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Testicular Lymphoma

A Retrospective, Population-based, Clinical and Immunohistochemical Study

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From a population-based registry, 35 patients with histologically verified testicular lymphomas were identified: diffuse large B-cell lymphomas (DLBCL) in 33 and peripheral T-cell lymphomas in two cases. Twenty-two patients had localized disease (Pe stage I and II). Twenty-eight patients received systemic chemotherapy, 17 of whom also received intrathecal prophylaxis, and 12 out of these 17 also received radiotherapy to the contralateral testis. In the Pe stage I/II group, 7 out of 21 patients in complete remission (CR) relapsed. In 5 of them the CNS was involved (isolated CNS relapse in three). Remarkably late relapses occurred (up to 127 months). Intrathecal prophylaxis seemed to reduce the frequency of relapses involving the CNS, but the relatively short follow-up (median 45 months, range 34–88, for censored patients) prevents firm conclusions regarding efficacy. The outcome for the stage IV patients was poor, with only 1 out of 11 patients in continuous CR. Immunohistochemical analysis of the DLBCL tumours revealed that 31% had the germinal centre B-cell-like phenotype. CD44 was expressed in all the tumours of stage IV patients but in less than half of the Pe stage I/II patients. A high intratumoural microvessel density was correlated with a high degree of Ki-67 positive tumour cells and an inferior overall survival.

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Testicular lymphoma accounts for about 1% of all lymphomas and is the most common malignant testicular tumour in men over the age of 60 (1). The diagnosis is usually obtained after orchidectomy and the dominant lymphoma subtype is DLBCL. Owing to the low incidence of the disease and the absence of prospective studies, the most appropriate therapy remains to be established. Early retrospective studies indicated a high rate of distant relapses in patients with localized disease treated by surgery alone or surgery plus loco-regional radiotherapy (RT), and therefore systemic, doxorubicin-based chemotherapy has been recommended (2–4). Nevertheless, a considerable relapse rate to extranodal sites, such as the central nervous system (CNS) or the contralateral testicle, has been recognized (1). In the Western Sweden Health Care Region, chemotherapy has been used for testicular lymphomas since the 1980s. In 1995, the recommendation was changed to a combined modality treatment (CMT), consisting of systemic doxorubicin-based chemotherapy, prophylactic intrathecal (IT) chemotherapy, and scrotal RT.

The propensity to disseminate to other extranodal areas seems to indicate a spreading behaviour of testicular lymphomas different from their nodal counterparts. The possible tumour properties governing this spread remain unknown but it has been suggested that the expression profile of CD44 and other adhesion molecules plays a role in tumour cell dissemination in cases of malignant lymphoma (5, 6). Furthermore, in solid tumours, it has been shown that increased intratumoural microvessel density (MVD) is correlated to increased metastatic potential and a worse prognosis (7). Information on MVD in malignant lymphomas is limited (8–10) and we are unaware of any study concerning MVD in testicular lymphomas. Furthermore, in a recent study based on the immunohistochemical profile, lymph node based DLBCL has been divided into two groups, germinal centre B-cell like (GCB) and non-GCB type, the former with a more favourable prognosis (11).

The aim of the present work was to investigate, retrospectively, the efficacy of the current treatment modality compared with other treatment strategies, as well as to examine what impact clinical and histopathological characteristics, in particular CD44 expression, MVD, and GCB properties, had on the prognosis in a population-based cohort of patients with testicular lymphoma.

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MATERIAL AND METHODS

Patients

Patients with testicular lymphoma, diagnosed in the Western Sweden Health Care Region (population 1.6 million) between 1985 and 2000, were included. The patients were identified from the population-based Regional Centre for Cancer Registration (1985–1994) and the Regional Lymphoma Registry (1995–2000). All male lymphoma cases registered between 1985 and 2000 were investigated and those with a histopathologically verified testicular lymphoma were included in the study. All available clinical files were collected from the different hospitals and centrally reviewed. Data concerning the patient's age, symptoms, disease extent, the level of lactate dehydrogenase (LD) at presentation, and the treatment modalities, and also the response rate, relapse pattern, and survival time were recorded.

