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Serum Ferritin Concentrations in Patients with Haematological Malignancies and Cancers of the Lung, Pancreas, Liver, Oesophagus, Stomach and Colon

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INTRODUCTION

Ferritin is an iron storage protein that occurs in apparently all eukaryotic cells. Small, but clinically important, amounts of ferritin are also found in serum. The recent development of sensitive radioimmunoassays (1) has greatly stimulated current research on serum ferritin in normal and pathological states. In particular, findings of markedly elevated levels of serum ferritin in many cancer patients (2, 3, 4) and of carcinofetal variants of this protein in tumour tissues (5, 6, 7, 8) have intensified our interest in ferritin as a possible tumour marker.

In the present communication, we evaluate the usefuleness of serum ferritin for diagnosis, monitoring or staging of haematological malignancies and cancers of the lung, pancreas, liver, esophagas, stomach and colon. We also discuss the potentiality of the combined use of serum ferritin with other tumor markers to increase the sensitivity.

MATERIALS AND METHODS

Ferritin was isolated from human liver by methods described elsewhere (9) with minor modifications using preparative electrophoresis at the final step of purification. In brief, the tissue was homogenized with four parts of distilled water and the homogenate was heated at 75°C for 10 min. After centrifugation at 10,000 × g for 30 min, the supernatant was passed through a Millipore filter (pore size 0.45 μm). Ferritin was precipitated from the filtrate with half-saturated ammonium sulfate (final concentration) and the

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precipitated protein was dissolved in a small volume of phosphate buffer (20 mmol/liter, pH 7.0). The solution was then subjected to gel filtration, first on a Sephadex G-200 column and then on a Sepharose 6 B column. Preparative-scale gel electrophoresis was used at the final step of purification. Ferritin preparations thus obtained were electrophoretically homogeneous.

The preparation of rabbit antisera against human liver ferritin was based on the method of Marcus and Zinberg (2). Anti-ferritin sera thus obtained did not cross react with other serum proteins or with other cellular components by double immunodiffusion techniques.

Immunoradiometric assays for serum ferritin using paper disc as a solid phase material were performed as previously described (10). Filter paper discs with a diameter of 5 mm (Toyo filter No. 51) were activated with BrCN according to the method of Ceska and Lundkvist (11). Sodium sulfate precipitated γ globulin fractions of rabbit antiserum prepared against purified ferritin was applied to affinity chromatography of BrCN activated Sepharose to which human liver ferritin was previously immobilized. After sufficient washing with P. B. S., antiferritin antibody fraction was eluted with 3 mol/liter of KSCN in P. B. S. and dialyzed against P. B. S. overnight.

The antibody solution thus obtained (1 mg/ml) was diluted in 200 ml 0.1 mol/liter $NaHCO_3$ containing 0.5 mol/liter NaCl. The solution was then incubated with activated paper discs (5 g) for 3 hrs at 4°C. The discs were then washed with 500 ml of the same buffer for 10 min and this washing was repeated one more time. The remaining reactive groups were blocked with 200 ml 0.05 mol/liter ethanolamine in 0.1 mol/liter $NaHCO_3$ containing 0.5 mol/liter NaCl for 3 hrs at room temperature. The discs were washed again two times with 500 ml 0.5 mol/liter $NaHCO_3$ and two times with 0.1 mol/liter acetate buffer for 30 min each time. Finally, the discs were washed two times with 500 ml of PBS-BSA buffer : bovine serum albumin (1 g/liter), normal rabbit serum (1 ml/liter), and sodium azide (20 mg/liter). Iodination of purified antiferritin antibody was performed according to the method described elsewhere (3).

The assay procedure was based essentially on the method of Ceska and Lundvist (11) modified by using the PBS-BSA buffer as the incubation buffer. Radioactivity was counted using an LKB Wallac autogamma scintillation counter. Standard curves of this assay system showed satisfactory linearity over the range of 0.25～100 ng/ml.

Serum samples were collected from the patients with leukemias and cancers of the lung, pancreas, liver, oesophagus, stomach and colon who were admitted to our hospital during 1974-1978.

Assays for CEA and AFP were carried out with the CEA-ROCHE kit

(ROCHE Co. Ltd.) and AFP kit (DAINABOT Co. Ltd.) respectively. Measurement of RNase was based on the method by Reddi[16] and Trypsin was measured by using RIA kit of HOECHST.

