

A systematic review of lactate dehydrogenase isoenzyme 1 and germ cell tumors

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Abstract

Objectives: To evaluate lactate dehydrogenase isoenzyme 1 (LD-1) as a tumor marker of germ cell tumors.

Methods: A literature search included a CancerLit and Medline computer search of articles regarding germ cell tumors and LD-1 published between 1963 to 99 and a manual search of reference lists, theses, and textbooks. Forty articles, letters to the editor, and abstracts on testicular germ cell tumors and 10 articles on ovarian germ cell tumors fulfilled inclusion criteria.

Results: Of 696 patients with testicular germ cell tumors, 423 (61%) had a raised serum LD-1 catalytic concentration (S-LD-1). Patients with seminoma have a raised S-LD-1 more often (63%) than those with nonseminoma (60%). S-LD-1 was raised less often in patients with stage I (48%) than in those with stage II (50%) and stage III (67%). S-LD-1, serum alpha fetoprotein concentration (S-AFP), and serum human chorionic gonadotropin concentration (S-hCG) were discordant. S-LD-1 predicted outcome in four studies: one study regarding relapse in patients with nonseminomatous testicular germ cell tumors stage I, and three studies regarding survival of patients with metastatic testicular germ cell tumors. In two of three studies, S-LD-1 was a better prognostic predictor for patients with metastatic testicular germ cell tumors than S-LD. Of 40 patients with ovarian germ cell tumors, thirty-five (88%) had a raised S-LD-1.

Conclusions: S-LD-1 is a useful serum tumor marker of testicular germ cell tumors. For patients with ovarian germ cell tumors, S-LD-1 was raised more often than for patients with testicular germ cell tumors. Further studies are required for a general recommendation regarding the use of S-LD-1 for germ cell tumors. © 2001 The Canadian Society of Clinical Chemists. All rights reserved.

Keywords: Lactate dehydrogenase isoenzymes; Testicular neoplasms; Ovarian neoplasms; Metanalysis; Lactate dehydrogenase; Human chorionic gonadotropin; Alpha fetoproteins

1. Introduction

Serum tumor markers are used to define the stage, predict the prognosis, monitor therapy, and detect a relapse [1,2]. For patients with metastatic testicular germ cell tumors, serum tumor markers may predict survival [3]. In the large International Germ Cell Cancer Collaborative Group study, the extent of tumor and levels of three serum tumor markers predicted the long-term survival [4]. The tumor markers were serum lactate dehydrogenase (*L*: lactate: NAD⁺ oxidoreductase, EC 1.1.1.27, S-LD) catalytic concentration, serum alpha fetoprotein concentration (S-AFP) and serum human chorionic gonadotropin concentra-

tion (S-hCG). So the fifth International TNM (T primary tumor, N regional lymph nodes, and M distant metastases) classification of 1997 used the site and the extent of the tumor lesions and the levels of the tumor markers to group the patients [5].

LD consists of five LD isoenzymes that combine two subunits, LD-A and LD-B, in different tetramers. LD isoenzyme 1 is a homotetramer of four LD-B subunits and LD isoenzyme 5, LD-5, of four LD-A subunits (Fig. 1). A sixth LD isoenzyme, LD-X, is found only in testicular postmeiotic, haploid germ cells and spermatozoa (Fig. 2). LD-X is a homotetramer of four LD-C subunits. Testicular and ovarian germ cell tumors have a predominance of S-LD-1 whereas other cancers have other LD isoenzyme patterns [6]. Studies showed that serum LD-1 catalytic concentration (S-LD-1) have advantages as tumor marker compared with S-LD in patients with testicular germ cell tumors [7–9].

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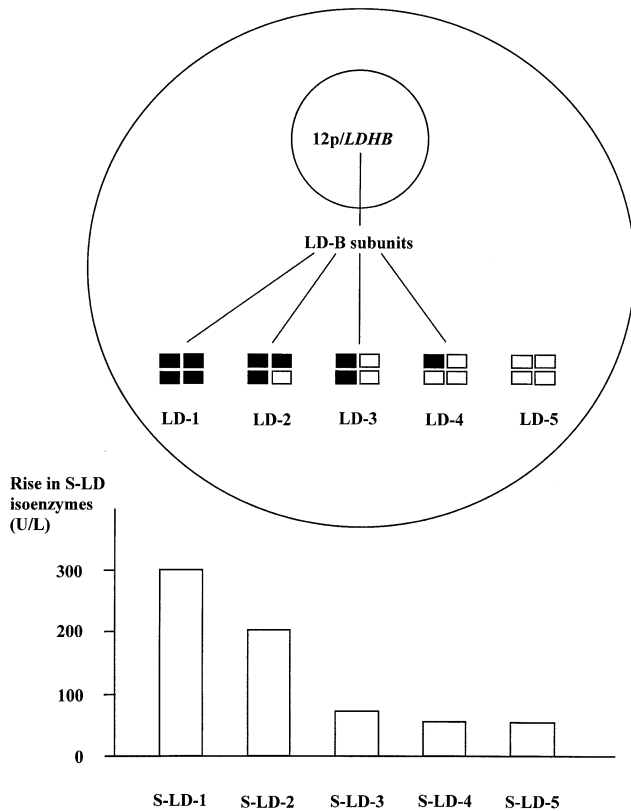


Fig. 1. Link between a karyotype aberration in the nuclei of testicular germ cell tumors, high copy number of the short arm of chromosome 12, 12p, with *LDHB*, the gene locus for LD-B subunit, high level of LD-B subunit in the cytoplasm of the tumor cells, and thereby of the anodal LD isoenzymes, and the median rise for the S-LD isoenzymes in 20 patients metastatic testicular germ cell tumors and a raised S-LD-1. *Acta Oncologica*, reprinted with permission [8].

However measuring S-LD-1 may be more complicated than measuring S-LD.

This systematic review overviewed the role of S-LD-1 as a tumor marker of germ cell tumors. As for testicular germ cell tumors, the review evaluated the biologic background for a raised S-LD-1, effects from different assay conditions for measuring S-LD-1, various aspects as tumor marker, and usefulness of S-LD-1 compared with that of the international classification. As for ovarian germ cell tumors, the review summarized the fraction of patients with a raised S-LD-1.

2. Material and methods

2.1. Literature search

The reviewer made a computer and a manual search. A CancerLit search selected articles published between 1963 and August 1999 using the search terms “lactate dehydrogenase” or “ld isoenzymex” or “ld-1” and “testis” or “testicular” or “testicle”. This search gave 288 articles. A MEDLINE search (Silver Platter (Spirs) version 4.0) of articles

