Eur J Cancer, Vol. 28, No. 2/3, pp. 410–415, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00

Serum Lactate Dehydrogenase Isoenzyme 1 and Tumour Volume are Indicators of Response to Treatment and Predictors of Prognosis in Metastatic Testicular Germ Cell Tumours

Finn Edler von Eyben, Ole Blaabjerg, Ebbe Lindegaard Madsen, Per Hyltoft Petersen, Christian Smith-Sivertsen and Bo Gullberg

44 patients with metastatic testicular germ cell tumours treated with cisplatin-based chemotherapy were evaluated for prognostic implications of clinical characteristics. 22 obtained complete remission by the initial chemotherapy, and 30 are disease-free. S-LDH-1 had an overall predictive value regarding the response of 80%, S-LDH of 64%, S-AFP of 62%, and S-hCG of 62%. In multivariate analysis regarding response, only tumour volume classified according to the Royal Marsden system ($P = 0.0036$) and S-LDH-1 ($P = 0.0069$) yielded information. Regarding survival, S-LDH-1 ($P = 0.0141$) and an estimate of total tumour mass ($P = 0.0171$) had most impact with additional information from S-hCG only ($P = 0.0536$). We conclude that S-LDH-1 may be used as a tumour marker in addition to S-hCG and S-AFP in patients with metastatic testicular germ cell tumour.
Eur J Cancer, Vol. 28, No. 2/3, pp. 410–415, 1992.

INTRODUCTION

FOR PATIENTS with metastatic testicular germ cell tumour, monitoring the clinical course with tumour markers is important. It is generally recommended to measure serum human chorionic gonadotropin (S-hCG), and serum alpha fetoprotein (S-AFP), with or without serum lactate dehydrogenase (S-LDH) [1]. The prognosis for patients with metastatic testicular germ cell tumours depends on the tumour burden after orchiectomy and before chemotherapy. Tumour marker levels at the beginning of cytotoxic treatment may also contribute in predicting survival [2, 3]. However, the prognostic impact of the three markers diverge from study to study [4, 5].

LDH is separated in five isoenzymes by electrophoresis. LDH isoenzyme 1 (LDH-1) has the highest migration towards to anode, and LDH isoenzyme 5 (LDH-5) the lowest migration. The isoenzymes are composed of four subunits with different combinations of two polypeptides, the A and B subunits. LDH-1 consists of four B subunits, and LDH-5 of four A subunits.

In patients with germ cell tumours, an increased S-LDH is mainly due to the anodal LDH isoenzymes, especially S-LDH-1 [6]. S-LDH-1 may also help to predict the outcome [6, 7]. Hence, we studied the impact on prognosis of patients with metastatic testicular germ cell tumours from tumour burden, S-LDH-1, S-LDH,S -AFP, and S-hCG.

Correspondence to F.E. von Eyben.
F.E. von Eyben is at the Department of Internal Medicine, Central Hospital, Nykøbing Falster, DK-4800 Nykøbing Falster, Denmark; E.L. Madsen is at the Department of Oncology and Radiotherapy; F.E. von Eyben, O. Blaabjerg and P.H. Petersen are at the Department of Clinical Chemistry, C. Smith-Sivertsen is at the Department of Diagnostic Radiology, Odense University Hospital, Denmark; and B. Gullberg is at the Department of Community Health Sciences, Malmö General Hospital, Malmö, Sweden.
Revised 31 Oct. 1991; accepted 18 Nov. 1991.

PATIENTS AND METHODS

Patients

We studied 44 patients with metastatic testicular germ cell tumours treated at the Department of Oncology and Radiotherapy, Odense University Hospital, between January 1980 and December 1985. All had evidence of disease at the start of chemotherapy (Table 1). 11 patients treated during the same period but without serum samples available from the time of the first and the second course of chemotherapy were not included in the study (Table 1).

Histological and staging procedures

All had histologically proven testicular germ cell tumour. The diagnosis was revised according to the DATECA protocol [3] and WHO. The findings of the physical examination, chest X-ray, pedal lymphangiogram and computed tomography scans of the thorax and abdomen at start of chemotherapy for metastatic disease were re-examined by two physicians. The volume of measurable tumour lesions (V) was calculated by the formula for an ellipsoid of revolution [8]

$$V = \pi/6 \times D_1 \times D_2^2$$

where D_1 is the largest diameter and D_2 the perpendicular diameter. We estimated the total tumour mass in each patient by the sum of the tumour volumes.

