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Serum Ferritin as a Third Marker in Germ Cell Tumours*

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Abstract—*Serial measurements of serum ferritin have been assessed as an additional marker in a study of 12 patients with germ cell tumours. The standard markers, serum AFP and β HCG, were also assessed serially. During treatment elevated levels of serum ferritin were detected in 10 patients, elevated AFP in 10 patients and elevated β HCG in 6 patients. A poor prognosis was associated with persistently raised serum ferritin and either, or both, elevated AFP and β HCG levels. Decreasing levels of serum ferritin indicated favourable response to treatment; rising values were associated with recurrence or dissemination of tumour. Even if serum ferritin cannot be classed specifically as a tumour product, it may be useful in the early detection of residual or recurrent tumour.*

INTRODUCTION

ALPHA-FOETOPROTEIN (AFP) and the β subunit of human chorionic gonadotropin (β HCG) are well-documented markers of germ cell tumours. However, levels of both markers are normal in over 70% of patients with non-seminomatous tumour, clinical stage 1 [1–3] and in approximately 10% of patients with advanced disease [4, 5]. Although elevated initially, during chemotherapy marker levels may be normal even in the presence of residual tumour.

Previously, ferritin, the iron-storage protein [6–8], has been demonstrated by indirect immunofluorescence in cell smears from embryonal carcinoma [9] and, using the immunoperoxidase technique on tissue sections, in embryonal carcinoma, yolk-sac tumour, teratoma and seminoma [10]. Elevated levels of serum ferritin are found in a variety of benign and malignant conditions, but have been related to a poor prognosis in testicular germ-cell tumours [11]. The function and origin of serum ferritin are still not fully elucidated; increased levels may result from various processes, including non-specific anaemia and reticuloendothelial iron malutilisation, tissue damage, inflammation and infection, as well as from production by the tumour.

We have monitored serum levels of ferritin, in addition to serum levels of AFP and β HCG, in 12 patients with germ-cell tumours throughout their course of treatment and find that serum ferritin may give good indication of response to therapy, recurrence of tumour and widespread disease, and may be useful as a third marker in the management of these patients.

PATIENTS AND METHODS

Twelve patients selected at random were studied throughout the course of treatment (Table 1); 2 females with malignant ovarian tumours, 9 males with non-seminomatous tumours including a mediastinal tumour, and one male with seminoma. Classification of tumours was according to the World Health Organisation, 1977.

There have been changes in the treatment regimes during the period of study but, in general, radiotherapy was the initial treatment after surgery and then follow-up where necessary was with chemotherapy, except in the case of pure seminoma, where radiotherapy alone was employed. Combination chemotherapy included vinblastine and bleomycin and, in some cases, also *cis*-platinum. Throughout treatment, routine haematological and biochemical investigations were performed.

Post-operative AFP levels were determined

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by single antibody radioimmunoassay (RIA) and polyethylene glycol separation—[^{125}I]-labelled AFP supplied by Abbott Diagnostics, anti-AFP supplied by Dako (Copenhagen). The assay range was 10–250 ng/ml; samples requiring dilution were diluted with normal male plasma (AFP < 10 ng/ml). β HCG was assayed using a commercial kit by the double-antibody technique (Nuclear Medical Supplies). The assay range is 3–100 IU/l; samples requiring dilution were diluted with β HCG-free serum. Normal β HCG values are < 3 IU/l. Serum ferritin was determined by non-competitive immunoradiometric assay (Hoechst, Behringwerke, Germany). The assay range is 10–500 ng/ml; samples requiring dilution were diluted with serum. Normal values are < 360 ng/ml.

Apparent half-life values were calculated according to the formulae: $X_t = X_{0e}^{-0.139t}$ for AFP; and $X_t = X_{0e}^{-1.05t}$ for β HCG where X_0 is the initial marker concentration and X_t is the concentration at time t in days [12, 13]. The rate of serum AFP decline lies within the normal biologic half-life range (4–6 days) in the absence of AFP synthesis by tumour. Normal half-life values for β HCG are < 20 hr. Values greater than these indicate the persistence of tumour.

