

The diagnostic value of serum leucine aminopeptidase

G. MERICAS, E. ANAGNOSTOU, ST. HADZIYANNIS,
AND S. KAKARI

From the Departments of Medicine and Chemical Pathology, the Evangelismos Hospital, Athens

SYNOPSIS Serum leucine aminopeptidase determination was found to be a useful screening procedure for hepatobiliary disease in jaundiced and unjaundiced patients. Values under 1,000 units are of no help in the differential diagnosis of jaundice but values above 1,000 units are highly indicative of biliary obstruction. The differentiation of intra- from extrahepatic obstruction as well as of malignant from benign jaundice cannot generally be established by this single test.

A number of studies relating to the clinical value of serum leucine aminopeptidase determinations have already appeared (Arst, Manning, and Delp, 1959; Bressler, Forsyth, and Klatskin, 1960; Göggele, Creutzfeldt, and Murucas, 1960; Miller and Worsley, 1960; Pineda, Goldbarg, Banks, and Rutenburg, 1960a; Winsten and Seller, 1961). It is now accepted that serum leucine aminopeptidase activity does not suffice for early diagnosis of cancer of the pancreas as had been thought originally (Rutenburg, Goldbarg, and Pineda, 1958). The level of this enzyme is raised in serum almost exclusively in the presence of hepatobiliary and pancreatic disease (Arst *et al.*, 1959; Hammond, Rosenak, and Khoo, 1960; Banks, Pineda, Goldbarg, and Rutenburg, 1960). Concerning the differential diagnosis of jaundice there are conflicting views. It is the purpose of the present paper to report findings on 400 determinations of serum leucine aminopeptidase activity performed between May 1961 and January 1962.

MATERIALS AND METHODS

Serum leucine aminopeptidase activity was assayed by a method adopted by the Biochemical Department of Messrs. Boehringer and Soehne (Mannheim) derived from those of Green, Tsou, Bressler, and Seligman (1955), Goldbarg and Rutenburg (1958), and Braun-Falco and Salfeld (1957). Undiluted serum, 0.05 ml., was incubated with 2.5 ml. of the buffered substrate solution (L-leucyl- β -naphthylamide hydrochloride) at 37°C. for one hour. The liberated β -naphthylamine was coupled immediately with diazotized 3-chloro - 4-nitraniline to a red azo dye. The colour density of the azo dye was measured with a Junior Coleman spectrophotometer at 492 μ .

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Serum leucine aminopeptidase units were expressed in terms of micrograms of β -naphthylamine liberated from the substrate by the enzymatic action of 1 ml. of serum after one hour's incubation at 37°C.

Serum leucine aminopeptidase activity was assayed in 400 sera obtained from 50 healthy persons (controls) and 195 in-patients; most of the patients were suffering from hepatobiliary diseases.

RESULTS

NORMAL VALUES Normal values obtained from 50 controls (25 males and 25 females) are shown in Table I. Significant differences have not been observed between the two sexes. The upper limits of normal were taken as the mean plus 2 standard deviations, that is 400 units.

HEPATOBIILIARY DISEASES In a group of 117 patients with a variety of hepatobiliary diseases, 95 of them were jaundiced and 22 were not jaundiced. Between the unjaundiced patients there were cases of cholelithiasis, hepatic metastases, infectious hepatitis, and calculous cholecystitis. Serum leucine aminopeptidase activity was increased in 91 (95.6%) of the jaundiced and 14 (63.6%) of the unjaundiced patients, (Table II). There was no correlation between serum leucine aminopeptidase activity and bilirubin in jaundiced patients. Serum analysis, biopsy, operation, and clinical diagnosis beyond any doubt were made in the case of 80 of these patients. Individual serum leucine aminopeptidase values for each case of this group are shown in Table III. In Fig. 1 a comparison is made between leucine aminopeptidase and alkaline phosphatase in cases of obstructive jaundice.

