Leukemia Risk Following Hodgkin's Disease: Relation to Cumulative Dose of Alkylating Agents, Treatment With Teniposide Combinations, Number of Episodes of Chemotherapy, and Bone Marrow Damage

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Purpose: The development of leukemia is one of the most serious long-term complications of modern treatment for Hodgkin's disease (HD). This study was undertaken to examine the relation between risk of leukemia and various treatment factors (including cumulative dose of cytostatic drugs and interaction with radiotherapy [RT]), while also assessing the effect of treatment-induced bone marrow damage.

Patients and Methods: We conducted a case-control study in a cohort of 1,939 patients treated for HD between 1966 and 1986 in the Netherlands. Detailed information from the medical records was obtained for 44 cases of leukemia and 124 matched controls, in whom leukemia had not developed.

Results: The cumulative dose of mechlorethamine was the most important factor in determining leukemia risk. As compared with patients who received RT alone, patients treated with six or fewer cycles of combinations including nitrogen mustard (mechlorethamine) and procarbazine had an eightfold increased risk of developing leukemia ($P = .08$), while patients who received more than six of such cycles had a greater than 40-fold excess risk ($P < .001$). Treatment with lomustine or a combination of teniposide and cyclophosphamide also significantly increased the risk of leukemia. Patients who had

received chemotherapy (CT) during two or more time periods had a nearly 40-fold increased risk of leukemia as compared with patients treated only once. The extent of RT did not further increase leukemia risk among patients who also received CT. A significantly increased risk of leukemia was found among patients with low platelet counts, both in response to initial therapy and during follow-up. Patients who experienced 2 or more half-year periods with platelet counts less than $75 \times 10^6/\text{mL}$ had an approximately fivefold risk of developing leukemia, and a similar risk increase was found for patients who responded to initial treatment with a $\geq 70\%$ decrease of platelet counts (as compared with patients who had a $\leq 50\%$ decrease).

Conclusion: In addition to mechlorethamine, lomustine and teniposide combinations were also linked to an elevated risk of developing leukemia. Since the number of CT episodes was found to be a strong determinant of leukemia risk, it is important that new therapies for HD continue to yield high initial cure rates. Further studies are warranted to investigate whether patients at high risk for developing leukemia may be identified from the response of their platelets to initial therapy for HD.

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SINCE THE FIRST reports of leukemia following Hodgkin's disease (HD)^{1,2} in the 1970s, numerous studies have convincingly shown that patients who have received chemotherapy (CT) with alkylating agents are at strongly increased risk to develop leukemia, specifically the acute nonlymphocytic type.³⁻¹² The CT-related risk of leukemia has been reported to be more than 100-fold higher than the risk in the general population, resulting in an actuarial risk of 8% to 12% after 15 years of follow-up.^{5,9,10} The highest risk of leukemia has generally been reported to occur between 2 and 10 years following the first treatment. It has been consistently reported that irradiation alone is associated with little or no increased risk of leukemia.

Although leukemia following HD appears to be the most studied malignancy induced by treatment, several questions of interest remain unanswered, because of conflicting results or lack of sufficient data. First, the literature is inconsistent with respect to the important question of whether combined modality treatment increases the risk of leukemia over that induced by CT alone. Enhanced risk with extensive radiotherapy (RT) was reported in some studies,^{6,7,13-15} but not found in others.^{9,10,16,17} Con-

flicting results have also been reported with respect to the relative leukemogenicity of different CT regimens,^{16,17} and only one study to date has attempted to unravel the independent and combined effects of different cytostatic agents on leukemia risk.¹⁶ Several studies have indicated that factors other than CT and RT also affect leukemia risk. Splenectomy,^{16,18} advanced stage,^{16,19,20} and older age (particularly > 40 years)⁷⁻¹⁰ have been identified as risk factors in some studies, but these findings have not been uniform.

To date, no study has examined whether treatment-

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related severe acute bone marrow toxicity or long-term impaired bone marrow function are associated with increased leukemia risk. This issue is of interest, since recent experimental data suggest prolonged effects of CT on bone marrow function.²¹

We report here on the results of a case-control study in which the characteristics of leukemia cases developing in a large series of HD patients ($N = 1,939$) were compared with matched controls in whom leukemia did not develop. This study was designed to evaluate the dose-dependent effects of different cytostatic agents, extent of RT, stage, splenectomy, and bone marrow damage on leukemia risk.

PATIENTS AND METHODS

Study Population

The case-control study was conducted in a cohort of 1,939 patients with HD who were admitted to the Netherlands Cancer Institute (NKI) in Amsterdam ($n = 921$) or the Dr Daniel den Hoed Cancer Center (DDHK) in Rotterdam ($n = 1,018$) between 1966 and 1986. Patients were identified through the institutes' hospital tumor registries. Methods used to assess second cancer risk in the cohort have been extensively described elsewhere.²² In brief, the recent medical status of all patients was collected directly from the medical records or, in case of patients lost to follow-up, by mailing a questionnaire to specialists in other hospitals and to general practitioners. For 97% of the cohort, we obtained medical status up to at least January 1, 1989. Forty-three cases of leukemia or myelodysplastic syndrome were observed in the cohort. For the case-control study, we added one case that developed after the patient's follow-up for the cohort study had been completed. For all but one of the case patients, the leukemic condition was reclassified on the basis of peripheral-blood samples, bone marrow aspirates, and biopsy specimens; all cases of myelodysplastic syndrome were reviewed independently by two hematologists. The one case of acute nonlymphocytic leukemia for which slides were not available for review was retained in the study on the basis of several clear pathology reports, supplemented by strong clinical data.

