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Original Research

Intravenous but not intrathecal central nervous system–directed chemotherapy improves survival in patients with testicular diffuse large B-cell lymphoma

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Abstract ***Background:*** Testicular lymphoma is a rare malignancy affecting mainly elderly men, the majority representing diffuse large B-cell lymphoma (DLBCL). Its relapse rate is higher than that of nodal DLBCL, often affecting the central nervous system (CNS) with dismal prognosis.

Patients and methods: We searched for patients with testicular DLBCL (T-DLBCL) involvement from the pathology databases of Southern Finland University Hospitals and the Danish Lymphoma Registry. Clinical information was collected, and outcomes between treatment modalities were evaluated. Progression-free survival (PFS), disease-specific survival (DSS) and overall survival (OS) were assessed using Kaplan–Meier and Cox proportional hazards methods.

Results: We identified 235 patients; of whom, 192 were treated with curative anthracycline-based chemotherapy. Full survival data were available for 189 patients. In univariate analysis, intravenous CNS-directed chemotherapy, and irradiation or orchiectomy of the contralateral testis translated into favourable PFS, DSS and OS, particularly among the elderly patients

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(each $p \leq 0.023$). Intrathecal chemotherapy had no impact outcome. In multivariate analyses, the advantage of intravenous CNS-directed chemotherapy (hazard ration [HR] for OS, 0.419; 95% confidence interval [CI], 0.256–0.686; $p = 0.001$) and prophylactic treatment of contralateral testis (HR for OS, 0.514; 95% CI, 0.338–0.782; $p = 0.002$) was maintained. Rituximab improved survival only among high-risk patients (International Prognostic Index≥3, $p = 0.019$). The cumulative risk of CNS progression was 8.4% and did not differ between treatment modalities.

Conclusion: The results support the use of CNS-directed chemotherapy and prophylactic treatment of the contralateral testis in patients with T-DLBCL involvement. Survival benefit appears resulting from better control of systemic disease rather than prevention of CNS progression.

1. Introduction

Primary testicular lymphoma is a rare malignancy with an estimated yearly incidence of less than 0.3/100,000 [1]. Testicular involvement is seen in 1–2% of patients with lymphoma. The median age of patients with testicular lymphoma is on the later 7th decade of life, and lymphoma is the most common testicular malignancy in men older than 50 years of age. Most lymphomas with testicular involvement represent diffuse large B-cell lymphoma (DLBCL) [1–4].

Common prognostic factors of DLBCL, such as International Prognostic Index (IPI) and high tumour burden, have been shown to correlate with survival also in testicular DLBCL (T-DLBCL). However, even after aggressive chemotherapy relapse rate of T-DLBCL is higher and overall prognosis of T-DLBCL is worse than those of nodal DLBCL, relapses often occur in the central nervous system (CNS), and the prognosis for patients with CNS involvement is dismal [5,6].

T-DLBCLs represent most commonly non–germinal centre B-cell (non-GCB) subtype [2]. BCL2 positivity is also common, whereas expression of BCL6 and MYC is infrequent [7]. Prognostic role of these three proteins remains to be shown. Molecular studies in T-DLBCL are still based on small series. Nevertheless, as recently elegantly summarised by Twa et al [8], T-DLBCL is likely to represent a biologically distinct lymphoma entity. In addition to the molecular features of the lymphoma cells, elements of the microenvironment have been shown to have prognostic impact [9,10] and therapeutic potential [11].

T-DLBCL is considered a curable disease with modern treatment comprising anthracycline-based immunochemotherapy, CHOP (cyclophosphamide, Adriamycin, vincristine, prednisolone) with CD20 antibody rituximab (R–CHOP). However, the optimal therapy is unclear. As for many DLBCL subtypes, no randomised trials specifically in T-DLBCL have been conducted, and treatment guidelines are based on phase II trials [12,13]. In addition to R–CHOP, eligible patients commonly receive CNS prophylaxis with either intravenous (i.v.) or intrathecal (i.t.) methotrexate (MTX) or cytarabine (AraC) [14]. Thus far, however, no prospective randomised studies on the benefit of this approach have been published. In a retrospective series of 373 patients from the prerituximab era, i.t. chemotherapy resulted in improved progression-free survival (PFS) without benefit in overall survival (OS) or disease-specific survival (DSS) rates [3]. Furthermore, the contralateral testis also serves as a sanctuary to lymphoma cells, leading to risk of testicular relapse after immunochemotherapy. Therefore, contralateral orchiectomy or irradiation is recommended for eligible patients [12,15]. Owing to these recommendations, T-DLBCL is underrepresented in prospective clinical trials not allowing the use of CNS prophylaxis or irradiation, which may be the reason the testes were not recognised as a risk factor for CNS recurrence in the CNS-IPI model [16].

