European Journal of Cancer Vol. 31A, No. 5, pp. 848–849, 1995
Elsevier Science Ltd
Printed in Great Britain

Letters

0959-8049(95)00014-3

Flow Cytometric and Cytophotometric DNA Analysis Cannot Predict Subsequent Tumour Recurrence in Pathological Stage IIA/B Non-seminomatous Testicular Germ Cell Tumour Patients Who Do Not Receive Adjuvant Chemotherapy

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MORE THAN half of the patients with clinical stage I (Lugano) non-seminomatous testicular germ cell tumours (NSGCT) and low stage lymph node metastasis (pathological stage IIA/B) are cured by retroperitoneal lymph node dissection (RPLND) [1, 2]. Two courses of adjuvant chemotherapy after RPLND decrease the risk of subsequent recurrence to less than 1%. However, the Testicular Cancer Intergroup Study showed that observation after RPLND for pathological stage II disease, with three courses of chemotherapy for the 30–50% who recur, yields equivalent overall survival [3]. A high volume of retroperitoneal lymph node metastasis may be predictive of subsequent tumour recurrence [1]. So far no reliable predictive factors for distant tumour recurrence after RPLND for stage IIA/B disease have been found to select patients for adjuvant chemotherapy.

In addition to histopathology, DNA analysis (cell cycle analysis by flow cytometry, single cell cytophotometry) helped to predict occult metastatic disease in clinical stage I NSGCT patients [4]. The same methods were applied to a group of pathological stage IIA/B NSGCT patients to predict tumour recurrence during observation without adjuvant chemotherapy.

Between January 1981 and December 1990, 59 clinical stage I NSGCT patients after RPLND were found to have metastatic lymph node involvement (pathological stage IIA or IIB) at Indiana University and did not receive adjuvant chemotherapy. Seventy-eight tissues blocks (formalin-fixed, paraffin-embedded tissue of the orchiectomy specimen) of 38 of these patients (15 of 38 with subsequent distant tumour recurrence, 23 of 39 without tumour recurrence) were obtained by correspondence (mean: 2.1 blocks per patient, range 1–4). In the remaining patients, the orchiectomy specimens were either not available because they had already been cleared from the files or the referring hospitals refused to send the blocks. Follow-up of the patients averaged 45.7 months (range 9–147) after RPLND and mean time to tumour recurrence was 8.2 months after RPLND (range 2–29).

In 18 of 23 patients (78%) without and in 14 of 15 patients (93%) with subsequent tumour recurrence, embryonal carcinoma was a major component of the primary tumour. In both groups approximately 50% of patients showed lymphatic invasion of their primary tumour. The patients without tumour recurrence had a mean of 2.7 retroperitoneal nodes containing metastasis at RPLND (standard deviation (S.D.) 2.4, range 1–6). The patients with subsequent distant relapse, however, were found to have a mean of 5.8 (S.D. 7.3, range 1–25) malignant lymph nodes ($P = 0.13$) at RPLND. Pathological stage IIB patients tended to develop subsequent distant metastases more often than patients with IIA disease (Table 1). DNA analysis by flow cytometry, including aneuploid S-phase analysis of the primary tumour stemline, did not allow prediction of subsequent chest or serological recurrence in these pathological stage II patients. All but 4 patients with tetraploid tumours had tumours with hyperdiploid stemlines. The DNA indices of patients with and without recurrence did not show any notable difference (1.46 (S.D. 0.17) for non-relapsers, 1.49 (S.D. 0.27) for relapsers). Nuclei with a very high DNA content (5c exceeding rate) were detected by single cell cytophotometry. There was a trend towards increasing chance of recurrence with a higher percentage of hyperpentaploid nuclei and higher amounts of aneuploid nuclei (2c deviation index), but this was not statistically significant (Table 1).

Table 1. Histopathological, flow cytometric (FC) and single cytophotometric (IA) results in pathological stage II patients with and without tumour recurrence (two-tailed Students t-test)

	Patients with tumour recurrence ($n = 15$)	Patients without tumour recurrence ($n = 23$)	P value
Mean number of malignant lymph nodes (S.D.)	5.8 (7.3)	2.7 (2.4)	0.13
Pathological stage IIA patients	8 (53%)	17 (74%)	0.20
Pathological stage IIB patients	7 (47%)	6 (26%)	0.20
DNA index by FC (S.D.)	1.49 (0.27)	1.46 (0.17)	n.s.
% aneuploid S-phase by FC (S.D.)	40.8 (13.9)	36.6 (19.7)	0.46
2c deviation index by IA (S.D.)	3.56 (3.1)	1.97 (1.3)	0.09
% 5c exceeding rate by IA (S.D.)	9.69 (8.5)	6.38 (4.2)	0.19

S.D., standard deviation; n.s., non-significant. $P < 0.05$ was used as a measure of significance.