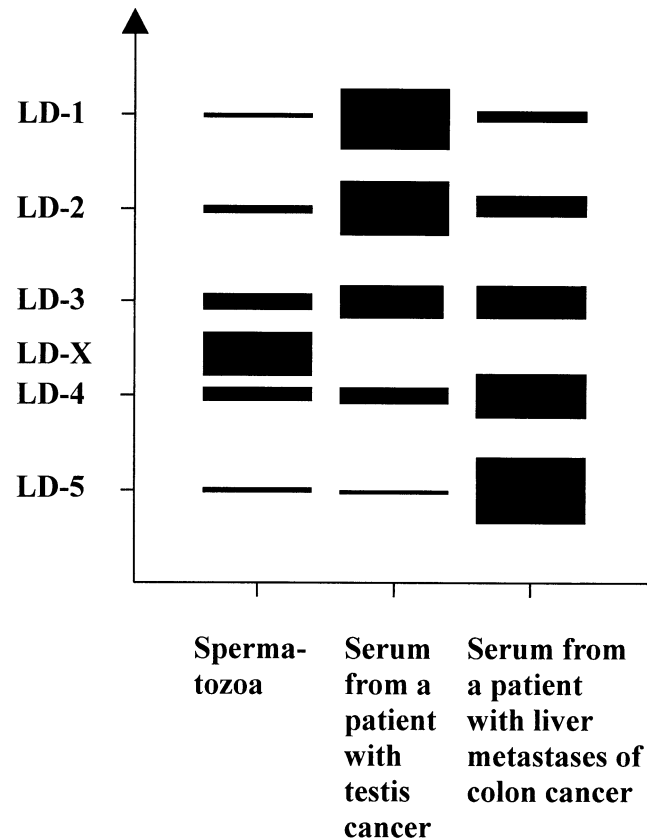


Fig. 2. Schematized LD isoenzyme pattern by electrophoretic separation: spermatozoa and sera of two patients with a highly raised S-LD: one with metastatic testicular germ cell tumor and one with colon cancer and liver metastases without presence of germ cell tumor [43,49].

from 1966 to August 1999 used the medical subject heading (MeSH) terms “lactate dehydrogenase” and “testicular neoplasms”. This search gave 136 articles. Another MEDLINE search used the search terms “lactate dehydrogenase” and “germ cell and embryonal neoplasms” and gave 56 articles. The two MEDLINE searches did not add articles to the Cancerlit search.

The manual search looked for other articles in reference lists, theses, review articles, conference reports, and textbooks. Leading oncology centers did not give more data.

As to the clinical aspects of testicular germ cell tumors, this review selected articles with adult patients, histologic criteria for the diagnosis, and measurements of S-LD-1 as the treatment started. The review excluded double publications, reviews, articles with children and nongerml cell neoplasms and with measurements of S-LD but not S-LD isoenzyme(s), studies with animals, and laboratory studies. In one article, patients had a higher rise in S-LD-5 than in other LD isoenzymes contrasting the predominant rise of the anodal LD isoenzymes, LD-1 to LD-3, in all other studies [3]. The article did not detail the technical background for the inverse LD isoenzyme pattern. It might represent a technical error. So this article was also excluded. Based on the selection criteria, the Cancerlit search gave 23 articles and the

manual search gave 17 articles, letters to the editors, and abstracts.

Regarding ovarian germ cell tumors, the review selected articles using similar criteria. The CancerLit search gave one article and the manual search nine.

As for extragonadal germ cell tumors, a Cancerlit search gave no articles and a manual search gave four.

2.2. Methods

The reviewer analyzed the data of the published articles and undertook further analyses of patient data from the Danish studies.

For patients with testicular germ cell tumors, the review analyzed subgroups based on the main histology and stage. The review based staging on the anatomical site of the tumors only as did staging of the TNM classifications before 1997 [10].

The studies used different assays for measuring S-LD-1 (immunochemical assay or electrophoresis, disregard of serum samples with hemolysis or correction for hemolysis in all samples) [11,12]. The review compared the findings according to the differences in assay conditions. Raised serum marker values were those above the upper reference limit. The cut off limits for S-LD, S-AFP, and S-hCG used in this review were those of the recent TNM classification [5].

2.3. Statistical methods

The review combined the findings of the selected studies assuming that subgroups of patients had a common fraction of raised tumor marker values at diagnosis. Therefore, it assessed the results based on the fixed effects model for a systematic review [13],

$$r_i/n_i = \Theta + e_i$$

where in the i study, r_i = number of patients with a raised test, n_i = total number of patients, Θ = overall fraction, and e_i = random error.

The overall fraction with a raised S-LD-1 in the published series for groups of patients, $f\{R\}$, estimated the probability of the groups having a raised S-LD-1, $p\{R\}$

$$f\{R\} = \sum r_i / \sum n_i \approx p\{R\}$$

The review summarized the studies as previously described [14]. It calculated 95% confidence intervals (CI) for fractions of groups of patients [15], and compared subsets of articles and abstracts, and groups of patients. The review evaluated survival with the lifetable method, compared the survival of subgroups using the logrank test, and considered a p value <0.05 statistically significant. It assessed the relative risk for raised vs. normal values to predict the outcome as outlined by Morris and Gardner [16]. The studied outcome for patients with metastatic disease was

whether they died of tumor or not. For patients with stage I, the outcome was whether they had a relapse or not.

3. Results

3.1. Testicular germ cell tumors

3.1.1. Clinical aspects

Testicular germ cell tumors is the most common malignancy for men 20 to 35 yr of age. Patients with mal descended testes have an increased risk of testicular germ cell tumors. Most patients with these tumors are 50 yr old or younger. Through the latest two decades, the 5-yr survival of patients improved due to a progress in staging, monitoring, and therapy [17]. Today, patients in many countries have a 5-yr survival of $>90\%$.

At the start of treatment, nearly all patients have the testis with the primary tumor removed (orchiectomy). Testicular germ cell tumors have two main histologies, seminoma and nonseminomatous tumors, and three main stages. Stage I is a primary tumor without metastases, stage II refers to regional lymph node metastases, and stage III to distant metastases, according to the third edition of the TNM classification [10].

Oncologists base the postsurgical treatment on the main histology and stage of the tumor. For stage I patients, the treatment is changing in recent years. Earlier, most stage I patients underwent radiotherapy or chemotherapy after the initial orchiectomy. These patients have a low relapse rate. Alternatively, patients with stage I may be followed without these treatments before they relapse, the surveillance program [17]. 20 to 30% of these patients relapse. Treated at relapse nearly all patients are cured [18–20]. So 70% of the patients with stage I followed with surveillance avoid postsurgical treatment and its long-term side effects. Denmark uses surveillance for all patients with nonseminomatous tumors and those with seminoma who have a low risk ($<20\%$) of a relapse [18,21]. The program is increasingly used internationally [17]. It requires a regular monitoring of serum tumor markers for the first five years after orchiectomy [22].

Clinical features at the time of orchiectomy point out a subgroup of patients with stage I who have a raised risk of a relapse. Some centers give high-risk patients adjuvant therapy after the orchiectomy. Danish patients with seminoma and high-risk receive adjuvant radiotherapy. Some centers in other countries give adjuvant chemotherapy to patients with nonseminomatous tumors and a high-risk [23].

Since the late 1970s, oncologists treat patients with metastatic testicular germ cell tumors with platin-based combination chemotherapy. It cures most patients, especially those with low tumor volume. Those with normal or slightly raised serum tumor marker values, the S0 and S1 categories of the recent TNM classification, have a higher cure rate than those with higher values, the S2 and S3 categories [4].

The TNM classification prognosticates patients with metastatic testicular germ cell tumors in three serum tumor marker categories. Many oncologists consider prognostic factors as they decide the type of chemotherapy and give poor-risk patients more intensive chemotherapy than good-risk patients [24].

Patients who have residual lesions and normal S-AFP and S-hCG values after chemotherapy may have residual teratoma and need surgery before they are cured [23]. Patients with residual lesions with other histologic types of germ cell tumors need further chemotherapy. Oncologists may also use S-LD in estimating whether residual lesions after chemotherapy represent germ cell tumor apart from teratoma, teratoma, or fibrotic/necrotic tissue [25]. Most patients who die of tumor do so within two years after diagnosis.