RESULTS

Correlation of AFP, β HCG and ferritin with clinical course of disease

At some stage throughout the course of the disease, elevated levels of AFP were found in 10 of the 12 patients; elevated β HCG in 6 patients; and elevated serum ferritin in 10 patients (Table 1). In only one patient (3) were all three values normal; this case of pure seminoma received radical radiotherapy and the patient remained well on follow-up with no evidence of disease.

Serum ferritin levels were also normal in patients 1 *and* 2.

Patient 1 (Fig. 1) showed an initial elevation of AFP which decreased and was maintained within the normal range after orchidectomy. All further investigations proved negative, the patient appeared well, but a sudden rise in β HCG occurred one month prior to death from brain metastases.

Patient 2 (Fig. 2), a young girl with yolk-sac tumour, had elevated AFP levels throughout intensive chemotherapy. Half-life values were never less than 5 days and often greater then 10 days. Excision of tumour from the anterior abdominal wall was attempted; however, the

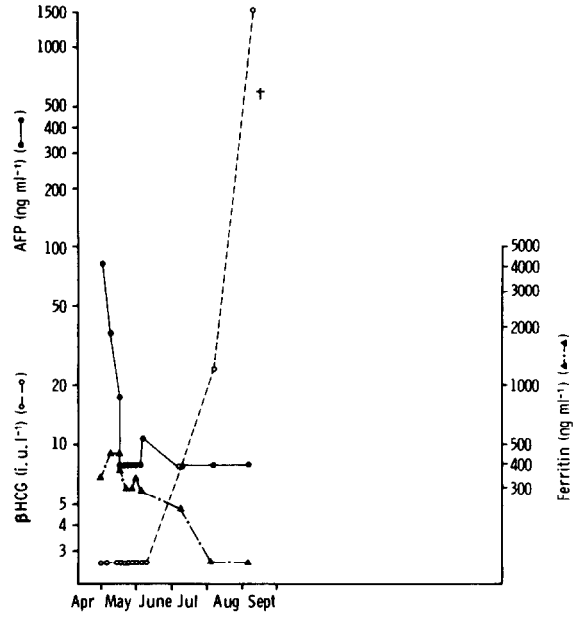

Fig. 1. *Patient* 1. *In all the figures:* † *denotes death of patient;* ↓ ■ *denote chemotherapy;* □ *denotes radiotherapy.*

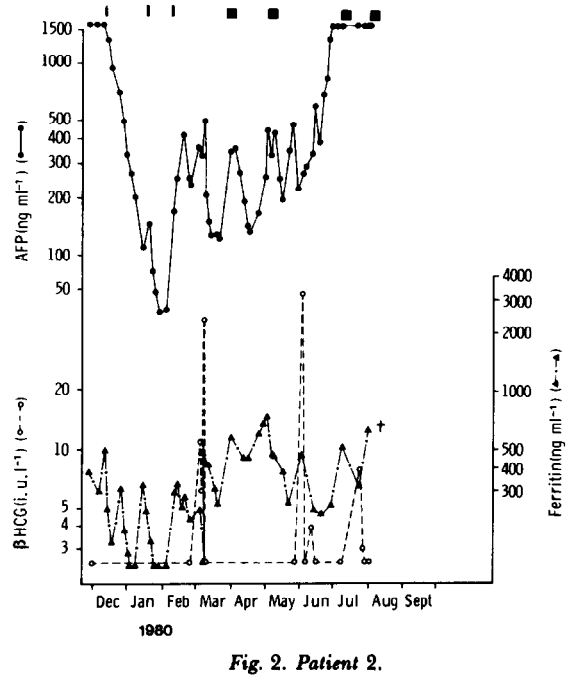

Fig. 2. *Patient* 2.

lesion was found to be extensive and the patient died as a result of massive recurrence in the peritoneal cavity. Although ferritin is marginally elevated at times, levels did not aid management of this patient.

Increased ferritin levels correlated with progression of disease in two patients.