TABLE I
NORMAL VALUES FOR SERUM LEUCINE AMINOPEPTIDASE ACTIVITY

	No. of Patients	Age (yr.)	Serum Leucine Aminopeptidase Activity (units/ml.)		
			Range	Mean	± S.D.
Males	25	20-70	130-380	253	71.3
Females	25	12-65	130-370	243	77.4
Total	50	12-70	130-380	248	75.4

TABLE II
SERUM LEUCINE AMINOPEPTIDASE LEVELS IN HEPATOBILIARY AND MISCELLANEOUS DISEASES

	No. of Patients	Serum Leucine Aminopeptidase Activity				
		Normal		Raised		
		No.	(%)	No.	(%)	
Miscellaneous diseases	50	47	(94%)	3	(6%)	
Hepatobiliary diseases	Jaundiced	95	4	(4.4%)	91	(95.6%)
	Not jaundiced	22	8	(36.4%)	14	(63.6%)
	Total	117	12	(10.3%)	105	(89.7%)

TABLE III
SERUM LEUCINE AMINOPEPTIDASE ACTIVITY IN HEPATOBILIARY AND PANCREATIC DISEASE

Diagnosis	No. of Cases	Individual Values (Leucine Aminopeptidase units/ml.)	Mean
Acute infections and homologous serum hepatitis	20	420, 425, 425, 435, 460, 520, 525, 605, 605, 670, 680, 705, 710, 720, 740, 760, 765, 790, 790, 1,000	620
Cirrhosis	14	260, 315, 395, 405, 420, 470, 475, 575, 575, 600, 605, 630, 840 ¹ , 1,050 ¹	540
Drug hepatitis	2	525, 705	615
Chronic cholangiolitic hepatitis	2	1,390, 1,655	1,520
Calculous cholecystitis without jaundice	10	210, 260, 285, 315, 345, 370, 370, 380, 400, 420,	335
Calculous cholecystitis with jaundice	5	630, 660, 680, 735, 870	715
Cholecholelithiasis	4	450, 810, 945, 1,160	840
Cancer of the pancreas (all patients jaundiced)	14	605, 700 ² , 720, 910, 1,050 ² , 1,050 ² , 1,250, 1,250, 1,630 ² , 2,000 ² , 2,320 ² , 2,420 ² , 2,780 ² , 2,900 ²	1,540
Hepatic metastases	9	480, 760, 790, 900, 1,050, 1,080, 1,210, 1,420, 1,840	1,060
Total	80		

¹Biliary cirrhosis.

²With hepatic metastases.

CONGESTIVE HEART FAILURE Of 15 patients with congestive heart failure serum leucine aminopeptidase was increased in eight of them (420, 425, 425, 460, 475, 480, 550, and 575 units). Some of the cases with normal values suffered from severe right heart failure and the liver was greatly enlarged.

MISCELLANEOUS Of 50 patients with a variety of diseases not involving the hepatobiliary system and pancreas, serum leucine aminopeptidase activity was

normal in 47 and slightly increased in three of them (420, 425, 460 units).

SERIAL DETERMINATIONS In cases of acute infective hepatitis serum leucine aminopeptidase activity returned to normal usually late during the post-icteric phase. Figure 2 shows the results of serial leucine aminopeptidase determinations and other tests of liver function in a patient with homologous serum hepatitis. In cases of malignant obstruction

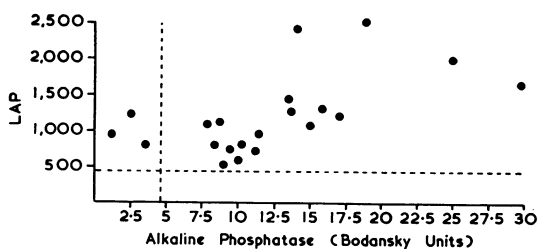


FIG. 1. *Leucine aminopeptidase and alkaline phosphatase levels compared in obstructive jaundice.*

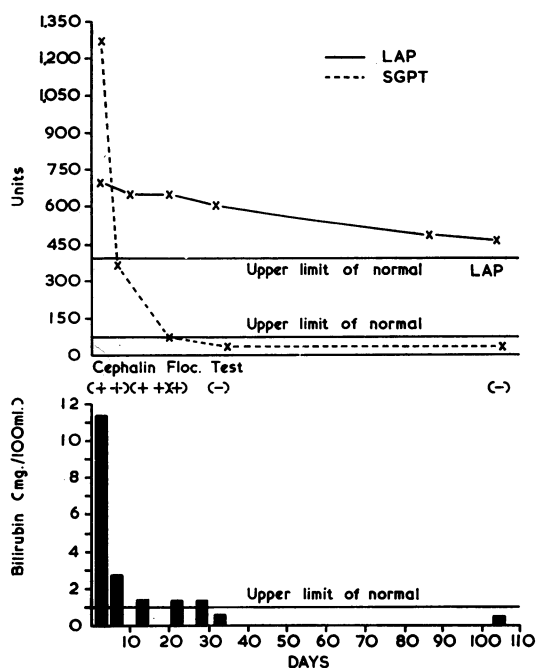


FIG. 2. *Results of serial leucine aminopeptidase determinations and other tests of liver function in a patient with homologous serum hepatitis.*