3.1.2. Biology

Testicular germ cell tumors have characteristic chromosomal abnormalities with a raised chromosome number, mainly between 50 and 90, and a high copy number of the short arm of chromosome 12, 12p [26]. The tumors often have an isochromosome i(12p) where two short arms of the chromosome replace two long arms. The tumors often have this isochromosome as a relevant karyotypic aberration [27]. Seminomas and nonseminomatous tumors, ovarian germ cell tumors [28], and cerebral germ cell tumors (germinomas) often have this isochromosome. In a review of 20,007 neoplasms, 119 of 135 tumors with i(12p) were testicular germ cell tumors [29]. Of 16 nongerm cell tumors with i(12p), 12 were adenocarcinomas and four acute myeloid leukemias. So germ cell tumors and nongerm cell malignancies differ in chromosomal pattern [27,30].

The S-LD isoenzyme pattern of patients with testicular germ cell tumors diverge from that of normal testis tissue and spermatozoa. The patients have a predominance of the anodal LD isoenzymes and no LD-X in the blood (Fig. 2) [8,31].

Ten articles analyzed the biologic background for a raised S-LD-1 in patients with testicular germ cell tumors [8,32–40]. The gene locus for the LD-B subunit, *LDHB*, is localized to the region 12p12.1 to 12p12.2 of the short arm of chromosome 12 [41]. In patients with measurable tumor lesions, S-LD-1 correlated with the total copy number of 12p in the tumor lesions [37], and with the sum of mRNA for *LDHB* in the tumors [32]. Intratubular germ cell neoplasia, microinvasive tumor, and a macroscopically overt seminoma had a high expression of *LDHB* in another study [35]. So the expression of LD-B subunits is an early event in the tumorigenesis of testicular germ cell tumors. In patients with testicular germ cell tumors, S-LD-1 rose more than the other S-LD isoenzymes (Fig. 1) [8].

Three articles described LD-1 in tissue of testicular germ cell tumors [38–40], using immunohistochemical examinations and electrophoresis. Tissues from 11 seminomas had higher LD-1 and LD-2 catalytic concentrations than normal

testis tissue [38]. For patients with seminoma stage I, S-LD-1 measured before orchiectomy correlated with the extent of tumor necrosis [42]. It was not the case for patients with nonseminomatous tumors. So for patients with seminoma, tumor necrosis contributes to the rise in S-LD-1.

LD-X consists of four LD-C subunits. The gene locus for the LD-C subunit, *LDHC*, is localized to chromosome 11. Only mature postmeiotic germ cells of the testis and spermatozoa have LD-X (Fig. 2) [43]. The increased ploidity of testicular germ cell tumors blocks the expression of *LDHC*. Thus, a testicular intratubular germ cell neoplasia, a microinvasive tumor, or a seminoma did not have LD-X [35]. Neither did tissue of 11 seminomas show LD-X [38]. As an exception, a seminoma transplanted to nude mice had LD-X in a single tumor [36].

Gene locus for the LD-A subunit, *LDHA*, is localized to the short arm of chromosome 11. Testicular germ cell tumors have a relatively low copy number of this chromosome but so far, no study has analyzed whether the tumors express *LDHA* more than other tissues.

Two articles measured LD isoenzymes in nude mice with transplants of human testicular germ cell tumors. Nude mice had human LD-1 in the blood [33,34]. In one article with transplants of a tumor of embryonal carcinoma and teratoma [33], the human S-LD-1 values in nude mice correlated with the sizes and weights of the tumor lesions. The second article described transplants of two other human germ cell tumors: a testicular endodermal sinus tumor and a sacrococcygeal tumor [34].

3.1.3. Analytical assays for S-LD-1

Seven articles dealt with conditions for the measurement of S-LD-1 in patients with testicular germ cell tumors [8,12, 44–48]. Four articles described an immunochemical assay [12,44–46]. This assay precipitates LD isoenzymes 2 to 5 in the serum samples and measures S-LD-1 as a LD catalytic concentration. Odense University Hospital measured the isoenzyme with an immunochemical assay whereas other centers used an electrophoretic measurement. Three articles described an electrophoretic assay [8,49,50]. This assay measures LD-1 as an S-LD-1/S-LD fraction. A simultaneous measurement of S-LD allows the laboratory to estimate S-LD-1 as a catalytic concentration. The two methods gave consistent results in patients with testicular germ cell tumors [9].

Hemolysis in the serum samples may increase S-LD-1 [12,46]. The impact from hemolysis may cause spuriously raised S-LD-1 and serum hemoglobin concentration in the serum samples [46]. Most centers disregard serum samples with obvious hemolysis [8]. S-LD-1, however, may be corrected for the positive bias from hemolysis according to the serum hemoglobin concentration [12,46]. In 5% of full blood samples mailed to a central center, the rise was more than half the upper limit of reference values [12].

In studies from Odense University Hospital, S-LD-1 measurements were corrected for the impact whereas other

centers disregarded serum samples with visually obvious hemolysis. The studies from the different centers had similar proportions of patients with a raised S-LD-1 in the published articles despite the differences in the LD isoenzyme assays ($p = 1.00$ for stage I, $p = 0.23$ for stage II, and $p = 0.24$ for stage III). So the review does not indicate a preference from one assay for S-LD-1 for the other [51]. Neither does this comparison support a routine correction for hemolysis in the serum samples.

One study defined analytical quality specifications based on a series of reference men, immunochemical measurement of S-LD-1 with or without correction for hemolysis according to the concept of clinical needs [44,52]. The maximum allowable positive analytical bias for measurements of S-LD-1 was 5 U/L and the maximum allowable imprecision was 11%.

A conventional upper limit of reference values for S-LD-1 of 180 U/L was used in an old study [49]. In contrast, recent studies used 114 U/L as the upper limit of reference values. A group of reference men who underwent a surgical exploration of the testis because a tumor was suspected had a log-normal distribution of S-LD-1 and the range of reference values was 50 to 112 U/L limit [44]. A series of patients cured for testicular germ cell tumors also had a similar low upper limit of S-LD-1 values [8]. The cut off limit 1.0x and 10x upper limit of reference values were the best limit to point to poor and very poor outcomes for patients with metastatic testicular germ cell tumors (Fig. 3A) [53]. An upper limit of reference values for S-LD-1 of 110 U/L increases the sensitivity but also the fraction of raised values not related to testicular germ cell tumors, the false positive values. Two studies used the low limit as the limit to imply an increased risk of relapse for patients with stage I [54,55]. Two studies of patients with metastatic testicular germ cell tumors using the low limit had a higher relative risk of death of tumor than a study using a high limit (Fig. 3B) [7,8,49].

The International Federation of Clinical Chemistry recommends a standardized LD assay [56]. Most laboratories do not follow this recommendation. The federation has not published a recommendation for the LD-1 assay.

Serum 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) catalytic concentration corresponds grossly to the sum of S-LD-1 and S-LD-2. In two articles, S-LD-1 correlated only loosely with serum hydroxybutyrate dehydrogenase catalytic concentration [47,48]. Therefore, hydroxybutyrate dehydrogenase may not be a useful alternative to S-LD-1.

3.1.4. LD-1 and diagnosis

Ten articles described S-LD-1 and the diagnosis of testicular germ cell tumors [3,6,8,32,50,57–61]. Generally, S-LD-1 is measured in a blood sample from an arm vein. These veins have lower S-LD-1 values than veins directly from the tumor [32].