The tumours were classified in three stages: stage 1, primary tumour only; stage 2, regional lymph node metastases; stage 3, distant metastases. We also classified tumour volume using the Royal Marsden Hospital system [2]. All assessments were made without prior knowledge of what the outcome would be.

Tumour marker determinations

Blood specimens for marker determinations were obtained 1–3 days before the first course of chemotherapy. We measured S-LDH-1, S-LDH, S-AFP, and S-hCG as reported earlier

Table 1. Characteristics of included and excluded patients with metastatic testicular germ cell tumours

Feature	Patients included	Excluded	*P*-value
Age at orchiectomy	29 (17–72)	31 (22–49)	0.2424
Seminoma	7	1	
Non-seminoma	37	10	0.9835
Stage 1 (relapse) and 2	29	6	
Stage 3	15	5	0.7144
Small tumour volume	30	8	
Large and very large tumour volume	14	2	
NI	0	1	0.7479
Prior radiotherapy	4	2	
No radiotherapy	40	9	0.6888
S-LDH			
Normal (< 450 U/l)	17	3	
Raised	26	7	
NI	0	1	0.8587
S-AFP			
Normal (< 20 kU/l)	24	5	
Raised	20	6	0.8375
S-hCG			
Normal (< 30 U/l)	22	5	
Raised	22	5	
NI	0	1	1.00

NI denotes patients where information is missing. S-LDH was determined in all but 1 patient.

[9]. The serum haemoglobin concentration was determined spectrophotometrically in all samples. We reduced the measured isoenzyme activities by 0.2 U/l for each mg/l haemoglobin as to minimise the impact from haemolysis. We used the reference intervals from an earlier study [9]. All measurements were made without prior knowledge of what the outcome would be.

Treatment

42 patients had standard dose cisplatin, vinblastine and bleomycin (PVB) as the first chemotherapy regimen. 1 had a combination of high dose cisplatin and etoposide (PE). 1 had a high dose cisplatin regimen with etoposide, bleomycin, and vinblastine (PEBV). 5 patients with only minimal response after 2–3 courses of PVB were also switched to these regimens. Patients with seminoma stage 2 group underwent high voltage irradiation after 2–3 courses of PVB. Residual lesions after the induction courses were resected. Only the patients with remaining viable tumour were given further chemotherapy.

Patients with complete remission (CR) were followed without further treatment unless they had a relapse.

Assessment of response

Complete remission (CR) implied disappearance of all tumour lesions and cancer related symptoms for at least 1 month. Patients without CR had incomplete remission (IR). We classified all patients with residual lesions after chemotherapy as IRs.

Patients with seminoma stage 2 were not evaluated for response to the initial chemotherapy.

Table 2. Relation between tumour markers and response to initial chemotherapy

Tumour marker	Prediction of CR from a normal test	Prediction of IR from a raised test	Sensitivity	Specificity	Overall predictive value
S-LDH-1	0.89	0.71	0.73	0.88	0.80
S-LDH	0.79	0.58	0.50	0.82	0.64
S-AFP	0.68	0.55	0.59	0.65	0.62
S-hCG	0.71	0.55	0.55	0.71	0.62

The calculations are based on 39 patients evaluable for response to the chemotherapy. 5 patients with seminoma stage 2 were excluded.

Relation between marker status and response to chemotherapy

The predictive value of a normal test was defined as the number of evaluable patients with CR and a normal test divided by the total number with a normal test. The predictive value of an increased test was the number of IRs with an increased test divided by the total number with an increased test. The sensitivity of a test regarding remission was the number of CRs with a normal test divided by the total number with CR. The specificity was the number of IRs with a raised test divided by the total number with IR. We calculated the efficacy for a test as predictor of response as the sum of the CRs with a normal test and the IRs with a raised test divided by the total number of patients (Table 2) [10].

Follow-up

All patients were monitored regularly from initiation of chemotherapy with case history, physical examination, chest X-ray, CT scans, and determination of S-LDH, S-AFP, and S-hCG. The patients were followed up until May 1991.