Patient 4 (Fig. 3) presented with a mass in the left upper abdominal quadrant and another mass in the left testis. Histology revealed

Table 1. Elevated levels of AFP, β HCG and ferritin in 12 patients with germ-cell tumours

Patient	Sex	Age	Histology of 1° tumour	Treatment* SU	IR	CT	Survival time (months) from diagnosis (assessed January 1981)	Serum† AFP	β HCG	Ferritin
1	M	24	EC + T/YST/C	+			5 (dead)	+	+	−
2	F	16	YST	+		+	10 (dead)	+	−	±
3	M	29	S	+	+		15 (remission)	−	−	−
4	M	44	S/EC	+	+	+	7 (dead)	+	+	+
5	M	30	MT	+	+	+	4 (dead)	+	−	+
6	M	23	EC + T/YST	+	+	+	14 (remission)	−	−	+
7	M	23	EC	+		+	19 (cerebral involvement)	+	+	+
8	M	20	S/EC	+	+	+	27 (remission)	+	−	+
9	F	7	EC + YST	+	+	+	31 (remission)	+	−	+
10	M	30	EC + T	+		+	31 (remission)	+	+	+
11	M	26	T + EC	+	+	+	50 (dead)	+	+	+
12	M	22	S/EC	+	+	+	35 (remission)	+	+	+

(EC = embryonal carcinoma; T = teratoma; YST = yolk-sac tumour; C = choriocarcinoma; S = seminoma; MT = mediastinal teratoma; SU = surgery; IR = irradiation; CT = chemotherapy.)

* + Denotes treatment.

† + Denotes elevated marker levels; − denotes normal levels.

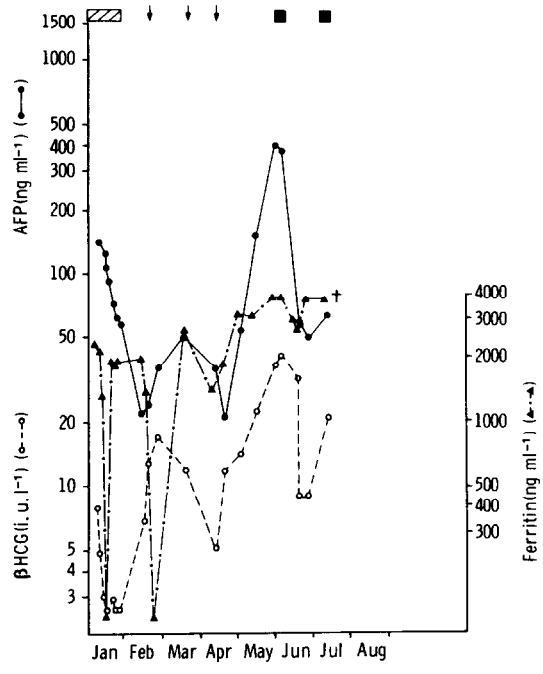

Fig. 3. Patient 4.

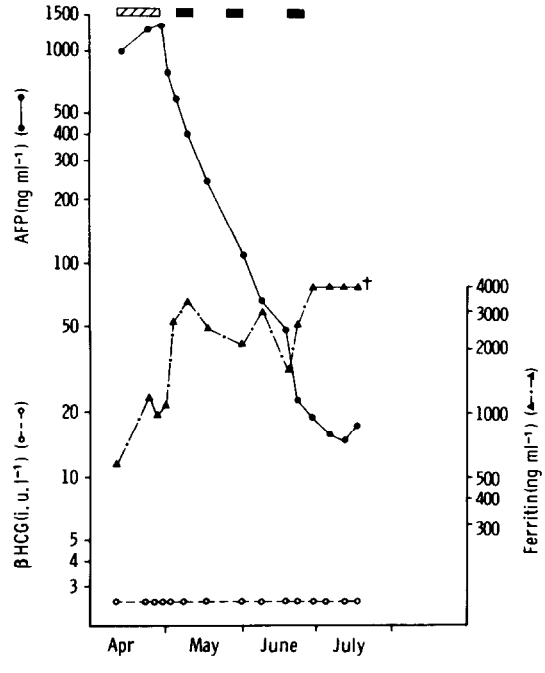

Fig. 4. Patient 5.

seminoma and embryonal carcinoma. Levels of AFP, β HCG and ferritin decreased after radiotherapy but rapidly rose again. In mid-February, because of pulmonary metastases, chemotherapy was initiated, consisting of vincristine, actinomycin D and cyclophosphamide The patient was anaemic and in poor general condition, and at the end of May received modified chemotherapy, consisting of vinblastine and bleomycin. From April until the patient's death in July, all three marker levels remained elevated with only minor fluctuation during two courses of third-line chemotherapy. Post-mortem examination disclosed metastatic tumour, involving the para-aortic lymph nodes forming a mass, the liver, the inferior vena cava, the lungs, the lumbar vertebrae, the right adrenal and the stomach wall. The spleen contained excessive haemosiderin.