Testicular germ cell tumors have a characteristic LD isoenzyme pattern with a rise mainly of anodal LD isoen-

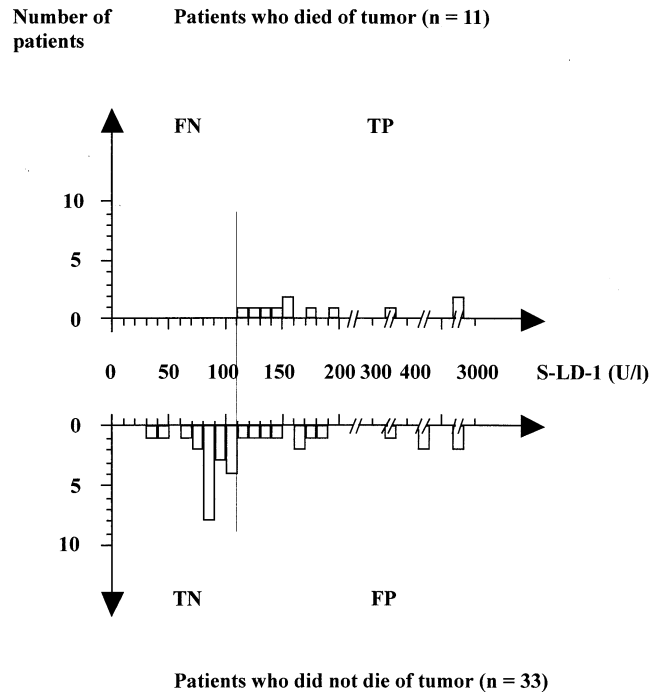


Fig. 3. Distribution of S-LD-1 values in patients with metastatic testicular germ cell tumors who died or survived. TN denotes true negative, low S-LD-1 values that correctly predicted survival, and FP false positive high S-LD-1 values that incorrectly predicted death of tumor. Clinical Chemistry Laboratory Medicine, reprinted with permission [53].

zymes. This isoenzyme pattern reflects that the tumors produce a high number of LD-B subunits (Fig. 1). Serum from a patient with a metastatic testicular germ cell tumor and a raised S-LD had a higher LD-1/LD fraction than adult testicular tissue [49].

In one study, S-LD-1, the S-LD-1/S-LD fraction, the S-LD-1/S-LD-2 ratio, and the S-LD-5/S-LD-1 ratio has been combined as a tumor marker of testicular germ cell tumors [50]. In another study, 7 of 20 patients with seminoma had a raised S-LD-1/S-LD fraction only, 1 had a raised S-LD-2/S-LD fraction, and 6 had a raised fraction of S-LD-1 and S-LD-2 [62]. Patients with metastatic disease, however, had a raised S-LD-1 more often than a raised S-LD-1/S-LD fraction or S-LD-1/S-LD-2 ratio [8]; nearly all patients with a raised S-LD-1/S-LD fraction or S-LD-1/S-LD-2 ratio also had a raised S-LD-1. Therefore, the S-LD-1 catalytic concentration is the best and most simple measurement of the three expressions of the isoenzyme as a serum tumor marker.

In one article of men with maldescended testes, S-LD-1 was not useful as a screen for testicular neoplasia [57]. A testis biopsy detected even tumors that were not clinically detectable, such as intratubular germ cell neoplasia, and was a better screen for neoplasia among these men [63].

Of 696 patients with testicular germ cell tumors, 423 (61%, CI 57–64) had a raised S-LD-1 (Table 1). It was raised equally often in patients with seminoma (154 of 245 patients, 63%) and with nonseminomatous tumors (269 of

Table 1
Fraction of patients with a raised S-LD-1 in patients with testicular germ cell tumors

Patients	No evidence of disease	Evidence of disease								References
		Seminoma				Nonsemi-nomatous tumors				
		Stage				Stage				
	I	II	III	X	I	II	III	X		
	0/0	0/0	0/0	0/0	9/9	0/0	0/0	0/0	2/4	6
	0/0	0/0	0/0	0/0	0/0	0/0	0/0	25/46	0/0	67
	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2/3	68
	0/0	0/0	0/0	0/0	9/9	0/0	0/0	0/0	21/32	69
	0/0	0/0	1/2	1/1	0/0	0/0	1/8	2/3	0/0	65
	0/0	0/0	0/0	4/6	0/0	0/0	0/0	30/36	0/0	50
	0/0	0/0	0/0	0/0	6/8	0/0	0/0	0/0	25/30	31
	0/0	1/2	4/9	0/0	0/0	1/1	0/5	7/20	0/0	49
	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	15/15	70
	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	19/31	71
	0/0	0/0	0/0	0/0	13/20	0/0	0/0	0/0	7/11	62
	0/0	5/7	1/3	1/1	0/0	5/7	1/1	2/2	0/0	45
	0/0	4/9	7/9	1/1	0/0	2/6	5/13	9/12	0/0	72
	0/0	4/5	1/1	0/0	0/0	0/0	0/0	1/1	0/0	32
	0/0	1/3	3/3	0/0	0/0	1/3	1/5	8/13	0/0	37
	0/0	0/1	3/5	1/1	0/0	2/10	6/13	12/14	0/0	7
	0/0	4/5	1/1	0/0	0/0	2/8	1/1	4/5	0/0	66
	0/0	69/110	0/0	0/0	0/0	27/68	0/0	0/0	0/0	54
	0/0	0/2	2/2	0/1	0/0	1/1	1/1	7/7	0/0	64
	0/21	0/0	5/7	2/2	0/0	0/0	5/12	8/16	0/0	8
Total no. of patients	0/21 (0%, 0–16)	79/144 (55%, 46–63)	28/42 (67%, 50–80)	10/13 (77%, 46–95)	37/46 (80%, 66–91)	41/104 (39%, 30–49)	22/59 (37%, 25–51)	115/175 (66%, 58–73)	91/126 (72%, 64–80)	

The table shows the fraction of patients with testicular germ cell tumors who had raised S-LD-1 values by main histology and stage. Stage X denotes an unknown stage. The parentheses show the percentage and the 95% confidence interval.

451 patients, 60%). For patients with stage I, the fraction with a raised S-LD-1 depended on the histology of the tumor: Those with seminoma had a raised S-LD-1 more often (55%) than those with nonseminomatous tumors (39%). In contrast, S-LD-1 did not differ between the main histologic groups for patients with metastatic disease.

S-LD-1 is discordant with S-AFP and S-hCG and adds information to that of the other tumor markers. Patients with seminoma have raised S-LD-1 more often than a raised S-AFP and S-hCG (Table 2). In contrast, patients with nonseminomatous tumors have a similarly large fraction of raised values of the three serum tumor markers.

Table 2
Raised and normal S-LD-1, S-AFP and S-hCG in 378 patients with testicular germ cell tumors

Patients	LD-1		S-AFP		S-hCG		No. of patients	
	R	N	R	N	R	N	Seminoma	Nonseminomatous tumors
	X		X		X		0	30
	X		X			X	0	26
	X			X	X		14	12
	X			X		X	81	22
		X	X		X		0	25
		X	X			X	1	31
		X		X	X		7	13
		X		X		X	83	33
Total	95	91	1	185	21	165	186	–
Total no. of patients	90	102	112	80	80	112	–	192

The table shows the findings of six studies of patients with testicular germ cell tumors: 186 patients with seminoma and 192 patients with nonseminomatous tumors [7, 8, 37, 45, 54, 64]

R denotes raised values, N normal values, 30 patients with nonseminoma had raised values of S-LD-1, S-AFP, and S-hCG.

The LD isoenzyme pattern may be used in differential diagnosis regarding germ cell tumors. Patients with most nongerm-cell cancers and nonmalignant diseases differ in LD isoenzyme pattern [6,58,59]. Acute myocardial infarction and hemolytic anemia are among the few other diseases with a relatively high S-LD-1 but clinicians may easily sort out the two diseases due to other clinical features. Of patients with small cell carcinoma of the lung and with other cancers, only few had an LD-1 pattern [60].

