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Acknowledgements—We would like to thank Phillip St Louis and Lawrence Harrison for their excellent technical support.
This study was supported by the National Cancer Institute of Canada.

Eur J Cancer, Vol. 27, No. 11, pp. 1416–1423, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

HIV-Associated Lymphoma: Histopathology and Association with Epstein–Barr Virus Genome Related to Clinical, Immunological and Prognostic Features

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All 51 cases of HIV-related malignant lymphoma in Denmark diagnosed from 1983 to 1989 were reviewed. There were 12 Burkitt-type lymphomas, 30 immunoblast-rich lymphomas and 9 other lymphomas. Patients with immunoblast-rich lymphomas had significantly lower CD4 cell counts (median 60 vs. 188 × 10^6/l, $P < 0.05$), and more often a history of previous AIDS-defining illnesses (50% vs. 0%, $P < 0.005$), compared with patients with Burkitt-type lymphomas. Epstein–Barr virus (EBV) DNA was demonstrated in 14 of 19 immunoblast-rich tumours, and in 2 of 7 Burkitt-type lymphomas ($P = 0.10$). Compared with EBV DNA-negative tumours EBV DNA-positive tumours were associated with lower CD4 cell counts (median 39 vs. 188 × 10^6/l, $P = 0.01$). It is concluded that two main types of HIV-related malignant lymphoma exist. One is associated with severe immunosuppression, is often of immunoblast-rich morphology, and may be linked to EBV, whereas the other may occur in the absence of immunosuppression, is often of Burkitt-type morphology, and is probably not linked to EBV. In addition to these two main types, other non-Hodgkin lymphomas and Hodgkin's disease do occur.
Eur J Cancer, Vol. 27, No. 11, pp. 1416–1423, 1991.

INTRODUCTION

LIKE PATIENTS with genetically determined immune deficiency and patients with iatrogenic immunosuppression after organ transplantation [1–2], patients infected with human immunodeficiency virus (HIV) are at greatly increased risk for developing non-Hodgkin B-cell lymphomas [3–5], and in 1985 the Centers for Disease Control included non-Hodgkin lymphoma as an AIDS-defining illness [6]. In the USA non-Hodgkin lymphoma has been reported to be present in 2.9% of notified AIDS cases [7]. Among Danish AIDS patients reported in or before 1987, malignant lymphoma comprised approximately 5% of primary AIDS diagnoses, and in this population the cumulative risk for developing lymphoma within 18 months of the AIDS diagnosis was estimated to be 10% [8]. In a small group of patients on long-term antiretroviral therapy, Pluda *et al.* [9] estimated the probability of developing malignant lymphoma to be approximately 30% by 30 months of therapy and 45% by 36 months of therapy. As the AIDS epidemic progresses and as the

survival of AIDS patients is currently improving [10–11] an increase in the incidence of non-Hodgkin lymphoma must be expected.

Although it is well known that the vast majority of HIV-related lymphomas are of high grade B-cell type, consisting either of immunoblastic lymphoma or small non-cleaved lymphoma [4–6, 7, 12, 13], it has been difficult to determine clinical, immunological, aetiological or prognostic correlates between one or other pathological type of HIV-related lymphoma, and it is not known why the Burkitt type of malignant lymphoma is endemic among patients with HIV infection but not among patients with other immune deficiencies [7].

In a previous retrospective study of 27 cases of HIV-related lymphomas, we found that Epstein–Barr virus (EBV) DNA was detectable in a high proportion of tumours with a predominant population of immunoblasts whereas this virus was less frequently associated with Burkitt-type lymphomas, suggesting the existence of two main groups of HIV-related lymphoma with different pathogeneses [14]. To broaden our knowledge of the various pathological types of lymphoma we reviewed all cases of HIV-related lymphoma recognised in Denmark up to the end of 1989, and related the pathological type to clinical, immunological, and prognostic features.

PATIENTS AND METHODS

The study included all 51 patients diagnosed with HIV-associated lymphomas in Denmark from 1981 to December 1989. The patients' medical records were reviewed according to a standardised form. Clinical information obtained included age, sex, previous risk behaviour, date of occurrence of AIDS-defining illnesses or other serious HIV-related conditions, clinical signs at the time of lymphoma diagnosis, site and stage at presentation, type of therapy, response to therapy and date and cause of death. From late 1984 patients were prospectively tested for the presence of HIV antibodies. For patients who were diagnosed before antibody tests were available, frozen serum samples were analysed for HIV antibody retrospectively. When available, T-cell subsets, lymphocyte count, serum immunoglobulin levels, haemoglobin, platelet count, serum lactate dehydrogenase, serum HIV antigen, EBV antibody, and cytomegalovirus (CMV) antibody were noted.