Statistical analysis

We evaluated the relation between the clinical characteristics and the response to the initial chemotherapy. The findings were evaluated as discrete variables (normal vs. raised) with Fisher's exact test (Table 3), and as continuous variables with the Mann-Whitney U test (Table 4). The relation between variables was analysed with Pearsons correlation coefficient test. Statistical significance implied a *P*-value less than 0.05.

Selected characteristics were studied as to the prediction of overall survival (Tables 5 and 6). The survival from start of chemotherapy was calculated as a Kaplan–Meier estimate and differences in outcome between subgroups were evaluated with the logrank test.

Multivariate analyses were performed with the Cox's proportional hazard model [11]. The subgroups of stage and of tumour volume were entered in the analyses as three variables each. Markers were analysed simultaneously both as discrete and as continuous variables, as were both measures of tumour mass and staging. Covariates were selected in a stepwise (forward) fashion with use of the maximum-likelihood ratio. A *P*-value of 0.15 was adopted as limit for inclusion of a covariate.

RESULTS

Tumour load, S-LDH-1, S-LDH, S-AFP, and S-hCG

30 patients had small tumour disease according to the Royal Marsden Hospital classification, 5 had large volume disease, and

Table 3. Characteristics of 44 patients with metastatic testicular germ cell tumours and the impact of the characteristics on response

Feature	Total no. of patients	Evaluable no. of patients	CRs	*P*-value
Seminoma	7	2	1	
Non-seminoma	37	37	21	1.000
Stage 1 (relapse)	11	11	9	
Stage 2 and 3	33	28	13	0.0947
Small and large tumour volume	35	30	22	
Very large tumour volume	9	9	0	0.0002
Prior radiotherapy	4	4	3	
No radiotherapy	40	35	19	0.8145
S-LDH-1				
Normal (< 109 U/l)	20	18	16	
Raised	24	21	6	0.0003
S-LDH				
Normal	17	14	11	
Raised	26	24	10	0.0586
S-AFP				
Normal	24	19	13	
Raised	20	20	9	0.2494
S-hCG				
Normal	22	17	12	
Raised	22	22	10	0.2127
Total no. of patients	44	39	22	

5 patients with seminoma stage 2 were excluded in the evaluations of response. Evaluable patients without CR had IR. *P*-values show the distribution of CR and IR according to Fisher's exact test (two-tailed).

Table 4. Clinical characteristics according to response in 39 evaluable patients

Clinical characteristic	Median value	Range	*P*-value
Total tumour volume (cm^3)			
CR	3.394	0–125.7	
IR	27.72	0.5–1892.4	0.0053
S-LDH-1 (U/l)			
CR	94	34–1817	
IR	158	71–2702	0.0014
S-LDH (U/l)			
CR	441	279–1166	
IR	579	286–6800	0.031
S-AFP (kU/l)			
CR	16.5	4–1540	
IR	80	4–6600	0.210
S-hCG (U/l)			
CR	30.2	0–20000	
IR	70	0–1040000	0.138

The table shows the findings according to the level of response to chemotherapy (CR and IR). *P*-values are calculated according to Mann–Whitney U test (two-tailed).

Table 5. Clinical characteristics of 44 patients evaluated regarding impact on prognosis

Clinical characteristic	No. of patients	No. of survivors	*P*-value
Histology			
Seminoma	7	6	
Non-seminoma	37	24	0.5401
Tumour volume (Royal Marsden Hospital system):			
Small	30	23	
Large	5	4	
Very large	9	3	0.0011
Stage 1 (relapse)	11	8	
2	18	14	
3	15	8	0.2245
S-LDH-1			
Normal	20	18	
Raised	24	12	0.0007
S-LDH			
Normal	18	16	
Raised	26	13	0.0082
S-AFP			
Normal	24	18	
Raised	20	12	0.2607
< 500 kU/l	39	27	
> 500 kU/l	5	3	0.7928
S-hCG			
Normal	22	13	
Raised	22	17	0.2231
< 1000 IU/l	36	25	
> 1000 IU/l	8	5	0.6228
Raised S-LDH-1 and either raised S-AFP or raised S-AFP or both			
Absent	28	25	
Present	16	5	< 0.00001

P-values are calculated according to the logrank test (two-tailed).

