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Lactate Dehydrogenase as a Marker for Testicular Germ-Cell Tumours

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Abstract—*One hundred and eighty-four patients with testicular germ-cell tumours, 92 with seminoma and 92 with non-seminomatous germ-cell tumours (NSGCT) had total lactate dehydrogenase (LD) assay performed at the time of initial staging following orchidectomy. The proportion of patients with elevated plasma* LD *and the mean plasma LD increased with advancing stage and increasing tumour bulk for both seminoma and NSGCT. Of patients receiving cytotoxic chemotherapy,* 87.5% *with seminoma and* 63% *with NSGCT had elevated plasma LD with subsequent levels reflecting regression or progression of disease. Elevated plasma LD levels were seen in four of seven seminoma, and in* 18 *of* 30 *NSGCT patients relapsing after primary treatment.*

The LD assay provides useful information, and complements the routine measurement of alphafetoprotein (AFP) and beta human chorionic gonadotrophin (βhCG) in the management of patients with testicular germ-cell tumours.

INTRODUCTION

AFP and βhCG are established as markers in the management of patients with NSGCT. Serial estimations of AFP and βhCG are important for the detection of metastatic disease following orchidectomy alone or following adjuvant therapy with cytotoxic drugs or radiotherapy, and also for monitoring the response of established metastatic disease to cytotoxic chemotherapy [1, 2]. Approximately 75% of patients with metastatic NSGCT have elevated levels of one or both of these markers (1) and elevated levels of βhCG are seen in approx. 5–10% of patients with active seminoma [3, 4] but for the remainder an additional biochemical marker of disease activity would be of value.

In 1978, Von Eyben [5], in a series of 27 patients with advanced testicular tumours, reported a correlation between serum LD, and tumour burden calculated by the areas of all lesions demonstrated on chest radiographs and lymphograms. We have prospectively evaluated the role of LD as a tumour marker in the initial assessment, treatment and follow-up of patients with testicular germ-cell tumours.

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PATIENTS AND METHODS

Two hundred and fourteen patients with testicular germ-cell tumours were referred for treatment to the Department of Clinical Oncology, Edinburgh between January 1978 and June 1984. One hundred and eighty-four patients (86% of the total), including 92 with seminoma and 92 with NSGCT, had plasma LD assayed at the time of initial staging following orchidectomy. One hundred and seventy-six of them (seminoma, 87: NSGCT, 89) had serial LD assays performed during therapy, and 161 (seminoma, 85: NSGCT, 76) had serial LD assays performed during follow-up (minimum 6 months).

Histological classification

Histological material was reviewed in Edinburgh and was classified according to the British Testicular Tumour Panel classification [6].

Clinical staging

Patients were investigated routinely by chest radiography, lymphography, haematology and bio-chemistry, and since 1980 CT scanning of thorax and abdomen. The Royal Marsden staging system, [7] was employed (Table 1). According to these criteria, patients with lung metastasis ≥ 2 cm and node metastasis ≥ 5 cm were considered to have bulky disease.

Table 1. *Royal Marsden Hospital staging classification*

Stage I	No evidence of disease outside the testis
Stage II	Infradiaphragmatic node involvement This is subdivided according to the maximum diameter of metastases into the following substage categories: IIA Maximum diameter of metastases < 2 cm IIB Maximum diameter of metastases 2–5 cm IIC Maximum diameter of metastases > 5 cm
Stage III	Supra and infradiaphragmatic lymph node involvement This is subdivided as follows: Abdominal nodes: A, B, C as for Stage II Mediastinal nodes noted M+ Neck nodes noted N+ 0 = Negative lymphogram and abdominal CT scan
Stage IV	Extension of tumour to extralymphatic sites. The following suffixes define the extent and volume of metastatic spread: 0, A, B, C for abdominal nodes as for Stages I and II Mediastinal nodes noted M+ Neck nodes noted N+ Lung substage: L_1 Metastases ≤ 3 in number L_2 Metastases > 3 in number < 2 cm maximum diameter L_3 Metastases > 3 in number > 2 cm maximum diameter H+ Hepatic involvement. Other sites e.g. bone and brain are specified

Tumour markers

Serial estimations of plasma AFP, LD and serum βhCG, together with routine liver function tests, were performed at initial staging prior to radiotherapy, at each cycle of cytotoxic chemotherapy, and at each follow-up visit. LD isoenzyme studies have not been performed.