The morphology of the lymphomas was assessed using standard histopathological methods as described previously [14]. All cases were reviewed by two of the authors (G.P., S.J.H.-D.) to confirm the diagnosis and to classify the lymphomas. Classification was based upon the updated Kiel classification system [15] with modifications as reported previously [14]. When possible, cases were evaluated for the presence of EBV DNA by *in situ* nucleic acid hybridisation in routinely processed paraffin-embedded tissue material as described previously [14].

The censoring date for follow-up was 30 October 1990. All survival data refer to survival from time of diagnosis.

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Revised 12 July 1991; accepted 18 July 1991.

Statistical methods

Frequencies were compared using Fisher's exact test. Numeric variables were compared by the Mann–Whitney rank test. Life tables (Kaplan–Meier) were used to analyse survival, and were compared using the logrank test [16]. Cox proportional hazards regression analysis (BMDP) was used to identify independent predictors of survival among the variables studied. All significance levels correspond to those for two-tailed tests.

RESULTS

Incidence of malignant lymphoma

A total of 51 cases of HIV-associated malignant lymphoma were diagnosed between 1981 and the end of 1989. The yearly distribution of lymphomas with the corresponding total number of AIDS cases reported that year in parentheses was: 1983, 1(12); 1984 1(17); 1985 3(38); 1986 4(89); 1987 10(100); 1988 10(125); and 1989 22(171).

Patients' characteristics

38 (74%) patients were homosexual or bisexual men, 8 (16%) were heterosexuals, 2 (4%) were haemophiliacs, 1 (2%) was a recipient of contaminated blood transfusions and 2 (4%) had no identifiable risk behaviour. The 49 men and 2 women ranged in age from 19 to 78 (median 42) years.

The diagnosis of AIDS preceded the onset of lymphoma in 19 (37%) of the patients with a range from 5 to 828 (median 274) days. AIDS-defining illnesses for the 19 patients were: *Pneumocystis carinii* pneumonia, 11; oesophageal candidiasis, 3; cytomegalovirus pneumonia, 1; Kaposi's sarcoma, 4. 8 patients (16%) had a history of other HIV-related symptoms (CDC group IVA or CDC group IV C-2), 11 (22%) had persistent generalised lymphadenopathy as the only clinical sign of HIV infection (CDC group III). 13 (25%) had no previous history of HIV-related symptoms (CDC group II).

All patients were HIV seropositive. Among those patients who were examined for this, 19 (95%) of 20 were EBV seropositive, and 40 (93%) of 43 were CMV seropositive.

Clinical features

Although most patients presented with extensive disease involving multiple sites, the clinical picture was often dominated by symptoms and signs from single organ systems. 15 patients presented with abdominal pain as the main symptom. 6 of these patients developed acute obstruction or perforation of the small bowel, and 3 had large palpable abdominal tumours. 9 patients presented with symptoms from the central nervous system (CNS), 14 with lymph-node enlargement as the dominating symptoms and 4 presented with tumours located in the oral cavity. The number of patients in each stage at the time of diagnosis were: stage I, 2 (4%); stage IE, 8 (16%); stage II, 1 (2%); stage III, 7 (14%); and stage IV, 33 (65%) patients. CNS involvement was demonstrated in 9 (18%) of the patients, and bone marrow involvement was found in 7 (21%) of those patients evaluated. In total, extranodal involvement by lymphoma was found in 41 (80%) patients. The sites of involvement in the 8 patients who presented with primary localised extranodal sites (stage IE) were CNS 3, gingiva 2, testis 1, large bowel 1 and rectum 1. 39 (76%) patients had constitutional symptoms (B-symptoms) at presentation.

Therapy

In 9 cases malignant lymphoma was first diagnosed at autopsy. Among the remaining 42 patients, 9 were in such a bad condition

that they were not considered eligible for therapy. 31 of the 33 patients who received therapy were treated with standard chemotherapeutic regimens. Most regimens included cyclophosphamide, doxorubicin hydrochloride, oncovine and prednisolone (CHOP). In addition, 6 of these patients were treated with high-dose methotrexate, and 3 received methotrexate and cytosar intrathecally. The median number of treatment cycles was 6 (range 1–12). 2 patients with localised disease were treated with radiation therapy alone.

15 (45%) of the 33 patients who received therapy had progressive disease in spite of treatment, and 5 patients (15%) had a partial response. 12 (36%) patients showed a complete response, but 4 had relapses later on. In 1 case information concerning response to therapy was not available. 8 of the patients who had favourable responses to therapy developed progressive disease later on.

22 patients were treated with zidovudine at some time during the course of their HIV infection. In 15 cases therapy was initiated before the diagnosis of malignant lymphoma.

