital, 784 Massachusetts Avenue, Boston, MA 02118, U.S.A.

Table 1. CEA,  $\beta$ -NAH, serum bilirubin, alkaline phosphatase and AST in benign and malignant liver disease

Patient No.	CEA (ng/ml)	$\beta$ -NAH (U/l)	Serum bilirubin (mg%)	Alkaline phosphatase (U/l)	AST (U/l)
<i>Malignant biliary obstruction</i>					
1	162	65.1	4.8	990	114
2	29	42.3	4.0	256	78
3	14.7	31	8.1	1025	289
4	9.7	68.3	29.1	334	70
5	9.5	34.6	3.5	425	36
6	8.6	46.3	19.5	329	186
7	8.6	73.2	14.6	1374	192
8	8.2	41.6	12.3	540	288
9	7.0	33.3	12.0	400	107
10	6.7	50.1	9.0	475	225
11	5.4	29.3	15.0	475	67
12	5.4	38.3	2.5	153	76
13	4.6	47.3	6.0	456	84
14	4.1	47.3	21.0	420	101
15	3.9	39.9	3.3	985	107
16	2.1	73.2	>20	371	61
<i>Benign biliary obstruction</i>					
17	15.1	26.6	3.7	600	100
18	8.6	26.6	10.0	—	—
19	8.4	23.8	8.4	—	35
20	7.3	30.6	2.3	675	48
21	7.3	16.7	5.3	253	56
22	7.0	26.6	3.1	740	30
23	6.6	18.3	3.1	1575	210
24	5.2	19.8	6.8	1200	192
25	4.5	23.3	6.4	178	117
26	2.5	17.3	1.9	336	269
<i>Viral hepatitis</i>					
27	5.4	5.7	2.7	354	243
28	3.3	14.0	0.8	298	33
29	2.6	26.3	8.7	192	>1500
30	2.2	29	1.1	356	570
31	1.4	21.3	1.5	156	256
<i>Alcoholic liver disease</i>					
32	14.1	20.0	24.0	228	104
33	12.1	29.3	1.8	189	63
34	10.0	28.3	7.4	263	111
35	9.3	34.0	9.5	675	124
36	6.2	24.6	0.6	108	72
37	5.3	22.9	5.7	170	74
38	4.5	11.3	2.2	100	38
39	4.4	16.0	0.9	143	34
<i>Hepatic metastases</i>					
40	115000	21.6	0.5	135	40
41	56000	26.6	0.7	—	39
42	7800	14.3	0.3	127	15
43	774	33.3	0.9	217	27
44	572	80.6	3.2	600	102
45	278	32.6	1.2	283	39
46	101.4	42.3	0.8	164	43
47	78.3	36.3	1.5	355	190
48	42.3	44.6	0.2	423	26
49	6.9	68.6	3.4	365	156
50	5.3	18.0	0.8	92	13
51	2.3	23.3	0.4	87	30

patients with benign and malignant liver disease to determine if they were related and to learn how the latter assay might aid in the clinical distinction between the two disorders, and in the interpretation of plasma CEA levels.

## MATERIAL AND METHODS

### Patients

Fifty-one patients (26 women and 25 men) were studied: 16 with malignant extrahepatic biliary obstruction (15 pancreatic cancer and one cholangio carcinoma); ten with benign extrahepatic biliary obstruction; five with viral hepatitis; eight with alcoholic liver disease and 12 with liver metastases (primaries — eight colon, two breast, two lung). Liver function tests, including serum bilirubin, alkaline phosphatase and aspartate aminotransferase (AST), were measured simultaneously with measurements of CEA and  $\beta$ -NAH (Table 1). These parameters were also measured in 19 adult healthy controls. The normal range for serum bilirubin was 0.2–1.2 mg%, for alkaline phosphatase 30–115 U/l and for AST 0–41 U/l. A further 25 patients with metastases other than in the liver had CEA and  $\beta$ -NAH measurements. Informed consent was obtained from all participants in the study after the nature of the study had been fully explained.

### CEA assay

Plasma specimens were assayed for CEA in blind duplicate by radioimmunoassay using the CEA-Roche Test Kit (Hoffman LaRoche Inc., Nutley, NJ). Plasma values greater than 5 ng/ml were considered elevated. Reagents were kindly provided by Roche Laboratories.