Method of LD assay and statistical methods

Plasma LD was estimated, on a centrifugal analyser, using the optimised standard method [8, 9]. The upper limit of normal for the LD assay was 395 u/L. The mean plasma LD values for the individual stages of disease following logarithmic transformation of the individual LD results were compared using *t*-tests.

Treatment policy

(*a*) *Seminoma.* Stage I and II patients were treated with para-aortic and bilateral pelvic node radiotherapy, and in addition stage III patients received mediastinal and supraclavicular irradiation. From 1982, patients with bulky (≥ 5 cm) nodal disease were given prior chemotherapy with *cis*-platinum alone or combined with bleomycin and etoposide (BEP).

(*b*) *NSGCT.* Stage I and IIA patients received para-aortic and bilateral pelvic node radiotherapy and those in all other stages cytotoxic chemotherapy. Prior to 1980, this consisted of vinblastine and bleomycin (VB), and from 1980 until 1983, *cis*-platinum, vinblastine and bleomycin (PVB). Since 1983, patients with small volume disease (lung metastasis < 2 cm and/or node metastasis < 5 cm) have received BEP, and those with bulky metastatic disease alternating cycles of BEP and PVB.

Response criteria

CR was defined as the complete disappearance of all clinical, radiological and biochemical evidence of disease, and partial response (PR), a decrease of 50% or more in the size of the products of maximum perpendicular diameters of all measurable lesions.

RESULTS

1. *LD levels immediately following orchidectomy*

(i) *Seminoma.* The plasma LD levels of 91 patients with seminoma are given in Table 2. Elevation of plasma LD was seen in 9 of 59 (15%) stage I, 7 of 23 (30%) stage II A/B and stage III A/B, and 9 of 10 (90%) stage II C patients. The mean plasma LD was higher in stage II A/B compared with stage I ($P < 0.02$) and in stage II C compared with stage II A/B ($P < 0.001$). (Fig. 1).

(ii) *NSGCT.* The plasma LD levels of 92 patients with NSCGT are given in Table 3. Elevated values of plasma LD were seen in one of 38 (2.5%) stage I, 5 of 21 (24%) stage II A/B, 2 of 5 (40%) stage II C, 3 of 8 (37.5%) stage IV A/B L1/2, and all 18 (100%) stage IV C/L3/H+ patients.

Stage II A/B patients had higher mean plasma LD levels than stage I patients but this difference did not reach statistical significance ($P < 0.1$). Stage IV patients with bulky metastatic disease had significantly higher mean LD levels than those with small volume disease ($P < 0.001$), as did those with two or more sites of bulky disease compared with those with one site of bulky disease ($P < 0.02$). There was no significant difference between the mean plasma LD in patients with small volume blood-borne metastatic disease (IV A/B L1/2) compared with small volume node metastases (IIA/B) ($P < 0.5$) – Fig. 2.

2. *LD as a monitor of response to radiotherapy*

One patient with stage IIA NSGCT had an elevated pre-treatment plasma LD which failed to return to normal following para-aortic and pelvic node radiotherapy. He was noted to have small

Table 2. Seminoma — plasma LD at initial staging following orchidectomy

Stage	Number of patients	Number with elevated LD (%)	Mean LD (u/l.)	Standard error	
I	59	9 (15)	312.95	10.89	} p < 0.02
IIA/B	22	7 (32)	425.50	59.23	} p < 0.001
IIC	10	9 (90)	1140.60	205.13	
IIIA/B	1	–	–	–	