Histopathology

Tissue from 52 patients with a previous diagnosis of malignant lymphoma were examined for classification. In 1 case the diagnosis of malignant lymphoma could not be confirmed using morphological, phenotypic or genotypic criteria.

The 51 tumours fell into three groups based on histopathological criteria: (1) 30 tumours with a predominant population of immunoblasts, monomorphic or more often polymorphic with plasmacytic or plasmablastic differentiation. 8 tumours showed more marked lymphocytic polymorphism and larger sized immunoblasts. All these tumours have been designated immunoblast-rich lymphoma. (2) There were 12 Burkitt-type lymphomas, some of which had the typical appearance of endemic Burkitt's lymphoma whereas others showed atypical features. (3) Of the remaining 9 cases, 2 were classified as anaplastic large cell lymphoma, 5 as centroblastic lymphoma and 2 as Hodgkin's disease.

Table 1 summarises the distribution and the staging within each histological group. Most primary localised extranodal lymphomas were immunoblast-rich lymphomas. The relationship between the morphological type and a number of clinical and immunlogical variables is shown in Table 2. There were several differences between the groups. Patients with immunoblast-rich lymphomas had significantly lower CD4 cell counts, lower lymphocyte counts and higher serum level of immunoglobulin A compared to patients with Burkitt-type lymphomas. 15 (50%)

Table 1. Histopathological categories of 51 cases of HIV-associated malignant lymphoma, and relation to clinical staging and constitutional symptoms

Lymphoma type	No. of cases	Staging					Constitutional symptoms present
		I	IE	II	III	IV	
Immunoblast-rich	30	1	7	1	1	20	23
Burkitt-type	12	0	1	0	2	9	8
Centroblastic	5	1	0	0	1	3	4
Anaplastic large cell	2	0	0	0	2	0	2
Hodgkin's disease	2	0	0	0	1	1	2
Total	51	2	8	1	7	33	39

Table 2. Immunological and clinical characteristics by histolopathological group

Variable	Group		
	Burkitt-type (n = 12)	Immunoblast-rich (n = 30)	Other (n = 9)
Lymphocyte count (× 10^6/l) median (range)	2800 (700–5400)	800 (100–16400)†	700 (500–1900)*
CD4 (× 10^6/l), median (range)	188 (40–1100)	60 (0–1800)*	53 (22–463)*
IgA (g/l), median (range)	2.9 (0.2–10.3)	4.0 (1.3–16.2)*	4.5 (2.7–11.9)‡
IgG (g/l), median (range)	19.3 (6.3–38.1)	18.3 (13.0–88.0)	19.2 (13.1–42.3)
Platelets (× 10^6/l) median (range)	255 (100–370)	161 (36–423)	181 (115–293)
HIV Ag (no. positive/ no. examined)	3/9 (33%)	13/18 (72%)	5/7 (71%)
Bone marrow involvement (no. positive/ no. examined)	4/9 (44%)	3/19 (16%)	0/6 (0%)
CNS involvement (n)			
Parenchymal	0 (0%)	4 (13%)	0 (0%)
Meningeal	3 (25%)	2 (7%)	0 (0%)
Prior AIDS diagnosis (n)	0 (0%)	15 (50%)†	4 (44%)
Autopsy diagnosis (n)	0 (0%)	9 (30%)‡	0 (0%)
CR without relapse (no. of patients/ no. evaluable)	0/8 (0%)	5/16 (21%)	3/8 (38%)

*$P < 0.05$, † $P < 0.005$, ‡ $0.05 < P < 0.10$ (compared to Burkitt-type lymphoma).

of the patients with immunoblast-rich lymphomas had a prior diagnosis of AIDS compared to none of those with Burkitt type lymphoma ($P < 0.005$). All lymphomas diagnosed first at autopsy were of immunoblast-rich type. All cases with parenchymal CNS involvement were immunoblast-rich lymphomas, whereas meningeal involvement was found in both immunoblast-rich and Burkitt-type lymphomas. The lymphomas which were not classified as either immunoblast-rich or Burkitt-type were similar to the immunoblast-rich lymphomas with respect to CD4 cell count, the serum level or immunoglobulin A, and with respect to the proportion of patients who had a prior diagnosis of AIDS. However, in contrast to the two other pathological groups bone marrow involvement or CNS involvement was not demonstrated. The probability of obtaining complete response without relapse tended to be lower for patients with Burkitt-type lymphomas.