Staging was performed according to a protocol concerning primary extranodal (Pe) lymphomas published by Musshof et al. (12) and modified by the Nordic Lymphoma Group. Isolated, unilateral testicular involvement, with or without local extension to epididymis or funicle, was regarded as Pe stage I and involvement of pelvic or abdominal lymph nodes as Pe stage II. Ann Arbor stages III E and IV were considered as stage IV. Furthermore, the cases were classified according to the International Prognostic Index (IPI) (13). Since LD values not were available for all cases, the patients were classified into two groups as a crude IPI, a low-risk (low and low intermediate), or a high-risk group (high intermediate and high).

Complete remission (CR) was defined as absence of disease signs one month after completion of the planned treatment. Patients treated only by surgery were considered as being in CR if no signs of disease were noted at that time. Partial remission (PR) was defined as a $>=50\%$ decrease of the tumour masses. Progressive disease (PD) was defined as any new signs of disease or tumour progression within one month after completion of the planned therapy.

Overall survival (OS) was measured from the date of diagnosis to the date of death or the latest check-up. Event-free survival (EFS) was measured from the date of diagnosis until the date of relapse, the disease progression, death from any cause, or the latest check-up.

Histopathology

Paraffin-embedded material, processed at six different laboratories, from surgically removed testes infiltrated by lymphoma was available from 33 patients. In addition, in one patient the tissue material was needle biopsies and in another there were specimens taken at autopsy.

All available blocks were resectioned and stained with Htx-eosin and May-Grünwald-Giemsa. From 31 patients, blocks with representative tumour infiltration were chosen for further immunostainings. The latter comprised the demonstration of CD20, CD79a, CD10, CD30, bcl 2, bcl 6, MUM 1, CD3, Ki-67, CD44, and CD34. All immunostainings were performed with the DAKO Envision system in a Techmate autostainer. The suppliers of the primary antibodies, the clone designations, and the applied, primary, antibody concentrations are accounted for in Table 1.

Stainings to demonstrate CD3, CD20, CD79a, CD10, CD30, CD44, bcl 2, and MUM 1 were registered as positive with at least 50% of the tumour cells exhibiting staining reaction. For bcl 6, a tumour was considered positive with 20% or more positive cells. The tumours were classified in accordance with the WHO classification (14). On the basis of the outcome of the stainings to demonstrate CD10, bcl 6, and MUM 1, the B-cell tumours were divided as described by Hans et al. (11) as being of germinal centre B-cell origin (CD10+ or bcl 6+ together with MUM 1-) or not (all other staining combinations).

The Ki-67 positive tumour cells in four fields of vision at a magnification of 800x were counted on a digital monitor image created by a Panasonic F15 CCD videocamera and their numbers were expressed as cells per mm^2. The density of the CD34-positive intratumoural microvessel profiles was determined as described previously (10, 15). Briefly, using the Leica Q Win measurement programme, the number and area of CD34-positive vessels with a profile area ranging between 10 and 1200 μm^2, corresponding to microvessels with a diameter of between 3.2 and 34.6 μm, were registered from a digital image at a magnification of 200x in 20 fields of vision. The vessel density was expressed as vascular profiles/mm^2 and referred to as MVD.

Statistics

Owing to the small number of Pe stage II patients, Pe stages I and II were put together in the outcome and statistical analyses. The survival data were estimated by the Kaplan–Meier method and comparisons between groups were made

Table 1

Data on antisera used in immunostainings

Epitope	Clone	Primary antibody concentration	Supplier
CD3	F7.2.38	1:100	DAKO
CD10	56C6	1:100	Novacastra
CD20	L26	1:400	DAKO
CD30	Ber-H2	1:100	DAKO
CD34	QBend10	1:50	DAKO
CD44	DF1485	1:40	DAKO
CD68	KP1	1:4000	DAKO
CD79a	JCB 117	1:100	DAKO
Bcl 2	124	1:100	DAKO
Bcl 6	PG B6p	1:10	DAKO
MUM 1	MUM 1p	1:50	DAKO
Ki-67	MIB-1	1:100	DAKO

by the log-rank test. The chi-square or Fisher's exact test was used when comparing categorical variables in 2×2 tables and the Mann–Whitney U-test was utilized for testing numerical data against categorical variables. A difference with a p-value <0.05 was considered as statistically significant. The Cox proportional-hazard model was used for multivariate analysis of survival data. Follow-up time was estimated by the reversed Kaplan–Meier method (16). SAS (SAS Institute Inc., Cary, NC) and StatView (Abacus Concepts, Inc., Berkeley, CA) software was used for the statistical analyses.