In the Nordic countries, the most applied current therapy for T-DLBCL consists of R–CHOP–/CHOP-like regimen in combination with systemic CNS prophylaxis (high-dose(HD)-MTX or HD-cytarabine) and prophylactic contralateral orchiectomy or irradiation. However, the efficacy of this approach has never been evaluated. In the present Nordic collaborative study looking at population-based cohorts from Denmark and Finland, we investigated the impact of different treatment eras on survival and risk of CNS progression in patients with T-DLBCL.

2. Methods

2.1. Patients

We searched for patients with T-DLBCL from the pathology databases of three Southern Finland University Hospitals and the Danish Lymphoma Registry. Only the patients with testicular involvement at primary diagnosis were included, and those with primary CNS lymphoma involvement were excluded. For the Finnish cohort, the clinical data were collected from patient

records, and a tissue microarray (TMA) from the diagnostic tumour samples was assembled. The TMA was subjected to standard diagnostic immunohistochemistry using antibodies against CD79, CD10, BCL-2, BCL-6 and MUM-1. Lymphoma diagnosis was reviewed according to the current World Health Organisation (WHO) classification and cell of origin (COO) defined according to the Hans algorithm [17] by experienced haematopathologists (M.-L.K- .L. and P.K.). Accordingly, the samples were scored positive for CD10, BCL-6 and MUM1, if 30% or more of the tumour cells were stained with an antibody. For BCL2, the cut-off level was 50%.

For the Danish cohort, the data were collected from the Danish Lymphoma Registry [18]. CNS relapse was diagnosed on the basis of clinical symptoms together with radiological findings, positive cerebrospinal fluid (CSF) cytology, positive CSF flow cytometric immunophenotyping, tumour biopsy or combination of these.

2.2. Statistical methods

All statistical analyses were performed using IBM SPSS version 24.

The χ^2 test was used to assess the differences in the baseline prognostic factors. Cox univariate and multivariate regression analyses were performed to study the prognostic value of the factors. The Kaplan–Meier method was used to estimate survival rates, and the differences in these rates were compared with the log-rank test.

OS was defined as the time from the date of diagnosis until last follow-up or death from any cause, and DSS, from the date of diagnosis until last follow-up or death caused by lymphoma. PFS was measured from the date of diagnosis until relapse, progression or death from any cause. Probability values below 0.05 were considered statistically significant; all p-values were two-tailed.

The study and sampling were approved by the institutional review boards, ethics committees and Finnish National Supervisory Authority for Welfare and Health.

3. Results

3.1. Baseline and treatment characteristics

Clinical data were available from 235 patients diagnosed with T-DLBCL between 1987 and 2013. The median age at diagnosis was 71 years (range, 37–93 years). Two hundred thirty-three patients (98%) underwent orchiectomy. The disease was limited to the testis in 95 (41%) patients and regarded as primary testicular (PT)–DLBCL (stage I-II) in 146 (63%) patients according to standard staging criteria. In eight patients (11%) of the Finnish cases ($N = 75$), both testes were affected, which was considered as one extranodal site. The contralateral testis involvement was not recorded in the Danish Lymphoma Registry. Seven patients were excluded because of simultaneous CNS lymphoma.

One hundred ninety-one patients (82%) were treated with a curative intent according to institutional contemporary practice using CHOP or CHOP-like chemotherapy, 69 (36%) before and 120 (63%) in the rituximab (R) era. Seventy-six patients (40%) received i.v. CNS-directed chemotherapy. For 46 patients, the treatment plan was palliative or follow-up because of poor performance status (PS) or patient's choice. Of the 191 patients who received active therapy, information of death was not available for two patients. Thus, a population of 189 patients was used for survival analysis. Of these, 124 (65%) patients had PT-DLBCL.

