SERUM ALPHA-FETO-PROTEIN*

II. CORRELATION OF SERUM LEVELS WITH OTHER BIOCHEMICAL PARAMETERS IN PRIMARY CANCERS OF THE LIVER

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Quantitative immuno-assay methods have been able to detect alpha-feto-protein in the sera of about 78% of cases of primary cancer of the liver down to the lowest present practical limit of 0·1 mg./100 ml.[1]

Previous studies have shown that there are no biochemical[2] tests capable of completely distinguishing cases of primary cancer of the liver from other causes of liver dysfunction, especially when this takes the form of a space-occupying lesion, although there are patterns of results that are highly suggestive.[2-5] A summary of the biochemical tests[6] indicates which tests are similar and dissimilar when the means of results in cases with primary cancer of the liver are compared with a heterogeneous group of non-cancer patients with similar clinical presentations.[1] Tests showing no significant differences ($p>0.01$) between the cancer and non-cancer groups are: Thymol turbidity and flocculation, colloidal red, Takata-Ara, zinc sulphate turbidity, bilirubin, cholinesterase, mucoprotein, transaminases (SGOT and SGPT), sedimentation rate, lactic dehydrogenase (LDH), isoenzymes 3 and 4 (% of total), total serum protein, albumin (% and G/100 ml.), alpha$_2$-globulin (% and G/100 ml.), gamma-globulin (% and G/100 ml.), haemoglobin, prothrombin index, relation between alkaline phosphatase and bilirubin.

Tests showing significant differences between cancer and non-cancer groups ($p<0.01$) are:

1. *Higher in cancer group*: Cephalin cholesterol, alkaline phosphatase, cholesterol, aldolase, 5′-nucleotidase, lactic dehydrogenase and all isoenzymes (in units), glutathione reductase, isocitric dehydrogenase, alpha$_1$-globulin (% and G/100 ml.), beta-globulin (% of total), leucine aminopeptidase, hydroxybutyric dehydrogenase (SHBD), ratio SGOT/SGPT, ratio LDH/SHBD.
2. *Higher in non-cancer group*: LDH isoenzymes 1 and 2 (% of total), C-reactive protein, beta-globulin G/100 ml.†

These cases were initially suspected clinically, before biopsy, of having primary cancer of the liver, but the diagnosis was ruled out by biopsy or by the absence of serum alpha-feto-protein. Cases of viral and toxic hepatitis were excluded from this group on clinical grounds and secondary hepatic tumours by biopsy or postmortem examination. The relative number of cases in the cancer group (130) and in the non-cancer group (60) is a rough indication of the reliability of clinical acumen alone.

When the percentages of cases appearing in the abnormal range of any test were compared, no test was found to have any real discriminating power between the cancer cases and the heterogeneous non-cancer group.[6] This was not surprising, as most of the non-cancer cases had enlarged livers due to a space-occupying process of one type or another with related hepatitis.

It therefore appears that a clinical diagnosis receives only slight support from a wide spectrum of liver function tests. The exceptions to this are, of course, hepatic biopsy and the alpha-feto-protein test. In clinical practice it should be possible to diagnose nearly 80% of cases with the alpha-feto-protein test alone, which in the series under discussion would have at least halved the number of biopsies necessary to make a firm diagnosis.

An attempt was made to establish some correlation between serum alpha-feto-protein levels and the wide spectrum of biochemical parameters. In addition, differences in the biochemical results were sought between alpha-feto-protein positive and negative cases.

MATERIALS AND METHODS

We showed in a previous paper[1] that the range of serum alpha-feto-protein results was very wide, ranging from 0·1 mg./100 ml. to nearly 1,000 mg./100 ml. However, the distribution on a logarithmic scale was uniform, and so we have used the value of the logarithm (to the base 10) of the alpha-feto-protein result along the abscissa in all our graphs.

An IBM 360 computer was used to store all the biochemical data, to perform the statistical analyses and to plot the graphs.

For this study, only the initial results were used, so that the results reflect the undisturbed status of the patients before antitumour or symptomatic treatment, except in a few cases.

In each case the logarithm of the alpha-feto-protein result was plotted against the value of each of the tests listed above. The resultant scattergram for each test was used to gauge visually whether or not there was an obvious element of positive or negative correlation.