RESULTS

Patient characteristics

Thirty-five patients with a median age of 69 years (range 31–86) were identified as having proven testicular lymphoma. Staging procedures had been performed with abdominal and thoracic computed tomography in all but two patients and with bone-marrow biopsies in all but four patients. One patient was staged post mortem by autopsy.

The most common initial symptom was local swelling, accompanied by pain in some cases. Eighteen patients had involvement of the left testis, 14 of the right. Three patients had bilateral testicular enlargement; in two of them lymphoma involvement was verified in both testicles. All three patients had signs of disease dissemination.

Half of all the patients (18 out of 35) had Pe stage I, 4 had Pe stage II and 11 stage IV disease. Bulky disease was recognized in six patients; in four in the abdomen and in two in the testis. The LD level was normal in 17 patients, increased in 7 (one with Pe stage I, two with Pe stage II and 4 with stage IV) and was not available in 11 out of 35 patients. Still, 30 out of 35 patients could be classified according to the crude IPI; of these 23 had a low-risk profile.

A DLBCL was diagnosed in 33 out of 35 patients, while two young, stage IV patients had peripheral T-cell lymphomas, one of a small cell type and one of a large cell type.

Treatment

An overview of the main clinical data, treatment modalities, and outcomes is presented in Table 2. One patient received one course of chemotherapy after a lymphoma-positive, abdominal, fine-needle aspiration but died within one week; an autopsy disclosed a testicular lymphoma. In all the other 34 patients, orchidectomy was performed—unilateral in 30 and bilateral in four patients.

In five patients no further treatment was administered. Two patients received RT against para-aortic and iliac lymph nodes and scrotum, with 2 Gy fraction to a total dose of 40 Gy.

The other 28 patients received systemic chemotherapy; the standard CHOP regimen (cyclophosphamide, doxorubicin, vincristin, prednisone) in 22 and the CNOP regimen (cyclophosphamide, mitoxantrone, vincristin, prednisone) in 4 patients. Six to nine courses were administered to 18 out of 23 CHOP/CNOP-treated patients who completed the regimen and the remaining 5 patients received 3–5 courses. MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, bleomycin, prednisone) or four courses of the BEP regimen (cisplatinum, etoposide, bleomycin) were administered to one patient each, the latter owing to initially suspected seminoma. Additionally, three patients received RT against para-aortic and iliac lymph nodes (2 Gy fractions to 30-40 Gy) and 12 patients 2 Gy fractionated scrotal RT to 24 Gy (10 patients) and 40 Gy (2 patients).

Seventeen patients treated with systemic chemotherapy also received IT prophylaxis with 12 mg methotrexate; six times in 15 patients, two and four times in 1 patient each.

Thus, 12 patients received CMT with a combination of doxorubicin-based, systemic chemotherapy, IT methotrexate and scrotal RT (or bilateral orchidectomy, 3 patients).

Outcome

Four patients were withdrawn from response evaluation owing to missing clinical data after surgery (2 patients), death of cerebral infarction one week after orchiectomy (1 patient) and death from pneumonia one week after the first CHOP course (1 patient). Thus, 31 out of 35 patients were evaluable for response. CR was recognized in all 21 Pe stage I and II patients but only in 2 out of 10 stage IV patients, in total 23 patients (74%). Six patients had primary refractory disease, while 2 reached a PR. Both PR patients subsequently experienced disease progression and, like the 6 patients with primary refractory disease, died within a year.

Seven out of 21 Pe stage I/II and one out of two stage IV patients relapsed; in all of them, extranodal relapse was noted and the CNS was involved in 6 patients (isolated CNS relapse in 3). One patient experienced an isolated relapse in the contralateral testis; six months later, extensive nodal recurrence supervened. All 8 patients died within 9 months after relapse. Four of the 23 CR patients subsequently died of other diseases, without evidence of lymphoma. Ten patients are still alive in continuous CR, 9 with initial Pe stage I/II and 1 with stage IV disease. The median follow-up for these patients was 55 months (range 34–149).

All 35 patients were included in the survival analyses. At a median follow-up of 88 months, the estimated median OS was 47 months (95% CI 15–67) and EFS 42 months (95% CI 8–64). There was a highly significant survival difference between stages Pe I/II vs. IV and IPI low-risk vs. high-risk groups, respectively (see Table 3).