EBV DNA was demonstrated in 18 (58%) of the 31 tumours in which *in situ* hybridisation was performed successfully (Table 3). More immunoblast-rich tumours were EBV DNA-positive compared with Burkitt-type tumours (74% vs. 29%), but the difference was not statistically significant ($P = 0.10$). Patients with EBV DNA-positive lymphomas had significantly lower CD4 cell counts and lower CD8 cell counts than patients with EBV DNA negative lymphomas. Localised extranodal disease and CNS involvement were significantly more common for EBV DNA-positive lymphomas. The probability of obtaining complete response without relapse during therapy tended to be

Table 3. Differences between EBV DNA-positive and EBV DNA-negative lymphomas

Variable	All lymphomas		Immunoblast-rich lymphomas	
	EBV+ (n = 18)	EBV− (n = 13)	EBV+ (n = 14)	EBV− (n = 5)
Histopathology no. of patients/no. examined				
Immunoblast-rich	14/19 (74%)	5/19 (26%)		
Burkitt-type	2/7 (29%)	5/7 (71%)		
Other	2/5 (40%)	3/5 (60%)		
CD4 (× 10^6/l), median, range	39 (0–540)	188* (25–1800)*	35 (0–208)	270* (120–1800)
CD8 (× 10^6/l) median, range	277 (0–3600)	700* (225–3600)	198 (0–500)	514* (270–3600)
Bone marrow involvement (no. positive/no. examined)	1/11 (9%)	4/10 (40%)	0/8 (0%)	2/4 (50%)
CNS involvement (no. of patients)	6 (33%)	0 (0%)**	4 (29%)	0 (0%)
Localised extranodal disease (no. of patients)	6 (33%)	0 (0%)**	5 (36%)	0 (0%)
Complete response without relapse (no.of patients/ no.evaluable)	4/10 (40%)	0/9 (0%)	2/7 (29%)	0/3 (0%)

* $P < 0.01$, † $P = 0.05$.

more common for EBV DNA-positive lymphomas, whereas bone marrow involvement tended to be more common for EBV-negative lymphomas. These differences were not statistically significant. There were no differences between EBV DNA-positive and EBV DNA-negative lymphomas with respect to patient's age, the presence of constitutional symptoms at diagnosis, platelet count or serum levels of immunoglobulins. The observed differences between EBV DNA-positive and EBV DNA-negative lymphomas could not be explained by differences in morphology.

Survival

None of the patients were lost to follow-up. By October 1990 44 (86%) patients had died. The cause of death in 22 cases was progressive malignant lymphoma alone, in 10 cases progressive malignant lymphoma and another disease (opportunistic infections 4, bacterial infections 4, disseminated Kaposi's sarcoma 2), and in 10 cases other diseases (opportunistic infections 6, bacterial infection 1, HIV encephalopathy 1, cerebral infarction 1, cerebral haemorrhage 1). In 2 cases the cause of death was not known. For the 7 patients who were still alive survival ranged from 217 to 1241 days (median 511 days).

The prognosis was generally poor with a median survival of less than 100 days (Fig. 1). Approximately 20% of the patients survived for at least 1 year. The influence of various factors on survival is summarised in Table 4. The 1 year probability of survival was 11% (95% confidence limits 0–24%) for patients with a prior diagnosis of AIDS compared with 32% (8–38%) for patients without a prior diagnosis of AIDS ($P = 0.02$). A significantly shortened survival was also found for patients who presented with extranodal disease compared with those who had nodal disease only, probability of survival at 1 year 16% (3–30) and 30% (5–55%), respectively ($P = 0.04$). Patients who had a CD4 cell count ≥ 200 × 10^6/l at the time of diagnosis had a 1 year probability of survival of 54% (25–83%) compared to 7% (0–15) ($P = 0.01$) for those who had a CD4 cell count less than 200 × 10^6/l. A platelet count below 130 × 10^6/l also predicted a poor survival ($P = 0.01$). Patients who had constitutional symptoms and patients who were more than 40 years of age tended to have a shortened survival, but the difference was not statistically significant.

The survival of patients with proven CNS involvement at the time of diagnosis was extremely poor. In 6 out of 9 patients the diagnosis was obtained at autopsy. Of the remaining 3 patients only 1 survived more than 3 months.

Survival in relation to pathologic type is shown in Fig. 2. Survival was similar among cases of immunoblast-rich lymphoma and among cases of Burkitt-type lymphoma ($P = 0.58$). Patients with other types of lymphoma had a better survival compared with the patients who had either Burkitt-type or immunoblast-rich lymphomas ($P = 0.04$). The 2 patients with Hodgkin's disease survived 18 and 567 days, respectively. Survival was not significantly influenced by the presence of EBV DNA detected by *in situ* hybridisation, the presence of HIV antigenaemia, serum levels of immunoglobulin A, immunoglobulin G, or lactate dehydrogenase, haemoglobin levels, or by whether the patients had received zidovudine therapy or not.

