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Serum Ferritin Determinations are of no Value in the Management of Patients with Disseminated non-Seminomatous Germ Cell Tumors*

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Abstract—*In this study the clinical significance of serum ferritin (SF) determinations was evaluated in patients with disseminated non-seminomatous germ cell tumors (NSGCT). The SF levels during the presence of active tumor but before institution of combination chemotherapy* (cis-*platinum, vinblastine and bleomycin, PVB) were within the normal limits in 38/47 NSGCT patients. During PVB treatment peaking SF levels were observed in relationship to the drug administration, whereas alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) serum levels decreased continuously. Other drugs also caused temporary increases in SF levels. Tumor recurrence was not recognized by SF increases in all eight patients tested. Based on the results we conclude that serial SF determinations are of no value in monitoring patients with NSGCT.*

INTRODUCTION

FERRITIN is an iron-containing protein which is widespread in the animal kingdom. In humans ferritin is present in major organs such as heart, liver and spleen [1]. Ferritin is also a major constituent of hemopoietic tissue and the mononuclear phagocytic system, where it plays a central role in the storing and recycling of iron for future use in the synthesis of heme and other iron-containing proteins [2]. Increased levels of serum ferritin (SF), not related with iron overload, have been demonstrated in a variety of pathologic states, including solid tumors [3]. The usefulness of SF as a tumor marker in patients with non-seminomatous germ cell tumors (NSGCT) is still a matter of debate. Two research groups advocated that SF determinations are meaningful in monitoring therapy in advanced disease, especially in the serodiagnosis of metastases [4-6]. However, other authors drew a contradicting conclusion from their results obtained in patients with disseminated testicular malignancies, including NSGCT [7].

These conflicting reports prompted us to perform studies on the clinical significance of SF in these patients. We included patients who were serum-negative for the conventional tumor markers alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) because here a new marker is urgently needed.

The questions addressed in the present paper are: (1) Are SF levels indicative for the presence of active tumor in NSGCT patients? (2) Are SF levels influenced by chemotherapy? (3) Are SF levels of value in the early recognition of tumor recurrence?

Based on our results we conclude that SF determinations are of no use in monitoring patients with NSGCT.

MATERIALS AND METHODS

Patients

The patients and the serum samples were selected from a patient population of more than

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120 individuals with disseminated NSGCT and more than 10,000 serum samples (stored at -20°C). The vast majority of patients underwent orchidectomy elsewhere and were referred to our hospital for staging and chemotherapy. Serum samples were drawn before any therapy was started. The clinical histories of the patients were carefully screened for blood transfusions, which are known to influence SF levels. A detailed description of our routine treatment of these patients has been given elsewhere [8]. All patients received remission-induction chemotherapy consisting of four cycles of a combination of *cis*-diamminedichloroplatinum (P), vinblastine (V) and bleomycin (B), according to Einhorn and Donohue [9]. One cycle includes one week of hospitalization, during which the drug was given, and two weeks at home. Serum samples were collected three times a week during hospitalization and thereafter at every outpatient visit. In almost every serum sample AFP, HCG and SF were measured. In the nine patients in whom a longitudinal study was undertaken in total more than 1000 AFP, HCG and SF determinations were performed.

Methods

Serum levels of AFP were measured with an enzyme-linked immunosorbent assay (ELISA) (AFP-EIA, Abbott Diagnostic Products GmbH, Wiesbaden, F.R.G.) with a sensitivity of 0.5 μg/l and an upper limit of normality of 20 μg/l. Serum HCG was estimated with a radioimmunoassay (Institute National des Radioelements, Fleures, Belgium), which is characterized by a cross-reactivity with the β-HCG subunit and with luteinizing hormone of 5 and 2%, respectively. The upper normal limit was established at 2 μg/l. SF levels were determined with a commercial ELISA (Ferrizyme, Abbott Diagnostic Products GmbH) with a sensitivity of 5 μg/l, and with normal values for healthy males (excluding blood donors) within a range of 16–250 μg/l.

RESULTS

Although a large number of NSGCT patients was available for study, we chose a strategy in which a restricted number of patients was thoroughly examined. More than one phenomenon could be studied within a single patient. The experiments were divided in three groups, the numbers 1-3 corresponding with the questions 1-3 from introduction.

(1) *SF levels before chemotherapy*

SF levels were determined in samples of NSGCT patients ($n = 47$) with disseminated disease and before the institution of PVB chemotherapy. Eleven patients were negative and 36 positive for the conventional tumor markers. The results showed that SF levels were above normal range in 3/11 (27%) AFP- and HCG-negative patients and in 6/36 (17%) marker-positive patients.