In the Pe stage I/II group, 1 out of 12 patients treated with systemic and prophylactic IT chemotherapy experienced a CNS relapse, whereas 4 out of 6 patients treated

Table 2

Patient characteristics, treatment modalities and outcome

Pat No	Age, years	Diagnosis	Stage	IPI	Surgery	Further therapy	Chemo-therapy	Response	Relapse	EFS months	Current status	Cause of death
1	86	DLBCL	na	na	Uni	No		na		2	Dead	?
2	83	DLBCL	I	na	Uni	No		CR	N+En	5	Dead	Ly
3	78	DLBCL	na	na	Uni	No		na		0	Dead	?
4	71	DLBCL	IV	High	Uni	No		na		2	Dead	?
5	80	DLBCL	I	Low	Uni	No		CR	?	68	Dead	?
6	65	DLBCL	I	Low	Uni	RTn+RTs		CR		109	Dead	Other
7	74	DLBCL	IV	High	Uni	RTn+RTs		PD		5	Dead	Ly
8	67	DLBCL	IV	High	Uni	Chemo	CHOP 7	PD		5	Dead	Ly
9	79	DLBCL	II	na	No	Chemo	CHOP 1	na		1	Dead	Ly+ other
10	53	DLBCL	I	Low	Uni	Chemo	CHOP 9	CR	CNSp+ N+En	96	Dead	Ly
11	31	Peripheral T	IV	Low	Uni	Chemo	BEP	PD		3	Dead	Ly
12	46	DLBCL	I	Low	Uni	Chemo	CHOP 6	CR		149+	CCR	
13	70	DLBCL	I	Low	Uni	Chemo	CNOP 6	CR	CNSp	64	Dead	Ly+ other
14	58	DLBCL	I	Low	Uni	Chemo+IT	CNOP 6	CR	Te (+N)	45	Dead	Ly
15	46	Peripheral T	IV	Low	Uni	Chemo+IT	CHOP 3	PD		3	Dead	Ly
16	54	DLBCL	IV	Low	Uni	Chemo+IT	CHOP 8	PR	N *	11	Dead	Ly
17	76	DLBCL	IV	High	Uni	Chemo+IT	CHOP 2	PD		2	Dead	Ly
18	69	DLBCL	I	Low	Uni	Chemo+IT	CHOP 6	CR	CNSp/m	24	Dead	Ly
19	65	DLBCL	IV	High	Uni	Chemo+ RTn	MACOP-B	PR	CNSp *	10	Dead	Ly
20	69	DLBCL	IV	na	Bil	Chemo+ RTn	CHOP 6	PD		7	Dead	Ly
21	70	DLBCL	I	Low	Uni	Chemo+ RTs	CHOP 3	CR	CNSm+ En	127	Dead	Ly
22	48	DLBCL	I	Low	Uni	Chemo+ RTs	CHOP 3	CR		114+	CCR	
23	59	DLBCL	I	Low	Uni	Chemo+ RTs	CHOP 8	CR	CNSp	35	Dead	Ly
24	63	DLBCL	II	Low	Bil	CMT	CHOP 8	CR		29	Dead	Other
25	73	DLBCL	I	Low	Uni	CMT	CNOP 4	CR		88+	CCR	
26	74	DLBCL	I	Low	Uni	CMT	CHOP 4	CR		53	Dead	Other
27	66	DLBCL	I	Low	Uni	CMT	CHOP 6	CR		77+	CCR	
28	61	DLBCL	II	Low	Uni	CMT	CHOP 8	CR		62+	CCR	
29	81	DLBCL	I	Low	Uni	CMT	CHOP 3	CR		47+	CCR	
30	67	DLBCL	IV	High	Bil	CMT	CHOP 8	CR	CNSm+ En	8	Dead	Ly
31	78	DLBCL	I	Low	Bil	CMT	CNOP 6	CR		49	Dead	Other
32	69	DLBCL	I	Low	Uni	CMT	CHOP 6	CR		42+	CCR	
33	59	DLBCL	I	Low	Uni	CMT	CHOP 7	CR		40+	CCR	
34	74	DLBCL	II	Low	Uni	CMT+RTn	CHOP 6	CR		36+	CCR	
35	78	DLBCL	IV	High	Uni	CMT	CHOP 7	CR		34+	CCR	