Baseline characteristics of the 189 patients distributed according to i.v. CNS therapy are shown in Table 1 and according to PT-DLBCL versus advanced stage T-DLBCL are shown in Table S1. After a median follow-up of 80 months (range, 7–192 months), the 5-year PFS, DSS and OS rates were 52%, 71% and 60%, respectively (Figure S1A–C). Of the 102 registered deaths, the cause of death was reported for 81 patients. Lymphoma was the most common cause (56%). Major non-lymphoma–related deaths were other malignancy (10%), cardiovascular failure (7%) and infection (3%). As expected, classical risk factors in DLBCL were associated with survival also in this PT-DLBCL cohort (Table 2 and Figure S1D–I). Only twelve patients (6.3%) experienced CNS progression as their first recurrence. CNS progression was more common in patients with advanced stage (III-IV) and high IPI (3-5). All but one of them died from lymphoma. Additional six CNS progressions occurred at later relapses with fatal results. Of the baseline characteristics, stage, number of extranodal sited and IPI score were associated with the risk of CNS recurrence (Table 2). Information on adrenal gland or kidney involvement was available for 164 patients enabling CNS-IPI evaluation [16]. Their involvement was not associated with increased risk of CNS relapse (hazard ratio, 1.507; 95% confidence interval, 0.196–11.610; $p = 0.694$) and did not add to predictive impact of the IPI score.

3.2. Impact of rituximab on survival

One hundred twenty (63%) patients received immunochemotherapy. In the entire study population, immunochemotherapy was not superior to chemotherapy (Figure S2A–C). However, in the high IPI subgroup, immunochemotherapy translated into favourable survival (5-y DSS, 44% versus 14%, $p = 0.019$; Figure 1A–C).

3.3. Impact of CNS-directed chemotherapy on survival

Systemic CNS-directed chemotherapy, that is, i.v. MTX, HD-AraC or both, was given to 76 (40%) of the 189 evaluable patients; 51 of them were treated with immunochemotherapy. The timing and the dose of CNS-directed chemotherapy varied according to the protocol used, with the minimum cumulative dose of MTX being 1.5 g/m^2 and AraC being 8 g/m^2 for two cycles. There was no significant association with the usage of HD-MTX or HD-AraC and the PS, IPI score, stage or molecular subtype. CNS-directed i.v. chemotherapy was equally common in the rituximab and prerituximab eras (43% versus 36%, p = 0.443). However, the patients receiving i.v. CNS-directed chemotherapy were significantly younger than the ones treated with (R-)CHOP only or supplemented with i.t. chemotherapy (Table 1). In Kaplan–Meier analysis, i.v. CNS-directed chemotherapy translated into favourable survival (Figure 1D–F). When survival analysis of the CNS-directed chemotherapy was adjusted for age (cut-off, 70 years), the difference remained significant.

Table 1
Baseline characteristics of all patients treated with anthracycline-based chemotherapy according to whether patients received i.v. CNS-targeted therapy or not.