RESULTS

I. *Cut off level for abnormal elevation*

Serum ferritin concentrations in 182 normal subjects ranged between 8 and 243 ng/ml with the mean concentration of 124± 105 ng/ml (Fig. 1). The cut off level for abnormal elevation of serum ferritin, therefore, was defined as 229 ng/ml.

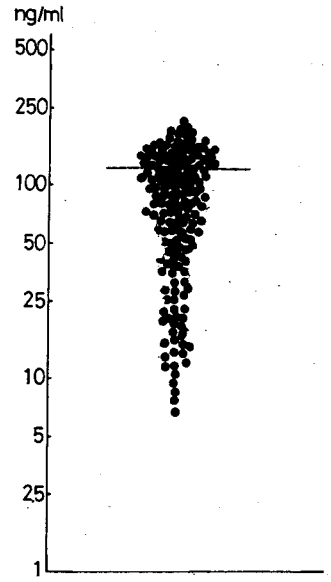

Fig. 1. Serum ferritin concemtrations in normal subjects.

II. *Serum ferritin concentration in haematological diseases*

Serum ferritin concentrations in patients with various haematological diseases were shown in Fig. 2. In 22 out 28 patients with acute myelogeneous leukemias, serum ferritin concentration exceeded the cut off level of abnormal elevation. In chromic myelogeneous leukemia, however, abnormal elevations were seen only in those who were in blastic crisis. It may be worthy of

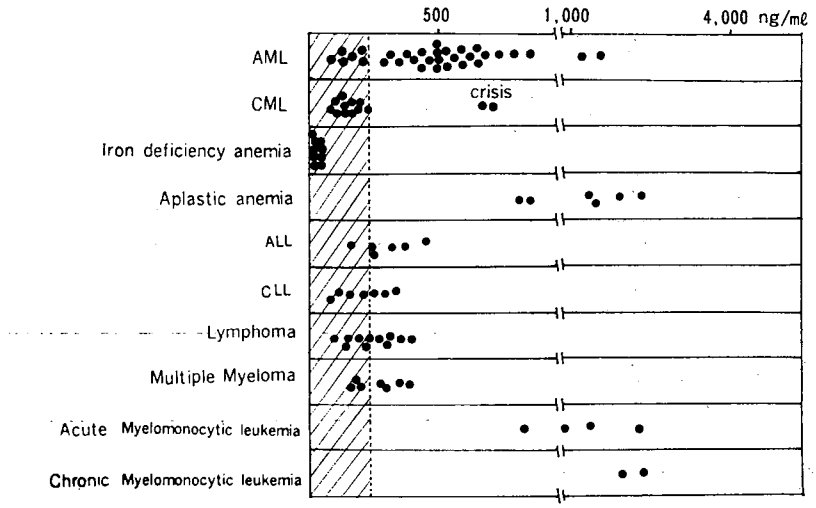

Fig. 2. Serum ferritin concentration in patients with various haematological malignancies.

special mention that serum ferritin concentrations in chronic and acute myelomonocytic leukemia were extremely elevated and were seen as high as few thousand ng/ml. Serum ferritin concentrations in lymphatic leukemia and multiple myeloma were also elevated although the incidence of abnormal elevation was rather lower than that of acute myelogeneous leukemia.

Among non-malignant haematological diseases, elevation of serum ferritin were observed in anemias whose iron store are increased in such cases as aplastic anemia.

In good contrast, serum ferritin concentrations in iron deficient anemia were consistently below the normal range.

III. *Serum ferritin concentration in lung diseases*

Serum ferritin concentrations in patients with lung cancer, pulmonary tuberculosis, sarcoidosis and acute pneumonia are shown in Fig. 3. Abnormal elevation of serum ferritin were found in 66% of the lung with the mean concentration of 570±543 ng/ml whereas in other lung diseases only 3.8% of the patients had elevated serum ferrtin.

The elevations of serum ferritin in lung cancer were reversible by the radical removal of tumors (Fig. 4). Two patients with continuous elevation after the operation eventually died of recurrence.