Abbreviations: IPI = see text, na = not available, Relapse = relapse after CR and progression after PR*, Uni = unilateral orchidectomy, Bil = bilateral orchidectomy, IT = intrathecal prophylaxis, RTs = scrotal radiotherapy (or bilateral orchidectomy, 3 patients), RTn = nodal radiotherapy, CMT = combined modality treatment see text, CHOP/CNOP = see text & number of courses, N = nodal, CNSp = brain parenchyma, CNSm = meningeal, En = extranodal sites outside CNS and testis, Te = contralateral testis, CCR = continuous complete remssion, Ly = lymphoma, Other = other disease, ? = unknown.

with systemic chemotherapy without IT prophylaxis suffered from relapses including the CNS ($p = 0.02$). The groups were comparable in respect of administered systemic chemotherapy, presence of bulky disease and LD levels. However, the median age was higher in the group who received CNS prophylaxis (69 vs. 56 years) and the follow-up time was significantly shorter for that group as well (median 45 months, range 34–88, for living patients). No difference in EFS or OS ($p = 0.3$) was recognized between the two groups. When looking at isolated CNS relapses, one

Table 3

Median values, confidence limits and statistical differences on survival versus stages and IPI

Event-free survival				Overall survival			
Stage I/II	Stage IV	IPI low	IPI high	Stage I/II	Stage IV	IPI low	IPI high
64*	3	64	2	67	11	67	8
47–114**	2–8	42–114	0–8	49–113	2–15	47–105	2–14
$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$				

*Denotes median value, months. **Denotes 95% confidence interval, months.

out of 12 vs. 2 out of 6 patients, respectively, experienced an isolated CNS relapse.

Immunohistochemistry and microvessel density

An extended immunostaining was performed in 29 of the 33 DLBCL cases. All these tumours were CD20+ and CD79a+. The results of the other immunostainings are presented in Table 4.

Nine out of 29 patients (31%) could be classified with a GCB and 20 out of 29 (69%) with a non-GCB phenotype. No difference in EFS or OS was noted between the two groups ($p = 0.4$).

There was a statistically significant difference in CD44 expression between the different stages and IPI risk groups. Eight out of 19 tested Pe stage I/II vs. 8 out of 8 stage IV patients were CD44+ ($p = 0.008$) and the fraction CD44+ regarding IPI low and high risk was 9/19 and 6/6, respectively ($p = 0.05$). However, no impact on EFS or OS ($p = 0.4$) was noted.

The mean $\pm$ SD for MVD was 80.8 ± 33.1 vessels/mm^2 tumour area (see Table 4). This was the only histopathological variable tested against survival that reached statistical significance in univariate analyses; a high MVD was correlated with a worse EFS and OS ($p = 0.03$). When tested in addition to stage or IPI in a bivariate analysis, MVD remained as an independent predictor of EFS ($p = 0.04$) and OS ($p = 0.04$). The mean $\pm$ SD for the number of Ki-67 positive cells was 228.8 ± 104.3 cells/mm^2 tumour area. The MVD was correlated positively with the Ki-67 expression ($r = 0.64$, $p = 0.0002$).

DISCUSSION

The 35 identified cases of testicular lymphoma correspond to 0.6% of all lymphomas diagnosed in our region between 1985 and 2000. The median age (69 years) was the same for the testicular lymphoma cohort as for all male DLBCL patients in the region.

Orchidectomy is the established diagnostic and first therapeutic procedure in cases of testicular lymphoma. The choice of further treatment is still a matter of debate, owing to the rare incidence of the disease and the absence of prospective, randomized studies.

Systemic, doxorubicin-based chemotherapy has been widely used also in patients with localized disease, since early retrospective reports indicated an improved outcome by adding chemotherapy to surgery $\pm$ RT (2–4), but in two recently published, retrospective studies no such benefit was noted (17, 18). However, in a very large, retrospective, multicentre study (19), Zucca et al. reported a survival benefit for patients treated with anthracycline-containing chemotherapy, regardless of disease stage, and a better outcome for patients who had received at least six cycles of chemotherapy. Nonetheless, a continuous risk of relapse has been observed, especially in the CNS and other extranodal sites, including the contralateral testis (19–22).