Characteristics			All	i.v. CNS-targeted therapy	No i.v. CNS-targeted therapy	p
			N (%)			
Number of patients			189	76 (40)	113 (60)	
Median age (range)			69 (37–88)	64 (38–88)	72 (37–86)	
Median follow-up (months) (range)			81 (6.6–192)	71 (6.6–186)	92 (14–192)	
Age		≤60 years	50 (26)	32 (42)	18 (16)	**<0.001**
>60 years	139 (74)	44 (58)	95 (84)			
Age		≤70 years	96 (51)	52 (68)	44 (39)	**<0.001**
>70 years	93 (49)	24 (32)	69 (61)			
ECOG		0–1	161 (85)	67 (88)	94 (83)	0.407
2–4	28 (15)	9 (12)	19 (17)			
Stage		PT-DLBCL	123 (65)	49 (64)	75 (66)	0.876
Advanced T-DLBCL	65 (34)	27 (36)	38 (34)			
No. of EN sites, median (range)			1 (1–10)	1 (1–5)	1 (1->5)	
Kidney or adrenal involvement			12 (6)	5 (7)	7 (6)	1.000
Missing			25 (13)	6 (8)	19 (17)	
IPI score	0–2		135 (71)	55 (72)	80 (71)	1.000
	3–5		53 (28)	21 (28)	32 (28)	
	Missing		1 (1)		1 (1)	
CNS-IPI score	0–1		89 (47)	38 (50)	51 (45)	0.973
	2–3		48 (25)	20 (26)	28 (25)	
	4–6		27 (14)	12 (16)	15 (13)	
	Missing		25 (13)	6 (8)	19 (17)	
Lactate dehydrogenase	Elevated		62 (33)	29 (38)	33 (29)	0.270
	Normal		124 (66)	47 (62)	77 (68)	
	Missing		3 (1)		3 (3)	
i.t. CNS-targeted therapy			63 (33)	16 (21)	47 (42)	**0.004**
Treated with rituximab			120 (63)	51 (67)	69 (61)	0.443
Treatment of contralateral testis			88 (47)	41 (54)	47 (42)	0.104
Relapse/progression			72 (38)	24 (32)	48 (43)	0.169
CNS relapse/progression			18 (10)	11 (14)	7 (6)	0.063
At first relapse			12 (6)	8 (11)	4 (4)	0.129
Deaths			102 (54)	24 (32)	78 (69)	**<0.001**
Lymphoma-specific			57 (30)	16 (21)	41 (36)	**0.023**
Other			45 (24)	8 (11)	37 (33)	**0.001**
Second malignancy			10	3	7	
Cardiovascular			7	2	5	
Infection			3	1	2	
Renal failure			1	0	1	
Pulmonary failure			1	0	1	
Diabetes			1	0	1	
Liver failure			1	0	1	
Unknown			21	2	19	

Statistically significant differences between the patient groups are in bold.
ECOG, Eastern Cooperative Oncology Group; PT-DLBCL, primary testicular diffuse large B-cell lymphoma; T-DLBCL, diffuse large B-cell lymphoma with testicular involvement; EN, extranodal; IPI, International Prognostic Index; CNS, central nervous system; i.v., intravenous; i.t, intrathecal.

Table 2
Cox regression analyses at the univariate level showing association of baseline characteristics and treatment parameters with outcome.

Characteristic	Hazard ratio (95% CI)							
	OS	p	DSS	p	PFS	*P*	CNS at 1st relapse	*P*
Age >70 at diagnosis	2.560 (1.689–3.879)	**<0.001**	1.834 (1.074–3.133)	**0.026**	2.158 (1.464–3.180)	**<0.001**	0.392 (0.106–1.453)	0.161
ECOG 0–1 versus 2–4	3.722 (2.323–5.964)	**<0.001**	5.850 (3.368–10.161)	**<0.001**	3.315 (2.100–5.235)	**<0.001**	3.681 (0.992–13.666)	0.051
Elevated LDH	2.139 (1.434–3.189)	**<0.001**	3.044 (1.803–5.141)	**<0.001**	2.235 (1.520–3.285)	**<0.001**	8.562 (2.311–31.721)	**0.001**
PT-DLBCL versus advanced T-DLBCL	2.323 (1.558–3.464)	**<0.001**	3.340 (1.961–5.688)	**<0.001**	2.104 (1.439–3.077)	**<0.001**	4.891 (1.471–16.263)	**0.010**
No. of EN sites (continuous)	1.411 (1.249–1.594)	**<0.001**	1.329 (1.233–1.570)	**<0.001**	1.390 (1.216–1.589)	**<0.001**	1.629 (1.333–1.991)	**<0.001**
More than one EN site	2.742 (1.741–4.317)	**<0.001**	2.484 (1.209–5.106)	**0.013**	3.233 (1.851–5.647)	**<0.001**	8.928 (2.808–380)	**<0.001**
IPI 0–2 versus 3–5	3.152 (2.094–4.743)	**<0.001**	5.080 (2.992–8.625)	**<0.001**	2.777 (1.880–4.103)	**<0.001**	11.888 (3.208–44.057)	**<0.001**
CNS-targeted therapy								
i.v.	0.405 (0.256–0.640)	**<0.001**	0.511 (0.287–0.912)	**0.023**	0.473 (0.312–0.716)	**<0.001**	2.535 (0.763–8.424)	0.129
i.t. only	1.129 (0.728–1.749)	0.588	0.940 (0.514–1.720)	0.841	1.066 (0.700–1.624)	0.764	0.031 (0.000–7.292)	0.212
Rituximab	0.889 (0.587–1.347)	0.579	1.198 (0.704–2.039)	0.504	0.962 (0.648–1.429)	0.851	0.881 (0.265–2.929)	0.836
Treatment of contralateral testis	0.543 (0.359–0.822)	**0.004**	0.410 (0.230–0.731)	**0.003**	0.530 (0.359–0.783)	**0.001**	0.325 (0.088–1.201)	0.092
Finnish versus Danish	1.244 (0.819–1.887)	0.306	1.079 (0.630–1.848)	0.782	1.090 (0.736–1.614)	0.669	0.590 (0.190–1.830)	0.363