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Revised 21 Dec. 1994; accepted 9 Jan. 1995.

In conclusion, neither histopathological evaluation nor DNA analysis predicted subsequent distant metastasis in pathological stage II NSGCT patients. Until predictors of subsequent distant (chest or serological) recurrence are found in pathological stage II patients, the options of immediate adjuvant chemotherapy or observation after RPLND must be presented to the patient. No known biological parameter mandates adjuvant chemotherapy in this group of patients.

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European Journal of Cancer Vol. 31A, No. 5, p. 849, 1995.

0959–8049/95 $9.50 + 0.00

0959-8049(94)00515-X

Exceptional Toxicity of 5-Fluorouracil and Interferon-α in a Patient with Hepatocellular Carcinoma

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WE OBSERVED severe mucosal toxicity and thrombocytopenia after a first course of fluorouracil (5-FU) and interferon-α (IFN-α) in a patient suffering from liver cirrhosis and hepatocellular carcinoma.

The patient was a 67-year-old man who, in 1992, had been diagnosed as having liver cirrhosis due to prolonged alcohol consumption. An inoperable hepatocellular carcinoma was diagnosed in April 1994. At the time of diagnosis he had a normal blood count, but the highest pretreatment value of alphafetoprotein (AFP) was 2500 U/l (normal values shown in Table 1).

Treatment with 5-FU, 500 mg/m^2 and IFN-α (Introna) 3 million units/day was given for 5 consecutive days (days 1–5). By the end of treatment, the patient started to have symptoms of stomatitis and was admitted on day 6 with severe mucosal bleeding of the oral cavity, epistaxis, fever and ascites. The thrombocyte count was 9 × 10^9/l. He had surface antibodies IgG, IgM and IgA against thrombocytes. Ultrasound examination showed a small, cirrhotic liver, a small spleen and some ascites. The leucocyte nadir of 1.1 × 10^9/l was observed on day 16. Bone marrow examination was performed when thrombocytes were recovering and showed normal haematopoiesis.

Table 1. Blood chemistry prior to, and 1 and 4 weeks after treatment with 5-fluorouracil and interferon-α

	Before treatment	Day 7	Day 28	Normal values
Hb (g/l)	103	90	106	128–168
WBC (×10^9/l)	7.6	3.0	12.8	3.7–10
Thrombocytes (×10^9/l)	158	9	65	150–400
GT (U/l)	839	364	273	<90
AFOS (U/l)	1147	597	649	110–300
Albumin (g/l)	29	18	16	37–50
Creatinine (μmol/l)	52	52	65	<135
Bilirubin (μmol/l)	29	38	46	<20
AFP (kU/l)	2500	800	812	<10–10

Hb, haemoglobin; WBC, white blood cell count; GT, glutamyltranspeptidase; AFP, alphafetoprotein.

The patient was treated with parenteral feeding, transfusions of thrombocytes (four times) and red cells (once), antibiotics and diuretics. His blood values prior to, on admission and on discharge from the hospital are presented in Table 1. He recovered and was discharged on day 30.

Haematologic and hepatic toxicities of IFN-α are usually dose related and occur more commonly with doses exceeding 10 MU. The incidence of severe bleeding and clotting disorders or grade 3–4 somatitis was less than 1% in a series of 800 treated cancer patients [1]. The tolerance of combined treatment is dose dependent; mucositis, myelosuppression and electrolyte waste are dose limiting [2]. Mucositis occurs usually at days 10–14 after combination treatment [3]. Liver cirrhosis decreases the metabolism of 5-FU, which is potentiated by IFN-α, and causes an exceptionally intense effect on mucosa and bone marrow, and slow recovery of blood values. Although IFN-α can usually be safely used with chemotherapy in the treatment of malignancies, the presence of a cirrhotic liver can cause exceptional severe synergistic toxicity, as seen in our patient. The presence of antibodies against thrombocytes in this patient indicates an immunological thrombocytopenia, but a direct inhibitory effect of IFN-α on promegakaryocytes in the bone marrow [4] cannot be dismissed.

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Received 1 Sep. 1994; accepted 30 Nov. 1994.

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