Besides patients with germ cell tumors, many patients with malignancies have a raised S-LD [3]. The LD isoenzyme pattern for most of these patients, however, differs from that of patients with germ cell tumors [61]. Patients with nongerm cell cancers without liver metastases most often have the highest LD isoenzyme rise in LD-3. Those with tumor lesions in the liver often have a relatively high rise of S-LD-5. In a patient with a history of seminoma and a high S-LD, the LD isoenzyme pattern pointed against presence of germ cell tumor (Fig. 2) [49].

3.1.5. LD-1 and staging

Twenty articles and abstracts described how S-LD-1 relates to the stage of testicular germ cell tumors [6–8,31, 32,37,45,49,50,54,62,64–72]. Patients with stage I tumors had a lower fraction with raised S-LD-1 (48%) than those with stage II (50%) and stage III (67%) (Table 1). Of the stage I patients, 39 to 55% had a raised S-LD-1 before the orchiectomy. Two thirds of the stage III patients have a raised S-LD-1 at the start of chemotherapy (66–77%). The stage was not stated for some patients with an S-LD-1 measurement. A large fraction of the patients with an unknown stage had a raised S-LD-1 like that of patients with metastatic disease. These patients had higher S-LD-1 values than those with no evidence of disease [8]. Similarly, S-LD-1 correlated with the sum of the volumes of measurable tumor lesions, an obtainable estimate of the total tumor burden [49].

For patients with stage I, a raised preorchiectomy S-LD-1 should normalize following the orchiectomy. A persistently raised S-LD-1 indicates metastatic disease, stage II or III.

Many patients have discordant values of the serum tumor markers such as raised S-LD-1 combined with normal values of S-AFP and S-hCG (Table 2) [8,45]. This combination of serum tumor marker values is especially frequent in patients with seminoma stage I. Due to the discordance, S-LD-1 adds to the serologic staging with S-AFP, and S-hCG of patients with germ cell tumors. No study changed the staging of patients with testicular germ cell tumors due to S-LD-1.

3.1.6. LD-1 and prognosis

Seven articles, letters to the editor, and abstracts analyzed whether S-LD-1 predicted the outcome for patients with testicular germ cell tumors [7–9,49,53–55]. S-LD-1 was a prognostic predictor in three of the articles. In a series of Danish stage I patients, the preoperative S-LD-1 pre-

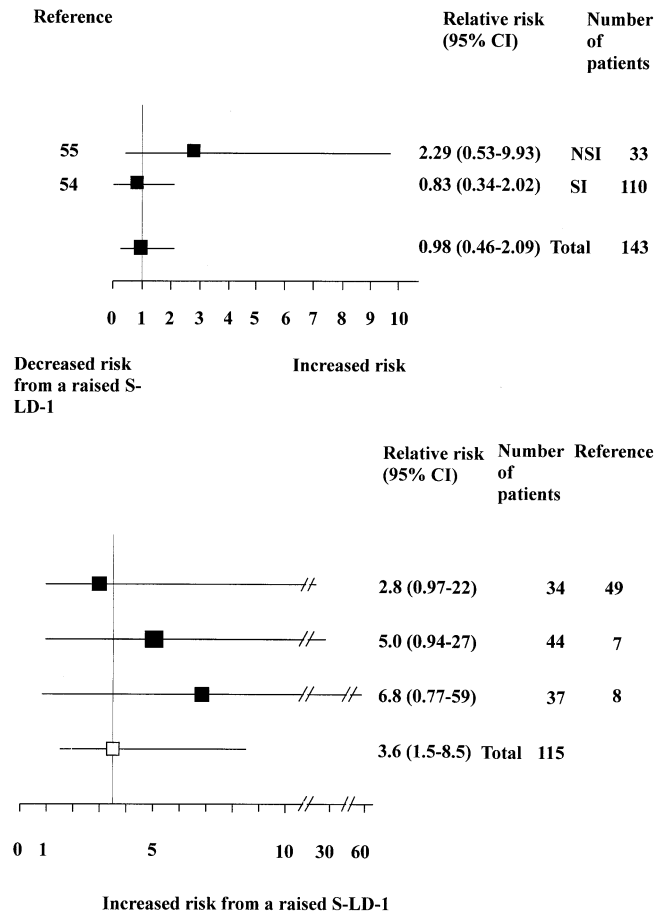


Fig. 4. (upper) Relative risk of relapse from a raised S-LD-1 in two series of stage I patients with nonseminomatous tumors (NSI) and seminoma (SI). (lower) Relative risk of death from a raised S-LD-1 in three series of patients with metastatic testicular germ cell tumors [7,8,49].

dicted the relapse for those with nonseminomatous tumors but not for those with seminoma [54,55]. Accordingly, the relative risk from a raised S-LD-1 should be stated separately for stage I patients with seminoma and nonseminoma (Fig. 4A).

Patient with metastatic testicular germ cell tumors and a normal S-LD-1 after the initial orchiectomy responded better to platin-based combination chemotherapy than those with a raised value [7]. In a multivariate analyses of risk factors, S-LD-1 predicted response to therapy and outcome. S-LD-1 had a prognostic prediction independent of the tumor burden and S-hCG - the other significant predictors. A raised S-LD-1 had a summary relative risk of death of 3.55 (CI 1.48–8.46) (Fig. 4B) [7,8,49]. Surprisingly, the recent patients had a larger prognostic discrimination from S-LD-1 than the patients treated in the 1980s. Two articles compared the prediction of prognosis by S-LD-1 with those of S-AFP and S-hCG [9,53].

Two cut-off points for S-LD-1, 1.0x and 10.0x upper limit of reference values, separated the patients in subgroups with very different prognoses as shown in Fig. 5 [7].

Patients with metastatic testicular germ cell tumors had a

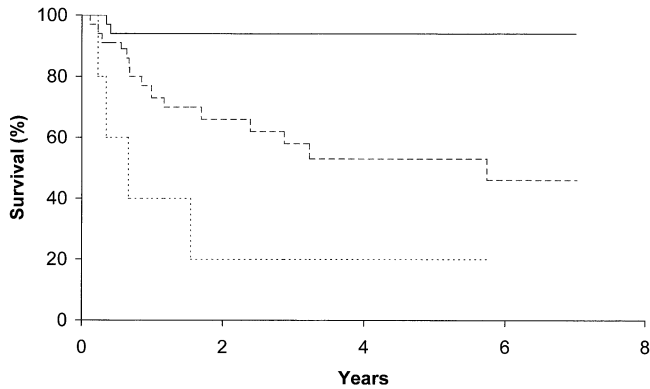


Fig. 5. Survival of 81 patients with metastatic testicular germ cell tumors according to the level of S-LD-1 (— denotes 39 patients with S-LD-1 < 1.0 × upper limit of reference values, - - - 37 patients with S-LD-1 1.0–10.0 × upper limit of reference values, and . . . 5 patients with S-LD-1 > 10.0 × upper limit of reference values. Year 0 denotes the day of orchietomy. British Journal of Cancer, reprinted with permission [9].

raised S-LD-1 more often than a high S category (S2–3) of S-LD, S-AFP, and S-hCG (Table 3) [8]. So more patients had an indication of a worse prognosis from a raised S-LD-1 than from raised values of S-LD, S-AFP, and S-hCG. Patients with metastatic testicular germ cell tumors had a larger difference in survival from S-LD-1 than from S-LD, S-AFP, and S-hCG.