Statistically significant differences are in bold.
CI, confidence interval; OS, overall survival; DSS, disease-specific survival; PFS, progression-free survival; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; LDH, lactate dehydrogenase; PT-DLBCL, primary testicular diffuse large B-cell lymphoma; T-DLBCL, diffuse large B-cell lymphoma with testicular involvement; EN, extranodal; CNS, central nervous system; i.v., intravenous; i.t, intrathecal.

Importantly, the benefit of CNS-directed chemotherapy was the most significant in the elderly patients (Figure S2D–F). When the analyses of the impact of CNS-directed therapy on outcome were restricted to patients who completed their CNS-directed therapy and responded favourably, association of i.v. CNS-directed chemotherapy with OS and PFS was sustained (Table S3).

When the patients were divided into four groups according to the use of rituximab and/or i.v. CNS-directed chemotherapy, we observed a trend towards a benefit of i.v. CNS-directed chemotherapy independent of the use of rituximab (Figure S2J-L).

Forty-seven patients (24%) received i.t. CNS-directed therapy only, more commonly in the rituximab era (42% versus 19%, p = 0.014) and in Denmark than in Finland (37% versus 7%, p < 0.001). In addition, 16 patients had received i.t. chemotherapy along with intensive i.v. CNS-directed chemotherapy, 13 with rituximab and three before rituximab era. In 113 patients treated with CHOP(-like) chemotherapy without i.v. CNS-targeted chemotherapy, the use of i.t. chemotherapy had no impact on survival (Figure 1G–I). However, there was a trend towards benefit of i.t. chemotherapy over no CNS-directed chemotherapy in the prerituximab era (Figure S2G–I).

3.4. Impact of therapy on CNS recurrence rate

Twelve patients (6%) experienced CNS progression as their first relapse (Table 1 and Figure S3). Of them, eight patients had received prophylactic i.v. therapy. Only one