In the Cox proportional regression analysis a CD4 cell count less than 200 × 10^6/l ($P = 0.001$), the presence of constitutional symptoms ($P = 0.001$), thrombocytopenia ($P = 0.001$), and a histopathological diagnosis of either Burkitt-type ($P = 0.002$) or immunoblast-rich lymphoma ($P < 0.001$) were independent predictors of a shortened survival. The presence of extranodal disease ($P = 0.09$), age ($P = 0.89$) or a prior diagnosis of AIDS ($P = 0.13$) were not independent predictors of a shortened survival.

Survival after therapy

The median survival of patients who received therapy was 138 days. Survival was significantly better for patients who showed remission following therapy. The 1 year probability of survival was 7% (0–16%) for patients who had progressive

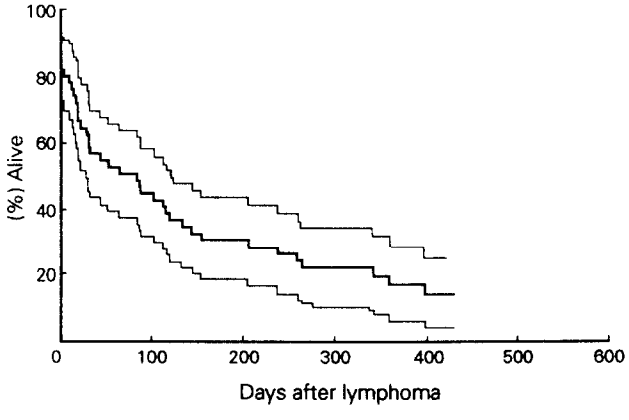

Fig. 1. Overall survival of 51 patients with HIV-related malignant lymphoma. Dotted lines show 95% confidence intervals.

Table 4. Clinical, immunological and histological variables and their influence on survival in 51 patients with HIV-associated lymphoma

Variable	Number of patients	1-year survival (95% CI)	P
Prior AIDS diagnosis			
Yes	19	11 (0–24)	
No	32	23 (8–38)	0.02
Constitutional symptoms			
Yes	39	14 (3–25)	
No	12	31 (6–57)	0.10
Extranodal sites			
Yes	41	16 (3–30)	
No	10	30 (5–55)	0.04
Histopathology			
Burkitt type	12	17 (0–38)	
Immunoblast-rich	30	12 (1–22)	
Other	9	42 (11–73)	0.04
Age			
> = 40 years	32	13 (2–23)	
< 40 years	19	25 (4–47)	0.08
CD4 cell count			
> = 200 × 10^6/l	13	54 (25–83)	
< 200 × 10^6/l	34	7 (0–15)	0.01
Platelets			
> = 130 × 10^6/l	28	27 (10–44)	
< 130 × 10^6/l	19	0 (0–24)	0.01
Haemoglobin			
> = 7 mmol/l	24	21 (9–48)	
< 7 mmol/l	25	10 (0–21)	0.23
Immunoglobulin A			
> = 4 g/l	22	16 (2–31)	
< 4 g/l	23	26 (6–46)	0.30
Immunoglobulin G			
> 19 g/l	23	22 (6–37)	
< = 19 g/l	23	17 (0–34)	0.80
Lactate dehydrogenase			
> 400 U/l	28	20 (4–35)	
< = 400 U/l	9	33 (7–60)	0.12
HIV Ag positive			
Yes	19	19 (0–38)	
No	15	17 (0–34)	0.68
EBV DNA positive			
Yes	18	22 (5–40)	
No	13	15 (0–35)	0.74
Zidovudine therapy			
Yes	22	14 (0–28)	
No	29	21 (3–39)	0.93

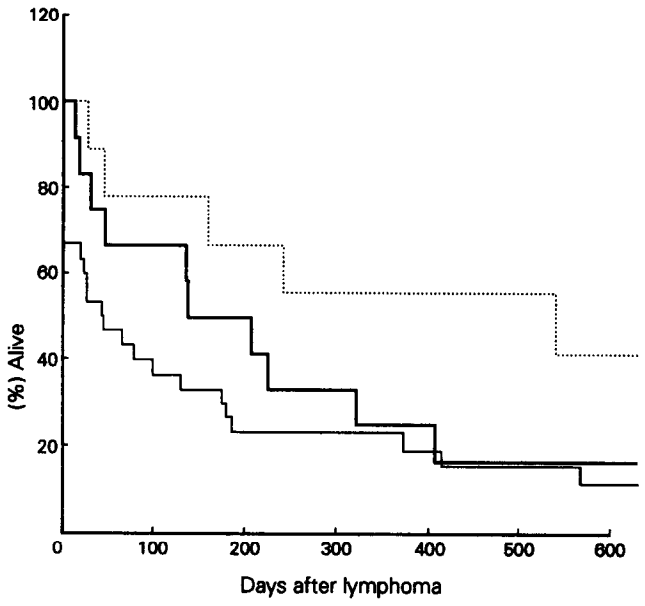

Fig. 2. Survival of patients with HIV-related malignant lymphomas in relation to histopathological type of the lymphomas. — = Burkitt's (n = 12), ... = immunoblastic (n = 30) and ---- = other lymphoma (n = 9)