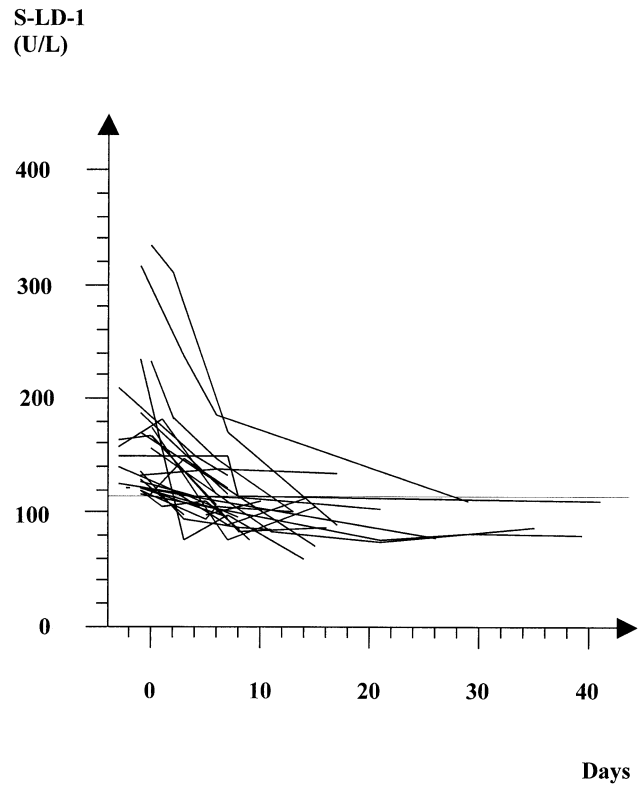
3.1.7. LD-1 and monitoring

Patients with testicular germ cell tumors were monitored with S-LD-1 in nine articles [6,8,45,49,50,62,64–66]. S-LD-1 was useful in the monitoring: Changes in S-LD-1 follow changes in tumor burden during the clinical course of patients. As the tumor burden gets smaller, S-LD-1 becomes lower. It shows that the tumor causes the rise of S-LD-1.

Table 3
Tumor markers according to the TNM classification for 81 patients with metastatic testicular germ cell tumors [7,9]

Serum tumor marker	Categories of serum tumor markers	No. of patients	p value
S-LD-1	<112 U/L	39 (48%)	<0.00001
	112–1120 U/L	37 (46%)	
	>1120 U/L	5 (6%)	
S-LD	<450 U/L	32 (40%)	0.00032
	450–675 U/L	21 (25%)	
	675–4500 U/L	22 (29%)	
S-AFP	<24 µg/L	50 (62%)	0.68
	24–999 µg/L	26 (32%)	
	1000–10,000 µg/L	4 (5%)	
S-hCG	>10,000 µg/L	1 (1%)	0.02
	<30 IU/L	39 (48%)	
	30–4999 IU/L	33 (41%)	
	5000–50,000 IU/L	4 (5%)	
	>50,000 IU/L	5 (6%)	

The cut off limits were those of the recent TNM classification [5]. The P values reflect the differences in survival between subgroups.



(B)

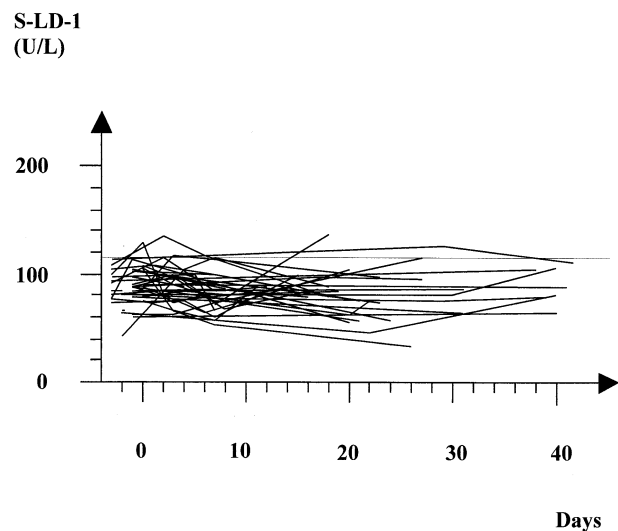


Fig. 6. (A) Decay of S-LD-1 in patients with nonseminomatous tumors stage I and a preoperative raised S-LD-1. Day 0 denotes the day of the orchietomy. (B) Clinical course of S-LD-1 in patients with an initially normal S-LD-1. Acta Oncologica, reprinted with permission [54].

In stage I patients with raised S-LD-1, the isoenzyme became normal after the orchietomy following a half-life of median 2.8 days [45]. S-LD-1 normalized within 4 to 50 days after surgery in eight of 10 evaluable patients. Several

Table 4
Fraction with a raised S-LD-1 in patients with ovarian germ cell tumors

Dysgerminoma					Nondysgerminoma					Reference
Stage					Stage					
I	II	III	IV	X	I	II	III	IV	X	
1/1	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	6
1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	75
0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	80
0/0	1/1	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	77
0/0	0/0	0/0	0/0	8/8	0/0	0/0	0/0	0/0	3/3	74
0/0	0/0	1/2	0/0	0/0	0/0	0/0	0/1	2/2	0/0	81
0/0	0/0	0/0	0/0	3/3	0/0	0/0	0/0	0/0	10/13	79
1/1	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	78
1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	76
0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	73

Stage X denotes patients with an unknown stage.

studies found this decay (Fig. 6A) whereas S-LD-1 remained stable in patients with an initially normal S-LD-1 (Fig. 6B).

In patients with stage II or III tumors and a raised S-LD-1, the isoenzyme decreased after orchiectomy, but remained raised before the postsurgical therapy in most of these patients [45,62]. S-LD-1 normalized in these patients as they achieved a complete remission after chemotherapy [8,50]. In contrast, S-LD-1 remained raised or increased in patients with residual or progressing tumors. Measured shortly before the patients' death, those who died of tumor had higher values of S-LD-1 than those who died of other causes [64].

Two articles were available regarding S-LD-1 in relation to relapse [44,66]. One article described the clinical course of S-LD-1 from diagnosis to relapse [66]. In a quarter of the patients, a raised S-LD-1 was the first sign of relapse. Especially patients with a relapse of seminoma often had a raised S-LD-1 as an early sign of the relapse. A series of increasingly raised S-LD-1 values in a patient suggests a progress of the tumor. The other article defined analytical quality specifications for the S-LD-1 assay based on clinical goals for monitoring S-LD-1 in stage I patients followed with surveillance [44].

Monitoring serum tumor markers aims to detect a relapse early in the clinical course. Patients with testicular germ cell tumors have a well-known relapse pattern. As for stage I patients who relapse, nearly all with nonseminomatous tumors and most with seminoma relapse within the first two years after orchiectomy. The time schedule for measuring the tumor markers reflects the goal for the monitoring, to detect a relapse early in the clinical course. Today, Danish oncologists measure tumor markers before the orchiectomy, in connection with staging and start of treatment at the oncological center, at two months intervals for the first year after surgery, every six months for the next two years, and once yearly for the following two years.

Two articles described how different physicians have reacted in treatment in relation to a raised S-LD-1. Zondag

changed the treatment for the patients due to a raised value [6]. In contrast, Odense University Hospital orders additional tests in this setting and changes the treatment only if the tests confirm that the tumor has progressed [66]. This approach reflects a concern for false-positive values, and for the toxicity of platin-based chemotherapy.

3.1.8. LD-1 and the international classification

For patients with metastatic testicular germ cell tumors, S-LD-1 had a greater discrimination of the prognosis than the international classification, and S-LD. Based on S-LD-1, the survival of the good and the poor risk subgroups of 81 patients differed more than the subgroups according to the classification of the International Germ Cell Cancer Collaborative Group (Figs. 5 and 7) [9]. Similarly, S-LD-1 had a better receiver operating character curve regarding long-term survival for these patients than the international classification [53]. So S-LD-1 may be a useful alternative to the international classification.