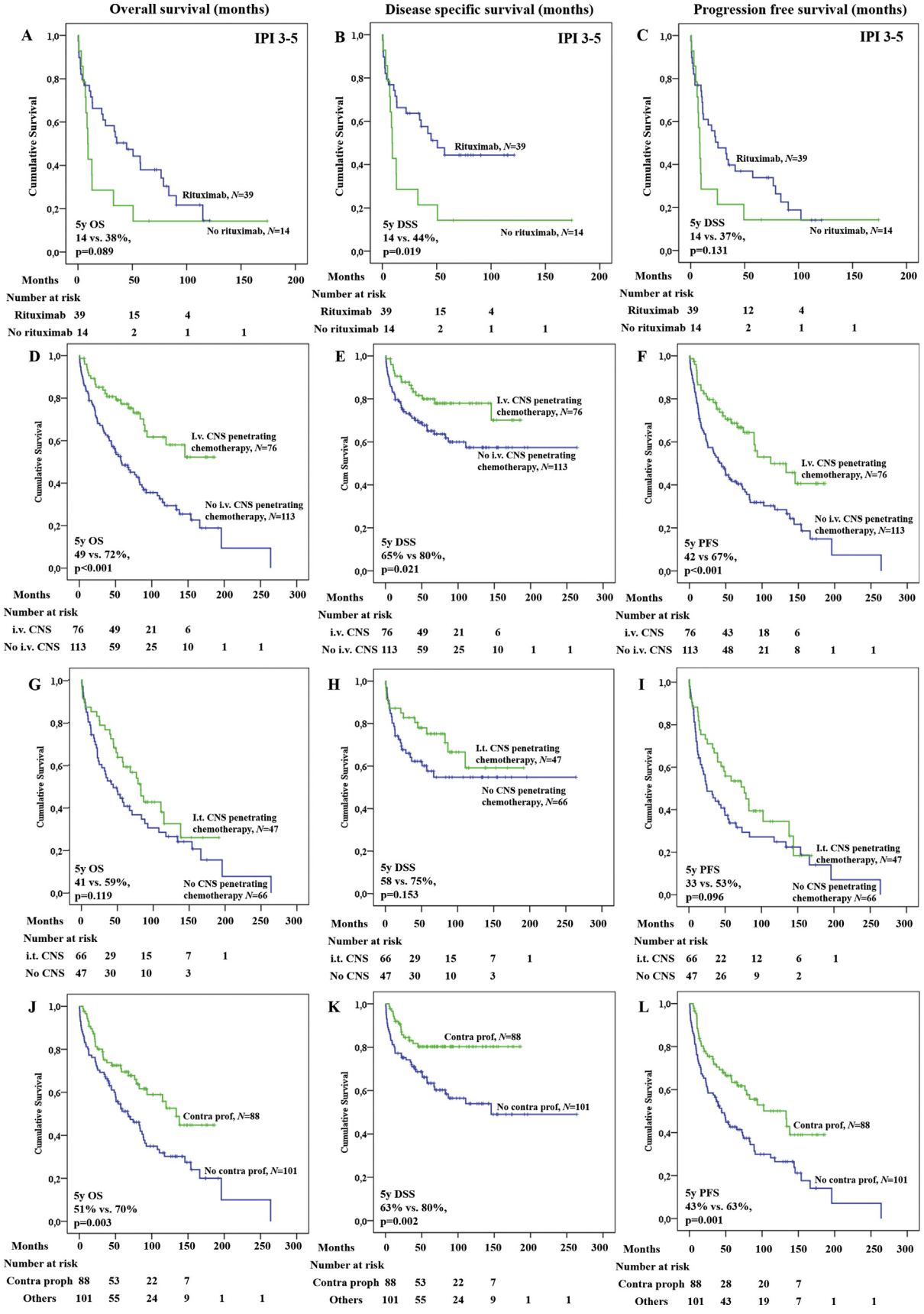

Fig. 1. The impact of treatment on survival among the patients with high risk (IPI, 3–5) disease. Rituximab was associated with improvement in overall survival (OS), disease-specific survival (DSS) and progression-free survival (PFS) (A–C). Intravenous central nervous system (CNS)–penetrating chemotherapy was associated with improved survival (D–F), whereas i.t. CNS-penetrating chemotherapy has no survival effect (G–I). Prophylactic treatment of the contralateral testis by either orchiectomy or radiotherapy is associated with improved survival on all end-points studied (J–L). IPI, International Prognostic Index; i.t., intrathecal; i.v., intravenous.

of the patients experiencing CNS progression had received i.t. chemotherapy together with i.v. CNS-directed chemotherapy. None of the 47 patients treated with i.t. chemotherapy was diagnosed with CNS progression. However, in the univariate analysis, the impact of i.t. therapy on the risk of CNS recurrence was not significant (Table 2).

3.5. Impact of contralateral orchiectomy or testis irradiation on survival

The contralateral testis was prophylactically treated in 88 (47%) patients, by orchiectomy in 13 and radiation in 75 patients. As the baseline characteristics were balanced between the subgroups, advanced age or poor PS did not explain the low frequency of this treatment in our material. Treatment of the contralateral testis was more common in the rituximab era (53% versus 36%, $p = 0.035$) and in Denmark than in Finland (53% versus 33%, $p = 0.002$), and there was also a strong association with i.t. chemotherapy (65% versus 37%, $p < 0.001$). Contralateral testis relapses were reported in four of 60 patients (7%), who did not receive prophylactic therapy. In addition, prophylactic therapy of the contralateral testis translated into favourable survival (Figure 1J-L).

3.6. Cox regression analyses

In univariate analyses (Table 2), significant prognostic factors for OS, PFS and DSS were all IPI factors, IPI score, i.v. CNS-directed chemotherapy and prophylactic treatment of contralateral testis. Rituximab, i.t. CNS-directed chemotherapy or country (Finland *versus* Denmark) had no significant influence on survival. The prognostic impact of i.v. CNS-directed chemotherapy and prophylactic treatment of contralateral testis on OS, DSS and PFS was sustained when adjusted by IPI factors (Table S2). All of the variables that had a significant impact on outcome were included in the multivariate analyses. Apart from stage, IPI factors, i.v. CNS-directed chemotherapy and prophylactic treatment of contralateral testis remained significant for OS and PFS, whereas age >70 years had no significant influence on DSS (Table 3 and Table S4).