The relationship between the stage of lung cancer and serum ferritin concentration was not clear. There were no increase in either mean con-

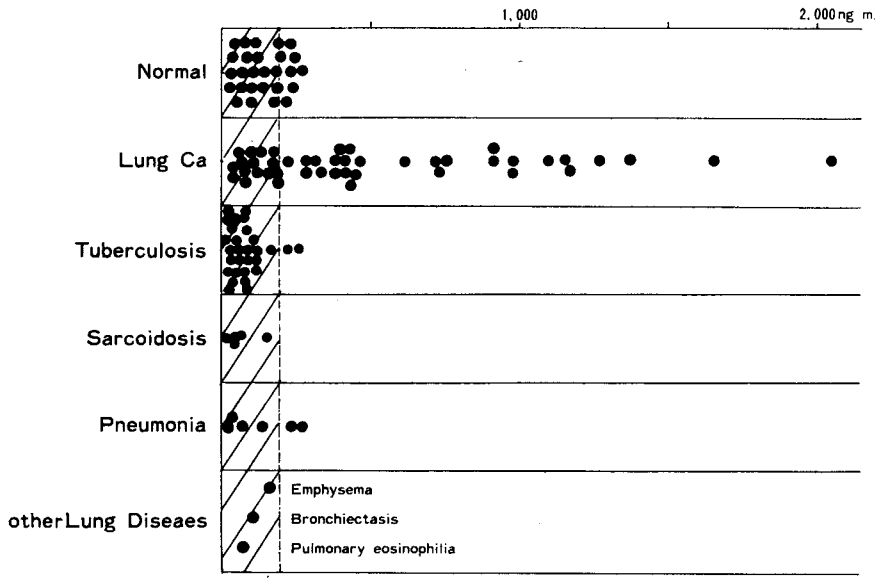

Fig. 3. Serum ferritin concentrations in various lung diseases.

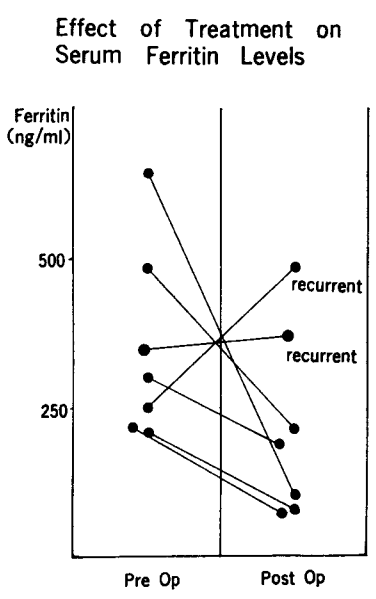

Fig. 4. Alteration of serum ferritin cincentrations by resection of tumors in lung cancer.

Table 1. *Serum Ferritin Concentration at Various Stages of lung Cancer*

Stage	No. of Subjects	Mean Value (ng/ml)	% of abnormal elevation (cut off 229 ng/ml)
I	6	642.3±594.8	54.5%
II	3	485.8±421.2	50.0%
III	19	593.2±411.6	63.3%
Total	28	594.5±562.4	62.2%

Table 2. *Serum Ferritin Concentrations in Different Histological Types of Lung Carcinoma*

Histology	No. of subjects	Concentration Mean (ng/ml) Ferritin
Adenocarcinoma	16	510.1±673.1
Squamous cell carcinoma	18	564.2±444.1
Anaplastic carcinama	11	508.3±452.1

centrations or incidences of abnormal elevation with the stages of lung cancer (Table 1). In other words, serum ferritin concentration and incidence of abnormal elevation at stage I were as high as those at stage III.

Serum ferritin concentrations in different histological types of lung cancer are listed in Table 2. There were no significant differences in mean values and incidences of abnormal elevation for each histological different lung cancer.

It is known that serum CEA levels are also often elevated in lung cancer. In this study, therefore, usefulness of a combination assay of ferritin and CEA for lung cancer was investigated. The relationship between serum ferritin concentrations and CEA levels in lung cancer is shown in Fig. 5. No correlation was observed between these two markers. The combined use of these independent markers is considered to be more meaningful for covering a wider range of lung cancer than the single assays. Table 3 shows the results of our combined assay method. Percent elevation of serum ferritin and CEA by their single assays were 61% and 47% respectively. However, when both markers were considered, approximately 90% of patients were detected to have abnormal values in either of these two markers.