Table 5. Predictors of survival in patients with malignant lymphoma eligible for therapy

Variable	No. of patients	1-year probability of survival (95%CI)	P
Prior AIDS diagnosis			
Yes	10	20 (0–45)	
No	23	32 (13–52)	0.04
Extranodal sites			
Yes	24	29 (7–50)	
No	9	33 (7–60)	0.24
Constitutional symptoms			
Yes	24	22 (5–39)	
No	9	44 (7–82)	0.28
Histology			
Burkitt-type	8	25 (0–55)	
Immunoblast-rich	17	21 (3–40)	0.15
Other	8	47 (13–80)	
CD4 cell count			
> = 200 × 10^6/l	11	51 (20–82)	
< 200 × 10^6/l	22	27 (7–46)	0.04
Platelets			
> = 130 × 10^6/l	20	38 (16–60)	
< 130 × 10^6/l	10	0 (0–45)	0.05
Age			
> = 40 years	19	21 (5–37)	
< 40 years	14	34 (7–62)	0.07
EBV DNA-positive			
Yes	10	40 (13–67)	
No	9	22 (0–49)	0.34

disease despite treatment compared with 56% (29–84%) for patients who showed a complete response following therapy ($P < 0.001$). A prior diagnosis of AIDS, and a CD4 cell count < 200 × 10^6/l were significantly associated with a shortened survival (Table 5). A platelet count < 130 × 10^6/l and age more than 40 years also tended to be predictive of a shortened survival.

In the Cox regression analysis a CD4 cell count less than 200 × 10^6/l ($P = 0.03$), the presence of constitutional symptoms ($P = 0.05$), a prior diagnosis of AIDS ($P = 0.005$), and a histopathological diagnosis of either Burkitt-type ($P = 0.001$) or immunoblastic-rich lymphoma ($P < 0.001$) were independent predictors of a shortened survival.

Table 6 shows individual data for patients who survived

Table 6. Characteristics of 8 HIV-seropositive patients who survived for at least 360 days after diagnosis of malignant lymphoma

Patient no.	Survival (days)	Histology	Stage	CD4 cell count ($\times 10^6$/l)	EBV DNA-positive	Constitutional symptoms
1	745	IB	IV	200	Yes	No
2	360	IB	I	60	ND	Yes
3	>511	ALC	IV	470	ND	No
4	>567	HD	III	400	ND	Yes
5	>952	CB	I E	20	Yes	No
6	>1241	IB	III	210	Yes	No
7	674	ALC	III	40	Yes	No
8	398	BUR	IV	340	No	Yes

IB = immunoblast-rich, ALC = anaplastic large cell, HD = Hodgkin's disease, CB = centroblastic, BUR = Burkitt-type, ND = not done. Only patient 7 had a prior diagnosis of AIDS.

for at least 360 days emphasising that no single parameter, immunological or clinical, can predict the long-term outcome.

DISCUSSION

The first cases of malignant lymphoma in homosexual men at risk for AIDS were reported in 1982 [17, 18]. Since then HIV-related lymphoma has been the subject of several larger reports from the USA [7, 19–24], Europe [25] and Australia [26]. The present study differs from these previous reports by including all cases from a country, and by relating histopathological findings and clinical, immunological and prognostic features of HIV-related malignant lymphoma to the presence of EBV DNA genomes in tumour cells. The study population is likely to represent almost all cases of HIV-related lymphoma in Denmark in the period. The necropsy frequency among deceased AIDS patients is high (67%) [8], and a large proportion of patients with malignant lymphomas from the period 1984–1988 have been tested for the presence of HIV antibody retrospectively [27].

When all cases in our series were analysed together the clinical findings were similar to those previously reported [3–5, 19, 21–23]. Extranodal disease was present in 80% of patients at diagnosis, the principal extranodal sites being bone marrow, CNS and the gastrointestinal tract, including the oropharynx and rectum. The clinical findings emphasises that given the wide range of presentations of malignant lymphoma the diagnosis should be considered in any HIV-infected patient presenting with progressive lymphadenopathy, tumours at any site, unexplained wasting, chronic fever, abdominal pain or symptoms from the CNS. The distribution of patients according to previous risk behaviour was similar to that observed among Danish AIDS patients generally [8] confirming that the occurrence of malignant lymphoma is not associated with any particular group at risk for AIDS. Patients with malignant lymphoma were significantly older than other AIDS patients (median 42 years vs. 37 years [8]). A similar age difference has been observed in Australia [26].