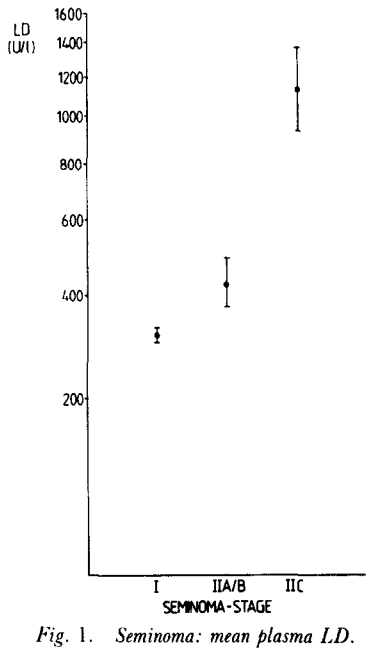

Fig. 1. Seminoma: mean plasma LD.

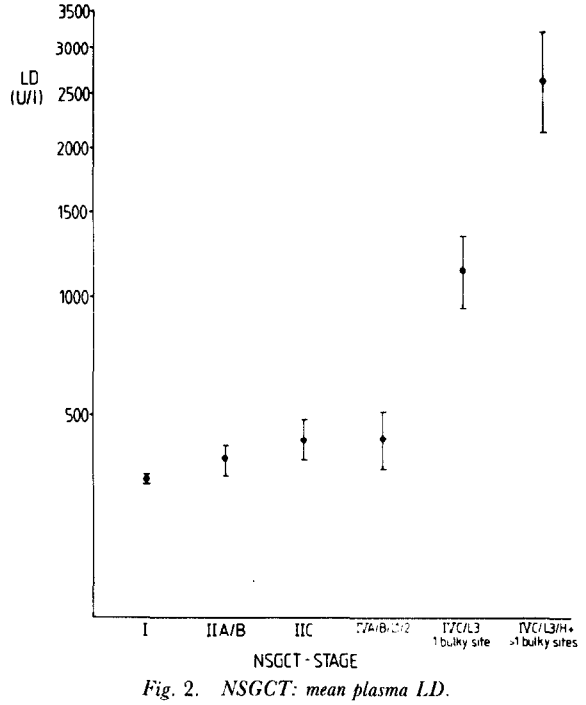

Fig. 2. NSGCT: mean plasma LD.

Table 3. NSGCT — Plasma LD at initial staging following orchidectomy

Stage	Number of patients	Number with elevated LD (%)	Mean LD (u/L)	Standard error	
I	38	1 (2.5)	301.82	9.73	} p < 0.1
IIA/B	21	5 (24)	363.81	34.32	
IIC	5	2 (40)	419.60	61.44	
IIIA/B	1	1	–	–	
IIIC	1	–	–	–	
IVA/B L1/2	8	3 (37.5)	424.00	88.80	} p < 0.001
IVC/L3 (1 site of bulky disease)	9	9 (100)	1145.33	198.30	} p < 0.02
IVC/L3/H+ (2 or more sites of) bulky disease)	9	9 (100)	2659.44	523.65	

volume supraclavicular node metastases 2 months after the end of radiotherapy. Following mediastinal and supraclavicular radiotherapy, the plasma LD fell to normal and the patient remains disease-free 4 yr after his initial radiotherapy. Elevated plasma LD levels in the remaining seven seminoma and 19 NSGCT patients receiving radiotherapy had fallen to normal within 2 months of the completion of therapy.

3. *LD as a monitor of response to cytotoxic chemotherapy*

(i) *Seminoma.* Eight patients with seminoma received cytotoxic chemotherapy, and of these seven (87.5%) had elevated levels of plasma LD which fell to normal during therapy. Normal values were obtained after the first cycle in five, after two cycles in one, and after three cycles in one. One patient eventually developed progressive drug-resistant disease, and this was reflected by a progressively rising plasma LD.