### $\beta$ -NAH assay

To 50  $\mu$ l of 0.0074 M *P*-nitrophenyl-2-acetamido-2-deoxy-D-glucopyranoside in water, 50  $\mu$ l of 1 M citrate buffer, pH 4.0, and 100  $\mu$ l of test serum were added. The mixture was incubated at 37°C for 30 min. The reaction was stopped by

adding 3 ml of 0.2 M glycine buffer, pH 10.7, and the absorbance of the nitrophenolate ion produced was measured at 400 nm. *P*-Nitrophenol (Sigma Chemical Co., St. Louis, MO) was used as standard. A serum blank was used with each assay. This was of special importance when icteric specimens were analyzed. The amount of enzyme was defined as micromoles of substrate hydrolyzed per minute per liter of serum (U/l) [9].

## RESULTS

CEA,  $\beta$ -NAH, serum bilirubin, alkaline phosphatase and AST values are shown in Table 1 for 51 patients divided into five groups: (a) malignant extrahepatic biliary obstruction; (b) benign extrahepatic biliary obstruction; (c) viral hepatitis; (d) alcoholic liver disease; and (e) hepatic metastases. The mean  $\pm$  S.D. values of the various measurements for the five patient groups and the healthy controls are shown in Table 2. Figure 1 depicts  $\beta$ -NAH levels for each person and shows the following: (a) more than half of the patients in all disease groups had  $\beta$ -NAH values  $>2$  S.D. above the mean of the controls; (b) there were no significant differences in  $\beta$ -NAH levels among the three groups with benign disease (see also Table 2); and (c) 15/16 patients with malignant biliary obstruction had higher  $\beta$ -NAH levels than those with benign obstruction. The mean  $\beta$ -NAH value for the group with malignant obstruction was significantly greater than that for the group with benign obstruction ( $P < 0.001$ ). Mean CEA values, on the other hand, were not significantly different for both groups (Fig. 2). Median CEA values for the malignant and benign groups were 8.2 and 7.3 ng/ml respectively. When patients with benign and malignant extrahepatic biliary obstruction were matched for similar CEA levels, higher  $\beta$ -NAH levels were observed for the patients with malignant obstruction in all six instances (Fig. 3). When patients with benign and malignant liver disease were matched for similar

Table 2. Mean  $\pm$  S.D. of  $\beta$ -NAH and CEA values in benign and malignant liver disease and in healthy controls compared with serum bilirubin, alkaline phosphatase and AST

Liver function test	Normal controls	Biliary obstruction Malignant	Benign	Viral hepatitis	Alcoholic liver disease	Liver metastases
CEA	1.5 $\pm$ 1.1	18.1 $\pm$ 38.9	7.2 $\pm$ 3.3	6.9 $\pm$ 8.5	8.2 $\pm$ 3.7	*
$\beta$ -NAH	15.8 $\pm$ 3.5	47.6 $\pm$ 14.7†	23.1 $\pm$ 4.7†	19.3 $\pm$ 9.5	23.3 $\pm$ 7.4	36.9 $\pm$ 20.1
Bilirubin	0.5 $\pm$ 0.2	11.5 $\pm$ 7.8	4.9 $\pm$ 2.8	2.9 $\pm$ 3.3	6.5 $\pm$ 7.8	1.2 $\pm$ 1.1
Alkaline phosph.	58.2 $\pm$ 12.7	563 $\pm$ 340	695.2 $\pm$ 483	271 $\pm$ 92.6	234 $\pm$ 187	259 $\pm$ 163
AST	13.7 $\pm$ 15.9	130 $\pm$ 81	117 $\pm$ 87	507 $\pm$ 669	77.5 $\pm$ 33.1	60.0 $\pm$ 57.8

\*Because of the wide range of values (see Table 1) the mean  $\pm$  S.D. is not given.

†Differences in  $\beta$ -NAH levels between patients with malignant and benign biliary obstruction are significantly different ( $P < 0.001$ ).

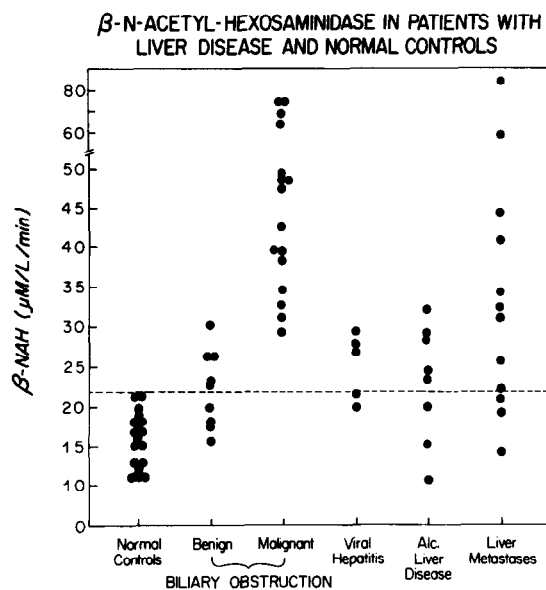


Fig. 1.  $\beta$ -NAH levels in normal controls and in patients with liver disease.