Table 6. Clinical characteristics according to survival of 44 patients

Clinical characteristic	Median value	Range	*P*-value
Total tumour volume (cm^3)			
Survivors	3.96	0–850	
Non-survivors	13.72	1.9–1892.4	0.042
S-LDH-1 (U/l)			
Survivors	97.5	34–2702	
Non-survivors	157	102–2344	0.0034
S-LDH (U/l)			
Survivors	422	279–5000	
Non-survivors	593	429–6800	0.016
S-AFP (kU/l)			
Survivors	12.5	4–6600	
Non-survivors	30	5–2200	0.266
S-hCG (U/l)			
Survivors	30	0–140000	
Non-survivors	37.5	0–1040000	0.737

P-values are calculated according to the Mann–Whitney U test (two-tailed).

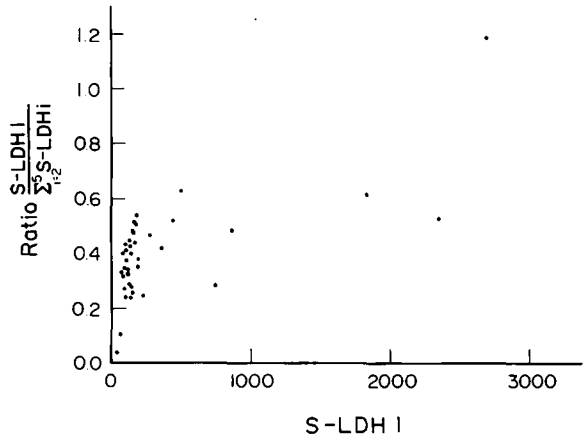

Fig. 1. The ratio between S-LDH-1 and the other S-LDH isoenzymes (S-LDH-2 to S-LDH-5) in relation to S-LDH-1 in 43 evaluable patients.

9 very large volume disease. The estimated tumour volume had a median of 9.2 cm^3 (range 0–2074 cm^3).

Table 3 shows the number of patients with raised tumour markers. S-LDH-1 had a median of 120 U/l (range 34–2702 U/l). S-LDH had a median of 463 U/l (range 279–6800 U/l), S-AFP had a median of 17 kU/l (range 4–6600 kU/l). S-hCG had a median of 31 U/l (range 0–1040000 U/l).

Histopathologic findings and tumour markers

The S-LDH-1 activities in the seminoma group (median 125 U/l, range 76–2702 U/l) conformed with those in the non-seminomatous group (median 115 U/l, range 34–2344 U/l, $P = 0.92$, Mann–Whitney U test, two-tailed). So did the proportion with a raised S-LDH-1 in the two groups ($P = 1.0$, Fisher's exact test, two-tailed). S-LDH in the seminoma group (median 441 U/l, range 281–5000 U/l) did not differ significantly from that of the non-seminomatous group (median 466 U/l, range 279–6800 U/l, $P = 0.88$ Mann–Whitney U test, two-tailed).

A raised S-AFP was less frequent in patients with seminoma than with non-seminomatous tumours (0% vs. 65%, $P = 0.0181$, Fisher's exact test, two-tailed). The seminoma group had also a slightly lower S-hCG (median 0 U/l, range 0–270 U/l) than the non-seminomatous group (median 35 U/l, range 0–1040000 U/l, $P = 0.063$, Mann–Whitney U test, one-tailed).

Relation between S-LDH-1, S-LDH, S-AFP, S-hCG, and tumour volume

Tumour volume correlated with S-hCG ($P = 0.046$, Spearman rank correlation coefficient test, two-tailed) but not with the other markers. Log LDH-1 correlated with log S-LDH ($P = 0.003$) but otherwise the markers did not correlate significantly.

Patients with a normal S-LDH-1 had a relatively low ratio between the activity of S-LDH-1 and the activity of the other S-LDH isoenzymes (LDH-2 to LDH-5) (median 0.34). The ratio was higher in the patients with raised S-LDH-1 (median ratio 0.47, $P = 0.0004$, Mann–Whitney U test, two tailed) (Fig. 1).

Response and survival

22 had CR from the initial chemotherapy. 17 had IR. 5 with seminoma stage 2 underwent irradiation after the initial chemotherapy before they were evaluated for response.