Patient 5 (Fig. 4) had a germ-cell tumour of primary mediastinal origin. Initially AFP was greatly elevated, but decreased after radiotherapy and subsequent chemotherapy; however, the AFP half-life of 12.2 days suggested residual tumour. His general condition deteriorated during radiotherapy and chest X-ray showed increasing bulk of disease. The level of serum ferritin rose during and after radiotherapy, and remained elevated throughout chemotherapy, fluctuating only slightly. One month prior to the patient's death, serum ferritin was extremely high. In addition to mediastinal tumour, post-mortem examination disclosed compression of the lungs by secondary tumour deposits, and metastases in lymph nodes and brain; although the liver was enlarged, it showed no evidence of metastases.

Decreasing serum ferritin levels coincided with apparently successful treatment of four patients.

In one case (6) (Fig. 5) both AFP and β HCG values were normal and serum ferritin became elevated. At presentation there was a three-month history of swelling of the right testis, and right inguinal orchidectomy was performed. Histology revealed embryonal carcinoma with teratoma and prominent yolk-sac elements; the tumour was confined to the testis. Radical radiotherapy followed. In June, 1979, a mass was reported in the left lung which subsequently disappeared. In January, 1980, the mass reappeared; this was the only notable disease. Serum ferritin rose during January and early February; although falls were seen in response to chemotherapy, values remained high until April. Towards the end of April the mass had reduced in size and serum ferritin was only marginally elevated. In view of the persisting chest X-ray abnormalities, thoracotomy was performed in August, revealing mature teratoma in the lung, and the patient has since remained well.

Patient 7 (Fig. 6) had embryonal carcinoma of the left testis and at presentation had a large intra-abdominal mass causing deviation of the left ureter, and also pulmonary metastases on

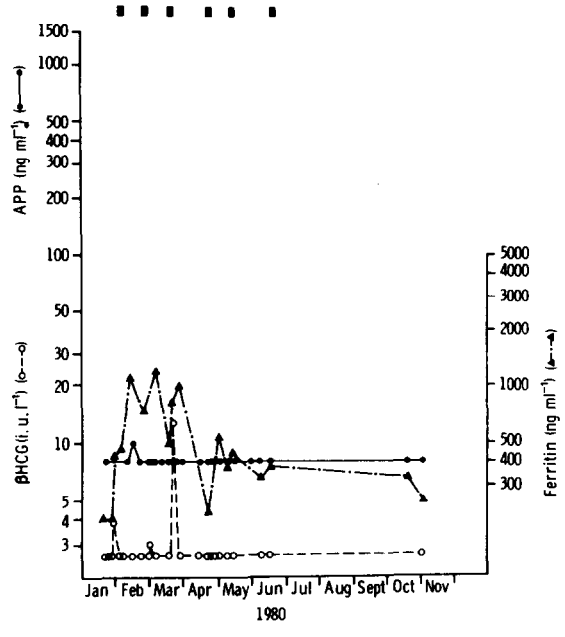

Fig. 5. Patient 6.