Our series of 51 patients with HIV-related lymphoma could be classified into three major groups based on the morphological features of the lymphomas. The first group consisted of patients with Burkitt-type lymphoma. This group had relatively less impaired immune function compared to the other groups, had no history of previous HIV-related malignancy or opportunistic infections, and had localised extranodal disease only rarely. The second group included patients with immunoblast-rich lymphomas of monomorphic or polymorphic type. These patients had severe impairment of immune function, had a high incidence of opportunistic infections or Kaposi's sarcoma before the diagnosis of lymphoma, and commonly had localised extranodal disease. The third group consisted of patients with other histological types of lymphomas. This group shared some features with the immunoblast predominant lymphomas. They had severely depressed immune function and a high frequency of opportunistic infections and Kaposi's sarcoma before the diagnosis of lymphoma, but involvement of CNS or bone marrow was not demonstrated in any of the patients who were examined for this.

The observation that different histopathological categories of HIV related malignant lymphoma correlate with clinical and immunological features has been suggested by others. Kalter *et al.* [4] noted less severe immune suppression and higher performance status at diagnosis for patients with diffuse undifferentiated lymphoma (Burkitt-type lymphoma) compared with patients with diffuse large cell lymphoma (immunoblast type lymphoma). In a series of 41 patients with HIV related lymphoma Boyle *et al.* [26] found that patients with Burkitt-type lymphoma had better preserved immune function than patients with immunoblastic type lymphoma, and none of 11 patients with Burkitt-type lymphoma had a previous AIDS diagnosis. A tendency for patients with immunoblast-rich lymphoma to present with localised extranodal disease, including primary CNS lymphoma, has been observed in several series [21, 24, 26].

It appears that HIV-associated malignant lymphoma is a heterogeneous group with possible differences in the pathogenesis of the lymphomas. Of interest is our notion of a relationship between the presence of EBV DNA in tumour cells and immunological and histopathological features of the lymphomas. EBV DNA tended to be more commonly observed in the various immunoblast predominant lymphomas, and was confined to the most immunocompromised patients, suggesting a possible pathogenetic role for EBV in the development of at least some of the large cell lymphomas. This is consistent with the hypothesis implicating expression of the EBV-encoded latent gene products, latent membrane protein (LMP) and EBV nuclear antigen 2 (EBNA 2), as an important factor in EBV-associated lymphomagenesis in patients with immune defects [28–30]. LMP can serve as a target for cytotoxic T-cells, facilitating control of the disease process in patients with preserved immune function. However, during late phases of HIV infection EBV cytotoxic T-cell function is strongly impaired [31], which may then lead to uncontrolled proliferation of EBV infected cells, and subsequently malignant transformation.

In contrast, immune suppression does not seem to be an important factor for the development of HIV-related lymphoma of the Burkitt type, which may explain that this type of malignant is endemic among HIV-infected patients but not among patients with other immune deficiencies [7]. Many of the patients with Burkitt-type lymphomas had normal CD4 cell counts, and EBV DNA was infrequently demonstrated by *in situ* hybridisation. HIV-associated Burkitt-type lymphoma has been reported to have chromosome rearrangements [mainly t(8;14) translocation] similar to those of other Burkitt-type lymphomas [32, 33]. This may lead to increased or inappropriate production of the c-*myc* protein, which is known to have oncogenic potential. The

polyclonal B-cell activation seen in patients with HIV infection could increase the risk for gene rearrangements and thereby c-*myc* protein activation. Since B-cell activation is an early feature of HIV infection, translocation may occur by chance at any stage of HIV infection, and because the tumour cells do not express LMP, the disease process may progress even in patients with normal T-cell function.

Median survival for patients with HIV-related lymphoma has been short in all series reported. In the present study median survival was approximately 3 months, which is slightly shorter than the median survival of 4–6 months reported in other series [4, 13, 19, 21–23, 25, 26]. Our attempt to identify factors of prognostic importance showed that a CD4 cell count less than 200×10^6/l, the presence of constitutional symptoms, and a histopathological diagnosis of either Burkitt-type lymphoma or immunoblast-rich lymphoma were independent predictors of a shortened survival. A previous diagnosis of AIDS was an independent predictor of a shortened survival only in patients who received therapy. Age or the presence of extranodal disease were not independent predictors of outcome. The prognosis for patients with CNS involvement at the time of diagnosis was uniformly poor.

From our results a shorter survival for patients with EBV DNA positive lymphomas might have been anticipated since these patients had significantly lower CD4 cell counts than patients with EBV DNA-negative lymphomas. However, that was not the case, suggesting that EBV-positive lymphomas have factors counteracting the influence of severe immune suppression. We can not explain this with certainty from our data, but although the number of evaluable patients was small, it appears that complete response following therapy may be more common in patients with EBV DNA-positive lymphomas, indicating that these tumours may be more sensitive to chemotherapy.