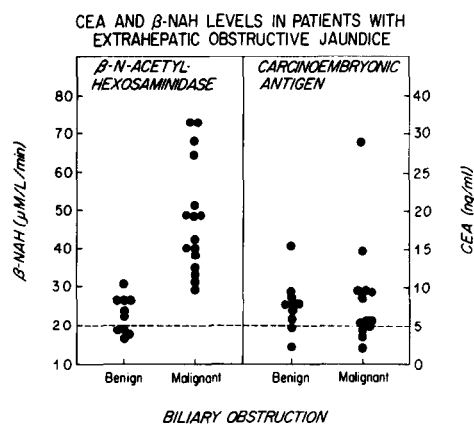


Fig. 2.  $\beta$ -NAH and CEA levels in patients with benign and malignant biliary obstruction. (The pancreatic cancer patient with a CEA level of 162 ng/ml is not included in the figure.)

alkaline phosphatase levels, significantly higher  $\beta$ -NAH levels were found in the patients with malignancy in all 11 instances (Fig. 4). Elevated CEA levels were observed more frequently in benign disease than elevated  $\beta$ -NAH. The highest CEA levels were seen in patients with liver metastases.

$\beta$ -NAH levels correlated significantly with bilirubin in patients with malignant biliary obstruction ( $P < 0.01$ ) and with all three liver function tests in the liver metastases group ( $P < 0.01$ ). In the group of patients with metastatic cancer but with no evidence for liver involvement [CEA values range from 16,000 to 0.4 ng/ml (mean 872.5 ng/ml; median 6.4 ng/ml)] had a mean  $\beta$ -NAH value of  $18.3 \pm 5.2$ , which was not significantly different from the control population. There was no correlation between the

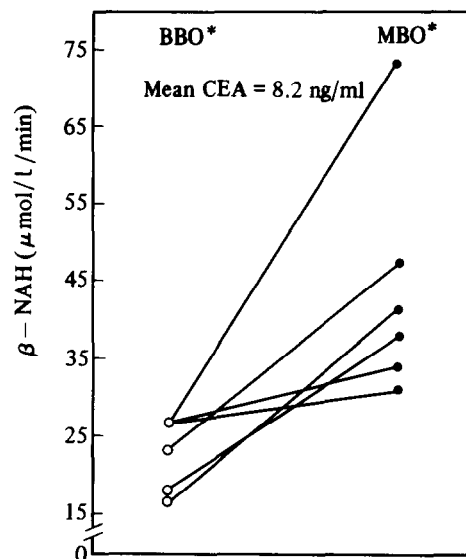


Fig. 3.  $\beta$ -NAH values in patients with benign and malignant biliary obstruction, matched for similar CEA values. The matched patients are joined by a line. \*BBO = benign biliary obstruction; \*MBO = malignant biliary obstruction. The mean CEA is 8.2 ng/ml for each group.

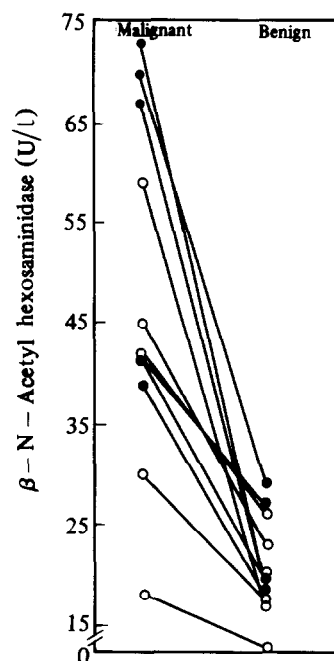


Fig. 4.  $\beta$ -NAH values in patients with benign and malignant liver disease, matched for similar alkaline phosphatase levels. ● Biliary obstruction (benign or malignant); ○ non-obstructive liver disease.

CEA and  $\beta$ -NAH values in any of the six patient groups, clearly showing the differing nature of the two markers.