serum leucine aminopeptidase activity was persistently raised. The same was found to occur in cases of chronic cholangiolitic hepatitis and biliary cirrhosis.

DISCUSSION

The results of the present study (Tables II and III) support the well-established view that serum leucine aminopeptidase is a specific and sensitive index of hepatobiliary disease (Arst *et al.*, 1959; Bressler *et al.*, 1960; Miller and Worsley, 1960; Banks *et al.*, 1960).

It is interesting to note that serum leucine aminopeptidase levels were elevated not only in the jaundiced patients but also in those not jaundiced, though to a much lower extent (Table II). It must also be emphasized that in some cases of the anicteric group serum leucine aminopeptidase activity was raised whereas results of the commonly performed liver function tests (alkaline phosphatase and transaminases included) were normal. The above finding was observed in cases of acute hepatitis in the post-icteric phase (Fig. 2), in one case of hepatic metastases, and in two cases of choledocholithiasis; on the other hand most anicteric patients with calculous cholecystitis (Table III) had a normal serum leucine aminopeptidase level. Similar findings have also been reported by Pineda, Goldberg, and Rutenburg (1960b).

Some authors (Göggel, Kreutzfeldt, and Murucas, 1960; Pineda *et al.*, 1960a and b; Shay, Sun, and Sipler, 1960) believe that biliary obstruction—intra- or extrahepatic—is mainly responsible for raising serum leucine aminopeptidase levels though hepatic damage may also contribute to a lesser degree. Since leucine aminopeptidase is normally found in bile (Pineda *et al.*, 1960a and b) it is possible that biliary obstruction impairs the excretion of this enzyme resulting in raised levels in serum. Against this concept and in favour of an overproduction theory, is the finding of raised serum leucine aminopeptidase activity in anicteric patients. Regardless of the pathogenetic mechanism of raised serum leucine aminopeptidase levels the interesting clinical viewpoint is whether a such highly elevated level in serum reflects the existence of cholestasis and whether this may be helpful in the differential diagnosis of jaundice. In our study values under 1,000 units were commonly observed in all groups of hepatobiliary disease. Values above 1,000 units have been found only in those cases in which biliary obstruction existed (cancer of the pancreas with jaundice, hepatic metastases, calculous biliary disease, chronic cholangiolitic hepatitis). It is therefore logical to assume that serum leucine aminopeptidase values under 1,000 units simply indicate the involvement of the hepatobiliary tract and that values above 1,000 indicate the existence of biliary obstruction, whatever its cause (malignant or benign) and localization (intrahepatic or extrahepatic). Further differentiation of the cause and the localization of biliary obstruction is not usually possible even with serial determinations.

It must be noted that extremely high values (above 2,000 units) were observed only in cases of obstructive jaundice due to cancer of the pancreas with hepatic metastases. This has been noted by other authors (Rutenburg, Goldberg, and Pineda, 1958;

René and Meelinkoff, 1960); whether this is due to a complete continuous biliary obstruction that only carcinomas can create or is due to the neoplastic tissue itself is not yet known.

Despite a certain amount of disagreement, some authors (Göggel *et al.*, 1960; Banks *et al.*, 1960) consider that the serum leucine aminopeptidase test is a more sensitive index of biliary obstruction than the commonly performed non-specific serum alkaline phosphatase determination. In our study serum leucine aminopeptidase activity was always abnormal when alkaline phosphatase was elevated but not vice-versa. The scatter diagram (Fig. 1) shows no significant differences between leucine aminopeptidase and alkaline phosphatase in cases of obstructive jaundice if values of 1,000 leucine aminopeptidase units and 12 alkaline phosphatase Brodansky units are respectively taken as indicative of obstruction.

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