(2) *SF levels during chemotherapy*

Serial SF determinations during the four cycles of remission induction chemotherapy were performed in nine NSGCT patients; two of them were negative and seven positive for the conventional tumor markers. The SF levels before chemotherapy in these nine patients were 4, 12, 52, 75, 98, 150, 381, 675 and 900 μg/l. All nine profiles displayed peaking SF levels, which appeared to be dependent on the PVB administration. Figure 1 shows an example of this phenomenon. It clearly demonstrates that during the five days of each of the four cycles the patient is hospitalized for the drug infusion, the SF values are increasing. This is followed by a rapid decrease once the drug administration is stopped. Figure 1 also illustrates the discordant profiles of SF and serum HCG levels during the PVB treatment. Such a different behavior between SF and the conventional tumor markers AFP and/or

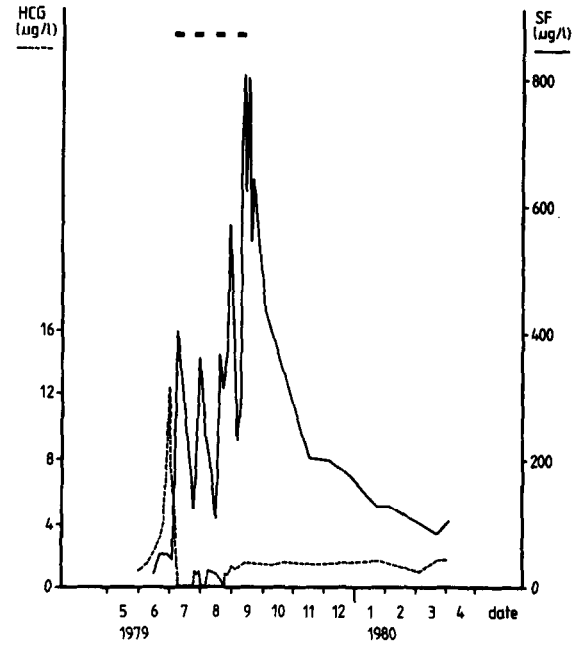

Fig. 1. SF and HCG profiles in an NSGCT patient treated with four cycles of PVB chemotherapy. A 41-yr-old NSGCT patient was referred to our hospital with extensive retroperitoneal lymph node and lung metastases. The orchidectomy had been performed elsewhere on 05-28-79. Four cycles of PVB chemotherapy were given (indicated in the figure by horizontal bars). Maintenance therapy, consisting of three-weekly alternating doses of P and PV, was started on 09-18-79 and ended on 09-09-80. A restaging laparotomy on 03-18-80 only revealed necrotizing tumor tissue. AFP levels remained normal during the course of disease (see also text).

HCG could be noticed in all seven marker-positive patients.

The influence of drugs other than PVB on the SF levels was investigated in seven patients who received rescue therapy after tumor relapse. The regimen consisted of sequential treatment with P, actinomycin D and VP 16-213 (etoposide) (four patients), P, VP 16-213 and cyclophosphamide (two patients) or P, adriamycin and VP 16-213 (one patient). In all instances the cytotoxic drugs influenced the SF levels. In Fig. 2 SF and AFP profiles are shown in an NSGCT patient with a complicated clinical course, which made a multiple drug regime necessary.

(3) *SF levels during tumor recurrence*

In eight patients who relapsed after four cycles of PVB, SF levels were studied before and during the tumor recurrences. Changes in SF levels never indicated the recurrent tumor correctly. Figure 3 shows an example of a patient who experienced two tumor recurrences. In both occasions the significant increases in AFP were not accompanied by meaningful changes in SF levels. Figure 4 illustrates a misleading SF profile. Multiple pulmonary metastases were indicated by chest X-ray examination but not by increases in SF and AFP. Extensive chemotherapy and

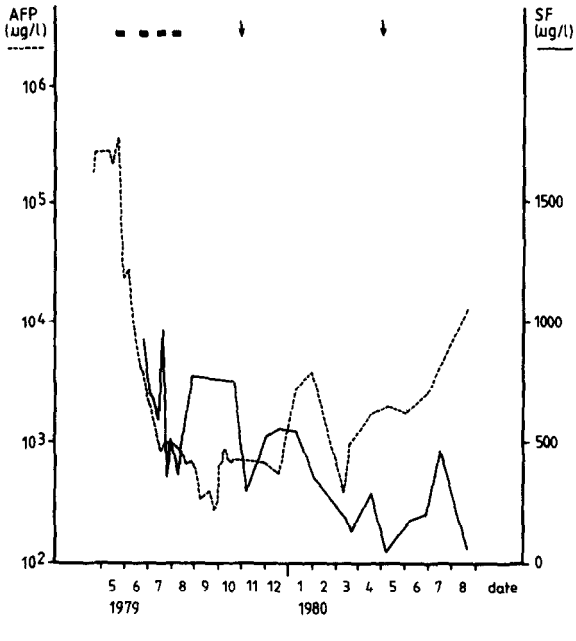

Fig. 2. SF and AFP profiles in an NSGCT patient with a multiple drug regime. In a 35-yr-old NSGCT patient extensive retroperitoneal lymph node metastases were discovered after orchidectomy. The patient was referred to our hospital and remission-induction chemotherapy consisting of four cycles of PVB (indicated in the figure by horizontal bars) was started on 05-14-79. Restaging laparotomy on 09-27-79 revealed vital tumor tissue and a three-weekly alternating V and PV chemotherapy (indicated in the figure by vertical arrows) was started on 11-06-79 and maintained until 05-06-80. HCG levels remained normal during the course of disease.