## DISCUSSION

These studies show that measurement of serum  $\beta$ -N-acetyl hexosaminidase activity is of potential clinical use in distinguishing patients with

extrahepatic obstructive jaundice of unknown etiology. Since CEA measurements may be normal or only mildly elevated in these patients the use of this assay for distinguishing between malignant and benign obstruction is limited (Fig. 2).  $\beta$ -NAH levels on the other hand were significantly higher in patients with malignant obstruction (Table 1, Fig. 2). When matched for similar CEA values, patients with malignant obstruction showed much higher values of  $\beta$ -NAH than those with benign obstruction (Fig. 3). (CEA levels in the benign obstruction patients were higher than expected, probably due to cholangitis accompanying the obstruction.) Hultberg *et al.* [9] compared the levels of  $\beta$ -NAH in the circulation of normal subjects with those with liver disease (primary biliary cirrhosis, chronic active hepatitis and alcoholic cirrhosis). The highest values of  $\beta$ -NAH were found in his 'cholestasis' group, which included patients with malignant extrahepatic biliary obstruction, cholelithiasis, atresia of the biliary ducts and intrahepatic cholestasis due to metastases of breast carcinoma. All patients had their cholestasis for 'more than 1 week prior to sampling'. They suggested that clearance by the non-parenchymal cells was reduced because they had lost contact with much of the portal blood. The authors did not distinguish between benign and malignant cholestasis, nor did they measure CEA levels.

One possible reason why  $\beta$ -NAH levels are higher in patients with malignant rather than benign obstructive disease is the longer duration of illness in the former before they seek medical attention. We have observed  $\beta$ -NAH levels greater than 35 U/l in some patients with long-term benign obstruction due to biliary cirrhosis or sclerosing cholangitis (unpublished data). Experiments in rats with bile duct ligations also showed that increased plasma levels of  $\beta$ -NAH varied directly with the length of obstruction [12]. Injection of mice with the non-ionic detergent Triton WR 1339 also resulted in impairment of the clearance rates of CEA, at a level similar to that obtained by ligating the common bile duct [13], and Thomas and Jones [14] have suggested that reduced CEA clearance in rats with biliary obstruction may be caused by the detergent-effect of bile acids on the Kupffer cell membrane. As both CEA and  $\beta$ -NAH are cleared from the circulation by Kupffer cells this explanation for elevated levels in obstructive jaundice may apply to both glycoproteins.

As greater elevations of  $\beta$ -NAH levels were found in patients with hepatic metastases,  $\beta$ -NAH measurement may also help in detecting or monitoring hepatic metastases from non-CEA-

producing tumors, such as undifferentiated or very poorly differentiated colonic cancers. When pairs of patients with benign and malignant liver disease were matched on the basis of having similar alkaline phosphatase levels,  $\beta$ -NAH levels were always higher in the patients with cancer (Fig. 4). The reason for this is not known but one possibility is that the tumor itself may be responsible for the elevated  $\beta$ -NAH levels. For example,  $\beta$ -NAH activities are elevated in ovarian adenocarcinoma tissue compared to normal ovary [15]. Extracts of human colonic carcinoma demonstrate a higher proportion of the isoenzyme  $\beta$ -NAH B than  $\beta$ -NAH A, while normal colonic mucosa contained a higher proportion of the A form of the enzyme [16]. Lo and Kritchevsky [17] showed in patients with solid tumors (especially with cancer of the breast, colon and melanoma) that the activity of both major isoenzymes A and B was significantly greater in the malignant group than in the sera of healthy volunteers or patients with non-malignant ailments ( $\beta$ -NAH-B >  $\beta$ -NAH-A). The highest  $\beta$ -NAH levels were in two patients with pancreatic carcinoma. Measurement of the A and B forms of the enzyme using preparative agarose isoelectric focusing in pancreatic cancer patients and normal controls showed up to a six-fold increase in total activity in the cancer patients. The ratio of the isoenzymes did not change significantly [Saravis *et al.* unpublished results]. In widely disseminated lung carcinoma of various histological types urinary  $\beta$ -NAH levels were elevated, compared to controls and patients in whom the tumor was confined to chest alone [18]. Another possible explanation for the elevated  $\beta$ -NAH levels in patients with malignant liver disease is suggested by the report that macrophages that have been activated by BCG lose the receptor for mannose/*N*-acetyl glucosamine [19]. It is thus possible that a similar response with loss of receptor occurs in Kupffer cells in close proximity to an adenocarcinoma. This explanation seems more likely than the first as patients with similar tumors but with metastases other than in the liver had essentially normal levels of the enzyme.

In some patients with acute viral hepatitis  $\beta$ -NAH may not be significantly elevated (Table 1), in contrast to marked abnormalities of other liver function tests. One possible explanation is that the virus attacks hepatocytes specifically and an intra-hepatic obstructive phase may be too brief to damage the Kupffer cell membrane sufficiently to raise  $\beta$ -NAH levels. These cases tend to demonstrate the independent action of CEA,  $\beta$ -NAH, AST, alkaline phosphatase and bilirubin. The data demonstrate the effectiveness of  $\beta$ -NAH in discriminating between malignant and benign

extra-hepatic biliary obstruction when CEA levels are normal or only mildly elevated.

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