(ii) *NSGCT.* Fifty-four patients received cytotoxic chemotherapy, and the distribution of patients with elevated levels of AFP, βhCG and LD are given in Table 4.Thirty-four patients (63%) had elevated plasma LD either alone or together with AFP and/or βhCG. The addition of the LD assay increased the proportion of patients with one or more positive markers to 87% from 71% using AFP and βhCG alone.

Of 33 evaluable patients receiving cytotoxic chemotherapy who had elevated plasma LD, 21 achieved CR and 12 PR. One patient with a marginally elevated plasma LD (396 u/L) demonstrated a rising level of LD during therapy (maximum 606 u/L) which fell to normal following six cycles of chemotherapy. Normal levels of plasma LD were obtained in 18 of 20 (90%) of the remaining complete responders, and in 11 of the 12 (92%) partial responders within the first three cycles of chemotherapy (Table 5). Transient levels of plasma LD (426, 460 and 467 u/L) were seen in three patients during chemotherapy.

All 21 patients who later developed progressive drug-resistant disease demonstrated progressively rising plasma LD levels.

Of 53 evaluable NSGCT patients receiving cytotoxic chemotherapy, 11 of the 19(58%) with normal plasma LD achieved CR compared with 22 of the 34 (65%) with elevated plasma LD.

4. *LD as a monitor of relapse following prior therapy*

(i) *Seminoma.* Seven patients relapsed following radiotherapy. One patient who relapsed with small volume disease had normal plasma LD at relapse, and four of the six patients with large volume disease had elevated levels of plasma LD at relapse. Two patients were noted to have elevated plasma LD at the same time as clinical evidence of relapse, one had no LD assays performed prior to relapse and one patient demonstrated progressively rising levels of the enzyme LD for 6 months prior to clinical evidence of relapse following radiotherapy for stage IIB disease but the values fell following cytotoxic chemotherapy (Fig. 3).

During follow-up (minimum 6 months) transient elevation of plasma LD was seen in 24 of 85 (28%) patients (Table 6).

(ii) *NSGCT.* Thirty patients relapsed following prior radiotherapy or chemotherapy, and the proportion with elevation of markers is given in Table 7. Plasma LD was elevated, either alone or together with AFP and/or βhCG in 8 of 16 (50%) patients with small volume, and 10 of 14 (71%) with large volume disease at relapse. Two patients demonstrated elevated levels of plasma LD for 1, and 2 months prior to other biochemical or clinical evidence of relapse. Overall, the addition of the LD assay increased the proportion of patients with one

Table 4. *NSGCT — distribution of patients with elevated markers prior to cytotoxic chemotherapy*

Marker	Number elevated	(%)
AFP alone	6	(11)
βhCG alone	3	(6)
LD alone	9	(17)
AFP + βhCG	4	(7)
AFP + LD	4	(7)
βhCG + LD	9	(17)
AFP + βhCG + LD	12	(22)
Marker negative	7	(13)
TOTAL	54	

Table 5. *NSGCT — number of cycles of chemotherapy received prior to return of elevated plasma LD to normal*

Response	Number of cycles	Number of patients	(%)
CR*	≤ 3	18	90
	4–5	2	10
PR	≤ 3	11	92
	4–5	1	8

*Excludes one patient with marginal elevation only (396 u/L)