RESULTS

Fig. 1 shows a selection of the scattergrams produced in the case of alkaline phosphatase, haemoglobin, % alpha$_1$-globulin, mucoprotein, lactic dehydrogenase and 'liver' isoenzyme 5, zinc sulphate turbidity, % albumin and cholinesterase activity.

There is a uniform lack of obvious significant correlation of the alpha-feto-protein test with any other biochemical parameter, and the scatter of results is fairly uniform.

When the biochemistry of the cancer cases with a positive alpha-feto-protein result was compared with those having a negative alpha-feto-protein there were no results showing a highly significant ($p<0.01$) degree of correlation, but several showed a lesser degree ($0.01<p<0.05$), viz. colloidal red, haemoglobin and total protein were higher in the alpha-feto-protein positive group and mucoprotein, sedimentation rate, C-reactive protein and % alpha$_2$-globulin were lower (Table I).

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†There were 129 cases in the primary cancer of the liver group and 60 in the non-cancer group (including cases of bilharzia, tuberculosis, cirrhosis of the liver and chronic congestive cardiac failure).

When the biochemistry of the cancer cases with alpha-feto-protein results negative, below, and above 20 mg./100 ml., were compared (this being approximately the median for the range), some interesting tendencies were observed (see Table I) again at borderline significance levels ($0{\cdot}01<p<0{\cdot}05$). In the 3 groups, as the alpha-feto-protein changes from zero to less than 20 mg./100 ml. and then to more than 20 mg./100 ml., the following tendencies were observed (means in brackets for the 3 groups: 0; <20; >20).

Mucoprotein decreases (211 - 180 - 167)
Cholesterol increases (152 - 164 - 198)
Erythrocyte sedimentation rate decreases (33 - 28 - 17)
Serum protein increases (7·81 - 8·11 - 8·25)
% $alpha_2$-globulin decreases (108 - 102 - 93)
% gammaglobulin is constant (31·4 - 30·5 - 31·5)
Haemoglobin increases (12·7 - 13·7 - 15·0)

DISCUSSION

When it is considered that no single test is capable of distinguishing cases of primary cancer of the liver with certainty or even with a lesser degree of accuracy, it is not surprising that a specific test like alpha-feto-protein should not correlate with any other. In addition, multiple pathology was often present, e.g. bilharziasis or cirrhosis, which also tended to confuse the interpretation of results.

It should be stressed that not a single false positive alpha-feto-protein result has yet occurred in our series,[1] which is approaching 200 cases, although we have not yet had the opportunity of examining any cases with non-hepatic tumours having an embryonal element.[2]

There appears to be no obvious correlation of tumour size, rate of growth, degree of necrosis or the presence of metastases with the serum alpha-feto-protein, but this is still under scrutiny. There is a suggestion that the alpha-feto-protein level is present less often in cases without cirrhosis (approximately 50%) than those having cirrhosis (approximately 85%), but these results are being re-evaluated and will be reported on in a subsequent article in this series.

The enzyme tests are, as expected, higher on the average in cancer cases than in the non-cancer group since they reflect tumour growth and necrosis generally,[3] and would therefore not be expected to correlate with serum alpha-feto-protein levels, since there is a dissociation of the serum level of alpha-feto-protein from tumour size, etc.

Initially it was thought that alkaline phosphatase levels were elevated out of proportion to bilirubin levels in cases of primary cancer of the liver. Detailed investigation of this point revealed that although cancer cases had elevated alkaline phosphatase levels (80%) more often than the non-cancer group (46%), discriminant analysis was unable to separate the two groups when the bilirubin result was compared with the alkaline phosphatase result. There was, in addition, no correlation with the presence or the level of alpha-feto-protein.

It is interesting that the alpha-feto-protein negative group of primary liver cancer cases showed elevations of tests which are usually interpreted as being indicators of tissue necrosis or 'activity' of a disease process (i.e. sedimentation rate, mucoprotein, C-reactive protein, % $alpha_2$-globulin). In addition the total serum protein and haemoglobin levels were lower. Taken together, these findings, all of borderline significance ($0{\cdot}01<p<0{\cdot}05$), become more important. They may, in fact, indicate a more 'active' carcinogenic process in the alpha-feto-protein negative group. On the other hand, there are indications that the alpha-feto-protein negative