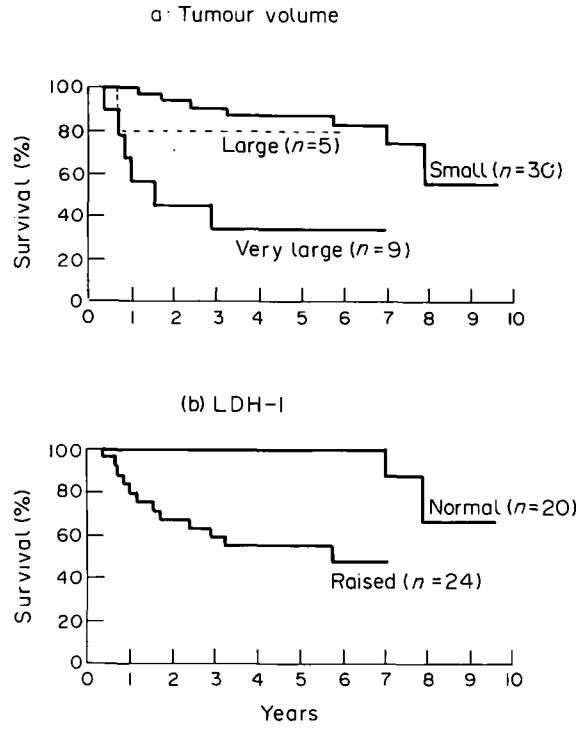

Fig. 2. Survival of 44 patients according to (a) the Royal Marsden Hospital system and (b) the S-LDH-1 activity.

Overall, 30 patients remain alive, and 14 died. The 3-year survival was 77%. Of the 22 patients with CR, 19 remain alive; 2 died without evidence of tumour at autopsy and 1 died with tumour. 7 patients with IR remain alive, 2 had pathological CR at laparotomy and after resection of an abdominal tumour lesion, respectively and 5 were saved with second-line chemotherapy. 10 patients with IR died with tumour, 4 patients with seminoma stage 2 remain alive; 1 died without evidence of tumour at autopsy.

Tumour markers and tumour mass in relation to response and survival

In univariate analysis of response, S-LDH-1 had a higher overall predictive value than the other three markers (Table 2). Small and very large volume according to the Royal Marsden Hospital system predicted high response rate ($P = 0.0216$) and low response rate ($P = 0.0002$), respectively (Table 3). Similarly, CR related to a low S-LDH-1, a small tumour volume, and a low S-LDH at the start of chemotherapy (Table 4).

In multivariate analyses of response to the initial chemotherapy, very large tumour volume ($P = 0.0036$) and S-LDH-1 as a discrete variable (raised vs. normal) ($P = 0.0069$) were predictors. Similarly, CR related to a low S-LDH-1, a low S-LDH, and a small total tumour volume at start of chemotherapy (Table 6).

In univariate analysis of overall survival, tumour volume predicted survival. The impact was similar when tumour volume was analysed according to our system using the sum of measurable tumour volumes, and according to the Royal Marsden Hospital system (Table 5, Fig. 2a). Three markers, S-LDH-1 (Table 5, Fig. 2b) and S-LDH as discrete variable and S-hCG as a continuous variable ($P = 0.0018$) were also significant predictors.

In multivariate analysis of overall survival, three variables

were predictors. S-LDH-1 as a discrete variable was most important ($P = 0.0141$). Tumour mass given as the sum of tumour volumes ($P = 0.0171$), and S-hCG as a continuous variable ($P = 0.0536$) also contributed in predicting outcome.

DISCUSSION

The principal findings of our study are the significant predictions of response and survival from S-LDH-1 in patients with metastatic testicular germ cell tumour treated with cisplatin-based combination chemotherapy. The predictive efficacy of S-LDH-1 surpassed that of the other markers. S-LDH-1 constituted an increasing proportion of the total S-LDH as the S-LDH-1 increased. The prognostic impact from S-LDH-1 remained in multivariate analyses without a separate gain from S-LDH. Our findings are consistent with previous reports regarding the prevalence of raised isoenzyme activity [6, 7, 12–14], response to chemotherapy [7], and outcome [6]. A few studies showed a higher prevalence of raised S-LDH-1 [15, 16], whereas an Australian investigation found a lower prevalence in the non-seminomatous group of patients [17]. Hydroxybutyrate dehydrogenase (HBD) was a significant prognostic factor for patients treated by Oliver *et al.*, both in univariate and multivariate analyses [18]. The HBD assay measures mainly the LDH-B subunit. Thus, HBD is predominantly due to LDH-1 in the absence of a raised LDH-2 and LDH-3.