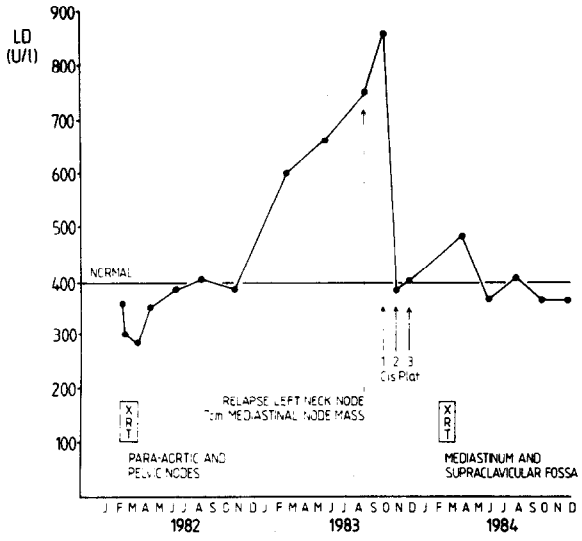

Fig. 3. Serial LD assays for patient with stage IIB seminoma who relapsed with supraclavicular and mediastinal node metastases following radiotherapy (see text).

Table 6. Maximum plasma LD in patients showing transient elevation during follow-up

Maximum plasma LD (u/L)	Seminoma Number of patients	(%)	NSGCT Number of patients	(%)
≤ 450	12	(52)	14	(50)
451–500	6	(26)	8	(29)
501–550	3	(13)	4	(14)
551–650	2	(9)	2	(7)
TOTAL	23		28	

Table 7. NSGCT — distribution of patients with elevated markers at relapse following prior therapy

Marker	Number elevated	(%)
AFP alone	6	(20)
βhCG alone	2	(7)
LD alone	7	(23)
AFP – βhCG	2	(7)
AFP – LD	4	(13)
βhCG – LD	2	(7)
AFP – βhCG – LD	5	(16)
Marker negative	2	(7)
TOTAL	30	

or more positive markers at relapse to 93% compared with 71% using AFP and βhCG alone.

During follow-up (minimum 6 months) transient elevation of plasma LD was seen in 28 of 76 (37%) patients (Table 6).

5. *Influence of pre-treatment plasma LD on survival of patients with NSGCT*

The 3-yr actuarial survival for patients with normal LD before treatment was 82%, compared with 68% for those with plasma LD 396–999 u/L, and 47% for those with plasma LD ≥ 1000 u/L, (χ^2 for trend = 11.66, $P = 0.001$) (Fig. 4).

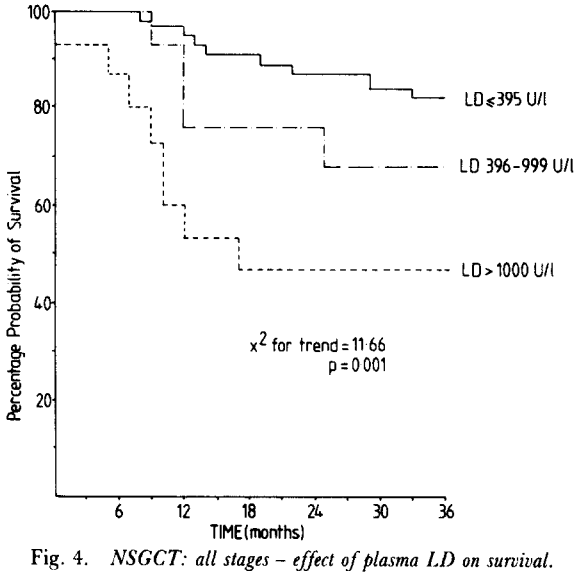

Fig. 4. *NSGCT: all stages – effect of plasma LD on survival.*

DISCUSSION

LD is a glycolytic enzyme found in a wide variety of tissues, with elevated plasma levels evident in myocardial infarction, megaloblastic anaemia, pulmonary infarction, acute renal and hepatic disease, a number of muscle disorders and many types of malignant disease. The LD assay has the advantage of being performed as part of routine liver function testing, and is a readily available and relatively cheap biochemical assay.

In 1978 Von Eyben [5] reported a correlation between tumour burden and serum LD in 27 patients with advanced testicular tumours, three of whom had seminoma. Two other series, Lieskovsky [10] and Lippert [11], have reported a correlation between serum LD and stage of disease and prognosis. Lieskovsky reported 21-stage B (infra-diaphragmatic node metastasis) and 17 stage C (supra-diaphragmatic node and blood-borne metastases) patients with NSGCT. Lippert [11] reported 19 stage A (confined to testis), 19 stage B and 42 stage C patients but their series included only 10 patients with seminoma. Bulk of metastatic disease prior to cytotoxic chemotherapy is an important prognostic indicator [12, 13], but the relationship between serum LD and bulk of disease was not established in these series.