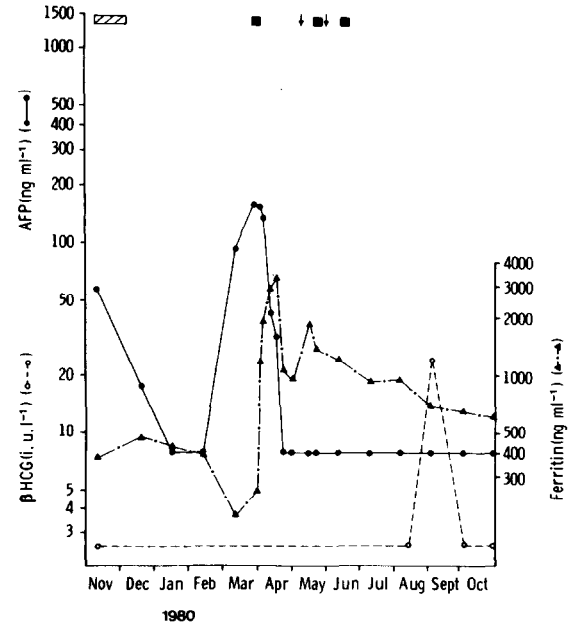

Fig. 7. Patient 8.

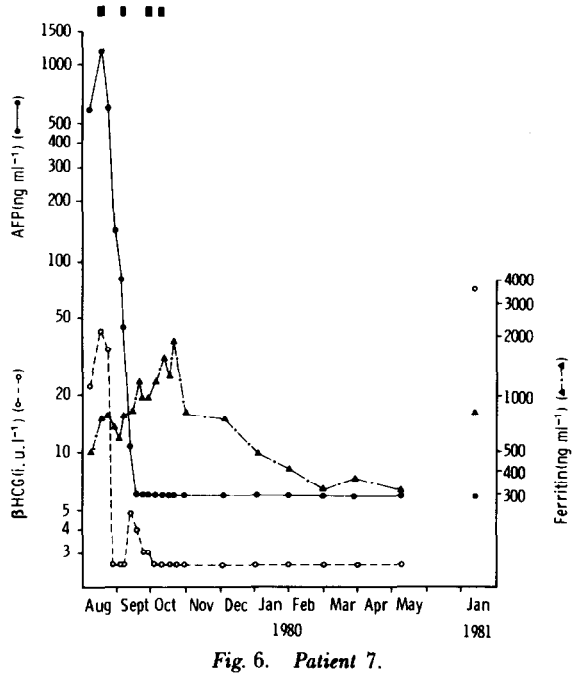

Fig. 6. Patient 7.

chest X-ray. AFP and β HCG were only marginally elevated, rose slightly and then rapidly declined during the first course of chemotherapy. Initially ferritin was slightly elevated and continued to rise over the following two months; levels then decreased gradually to the normal range. This patient was in remission for one year. However, recent elevations of β HCG and ferritin coincide with cerebral involvement.

In patient 8 (Fig. 7), a left inguinal orchidectomy disclosed a mixed tumour, partly seminoma, partly embryonal carcinoma, with involvement of one of the spermatic cord veins. AFP was moderately elevated, falling to normal after radiotherapy; the half-life of 23 days suggested residual tumour and levels rose again in February. Ferritin, originally on the high side of normal, decreased, rising slightly in late March, coinciding with pulmonary metastases. AFP levels returned to normal after chemotherapy, but the half-life, 9 days, was still prolonged. The respiratory system was clear by the end of April; ferritin, however, was still elevated. The patient was symptom-free and clinically well by the end of October. Ferritin levels decreased more slowly during chemotherapy and are only marginally elevated a year later.

Patient 9 (Fig. 8) presented with a ruptured malignant ovarian cyst with infiltration of bowel. Histology showed embryonal carcinoma with elements of yolk-sac tumour. Initial greatly elevated levels of AFP decreased during treatment, though half-life values between 5 and 10 days suggested possible residual tumour. Serum ferritin remained elevated for 18 months, but gradually returned to normal. β HCG estimations showed only slight fluctuations. This patient remains apparently disease-free.

In three cases the significance of persistently elevated serum ferritin in association with normal levels of AFP and β HCG is uncertain.