The patients with metastatic testicular germ cell tumors

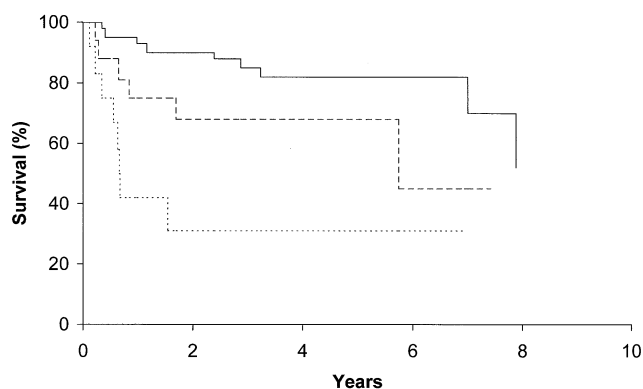


Fig. 7. Survival of 81 patients with metastatic testicular germ cell tumors classified according the International Germ Cell Cancer Collaborative Group study (— denotes 49 patients with a good prognosis, - - - 16 patients with an intermediate prognosis, and . . . 16 patients with a poor prognosis). British Journal of Cancer, reprinted with permission [9].

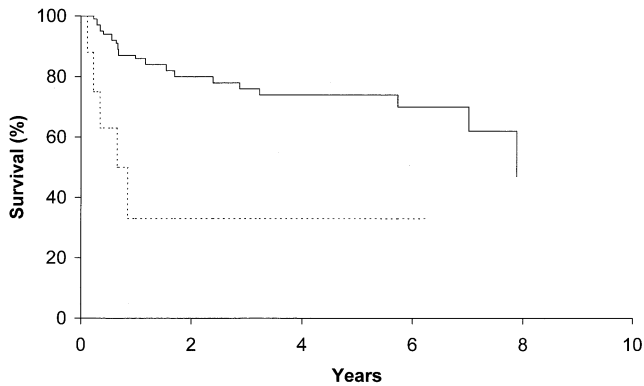


Fig. 8. Survival of 81 patients with metastatic testicular germ cell tumors according to S-LD-1/S-LD fraction (— denotes 73 patients with a low pretreatment S-LD-1/S-LD fraction (<0.375) and - - - 8 patients with a higher S-LD-1/S-LD fraction (>0.375). *British Journal of Cancer*, reprinted with permission [9].

who died of tumor more often had a raised S-LD-1 at start of treatment than a S-LD in the S1–S3 categories of the TNM classification [51]. This high sensitivity of S-LD-1 contributes to the clinical usefulness of S-LD-1.

Although S-LD and S-LD-1 in patients with testicular germ cell tumors have a large overlap of raised values [9], the two tumor markers differ in clinical practice. Accordingly, the S-LD-1/S-LD fraction significantly predicted survival (Fig. 8). While S-LD-1 was prognostically significant in a study of 37 patients, S-LD-5 was not ($p = 0.85$ log-rank test, Fig. 9) [8].

Three reports detailed the combination of a raised S-LD and a normal S-LD-1 for patients with a history of testicular germ cell tumors. In a series of three patients with seminoma, one had this combination of isoenzyme values due to a second malignancy [68]. In another series, one of seven patients with a raised S-LD and without evidence of disease had this combination [49]. This patient had a colon cancer with liver metastases and a highly raised S-LD with a predominance of the cathodal LD isoenzymes. In a third

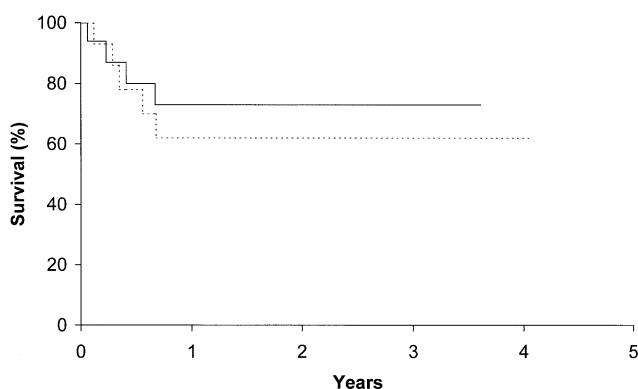


Fig. 9. Survival of 34 patients with metastatic testicular germ cell tumors according to S-LD-5 (— denotes 19 patients with a normal S-LD-5 and - - - 15 patients with a raised S-LD-5). *Acta Oncologica*, reprinted with permission [8].

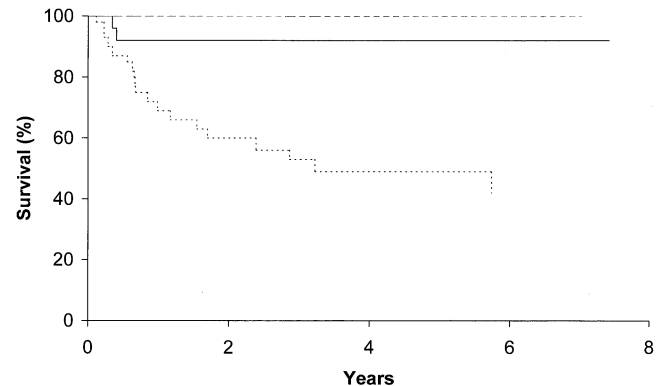


Fig. 10. Survival of 81 patients with metastatic testicular germ cell tumors according to the levels of S-LD and S-LD-1 (— denotes 31 patients with a normal S-LD and S-LD-1, - - - 8 patients with a raised S-LD and a normal S-LD-1, and . . . 42 patients with a raised S-LD-1 with a normal or a raised S-LD). *British Journal of Cancer*, reprinted with permission [9].

study of 81 patients with metastatic testicular germ cell tumors, eight of 49 (overall 10%) with a raised S-LD had a normal S-LD-1. The survival of this group was like that of other patients with a normal S-LD-1 (Fig. 10). So S-LD-1 may have a higher specificity for testicular germ cell tumors than S-LD.

In a patient with metastatic testicular germ cell tumor and a raised S-LD-1 and S-LD, S-LD normalized as S-LD-1 became normal [49].

3.2. Ovarian and extragonadal germ cell tumors

Compared with testicular germ cell tumors, few persons have ovarian or extragonadal germ cell tumors. Ovarian germ cell tumors have two main histologies, dysgerminomas and nondysgerminomatous tumors. Gynecological oncologists classify the tumors in four stages according to the classifications of the International Federation of Gynecologic Oncology. Stage I and II are local and regional stages. Stage III refers to IP metastases and stage IV to metastases outside the peritoneal cavity.

Ten articles described S-LD-1 and ovarian germ cell tumors [6,73–81]. Overall, 35 of 40 patients with ovarian germ cell tumors (88%, CI 73–96) had a raised S-LD-1. So patients with ovarian germ cell tumors had a raised S-LD-1 more often than patients with testicular germ cell tumors ($p = 0.0006$, Fisher's exact test). Patients with dysgerminoma had a raised S-LD-1 grossly as often (21 of 22, 95%) as those with nondysgerminomatous tumors (78%). The fraction of patients with a raised S-LD-1 was similarly high for those of the studies with more than ten patients and those published as single cases or in small series. Tissue from ovarian germ cell tumors had a high level of LD-1. In contrast, patients with ovarian nongerm cell tumors did not have the LD-1 isoenzyme pattern [82].