The present series of patients consists of 86% of all patients with testicular germ-cell tumours refer-

red to this centre between January 1978 and June 1984. It includes 92 patients with seminoma, and 92 with NSGCT. The Royal Marsden staging system [7] which subdivides each stage according to bulk of disease has been employed. For both seminoma and NSGCT with more advanced stage and increasing bulk of disease, there was greater elevation of the mean plasma LD. In particular, the mean plasma LD was higher for stage IIC than for stage IIA/B seminoma, and for stage IV C/L3/H+ compared with stage IV A/B L1/2 NSGCT. In addition, the bulky stage IV NSGCT patients with two or more sites of bulky disease had significantly higher mean plasma LD than did those with only one site of bulky disease. Of patients receiving cytotoxic chemotherapy for metastatic disease, 87.5% with seminoma and 63% with NSGCT had elevated plasma LD. The use of the LD assay increased the proportion of NSGCT patients with one or more positive markers to 87% compared with 71% using AFP and βhCG alone, and the plasma LD level during therapy reflected the regression or progression of disease. In patients who achieved both CR and PR to cytotoxic chemotherapy, the majority of patients had return of elevated plasma LD to normal following one to three cycles of chemotherapy. It was not possible to use the rate of fall of LD to differentiate between those who were, or were not, likely to achieve CR and PR. In a recent analysis, Horwich [14] found that the rate of fall of AFP and βhCG levels following chemotherapy for NSGCT was seldom of prognostic significance. However, the rapid fall of AFP, βhCG and LD following cytotoxic chemotherapy, even in patients who fail to be cured of their disease, does truly reflect a response to the drugs employed.

The 3-yr survival for all patients with NSGCT, irrespective of stage, was related to the plasma LD level at initial staging. For those receiving chemotherapy there was no significant difference between CR rates for those with elevated compared with those with normal plasma LD. Thus the pre-treatment plasma LD does not predict the outcome of chemotherapy.

During follow-up, transient elevations of plasma LD were observed, and it seems likely that the majority of these were due to leakage of cellular LD during specimen transit. In these cases several hours delay may have occurred before the plasma was separated from the blood cells as the sample had to be taken to the base hospital laboratory for analysis. Normally the samples are centrifuged within 1 hr of venesection.

A progressively rising plasma LD during follow-up is an indication for thorough investigation. Plasma LD was elevated in 71% of patients with large volume, in 50% with small volume, relapsed NSGCT, and in four of the seven relapsed seminoma patients. In addition the three seminoma patients with second relapses had elevated plasma LD. The use of the LD assay during follow-up is particularly useful for monitoring seminoma patients who are unlikely to have either elevated AFP or βhCG at relapse.

LD isoenzyme studies have not been performed in this series but in other series elevation of the LD-1 isoenzyme is the predominant abnormality in patients with testicular germ-cell tumours [15–17]. It is possible that quantitation of LD isoenzymes might provide a more specific marker than total LD assay but currently the methods available are not sufficiently precise. A further detailed study of the usefulness of LD-1 assay would be of value. Placental alkaline phosphatase is another useful marker for seminoma but is not yet generally available [18, 19] and its role requires further investigation.

Plasma LD is a valuable and readily available marker of testicular germ-cell tumours. Its plasma concentration may be related to both stage and bulk of disease. The proportion of NSGCT patients with elevated LD values is similar to that for AFP and βhCG. Serial assays of LD provide useful information which supplements the assay of AFP and βhCG, particularly for patients with seminoma. Its routine use as a tumour marker is recommended for all patients with testicular germ-cell tumours.

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