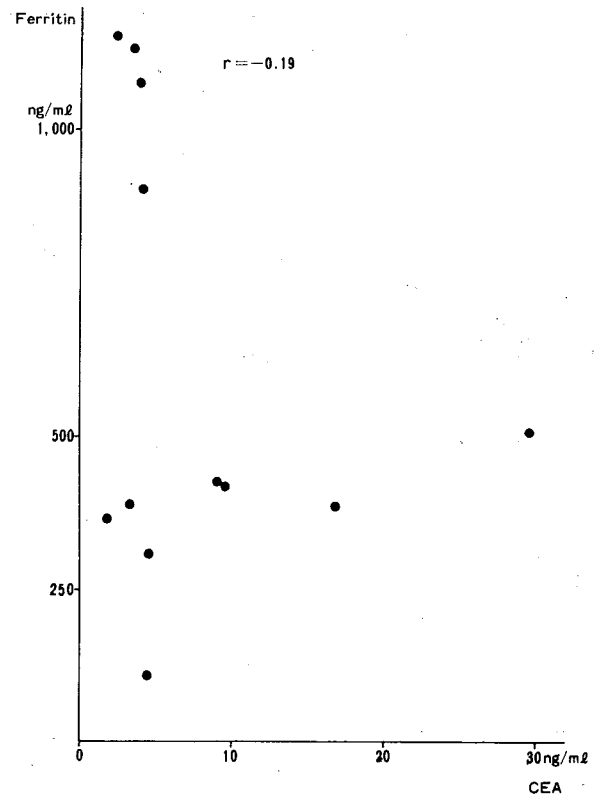

Fig. 5. Correlation between serum ferritin concentration and CEA.

Table 3. *Percentage of Elevated Serum Ferritin and Serum CEA Concentration in Patients with Lung Cancer*

Ferritin-CEA	% positivity (cut off level: 5 ng/ml for CEA 229 ng/ml for ferritin)
2 markers	42.1 (8/19)
Either one of 2 markers	89.5 (17/19)
Ferritin alone	61.8 (21/34)
CEA alone	47.5 (9/19)

IV. *Serum ferritin concentration in pancreatic diseases*

Fig. 6 shows serum ferritin concentration of patients with pancreatic cancer (adenotubular carcinoma), acute and chronic pancreatitis, pancreatic cysts and islet cell tumor.

Elevation of serum ferritin were observed in 31 out of 40 patients with

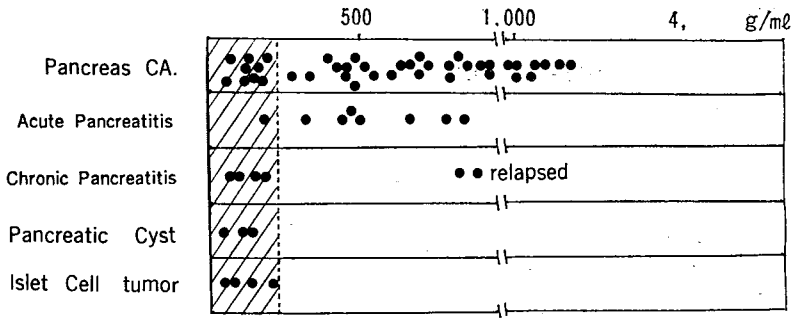

Fig. 6. Serum ferritins in pancreatic diseases.

pancreatic cancer and in 7 out of 8 patients with acute pancreatits. In chronic pancreatitis, serum ferritin concentrations were generally within a normal range although at relapses elevations were frequently observed. Serum ferritin concentrations in patients with pancreas cyst and in patients with islet cell tumor were not elevated.

Serum ferritin concentrations at various stages of pancreatic cancer are shown in Fig. 7. The incidences of abnormal elevation in stage I, II and III were 3/6 (50%), 7/9 (77.8%), 14/16 (87.5%) respectively and the mean serum ferritin concentrations increased as the stages advanced. The smallest tumor with a high serum ferritin concentration was measured $1.0 \times 1.5 \times 1.0$ cm when it was resected.