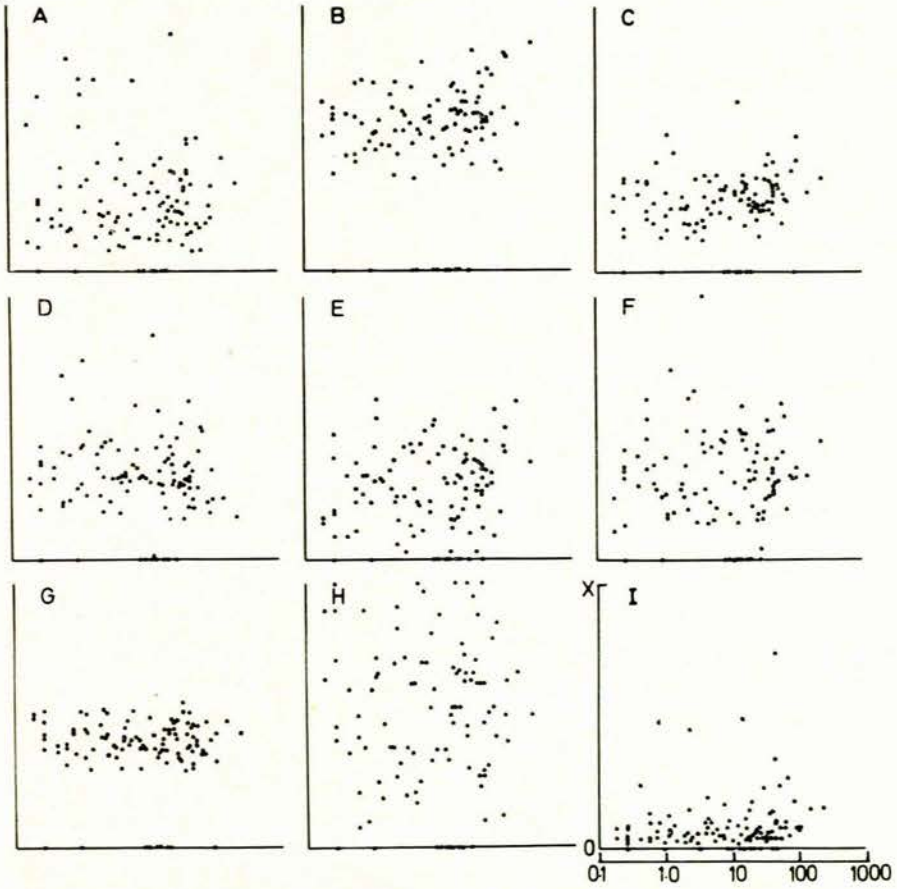

Fig. 1. Lack of obvious correlation between alpha-feto-protein and other biochemical tests. The scattergrams were produced by inking in an IBM Calcomp plotter output. The abscissa ranges from 0·1 to 1,000 mg./100 ml. of alpha-feto-protein on a logarithmic scale in every graph. The ordinates all range from 0 to a value X which is different in each case.
A: Alkaline phosphatase (X = 100 KA units). B: Haemoglobin (X = 25 G/100 ml.). C: % $alpha_1$-globulin (X = 20%). D: Mucoprotein (X = 500 mg./100 ml.). E: % lactic dehydrogenase isoenzyme 5 (X = 40). F: Zinc sulphate turbidity (X = 100). G: % albumin (X = 100%). H: Cholinesterase (X = 100% of normal activity). I: Lactic dehydrogenase (X = 10,000 units). Points falling on the abscissa do not have a result for the corresponding biochemical test.