Our findings concord with the previous observation that an increased S-LDH in these patients is due mainly to the anodal LDH isoenzymes [7, 19]. The increased LDH-1 production in testicular germ cell tumours may reflect a specific chromosomal abnormality present in about 80% of the tumours, an isochromosome of the short arm of chromosome 12, i(12p), and/or other chromosomal abnormalities with increased copy number of the short arm of chromosome 12. The gene locus for LDH-B, the subunit present as a tetramer in LDH-1, is on chromosome 12 in the region 12p12.2–12p12.1 [20]. Correspondingly, the tumours have a relatively high level of mRNA for LDH B [21]. The gene locus for the subunit LDH-A is localised on the short arm of chromosome 11 in the region 11p15.1–11p14. The copy number of the short arm of chromosome 12 in the tumour cells is much higher than that of the short arm of chromosome 11 [22, 24]. The total tumour copy nomber of the short arm of chromosome 12 related to the S-LDH-1 in peripheral arm vein blood [24]. So the imbalance in copy number of chromosome 11 and 12 may contribute to the predominance of the anodal LDH isoenzymes in patients with tumour lesions and raised S-LDH.

Total S-LDH was raised more often than S-LDH-1 in our patients. This may result from the fact that more disorders cause an increased total S-LDH than an increased S-LDH-1 [19]. A difference in origin for a raised S-LDH-1 and a raised S-LDH might have contributed to the greater prognostic impact from S-LDH-1 than from S-LDH.

Two classifications of tumour load (a quantitative sum of the measurable tumour volumes and the semiquantitative, Royal Marsden Hospital system) had a closely similar, high level of prognostic impact. Multivariate analyses favoured the Royal Marsden Hospital system as predictor of response, and the quantitative system as predictor of survival.

This study compares the information gained from S-LDH-1 in relation to that from the standard tumour markers, S-AFP and S-hCG, in the overall group of testicular germ cell tumours monitored with these markers. More seminoma patients had a raised S-LDH-1 than a raised S-AFP and S-hCG, as also found previously [6, 9, 21]. However, the study did not address whether the prognostic implications from a raised S-LDH-1 differ between patients with seminomatous and non-seminomatous tumours [25, 26].

S-LDH-1 and total tumour volume seem to express separate aspects of the aggressiveness of the tumours. Investigations of human disorders and experimental cell culture studies show that living cells release LDH-1 as they reach a point of irreversible damage [25]. So a high overall cell loss from the tumour is likely to cause the increased S-LDH-1 in patients with testicular germ cell tumour. The rate of cell loss may relate to the proliferation of the tumours and vary independently of the total tumour volume.

S-LDH pointed forward to the therapeutic and prognostic outcome for patients with metastatic testicular germ cell tumours in several multivariate investigations [8, 10, 26, 27]. The present study and the investigation analysing S-HBD [18] indicate that S-LDH-1 has a predictive potential at least as important as that of S-LDH, S-AFP, S-hCG. Thus, S-LDH-1 may be a useful addition to established strategies in separating patients with metastatic testicular germ cell tumours into two groups that are treated differently: a good risk group with a substantial cure rate from standard platin-based chemotherapy, and a poor risk group often given high dose platin combinations.