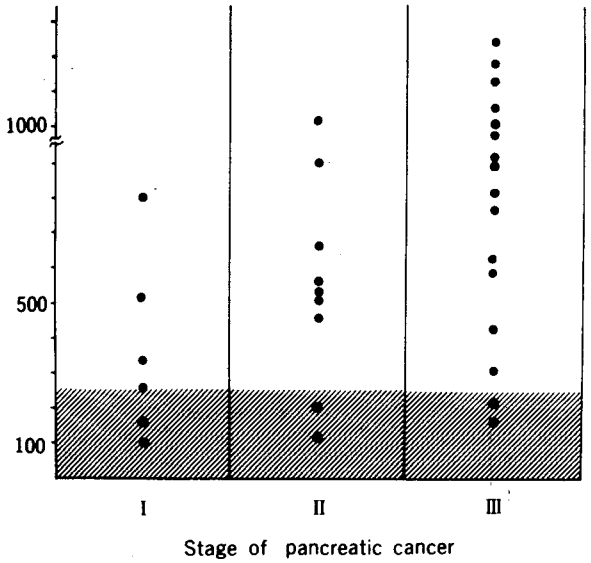

Fig. 7. Serum ferritin concentrations at various stages of pancreatic cancer.

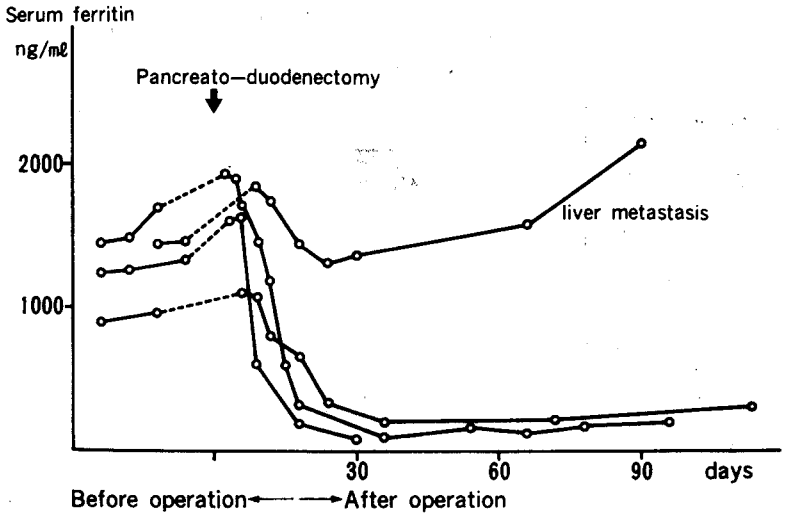

Fig. 8. Serial assay for ferritin before and after of pancreato-duodenectomy in pancreatic cancer.

Table 4. *Incidence of Abnormal Elevations in Combination Assays of Ferritin, RNase, CEA and Trypsin*

Tumor Marker	No. Tested	Positive	
Ferritin	25	17 (68.0%)	
RNase	25	16 (64.0%)	
CEA	25	13 (52.0%)	
Trypsin	25	9 (36.0%)	
		2 markers positive	1 marker positive
Ferritin·RNase	25	10	23
Ferritin·CEA	25	11	18
Ferritin·Trypsin	25	6	19
RNase·CEA	25	7	22
RNase·Trypsin	25	8	17
CEA·Trypsin	25	4	19
		3 markers posivive	1 marker positive
Ferritin·RNase·CEA	25	5	22
Ferritin·RNase·Trypsin	25	5	23
Ferritin·CEA·Trypsin	25	3	22
RNase·CEA·Trypsin	25	3	23
		4 markers positive	1 marker positive
Ferritin·RNase·CEA·Trypsin	25	2	23

Several patients with pancreatic cancer were followed with serial assays for ferritin (Fig. 8). Serum ferritin concentrations decreased to normal range within a few weeks after the operation when tumors were successfully removed.

Usefulness of combined assay of serum ferritin with RNase, CEA and Trypsin was also evaluated. In Table 4 the incidence of abnormal elevations in various combinations of these four markers are shown. The incidence of abnormal elevations of individual markers were 17/25, 16/25, 13/25 and 9/25 for ferritin, RNase, CEA and Trypsin, respectively. When one considers a combination of two given markers, the highest incidence of positive elevation of both markers (10/25) or either one of each marker (23/25) observed with the combination of ferritin-RNase. With a combination of 3 or 4 markers, the incidence of simultaneous positivity markedly decreased, suggesting that the combined assay with an excess of markers appear not to be of practical.