Four articles described S-LD-1 in patients with extragonadal germ cell tumors [34,80,83,84]. A raised S-LD-1 was

found in a patient with a retroperitoneal germinoma, a patient with a mediastinal germ cell tumor, a child with a yolk sac tumor and a girl with a sacrococcygeal tumor [34,83,84]. The fourth article dealt with children with sacrococcygeal germ cell tumors [80].

One article described S-LD-1 in germ cell tumors of the childhood [80]. Seven of eight children with yolk sac tumors (four sacrococcygeal, one ovarian, and three testicular tumors) had a raised S-LD-1.

4. Discussion

This review is a synthesis of widespread information and of biologic, analytic, and clinical findings. S-LD-1 had a consistent pattern as tumor marker of testicular germ cell tumors in studies from four continents, four decades, and 13 centers. S-LD-1 is the most important LD isoenzyme for patients with testicular germ cell tumors. The findings were consistent between studies using different LD isoenzyme assays. A raised S-LD-1 correlated with a relevant karyotypic aberration in testicular germ cell tumor, a high copy number of the short arms of chromosome 12—often in the form of *i*(12p). Nearly two thirds of the patients had a raised S-LD-1. S-LD-1, S-AFP, and S-hCG values were discordant. Patients with nonseminomatous testicular germ cell tumors stage I followed with surveillance who had a raised S-LD-1 had a higher risk of a relapse than those with a normal S-LD-1. Patients with metastatic disease and a raised S-LD-1 had a higher risk of death of tumor than those with a normal S-LD-1. S-LD-1 separated the patients in low-, intermediate-, and high-risk groups, and implied a larger difference in prognosis between the risk groups than the international classification, S-LD, S-AFP, and S-hCG [7]. Monitoring S-LD-1 may help to a correct staging and an early detection of a relapse. Most reported patients with ovarian germ cell tumors and evidence of disease had raised S-LD-1.

Genetic aberrations in the malignant transformation of testicular germ cells may explain the differences in the LD isoenzyme pattern. Tumors have a raised ploidy and the increased chromosomal number blocks the expression of *LDHC* [35], and explains why testicular germ cell tumors do not have LD-X. Rise of S-LD-1 in patients with testicular germ cell tumors was linked to the high copy number of 12p in the tumors. Accordingly, S-LD-1 belongs to a class of tumor markers that reflects characteristic chromosome aberrations of the tumors, *e.g.*, shown by cytogenetic examinations, and not specific histologies. The relation between 12p and S-LD-1 in testicular germ cell tumors explains why the isoenzyme was prognostically significant while S-LD-5 was not [8]. LD-5 is a homotetramer of LD-A subunits and the gene locus for the LD-A subunit, *LDHA*, is localized to chromosome 11. So S-LD-1 was a better serum tumor marker than S-LD [7,9]. In contrast, the established tumor

markers, LD, AFP, and hCG, are not expressions of genes of the short arm of chromosome 12 in the tumor lesions.

The findings of this review are relevant for general use. The reported patients had the range regarding histology and stage to whom clinical practice will apply serum tumor markers in future. Comparisons between S-LD-1, S-LD, and the TNM classification were carried out regarding 81 patients with metastatic testicular germ cell tumors who had distributions of S-LD, S-AFP, and S-hCG values like those reported by the International Germ Cell Cancer Collaborative Group [4]. Patients reported only in abstracts had a fraction with a raised S-LD-1 that did not differ much from those of the patients published in articles. Therefore, this review summarized both abstracts and articles that fulfilled the inclusion criteria.

The review had several limitations. It drew conclusions regarding testicular germ cell tumors from limited numbers of patients and events. Several studies reported S-LD-1 only as normal or raised isoenzyme values and may refer to the S-LD-1/S-LD fractions. For a fifth of the reported patients, the publications did not state the stage of the patients. The studies reported in the form of only abstracts did not detail the assay conditions for the measurements of S-LD-1.

Literature on LD-1 and germ cell tumors of other sites than testis is sparser than that of testicular germ cell tumors. So far, patients with ovarian germ cell tumors have been reported only as single cases and in small series. Comparisons between subgroups, however, indicate that the high fraction with a raised S-LD-1 for the patients reported as single cases or as small series was not due to their selection. The few data on S-LD-1 in the follow-up of patients with ovarian germ cell tumors do not allow any conclusions. As for extragonadal germ cell tumors, the literature does not allow any conclusions.

Two previous systematic reviews on S-LD-1 and S-LD had reservations for measuring LD isoenzymes in patients with germ cell tumors. One of the reviews claimed in the summary that a measurement of LD isoenzymes was not clinically useful [85]. The statement did not exempt germ cell tumors. Nevertheless, the present and the previous review evaluated the articles regarding germ cell tumors in a similar way. So the diverging conclusions of the two reviews did not reflect the reviewed articles. The other, earlier review preferred a routine use of S-LD and recommended a measurement of S-LD-1 in only patients with a history of germ cell tumors and an unexplained raised S-LD [3]. However, a raised S-LD may not be due to germ cell tumor even in patients with a history of testicular germ cell tumors and present tumor-suspected lesions; these lesions might be due to a second cancer. Correspondingly, two of three articles evaluating both S-LD and S-LD-1 during the last two decades gave priority to S-LD-1 for S-LD [7,9,53].

Further studies are indicated regarding S-LD-1 as tumor marker of testicular germ cell tumors. New studies may evaluate whether S-LD-1 predicts the outcome for patients with seminomas only. Other studies may validate the clin-

ical findings in large series of patients and the clinical aspects of false positive S-LD/S-LD-1 values. Genetic studies may elucidate further how parts of 12p contribute to the tumorigenesis and the clinical aspects of testicular germ cell tumors [86,87].

Further studies regarding patients with ovarian germ cell tumors may evaluate monitoring S-LD-1 in the clinical course. Ovarian germ cell tumors have relatively high fraction with a raised S-LD-1. It remains to be shown whether the raised S-LD-1 reflects a relatively high copy number of the short arms of chromosome 12 [28,88]. Studies of patients with extragonadal germ cell tumors and childhood germ cell tumors may show whether S-LD-1 is a tumor marker for these malignancies.

So far, books on testicular germ cell tumors and textbooks of genitourinary oncology have given only limited attention to S-LD-1 [24,89]. In contrast, this review supports a wider use of S-LD-1.

5. Conclusions

The literature on S-LD-1 and germ cell tumors had a consistent pattern that has implications for the use of S-LD-1 as a serum tumor marker. LD-1 is the most important LD isoenzyme as tumor marker of germ cell tumors. Regarding S-LD isoenzyme 1 variables as serum tumor marker of testicular germ cell tumors, S-LD-1 catalytic concentration should be preferred for the S-LD-1/S-LD fraction and the S-LD-1/S-LD-2 ratio. This review described how to use S-LD-1 in staging, prediction of prognosis, and monitoring of patients with testicular germ cell tumors. Classification of S-LD-1 values in three categories (normal, raised, and highly raised values) has prognostic implications for patients with metastatic testicular germ cell tumors. Serum hydroxybutyrate dehydrogenase catalytic concentration as alternative to LD-1 is poorly documented. Systematic reviews may highlight the evidence for clinical guidelines [3,90]. Regarding S-LD-1, the reported findings consistently suggested that the isoenzyme should be preferred for S-LD in routine use as tumor marker. The literature so far, however, is not sufficient support for recommending guidelines for the routine use of S-LD-1 as a tumor marker for patients with germ cell tumors. Therefore, further studies are required to validate the findings of this review.

Acknowledgments

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