TABLE I. CHEMISTRY OF CANCER CASES

Test	Normal value	Alpha-feto-protein negative (A)		Alpha-feto-protein <20 mg./100 ml. (B)		Alpha-feto-protein >20 mg./100 ml. (C)		Student's T test A/B	B/C	A/C	A/B+C
Thymol turbidity (units)	2	4·2	(3·0)	5·1	(7·6)	6·4	(3·8)	−0·62	−1·05	−2·55	−1·42
Thymol flocculation (units)	Neg.	3·0	(1·1)	2·8	(1·1)	3·2	(1·1)	0·33	−1·12	−0·36	−0·19
Colloidal red (units)	Neg.	3·3	(1·0)	3·5	(0·9)	3·6	(0·8)	−1·12	−0·49	−1·57	−1·67
Cephalin cholesterol (units)	Neg.	3·2	(1·0)	3·7	(1·3)	3·5	(0·9)	−1·08	0·69	−0·77	−0·99
Takata-Ara (units)	Neg.	2·3	(0·7)	2·1	(0·7)	2·1	(0·9)	1·23	−0·39	1·12	1·21
Zinc sulphate turbidity (units)	<12·5	33·0	(14·8)	33·2	(16·8)	34·9	(12·6)	−0·06	−0·58	−0·58	−0·51
Alkaline phosphatase (KA units)	<13·0	28·8	(12·6)	28·8	(18·3)	27·1	(14·1)	−0·01	0·55	0·52	0·47
Bilirubin (mg./100 ml.)	<1·2	3·5	(6·7)	2·2	(4·1)	3·5	(7·7)	1·09	−1·14	−0·01	0·88
Cholinesterase (% of normal)	>80	65·9	(85·4)	53·4	(24·5)	55·2	(22·6)	0·99	−0·39	0·84	1·27
Mucoprotein (mg./100 ml.)	<125	211·2	(79·9)	180·6	(61·2)	167·2	(62·6)	1·84	1·12	2·58	2·29
Total cholesterol (mg./100 ml.)	<200	152·6	(61·6)	164·8	(96·0)	198·9	(96·9)	−0·58	−1·81	−2·16	−1·29
Glutamic oxaloacetic transaminase (units)	<35	142·4	(107·7)	136·3	(124·2)	221·2	(256·4)	0·21	−2·22	−1·49	−0·99
Glutamic pyruvic transaminase (units)	<35	78·0	(52·5)	73·9	(115·4)	62·2	(44·2)	0·17	0·70	1·39	0·64
Aldolase (units)	<25	76·0	(75·1)	57·4	(58·2)	54·9	(29·0)	1·18	0·28	1·74	1·02
Sedimentation rate (Wintrobe – mm./hr)	<15	33·5	(20·2)	28·3	(18·6)	17·3	(16·2)	1·07	3·18	3·63	2·31
5′-nucleotidase (units)	<11	32·2	(23·4)	38·7	(58·5)	36·8	(29·1)	−0·53	0·21	−0·68	−0·51
LDH (units)	<280	817·9	(426·8)	830·1	(685·0)	1155·0	(1237·2)	−0·08	−1·68	−1·28	−0·91
% isoenzyme 1 (normal means)	18	10·2	(4·9)	11·7	(5·7)	10·8	(5·6)	−1·13	0·89	−0·40	−0·62
% isoenzyme 2 (normal means)	38	24·6	(7·7)	26·8	(8·1)	25·9	(9·1)	−1·12	0·67	−0·59	−0·74
% isoenzyme 3 (normal means)	27	20·9	(6·0)	20·6	(5·8)	21·3	(6·2)	0·21	−0·56	−0·23	−0·01
% isoenzyme 4 (normal means)	11	14·8	(4·7)	12·3	(5·0)	11·6	(6·1)	2·07	0·67	2·30	2·14
% isoenzyme 5 (normal means)	6	29·5	(13·5)	28·7	(14·4)	30·4	(12·9)	0·23	−0·66	−0·30	−0·17
Glutathione reductase (units)	<54	193·6	(112·4)	128·7	(78·0)	148·0	(126·1)	2·96	−0·96	1·52	2·26
Isocitric dehydrogenase (units)	<7	12·4	(6·5)	15·6	(15·4)	15·6	(10·7)	−0·96	−0·0	−1·32	−1·31
Total protein (G/100 ml.)	5·7–8·8	7·81	(0·82)	8·11	(0·88)	8·25	(0·79)	−1·45	−0·87	−2·28	−1·84
% albumin (normal range)	57–68	39·5	(6·0)	41·3	(6·1)	40·7	(6·2)	−1·26	0·51	−0·82	−0·78
% alpha$_1$-globulin (normal range)	2–5	5·6	(0·15)	5·4	(0·17)	6·0	(0·17)	0·54	−1·90	−1·03	−0·52
% alpha$_2$-globulin (normal range)	5–11	10·8	(2·2)	10·2	(2·2)	9·3	(1·8)	1·20	2·29	3·19	2·27
% beta-globulin (normal range)	8–16	12·6	(2·6)	12·5	(2·3)	12·5	(2·1)	0·21	−0·01	0·20	0·31
% gammaglobulin (normal range)	12–21	31·4	(6·4)	30·5	(7·8)	31·5	(6·5)	0·53	−0·71	−0·04	0·02
Leucine aminopeptidase (units)	<330	585·5	(420·5)	642·5	(531·5)	598·7	(333·5)	−0·47	0·51	−0·15	−0·26
Hydroxybutyric dehydrogenase (units)	<180	545·6	(381·8)	510·5	(459·0)	680·8	(838·3)	0·33	−1·31	−0·76	−0·36
Haemoglobin (G/100 ml.)	>15	12·7	(2·5)	13·7	(2·3)	15·0	(3·1)	−1·00	−2·44	−3·11	−1·93
Prothrombin (% of normal)	>85	75·4	(11·5)	76·1	(10·1)	75·3	(13·3)	−0·28	0·38	0·05	0·02
Number of cases		26		57		54					
Degrees of freedom								81	109	78	135