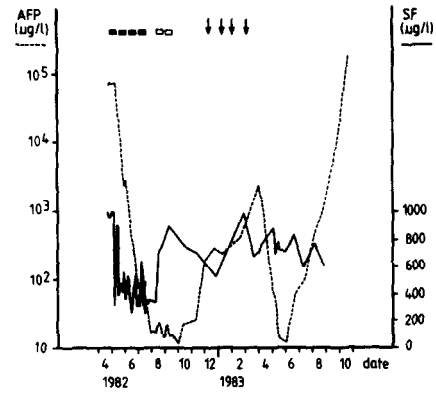

Fig. 3. SF and AFP profiles in an NSGCT patient with two tumor recurrences. A 41-yr-old NSGCT patient was admitted to our hospital with retroperitoneal lymph node and lung metastases after orchidectomy. Remission-induction consisted of four cycles of PVB (indicated in the figure by horizontal bars) and was followed by two consolidation cycles with PV, which started on 08-09-82 and 08-30-82 (indicated in the figure by open bars). Restaging thoracolaparotomy on 10-28-82 only revealed necrosis. However, a biochemical relapse became evident on 11-30-82. Single-agent therapy consisting of adriamycin was given on 12-12-82, 01-11-83, 02-01-83 and 03-03-83 (indicated in the figure by vertical arrows), but did not induce remission. Bone marrow was harvested on 03-08-83. Rescue therapy was started, consisting of VP 16-213 during three consecutive days, starting on 03-29-83, followed by high-dose VP 16-213 and high-dose cyclophosphamide, during three consecutive days, starting on 04-20-83. Bone marrow was re-infused on 04-29-83. A biochemical relapse became evident on 06-16-83 and the patient died in October 1983. HCG levels remained within the normal range throughout the course of the disease.

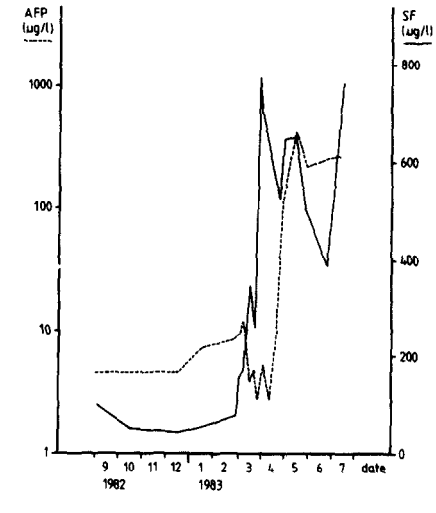

Fig. 4. Misleading SF profile in an AFP-positive NSGCT patient. At the age of 39 an NSGCT patient was admitted to our hospital with lung and skin metastases after orchidectomy elsewhere. Four cycles of PVB remission-induction chemotherapy was started on 04-05-82. Restaging thoracotomy was performed on 07-06-82 and revealed mature teratoma. The progression of lung metastases was histologically proven on 11-25-82. The bone marrow was harvested on 03-01-82. Rescue therapy consisted of high-dose VP 16-213 during three consecutive days (starting 03-02-83), high-dose VP 16-213 together with high-dose cyclophosphamide during three consecutive days (starting 03-21-83) and bone marrow re-infusion on 03-29-83. The lung metastases progressed and the patient died on 07-24-83.

autologous bone marrow re-infusion induced raised SF levels. Later on AFP levels increased, reflecting tumor progression, whereas the increased SF levels were thought to be due to the multiple blood transfusions.

DISCUSSION

Taking together (1) the impotence of SF to recognize active, disseminated tumor, (2) the peaking SF values during PVB chemotherapy whereas the established tumor markers AFP and HCG are constantly decreasing, (3) the influence of cytotoxic drug other than PVB on SF levels and (4) the failure of SF to detect tumor recurrence, we conclude that serial SF determinations are useless in monitoring NSGCT patients. This conclusion is in agreement with that reached by Engelmann *et al.* [7], but contradicts those made by Linkesch *et al.* [4, 5] and Grail *et al.* [6]. In our opinion Grail *et al.* [6] took insufficient notice of the influence of drug regimens on SF levels and they did not account for the extratumoral origin of SF.

Ferritin has been demonstrated in tissue sections of germ cell tumors of the testis, although different authors reported different percentages of ferritin-positive cells [10, 11]. An important source of SF is formed by cells of the mononuclear phagocytic system [2]. We hypothesize that the discordant behavior of SF vs AFP and HCG reflects different sites of synthesis and release. AFP and HCG are synthesized by the tumor, whereas SF levels in patients treated with chemotherapy mainly originate from a drug-induced release of ferritin from the mononuclear cells. Ferritin release from the necrotizing tumor is presumably less important.

The lack of tumor specificity of SF NSGCT patients emphasizes the need for investigations into new tumor markers in these patients. Perhaps hybridoma techniques could provide researchers with specific tumor markers in the near future.

In conclusion, SF determinations can safely be omitted in the management of NSGCT patients.

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