In the univariate analyses for CNS progression, significant prognostic factors were stage, elevated lactate dehydrogenase (LDH), number of extranodal sites and IPI score (Table 2), and significant prognostic factors were Eastern Cooperative Oncology Group PS and elevated LDH in a subgroup of patients with limited stage (Table S5), In the multivariate analyses, significant prognostic factors were number of extranodal sites and elevated LDH (Table 3 and Table S3).

When survival analyses were restricted to the patients with PT-DLBCL, i.v. CNS-directed chemotherapy remained a significant prognostic factor for OS, and i.v. CNS-directed chemotherapy and prophylactic treatment of contralateral testis remained a significant prognostic factor for PFS. Rituximab had no significant influence on survival (Table S6).

3.7. Biological risk factors

Archive tumour material was available for 74 Finnish patients. All cases were confirmed to be PT-DLBCL according to the updated WHO lymphoma classification [19]. According to the Hans algorithm, 56 tumours (76%) were non-GCB phenotype. In addition, 13 of the 18 GCB-DLBCLs were MUM-1 positive, indicating ABC phenotype [20]. Sixty patients were treated with a curative intent, 46 (77%) of them non−GCB-DLBCLs with inferior survival (5-y PFS, 53% versus 87%; $p = 0.05$).

Of the patients treated with a curative intent, the staining for BCL-2 and BCL-6 was available for 61 patients. BCL-6 positivity was observed in 23 (38%) cases with no correlation with survival. BCL-2 was positive in 47 (77%) of these 61 PT-DLBCLs. Although there was no statistically significant difference between the BCL-2-positive and BCL-2-negative cases, a trend towards worse prognosis was observed among BCL-2-positive cases (5-y OS 60% versus 83% $p = 0.118$, DSS 73% versus 90%, $p = 0.105$, PFS 57% versus 76%, $p = 0.112$). The immunohistochemistry for MYC was available for only 38 cases, one of which was positive.

4. Discussion

This is to our knowledge the largest cohort of patients with testicular DLBCL involvement reported so far covering both prerituximab and rituximab eras and characterised by a long-term follow-up and comprehensive clinical data. In our cohort, i.v. CNS-directed chemotherapy and prophylactic treatment of the contralateral testis were associated with favourable survival independently of classical risk factors. In the lack of prospective clinical trials, this type of real-life data may support clinical management decisions and guide the design of future clinical trials.

Rituximab has resulted in a marked improvement of the outcome in patients with DLBCL [21,22]. However, the benefit of immunochemotherapy in specific lymphoma subsets, for example, PT-DLBCL, has not been prospectively evaluated. In a phase II trial reported by Vitolo *et al.* [12], survival for patients with PT-DLBCL treated with rituximab was superior to that of the historical controls, whereas in

Table 3
Cox regression analyses at the multivariate level showing independent association of baseline characteristics and treatment parameters with outcome.

Characteristic	Hazard ratio (95% CI)							
	OS	p	DSS	p	PFS	*P*	CNS at 1st relapse	*P*
Age >70 at diagnosis	2.120 (1.363–3.297)	**0.001**	1.596 (0.892–2.885)	0.115	2.215 (1.439–3.409)	**<0.001**	0.684 (0.155–3.008)	0.615
ECOG 0–1 versus 2–4	1.896 (1.103–3.261)	**0.021**	2.847 (1.497–5.415)	**0.001**	1.726 (1.015–2.934)	**0.044**	1.438 (0.341–6.073)	0.621
Elevated LDH	1.854 (1.151–2.987)	**0.011**	2.212 (1.175–4.166)	**0.014**	2.331 (1.439–3.774)	**0.001**	5.488 (1.240–24.297)	**0.025**
PT-DLBCL versus advanced T-DLBCL	1.158 (0.679–1.976)	0.591	1.359 (0.671–2.753)	0.394	0.927 (0.543–1.581)	0.780	0.761 (0.131–4.419)	0.761
More than one EN site	2.138 (1.185–3.857)	**0.012**	1.691 (0.833–3.436)	0.146	2.036 (1.149–3.606)	**0.015**	5.665 (1.089–29.486)	**0.039**
CNS-targeted therapy i.v.	0.419 (0.256–0.686)	**0.001**	0.489 (0.258–0.925)	**0.028**	0.508 (0.324–0.797)	**0.003**	1.283 (0.325–5.074)	0.722
Treatment of the contralateral testis	0.514 (0.338–0.782)	**0.002**	0.391 (0.217–0.703)	**0.002**	0.513 (0.345–0.763)	**<0.001**	0.270 (0.071–1.025)	0.054