The statistic, 'Student's T', was calculated by standard methods with degrees of freedom (total cases − 2) and the probability (p) of two sets of data, e.g. (A) and (B+C), being significantly different was looked up in standard tables. For (e.g. 60 degrees of freedom) if 'Student's T' > 2·39 then $p < 0{\cdot}01$ and if 2·39 > 'Students' T' > 1·67 then $0{\cdot}05 > p > 0{\cdot}01$.

The mean and one standard deviation (in brackets) are given in the 3 categories.

The methods for the tests are the same as previously described,[2] except that the Beckman microzone electrophoresis technique is used now with corresponding changes in normal values.[8]

group has fewer cases of cirrhosis than the alpha-feto-protein positive group (50% *vs.* 85%).[6] The presence of a damaged liver might inhibit the production of abnormal proteins, thus causing the non-cirrhotic (and thus the alpha-feto-protein negative) group to have apparently higher levels of these abnormal proteins indicating 'activity'. However, this view is not supported by the data, as the cirrhotic and non-cirrhotic cases of primary liver cancer can be distinguished on the basis of results of the flocculations, zinc sulphate turbidity and gammaglobulin levels.[6] These differences do not appear at all (except for colloidal red) when the alpha-feto-protein negatives and positives are compared.

In spite of no obvious correlations when the scattergrams (of alpha-feto-protein level against the individual biochemical test) were studied, a cruder subdivision of cases into 3 groups—zero, less than 20 mg./100 ml. and greater than 20 mg./100 ml.—did reveal some, perhaps significant, tendencies. The mucoprotein, sedimentation rate and % alpha$_2$-globulin decreased progressively as the alpha-feto-protein level increased. There was little biochemical evidence of cirrhosis as the alpha-feto-protein level increased (*cf.* zinc sulphate turbidity and % gammaglobulin). The cholesterol levels increased steadily with increasing alpha-feto-protein levels. The significance of this is not yet understood but might be related to the cholesterol content of the tumour.

The amount of alpha-feto-protein present usually does not contribute much to the % alpha$_1$-globulins, so that it is felt that the levels are only related to the quantities of the usual alpha$_1$-globulins.

SUMMARY

There appears to be a reciprocal relationship between the serum alpha-feto-protein level and the indicators of 'activity' of a disease process, viz. mucoprotein, C-reactive protein, erythrocyte sedimentation rate and % alpha$_2$-globulin. On the other hand, the cholesterol, haemoglobin and total serum protein levels rose progressively. These findings could possibly, but not necessarily, be related to the suspected[8] lower incidence of concomitant cirrhosis in the alpha-feto-protein negative group of primary liver cancer cases.

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REFERENCES

1. Purves, L. R., Macnab, M. and Bersohn, I. (1968): S. Afr. Med. J., **42**, 1138.
2. Bersohn, I. (1957): *Ibid.*, **31**, 828.
3. Edmondson, H. A. and Steiner, P. E. (1954): Cancer (Philad.), **7**, 462.
4. Gutman, A. B., Tyson, T. L. and Gutman, E. B. (1936): Arch. Intern. Med., **57**, 379.
5. Popper, H. and Schaffner, F. (1957): *Liver: Structure and Function*. New York: McGraw-Hill.
6. Bersohn, I., Purves, L. R. and Geddes, E. W. (1969): S. Afr. Med. J. (in the press).
7. Abelev, I. (1968): Cancer Res., **28**, 1344.
8. Hess, B. (1963): *Enzymes in Blood Plasma*. London: Academic Press.