1. Smith RB, Haskell CM. Testis. In: Haskell CM, ed. *Cancer Treatment*. Philadelphia, WB Saunders, 3rd ed 1990, 779–797.
2. Medical Research Council Working Party on Testicular Tumours. Prognostic factors in advanced non-seminomatous germ-cell testicular tumours: results of a multicentre study. *Lancet* 1985, **i**, 8–11.
3. Væth M, Schultz HP, von der Maase H, *et al.* Prognostic factors in testicular germ cell tumours. Experiences from 1058 consecutive cases. *Acta Rad Oncol* 1984, **23**, 271–285.
4. Vogelzang NJ. Prognostic factors in metastatic testicular cancer. *Int J Androl* 1987, **10**, 225–237.
5. Hesketh PJ, Krane RJ. Prognostic assessment in nonseminomatous testicular cancer: implications for therapy. *J Urol* 1990, **144**, 1–9.
6. von Eyben FE, Skude G, Fossa SD, *et al.* Serum lactate dehydrogenase (S-LDH) and S-LDH isoenzymes in patients with testicular germ cell tumors. *Mol Gen Genet* 1983, **189**, 326–333.
7. Liu F, Fritsche HA, Trujillo JM, *et al.* Serum lactate dehydrogenase isoenzyme 1 in patients with advanced testicular germ cell cancer. *Am J Clin Pathol* 1982, **78**, 178–183.
8. von Eyben FE, Jacobsen GK, Pedersen H, *et al.* Multivariate analysis of risk factors in patients with metastatic testicular germ cell tumours treated with vinblastine and bleomycin. *Invasion Metastasis* 1982, **2**, 125–135.
9. von Eyben FE, Blaabjerg O, Hyltoft Petersen P, *et al.* Serum lactate dehydrogenase isoenzyme 1 as a marker of testicular germ cell tumor. *J Urol* 1988, **140**, 986–990.
10. Bajorin D, Katz A, Chan E, *et al.* Comparison of criteria for assigning germ cell tumor patients to "good risk" and "poor risk" studies. *J Clin Oncol* 1988, **6**, 786–792.
11. Kalbfliesch J, Prentice R. *The Statistical Analysis of Failure Time Data*. New York, John Wiley, 1980.
12. Lippert M, Papadopoulos N, Javadpour N. Role of lactate dehydrogenase isoenzymes in testicular cancer. *Urology* 1981, **18**, 50–53.
13. Vugrin D, Friedman A, Schwartz, Golbey R. Serum lactate dehydrogenase (LDH) isoenzyme 1 (LDH-1) and 2 (LDH-2) as tumor marker in germ cell tumours. *Proc AACR* 1980, **21**, 177.
14. Sugawara T, Furuhata T, Ogawa K, Hosaka M. A clinical study of testicular tumors—usefulness of serum lactic dehydrogenase (LDH). *Jap J Urol* 1986, **77**, 948–954.
15. Canal P, Villeneuve G, Bugat R, *et al.* La lactate deshydrogenase plasmatique et ses isoenzymes dans les tumeurs germinales non seminomateuses du testicule. *Pathol Biol* 1984, **32**, 245–250.
16. Zondag HA, Klein F. Clinical applications of lactate dehydrogenase isoenzymes: alternations in malignancy. *NY Acad Sci* 1968, **151**, 578–586.
17. Winkle DC, Clague AE, Gardiner RA. Elevated lactate dehydrogen-

ase isoenzyme 1 as a tumour marker in patients with germ cell testicular tumours. *Austr NZ J Surg* 1988, 58, 737–741.
18. Oliver RTD. Clues from natural history and results of treatment supporting the monoclonal origin of germ cell tumours. *Cancer Surveys* 1990; 9, 333–367.
19. von Eyben FE. Lactate dehydrogenase and its isoenzymes in testicular germ cell tumors: an overview. *Oncodevelopmental Biology and Medicine* 1983, 4, 395–414.
20. Human Gene Mapping. Ninth International Workshop on Human Gene Mapping. *Cytogenet Cell Genet* 1987, 46, 1–4.
21. von Eyben FE, Blaabjerg O, Petersen PH, *et al.* Lactate dehydrogenase isoenzyme 1 in testicular germ cell tumors. In: Oosterhuis JW, Walt H, Damjanov I, eds: *Pathobiology of Human Testicular Germ Cell Tumors. Recent Results in Cancer Research*, Springer Verlag, Berlin, 1991, 123, 85–92.
22. Castedo SMMJ, te Meerman GJ, Oosterhuis JW, de Jong B. Pathogenesis and oncogenesis of testicular germ cell tumors. Cytogenetic support for a unifying model. In: Castedo SMMJ. *Pathogenesis of Testicular Germ Cell Tumors. A Cytogenetical and Pathological Study*. Thesis, University of Groningen, 1988.
23. Lothe RA, Fosså SD, Stenwig AE, *et al.* Loss of 3p and 11p alleles is associated with testicular cancer tumors. *Genomics* 1989, 5, 134–138.
24. de Graaf WE, Marrink J, von Eyben FE, *et al.* Serum lactate dehydrogenase isoenzyme 1 activity in patients with testicular germ cell tumors relates to the total number of copies of the short arm of chromosome 12 in the tumor. The Mediterranean Conference on Tumor Markers, Nice, November 1991, in press (Abstract).
25. Fosså S, Fosså SD. Serum lactate dehydrogenase and human chorionic gonadotropin in seminoma. *Br J Urol* 1989, 63, 408–415.
26. Oliver RTD. A comparison of the biology and prognosis of seminoma and non-seminoma. In: Horwich A, Oliver RTD, eds. *Testicular Cancer*. London, Chapman Hall, 1991; 51–67.
27. Kristensen SR, Hørder M. Release of enzymes from quiescent fibroblasts during ATP depletion. *Enzyme* 1988, 39, 205–212.