Also in the present study, the relapse rate for the Pe stage I patients receiving anthracycline-containing chemotherapy after surgery was considerable; 6 out of 19 patients relapsed, with the CNS involved in 5. Remarkably late relapses were observed (up to 127 months). However, this phenomenon has also been recognized in some earlier reports (19, 22), in contrast with nodal DLBCL in which such late relapses are rare. Additionally, the CNS relapses in stage Pe I/II testicular lymphoma patients most often affect the brain parenchyma (17, 19) whereas the lepto-meninges and brain parenchyma seem to be affected equally often in stage IV patients (19). Also in the present series of Pe stage I patients, 3 CNS relapses occurred in the brain parenchyma (2 of which were isolated in the CNS), one in the lepto-meninges and one in both tissues (isolated).

The role of IT chemotherapy as CNS prophylaxis is controversial. Visco et al. (23) reported in a retrospective study one lepto-meningeal relapse in a subset of 16 patients receiving systemic chemotherapy and IT prophylaxis; the median follow-up was 49 months for living patients. In another retrospective study Zouhair et al. (24) noted relapses including the CNS in 4 out of 17 patients receiving IT prophylaxis but the same relapse fraction among patients not receiving prophylaxis. Further, Zucca et al. (19) recognized improved progression-free survival among a small subset of patients treated with IT prophylaxis but not a statistically significant reduction in the CNS relapse rate.

In a prospective study, Linassier et al. (25) followed 16 stage I/II patients, uniformly treated with three CHOP courses, regional node RT, IT prophylaxis, and brain RT. At a median follow-up of 73 months for surviving patients, 5

Table 4

Results of immunohistochemical stainings of 29 testicular diffuse large B-cell lymphomas

Pat No	CD10	Bcl 6	MUM 1	CD30	Bcl 2	CD44	MVD*	Ki-67**
1	NEG	POS	POS	NEG	POS	POS	41.9	231
2	NEG	POS	POS	POS	NEG	NEG	42.5	142
4	NEG	POS	POS	NEG	POS	POS	66.9	214
5	NEG	POS	POS	NEG	NEG	POS	106.9	352
6	NEG	POS	POS	NEG	POS	NEG	62.5	171
7	NEG	POS	POS	POS	POS	POS	86.3	154
8	NEG	POS	POS	NEG	POS	POS	46.9	147
9	NEG	NEG	POS	NEG	POS	POS	108.1	401
12	POS	POS	NEG	NEG	POS	POS	80	188
13	NEG	POS	POS	NEG	POS	POS	62.5	88
14	NEG	POS	NEG	NEG	NEG	NEG	71	183
16	POS	POS	POS	NEG	POS	POS	68.8	352
17	NEG	POS	NEG	NEG	NEG	POS	65	200
18	NEG	POS	POS	POS	POS	NEG	62.5	73
19	NEG	POS	POS	NEG	POS	POS	73.1	207
20	NEG	POS	POS	POS	POS	POS	43.8	257
21	NEG	POS	POS	NEG	POS	POS	146.3	446
23	POS	NEG	POS	NEG	POS	NEG	120.6	433
24	NEG	POS	NEG	NEG	POS	NEG	28.9	78
25	NEG	NEG	POS	NEG	NEG	POS	152.5	272
26	NEG	POS	POS	NEG	POS	POS	80.6	292
27	NEG	POS	NEG	NEG	POS	NEG	65.6	216
28	NEG	POS	POS	NEG	POS	NEG	123.3	236
29	NEG	NEG	NEG	NEG	POS	NEG	58	97
31	NEG	POS	POS	NEG	POS	POS	70	252
32	NEG	POS	POS	NEG	POS	NEG	56.3	242
33	POS	POS	POS	NEG	POS	POS	140	382
34	NEG	NEG	POS	POS	POS	NEG	101.9	121
35	POS	POS	POS	NEG	POS	POS	111.9	208
Positive	5	24	23	5	24	18		
Negative	24	5	6	24	5	11		

*MVD = microvessel profiles per sq.mm tumour area.
**No of Ki-67 positive cells per sq.mm tumour area.

out of 16 patients had relapsed, in one of whom the disease was isolated in the lepto-meninges.