Patient 10 (Fig. 9) underwent left inguinal

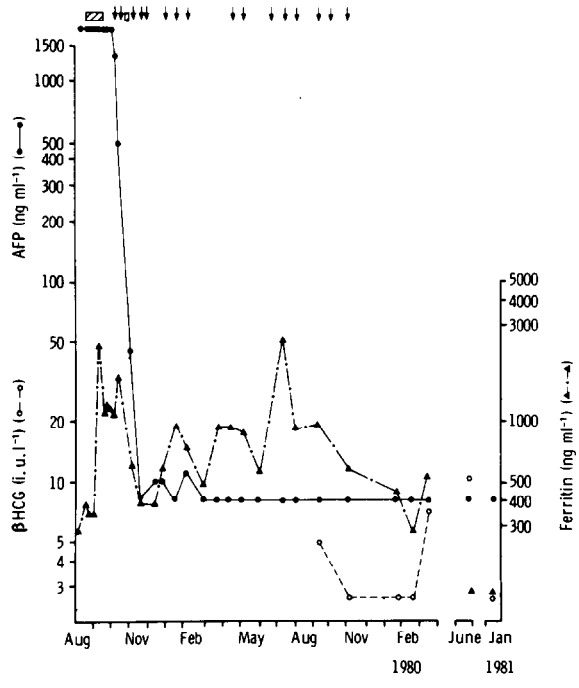

Fig. 8. *Patient* 9.

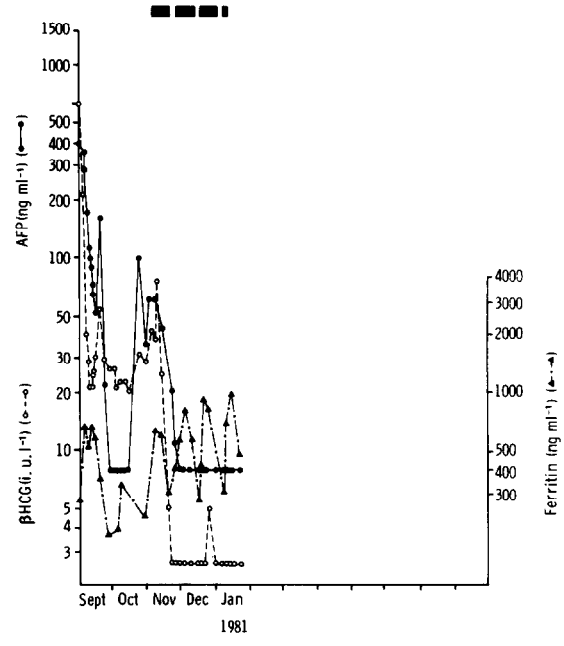

Fig. 9. *Patient* 10.

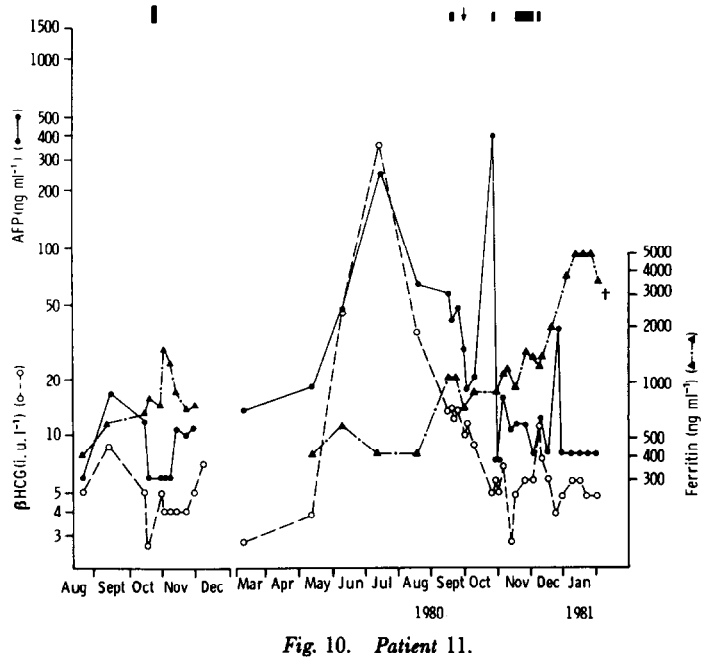

Fig. 10. *Patient* 11.

orchidectomy for testicular neoplasm with secondary hydrocele; histology showed embryonal carcinoma with trophoblastic elements. This patient was treated on the basis of having metastatic disease with raised tumour markers, but none of the clinical investigations, including CT scan, were abnormal; haemotology and biochemistry were normal. Levels of AFP and β HCG showed post-operative decrease and rose again, but during subsequent chemotherapy both marker levels returned to normal. Ferritin increased initially, a period of fluctuation followed and latterly levels are still high, though the patient is in apparent remission as he completes his chemotherapy regime.