Acknowledgements—Dr Bent Nørgaard Pedersen and cand. pharm. Jørgen Arends measured S-AFP and S-hCG and provided the specimens for S-LDH-1 measurement. Dr. Henrik Starklint revised the histological material. The Hans and Agnes Stener's foundation supported the study with a grant.

Eur J Cancer, Vol. 28, No. 2/3, pp. 415–420, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00

An Endocrine and Pharmacokinetic Study of Four Oral Doses of Formestane in Postmenopausal Breast Cancer Patients

M. Dowsett, A. Mehta, N. King, I.E. Smith, T.J. Powles, R.C. Stein and R.C. Coombes

43 postmenopausal breast cancer patients were treated orally with the aromatase inhibitor formestane (4-hydroxyandrostenedione) at daily doses of 62.5, 125, 250 or 500 mg for 4 weeks followed by 250 mg daily for a further 4 weeks. For some patients, 62.5 mg did not suppress serum oestradiol levels maximally. The doses of 250 and 500 mg did not differ in their effectiveness. Oestrone levels were suppressed by all doses of formestane but no consistent changes of aldosterone, cortisol or 17-hydroxyprogesterone occurred. Serum levels of sex hormone binding globulin fell by about 15% during treatment with 250 mg formestane reflecting its minor androgenic activity. The maximum concentration and area under the curve of serum formestane levels after the first dose varied in an approximately linear manner with dose. It is concluded that formestane is an effective, specific suppressant of oestradiol levels via the oral route requiring no more than 250 mg to be given daily.
Eur J Cancer, Vol. 28, No. 2/3, pp. 415–420, 1992.

INTRODUCTION

AROMATASE IS a cytochrome P_{450}-mediated enzyme complex which converts the androgens androstenedione and testosterone to oestrone and oestradiol, respectively. As such it is a pivotal enzyme in reproductive endocrinology and its manipulation provides a means by which a variety of sex steroid-dependent physiological and pathological processes may be altered. The possibility of treating oestrogen-dependent breast cancer with aromatase inhibitors to reduce the level of the patient's synthesis of oestrogen has been recognised for many years [1]. Aminoglutethimide was the first compound with which this approach was shown to be clinically effective [2–4]. However, aminoglutethimide has a number of clinically significant side-effects and it inhibits a number of other steroid hydroxylases, which necessitates its combination with glucocorticoid for maximum efficacy [5] and therapeutic safety [6]. There has therefore been a widespread search for an aromatase inhibitor which lacks this detrimental characteristic.

Formestane (4-hydroxyandrostenedione) was the first com-

Correspondence to M. Dowsett.
M. Dowsett, A. Mehta and N. King are at the Department of Academic Biochemistry; I.E. Smith is at the Medical Breast Unit, Royal Marsden Hospital, Fulham Road, London SW3 6JJ; T.J. Powles is at the Medical Breast Unit, Royal Marsden Hospital, Sutton, Surrey; R.C. Stein is at the Clinical Oncology Unit, St George's Hospital; and R.C. Coombes is at the Department of Medical Oncology, Charing Cross Hospital, London, U.K.
Revised 2 July 1991; accepted 10 Oct. 1991.