In the present study, 1 out of 12 Pe stage I/II patients receiving IT prophylaxis and systemic chemotherapy experienced a CNS relapse, which seems better than the outcome in the chemotherapy group without IT prophylaxis. However, the groups were not completely comparable. The median age was higher in the group who received IT prophylaxis and 3 patients died within 53 months of other diseases without lymphoma recurrence, which might have influenced the relapse rate. Most importantly, the follow-up time (median 45 months, range 34–88, for living patients) was too short for firm conclusions to be drawn regarding the long-term efficacy of IT prophylaxis.

In summary, on the basis of the present and earlier reports, the benefit of IT chemotherapy as CNS prophylaxis remains to be clarified. The propensity for late relapses emphasizes the need for long follow-up for patients with localized disease at presentation.

Considering the risk of relapse to the brain parenchyma, intravenous high-dose methotrexate could be an alternative for suitable patients but its role as CNS prophylaxis in testicular lymphoma remains to be determined. In a retrospective study of nodal, aggressive lymphomas (26), intravenous high-dose methotrexate and IT methotrexate were administered as CNS prophylaxis. One isolated CNS relapse among 11 patients with initial testicular involvement was recorded.

The addition of rituximab to the CHOP regimen has been shown to improve outcome in elderly patients with nodal DLBCL (27). However, when analysing the patient material with regard to the CNS relapse rate, no difference was recorded between the CHOP and rituximab/CHOP arms (28).

Scrotal RT in order to prevent relapse in the contralateral testis seems to be beneficial, at least for patients with localized disease (1, 19, 22), but the optimal RT dose has not been established. In the present study, one testicular

relapse occurred among patients who had not received scrotal irradiation and none in the irradiated group of patients. The role of loco-regional, nodal RT is still controversial and a positive impact on outcome has never been shown (1, 19, 25).

At present, however, it seems reasonable to suggest that Pe stage I and II patients are treated with the combined modality, including the CHOP regimen (at least six courses), IT prophylaxis and scrotal RT. For elderly patients not suitable for chemotherapy, surgery alone may be an option.

As regards the stage IV patients in our study, the outcome was very poor. The majority failed to achieve a CR and only one patient is still alive in a continuous first CR. Since the patient number was very small, no conclusions can be drawn from this series. However, the results are consistent with findings in other studies (1, 19, 21). Consequently, younger patients with disseminated disease should be offered an intensive treatment modality, including CNS prophylaxis.

In the study of nodal DLBCL by Hans et al. (11), 58% of the tumour cases were considered to be of the non-GCB phenotype, associated with a significantly inferior survival to those with the GCB phenotype. One possible explanation for the worse prognosis seen in testicular lymphoma might be a higher proportion of non-GCB patients. However, by applying the same criteria as Hans et al., approximately the same fraction (69%) of the tumour cases in this study had the non-GCB phenotype and, additionally, this attribute could not be demonstrated to be of significance for survival.

Tumour-cell expression of the adhesion molecule CD44 has been found to be correlated with dissemination in nodal DLBCL, and for patients with localized disease also correlated with an inferior OS (6). In their penetrative investigation of adhesion molecules expressed in unfixed testicular DLBCL, Horstmann and Timens (5) found that few adhesion molecules were expressed, but CD44 was present in 10 out of 12 cases. Stage and other clinical data were not available in that study. In the present, formalin-fixed material, CD44 positivity was not correlated with EFS, but there was a significant, positive correlation with stage (less than half of the Pe stage I/II but all stage IV patients tested were CD44+). The number of patients was too small to allow further analysis regarding possible different clinical behaviour between the CD44+ and the CD44− Pe stage I/II patients.

The small number of patients hampered the statistical analysis of the present material. Thus, the conditions for a complete, multivariate analysis could not be fulfilled, the material only permitting a bivariate analysis. The only histopathological variable that contained significant information on survival was MVD, which varied negatively with EFS, as well as with OS. This variable also appeared to be independent of stage and IPI. Its significant correlation with the Ki-67 marker supports the finding established in other tumour types (29) that cell proliferation is closely related to angiogenesis, indirectly assessed as MVD. MVD was higher in the testicular DLBCL than in this type of lymphoma in lymph nodes (10, 15). The reason for this may be tumour-specific features or may reflect differences in the vascularity of the organs in question (30). Although MVD in the present series was not correlated significantly with stage or IPI, the observation of prognostic information in the assessment of MVD may lead to speculations on the tumour vascularity in testicular lymphomas being an important prerequisite for dissemination.

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