It appears that both the degree of immunological defect, the characteristics of the lymphomas themselves, and, possibly, the tumour burden, are of importance for the outcome of malignant lymphoma. In accordance with our observation, Knowles *et al.* [21] noted a significantly longer survival of patients with large cell non-cleaved lymphomas (centroblastic lymphoma) than for patients with either immunoblastic or Burkitt-type lymphoma. In contrast, Kalter *et al.* [4] and Boyle *et al.* [26] reported a longer survival for patients with Burkitt-type lymphoma. In other series, no significant differences in survival were observed between the various pathological types [13, 19, 23]. The presence of AIDS-defining opportunistic infections or Kaposi's sarcoma before the diagnosis of lymphoma has relatively uniformly been reported as associated with shorter survival [3, 13, 22, 23, 26]. In some studies the importance of a prior AIDS diagosis has been reported to be independent of the CD4 cell count [13, 26]. In contrast, Kaplan *et al.* [23] found that a history of AIDS provided no additional information, when CD4 cell counts were available. Appropriate comparison of those studies which have attempted to determine prognostic factors is problematic for several reasons. There are differences in the selection of patients, some studies do not include immunological data and, finally, histopathological lymphoma classification may not have been performed in the same way from centre to centre. These differences probably explain some of the discrepancies observed.

Our study was not designed to evaluate the efficacy of therapy. Most patients were treated with the CHOP regimen. Approximately 50% of those who received therapy showed partial or complete responses, and some were still alive and disease-free 1 year after initiation of therapy. Similar response rates have been observed in other series [21–24]. The optimal therapy for patients with HIV-related lymphoma has not been ascertained. More intensive regimens of combination chemotherapy do not seem to prolong survival [23].

In conclusion, our data provide evidence for heterogeneity of HIV-related lymphomas. Further studies addressing the role of EBV in the pathogenesis and course of HIV-related malignant lymphoma should be encouraged.

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Acknowledgement—This study was supported by the Michaelsen Foundation, the Danish AIDS foundation, the Danish Medical Research Council and the EEC (European Federation of AIDS Research).

Eur J Cancer, Vol. 27, No. 11, pp. 1423–1429, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Treatment of Malignant Ascites due to Recurrent/Refractory Ovarian Cancer: the Use of Interferon-α or Interferon-α Plus Chemotherapy *in vivo* and *in vitro*

W.R. Bezwoda, T. Golombick, R. Dansey and J. Keeping

Intraperitoneal treatment with interferon (IFN) for malignant ascites due to advanced ovarian carcinoma refractory to chemotherapy gave an objective response rate of 36% (7/19 patients treated). *In vitro* studies demonstrated that cytotoxicity of peripheral blood monocytes/macrophages was stimulated by IFN. However, peritoneal exudate cells obtained after intraperitoneal treatment with interferon were not stimulated to kill autologous tumour cells. Clinical response was therefore most probably due to a direct inhibitory effect of IFN on growth of malignant cells rather than due to an immune modulatory effect. Using a newly established ovarian cancer cell line (UWOV1), synergy between the growth inhibitory/antitumour effects of IFN and cisplatin was demonstrated at clinically achievable concentrations of each agent. IFN plus cisplatin proved to be more effective than intraperitoneal cisplatin alone in control of peritoneal carcinomatosis. The response rate was 5/7 (77%) for combined modality therapy vs. 2/9 (22%) for intraperitoneal chemotherapy alone. Both *in vitro* and *in vivo* studies suggest a role for interperitoneal therapy for control of refractory ascites in ovarian cancer.
Eur J Cancer, Vol. 27, No. 11, pp. 1423–1429, 1991.

INTRODUCTION

WHILE COMBINATION chemotherapy has resulted in significant improvement in the response rate of ovarian cancer [1, 3], local tumour recurrence remains a major problem. Locoregional treatment with intraperitoneal drug instillation for the treatment and/or prophylaxis of this problem has been studied fairly extensively during the last 10 years [4–7]. The rationale for intraperitoneal therapy is based on the observation that drug concentrations at the tumour site (peritoneal cavity) are significantly higher following intraperitoneal instillation than the drug concentrations which can be achieved following systemic administration [8, 9]. Such increased drug concentrations should achieve a higher cell kill and therefore greater clinical efficacy.

However, many patients with recurrent ovarian cancer will already have been exposed to the most useful drugs before intraperitoneal therapy is given. Such prior exposure increases the risk of drug resistance. While dose escalation may be partly effective in overcoming drug resistance, alternative treatment approaches [10–12] need to be developed. A number of preliminary studies have indicated some potential for intraperitoneal interferon (IFN) [13, 14] for the treatment of peritoneal carci-

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Revised 22 May 1991; accepted 3 June 1991.