V. *Serum ferritin concentration in hepatic diseases*

Serum ferritin concentration in various hepatic diseases are shown in Fig. 9. In 11 out of 15 patients with hepatoma, serum ferritin levels were abnormaly elevated with a mean concentration of 432±295 ng/ml. Elevation of serum ferritin was also observed in 12 out of 14 patients with acute hepatitis, 5 out of 17 patients with chronic hepatitis and 6 out of 18 patients with cirrhosis.

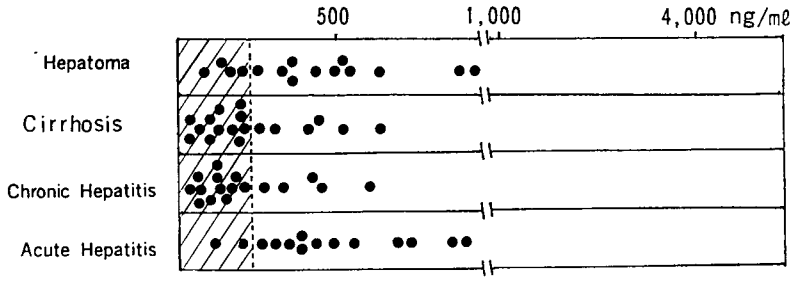

Fig. 9. Serum ferritin concentrations in hepatic diseases.

Table 5. *Percentage of Elevated Serum Ferritin and Serum AFP Concentration in Patients with Hepatoma*

Ferritin-AFP	% of positivity cut off level: 200 ng/ml for AFP 229 ng/ml for ferritin)
Either one of 2 markers	96% (24/25)
Ferritin alone	72% (18/25)
AFP alone	88% (22/25)

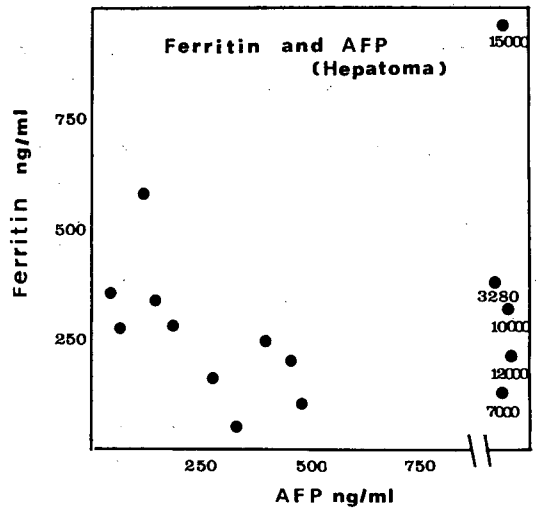

Fig. 10. Relationship between serum ferritin and AFP in hepatomas.

By combining the serum ferritin assay with AFP, the incidence of abnormal elevation which was detected by either one of these markers increased to 96% from the values of 72% and 88% for for ferritin and AFP, respectively (Table 5). No correlation was found between serum ferritin concentration and AFP levels in patient with hepatoma (Fig. 10).

VI. *Serum ferritin concentration in oesophagus, gastric and colon cancers*

In patients with cancers of the gastrointerstinal tract, serum ferritin concentrations were within the normal range unless liver metastasis occured (Fig. 11). In some patients with gastric or colon cancer serum ferritin concentrations were somewhat decreased, possiblly reflecting the diminution of storage iron due to bleeding from tumors.

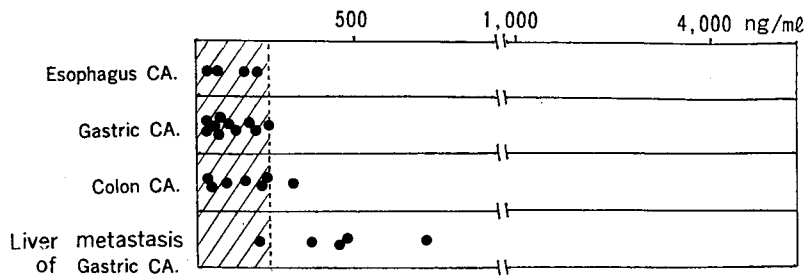

Fig. 11. Serum ferritin concentrations in cancers from gastrointestinal tract.