Statistically significant differences are in bold. Multivariate analysis showing the independent association of IPI with i.v. CNS-targeted therapy and contralateral testis treatment is shown in Table S2.
CI, confidence interval; OS, overall survival; DSS, disease-specific survival; PFS, progression-free survival; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; PT-DLBCL, primary testicular diffuse large B-cell lymphoma; T-DLBCL, diffuse large B-cell lymphoma with testicular involvement; EN, extranodal (as continuous variable; higher is worse); CNS, central nervous system; i.v., intravenous.

retrospective series, the impact of immunochemotherapy on the survival has been somewhat controversial [1,23,24]. We did not observe survival difference between the patients treated in the prerituximab and rituximab eras (Figure S2A–C). However, immunochemotherapy was associated with better outcome in the patients with intermediate high/high-risk IPI (Figure 1A–C). The results are comparable with the data reported from British Columbia ($N = 134$), where rituximab improved survival of the patients with advanced stage [24]. In our study, immunochemotherapy did not reduce the risk of CNS recurrence, which is also in line with the results from others [6,25], although contradicting the results from the RICOVER-60 trial [26].

More aggressive treatment including CNS-directed i.v. chemotherapy was associated with improved survival in the whole cohort and the subgroup of patients with PT-DLBCL. However, it is important to note that we did not observe reduction in the CNS recurrence rate, but rather a better control of the systemic disease, suggesting that R–CHOP alone may not be a sufficient therapy for the patients with testicular DLBCL involvement. As a limitation inherent to observational analyses, a potential patient selection bias must be considered when interpreting the present findings. We cannot exclude that the most fragile patients may not have undergone full diagnostic workflow at relapse, and thus, the number of CNS recurrences may be underestimated. In our cohort, the administration of CNS-directed i.v. chemotherapy was more common in younger patients, causing additional potential bias (Table 1). However, in the group treated with CNS-directed chemotherapy, survival of elderly patients was equal to that of younger patients. Furthermore, prognostic impact of i.v. CNS-directed chemotherapy on OS and PFS was independent of age and PS (Table 3) and was sustained among the subgroup of patients who received full chemotherapy and responded favourably (Table S3). Consistent with other reports [3,27], the use of i.t. chemotherapy did not correlate with improved survival.

In previous retrospective analyses, prophylactic irradiation of the contralateral testis has been associated with improved survival and reduced risk of contralateral testis relapse [15,28]. In our cohort, the use of contralateral testis prophylaxis was more common in the rituximab era, indicating increasing implementation of modern guidelines. It was also associated with an improvement in all end-points studied. Specifically, the risk of lymphoma-related death was significantly reduced, indicating reduced risk for systemic relapse and death. This improvement was retained in the multivariate analysis also after adjustment for factors such as use of rituximab, more aggressive chemotherapy or age.

In the lack of prospective trials, our results recommend aggressive chemotherapy with i.v. HD-MTX and/or HD-AraC for better control of systemic disease for eligible patients. In addition, treatment of the contralateral testis with either radiotherapy or orchiectomy

should be included in the management strategy of patients with T-DLBCL. Future studies should focus on T-DLBCL biology to provide less toxic enhancements to the R–CHOP backbone for fragile patients [8,11].

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Conflict of interest statement

S.M. has received honoraria from Pfizer, Takeda, Roche and Sanofi (not related to this study). P.- L.K-.L. has received research funding from Roche, Sanofi and Pfizer (not related to this study). S.L. has received honoraria and research funding from Roche, Takeda, Bayer and Janssen-Cilag (not related to this study). Other authors declare no conflicts of interest pertinent to the topic of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.04.004.

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