Patient 11 (Fig. 10) had persistent teratoma and embryonal carcinoma. Orchidectomy in November, 1976, was followed by radiotherapy. He remained well for 30 months until Summer, 1979, when tumour was found in the abdomen.

All 3 marker levels were rising and treatment with chemotherapy was commenced in

October. Chemotherapy was later stopped due to persistence of low blood counts, apparent absence of active disease and decreasing marker levels. Serum ferritin had increased from August until October, thereafter decreasing in response to chemotherapy. In June, 1980, both AFP and β HCG showed a continued increase; ferritin rose later. During further chemotherapy all three levels decreased; AFP and ferritin rose again, but AFP finally returned to the normal range, with only minor fluctuation. The patient died 50 months after orchidectomy; necropsy disclosed persistent embryonal carcinoma and bleomycin lung toxicity.

Patient 12 (Fig. 11) presented in early 1978. Orchidectomy revealed mixed seminoma/embryonal carcinoma. AFP and β HCG showed transient rises during radiotherapy. In November, 1978, tumour was found in a lymph node removed from the neck. Radiotherapy followed and chemotherapy began in April, 1979. Serum ferritin determinations were included in the study at this stage. AFP, β HCG and ferritin increased in September with only transient falls in October. AFP and β HCG half-life values were prolonged until December, 1979. Blood transfusions were given in the last few days of December. During further chemotherapy, AFP and β HCG levels returned to normal; serum ferritin, however, remained markedly elevated, with slight fluctuation. Although serum ferritin is still elevated by January, 1981, this patient was judged to be in remission; however, the nature of a persistent mediastinal mass awaits determination.

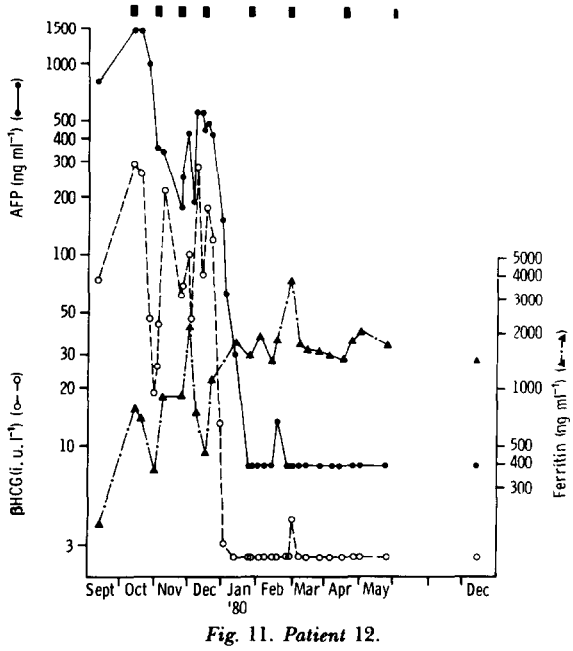

Fig. 11. *Patient* 12.

DISCUSSION

Elevated levels of serum ferritin were detected in 10 of 12 patients with germ-cell tumours studied during the course of their treatment; AFP was increased in 10 patients and β HCG in 6 patients. One of the patients with normal serum ferritin and histologically confirmed seminoma had normal levels of both AFP and β HCG; he responded well to therapy, gaining complete remission. In two patients ferritin levels increased with disease progression, correlating with the other markers, and in a further four cases levels fell with remission, serum ferritin being the only useful marker in one patient. In two more patients ferritin levels were never greatly elevated and thus essentially of little practical value; however, significantly elevated levels in two surviving patients indicate the need for careful surveillance.