DISCUSSION

The clinical implications of ferritin in serum have been recently studied by many investigators (12). An important and well accepted finding is that serum ferritin levels directly reflect the size of mobilizable iron stores in normal subjects as well as in patients with iron overload or iron deficiency, and, therefore, its assay provides useful information about the iron status in certain diseases (1). Another possible value of serum ferritin assays exist in its empirical use for cancer immunodiagnosis (3, 13), despite the fact that the mechanism accounting for the increase in serum ferritin levels in cancer is still uncertain.

In our present studies, elevations of serum ferritin levels were found in a wide variety of malignancies.

The highest incidence of elevation was observed in leukemia of myelogeneous origin but the use of serum ferritin assay as a primary dignostic test in this particular malignancy is not so significant in view of other easily avilable haematological parameters. Furthermore, a relatively high incidence of false positives in other haematological diseases, such as aplastic anemia, suggests that there is a certain limitation in using the assay primarily for diagnosis of hematological mailgnancy. Our present results and those of others (14) suggests, however, that ferritin assay in myelogenous leukemia may well be useful in monitoring the effects of treatment or in detecting the early stage of blastic conversion. The next highest incidence of elevated serum ferritin was observed in patients with hepatoma 11/15 (73%) or pancreatic cancer 31/40 (76%). The magnitude of the ferritin concentrations observed on initial diagnosis by other procedures suggests that serum ferritin may be a useful marker for the diagnosis of these cancers. Particularly in pancreatic cancers, the assay may be promising for the detection of cancer at an early stage since 3 out of 6 patients at stage I had elevated levels of serum ferritin. Its use for monitoring patients after the operative treatment also seems to be useful although the serum ferritin remained elevated for a few weeks even after complete resection of the tumor, possibly due to tissue damage by the operation. The major sources of false positives in the diagnosis of these cancers are acute massive inflammation, namely hepatitis or pancreatitis. However the elevation of serum ferritin is generally transient reflecting the tissue damage and differential diagnosis is not usually laborious.

In patients with carcinoma arising from epithelial origin of the alimentary tract such as oesophagus cancer, gastric cancer or colorectal cancer, the serum ferritin concentration does not usually exceed the upper limit of the normal range unless massive metastasis to other organs occurs. Further-

more, in these patients, serum ferritin levels may be grossly affected by diminution of storage iron due to bleeding from the tumor. Ferritin assay in these malignancies, therefore, is presently considered to be hardly useful for initial diagnosis.

The assessment of ferritin assay for the diagnosis of lung cancer is particularly meaningful since complications such as massive bleeding, liver damage, or iron overload due to repeated transfusions which influence serum ferritin levels, are rather rare in lung diseases. In the present studies, a relatively high frequency (61%) of elevated ferritin levels was observed in lung cancer and there were no significant differences in the mean values at various stages, suggesting that serum ferritin may be helpful for the diagnosis of lung cancer at an early stage (54% in stage I). In addition the low incidence of false positives in other lung diseases are indicative of the usufulness of the assay in differential diagnosis of a suspicious lung lesion.

Screening of malignancies is another matter. A promising way to increase the sensitivity would be to use ferritin assay in combination with other markers. For example, by simultaneous measurement of ferritin and CEA, approximately 90% of patients with lung cancer were detected to have abnormal values. This contrasted with 62% of ferritin and 48% of CEA alone. Similarly, the combined use of ferritin and AFP was useful to increase the sensitivity of diagnosis of heptaoma. In pancreas carcinoma, a combination of ferritin with RNase was found to be the most efficient to increase the sensitivity.

Despite the facts that elevation of serum ferritin in malignancies is thus clinically evident, the mechanism responsible for it is still unclarified. We have recently demonstrated the elevation of human ferritin in the circulation of nude mice inoculated with human tumors (15), suggesting that elevated serum ferritin could be derived from the tumor tissues themselves. However, with the advance of disease states, other mechanisms such as increased iron stores in the reticuloendothelial system due to impaired erythropoiesis, or tumor metastasis in the liver, may be involved in the elevation of serum ferritin.

Regardless of the origin of serum ferritin in cancer, as has been hitherto described, high levels of serum ferritin of unknown eitiology should be generally considered to have a diagnostic significance for malignancies.

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