Reports on the discordant behaviour of markers suggest that the neoplastic elements responsible for AFP and β HCG production may respond differently to treatment and that, due to heterogeneity in metastases, both may not be present in different sites[14, 15]. This may explain a return to normal values for AFP and β HCG but continued elevation of serum ferritin in those patients apparently successfully treated for recurrent tumour. A mass detected in the posterior mediastinum in one case (12) is assumed to be non-malignant as no change has occurred in nine months. The second patient (11) died recently and necropsy disclosed bleomycin lung toxicity, in addition to tumour infiltration. However, increased serum ferritin in this case could be due to infection or inflammation rather than progressive tumour. The third case (10) was treated on the basis of having metastatic disease with raised AFP and β HCG levels; however, none of the clinical investigations, including CT scan, were abnormal, and during chemotherapy both markers returned to normal. Serum ferritin levels showed similar fluctuations to AFP and β HCG during treatment but are currently moderately elevated in the presence of apparent clinical remission.

The finding that serum ferritin follows a similar pattern to AFP and β HCG in some patients would seem to imply that increasing levels are not simply due to tissue damage as a result of treatment. Tumour progression may also cause tissue necrosis. The effect of surgery on serum ferritin levels was studied in patients with breast cancer[16]. Ninety per cent of patients with pre-operative normal values showed an increase in serum ferritin concentration up to 10 days after surgery; a similar

percentage with elevated pre-operative levels also showed a post-operative rise and subsequent decline. Serum ferritin levels in our study did not always rise after treatment and more often showed some decrease, as with AFP and β HCG, to suggest the effectiveness of therapy. However, serum ferritin values in those patients judged to be in full remission only gradually decreased to normal several months after the last course of chemotherapy, whereas normal concentrations of AFP and β HCG were usually quickly attained. Some proportion of elevated serum ferritin may be due to cytotoxic therapy; however, in patients 7 and 12 elevated levels occurred many months after the last course of chemotherapy.

Other factors should be taken into consideration when serum ferritin levels do not coincide with the patterns of AFP and β HCG levels; elevated serum ferritin may reflect the disturbance to the reticuloendothelial system often associated with malignant disease and its treatment—patients 4, 11 and 12 required blood transfusions. Infection and inflammation may also play a role. There is, however, in addition, some evidence for increased serum ferritin production by the tumour. Indirect immunofluorescence with antisera against hepatoma ferritin demonstrated staining in cells from embryonal carcinomas and immature teratomas, but not in cells from benign tissue or seminomas[9]. Serum concentrations of AFP and ferritin were increased when large numbers of the tumour cells stained positively for both antigens, and decreasing levels were found after successful therapy. However, in cases of tumour recurrence AFP was not necessarily elevated in the serum, although detected previously in the primary tumour. Using antisera against normal human ferritin, Jacobsen *et al.* [10] described immunoperoxidase staining of ferritin in tissue sections of embryonal carcinoma, yolk-sac tumour, teratoma and seminoma. Ferritin was also demonstrated in carcinoma *in situ* in adjacent testicular tissue from patients with germ-cell tumours but not in germinal epithelium in a variety of non-malignant pathological conditions, including inflammation[17]. More direct evidence for the production of ferritin by tumour cells is provided in a study of nude mice inoculated with a variety of human carcinomas[18]. An anti-liver ferritin assay detected human ferritin in the sera of 15 mice with 10 different malignancies; serum ferritin levels increased with tumour growth and decreased rapidly after removal of tumour. When a radioimmunoassay based on more acidic isoferritins was employed, differences in serum ferritin estimation were noted; in some cases this assay detected an increase in serum ferritin of up to 5-fold the level detected by the anti-liver ferritin assay. Although such differences may simply be attributed to variation in standardisation of the protein, assays specific for the more basic isoferritins may underestimate serum ferritin concentration. The isoelectric profile of ferritin purified from a variety of neoplastic tissues differs from the corresponding normal tissue ferritin with the presence of extra bands of immunologically distinct isoferritins[19, 20]. The assay employed for serum ferritin estimation in the present study would not be capable of differentiation between normal and tumour tissue ferritin.

At the present moment, serum ferritin, when used in conjunction with AFP and β HCG, may be of value in the assessment of tumour response. It may not be a specific tumour product, however, in that, in some patients, it could also reflect various non-metastatic disorders.

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