From the 44 leukemia disorders, 32 were classified as acute nonlymphocytic leukemias; all cases of myelodysplastic syndrome ($n = 12$) were classified as refractory anemia with excess of blasts. For simplicity, all leukemic conditions will subsequently be referred to as leukemia.

For each case patient, three matched controls were selected from the cohort of HD patients. Controls had to have survived without a second cancer for at least as long as the interval between the diagnoses of HD and leukemia in the case patient. They were matched to the case patient on cancer center (for practical reasons), sex, date of birth (± 3 years), and date of diagnosis of HD (± 5 years). When more than three controls per case met these requirements, we selected those whose date of birth and then date of diagnosis of HD were closest to those of the corresponding case. Three controls each were found for 38 case patients, 2 controls each were found for four cases, and the remaining two cases were matched to only one control. Fifteen cases of acute nonlymphocytic leukemia, six cases of refractory anemia with excess of blasts, and 59 control subjects were also included in an international multicenter case-control study, which has been published elsewhere¹⁶; for the present study, more extensive data were collected on these patients.

Abstraction of Medical Records

For all study subjects, the full medical record was obtained for detailed data abstraction of all treatments received. When part of the treatment had been administered outside the two cancer centers, the two data abstractors went to peripheral hospitals to collect the relevant data. Information was collected on characteristics of HD (morphology, stage), all CT and RT given for HD, splenectomy, and indicators of bone marrow toxicity. For each course or cycle of CT, the details abstracted included the name and total dose of each drug used, the dates of administration, and whether it was given in combination. Regarding RT, we abstracted from the RT record the size and location of the fields irradiated. For each patient who underwent a splenectomy, information was collected on date and indication for splenectomy, blood cell counts (WBCs and platelets) immediately before splenectomy, highest counts in the 6-week period following splenectomy, and time to achieve these highest counts (in weeks). Acute bone marrow toxicity and long-term bone marrow damage are difficult concepts to measure retrospectively. With WBC and platelet counts as the only available indicators of bone marrow damage, we sought to develop an imperfect but practical approach to assess bone marrow damage after treatment. For each half-year period (January 1 to June 30, and July 1 to December 31) of follow-up, starting in the calendar year of diagnosis of HD, we abstracted the lowest counts of platelets and WBCs. In addition, we registered the counts before start of treatment. On average, WBC counts could be obtained from the medical records for 91% and 92% of all half-year periods in the follow-up of the cases and controls, respectively. Platelet counts were available for an average of 88% and 87% of all half-year periods in the follow-up of cases and controls, respectively.

After completion of each form by the data abstractors, all information abstracted was checked with the medical record by a physician in charge of study procedures and quality control.

Statistical Analysis

The relative risk of leukemia associated with specific exposures (eg, treatment and bone marrow toxicity) was estimated by comparing the case patients' history with that of their matched controls, using conditional logistic regression methods.²³ Relative risk estimates, P values, and 95% confidence intervals (CIs) for the relative risk estimates were calculated with the microcomputer program EGRET²⁴; comparisons between exposure categories were based on likelihood-ratio tests. Since all subjects had received RT, CT, or a combination of both, it was not possible to estimate the relative risk of specific treatments as compared with a reference category of subjects who had never been exposed to possible leukemogenic agents. Therefore, in our analysis of the relation between leukemia risk and CT, the comparisons were made either in relation to patients treated with RT, or to those treated without the specific cytostatic agent of interest. For case patients, we considered only the therapies and peripheral-blood counts in the period between the diagnoses of HD and leukemia; for each control patient, the analysis only took into account therapy and blood counts abstracted in a period of equal length, starting with the diagnosis of HD.

In evaluating the association between leukemia risk and CT, we took two approaches. First, patients were divided into mutually exclusive treatment groups, on the basis of all combination and single-agent CT given with alkylating agents. The relative risk associated with these treatments was assessed relative to RT only. Subsequently, we estimated the risk of leukemia associated with individual cytostatic agents, while adjusting for the cumulative dose of all other agents. For this analysis, we calculated for all study subjects the cumulative dose of mechlorethamine, procarbazine, lomustine,

chlorambucil, dacarbazine, cyclophosphamide, and teniposide. Teniposide was considered because recent literature has suggested that this agent has leukemogenic potential.^{25,26} For nine patients (5% of the study population), the dose information for one or more cycles was unavailable from the medical records. For seven patients, these doses could be estimated on the basis of the average dose given to the same patient in similar cycles for which all dose information was present. For one patient, for whom dose information on all four cycles was missing, we estimated the dose from the median dose for each agent per cycle in all other study subjects. To the one patient for whom both dose and number of cycles were missing for three agents, we assigned the median doses received in the study population. The relation between leukemia risk and the dose of a specific drug was evaluated by grouping the cumulative dose into evenly spaced categories, and estimating the relative risks between each category and the reference group of patients not treated with the drug.

In case of drugs for which the cumulative dose showed little variation between the study subjects (teniposide, lomustine, chlorambucil, dacarbazine, and cyclophosphamide administered in combinations), a categoric variable (yes/no) was used for further analyses. The dose-response effect of mechlorethamine was best fitted by including a binary term (yes/no) and a continuous variable (log-transformed cumulative dose) in the model. Since HD patients receive many alkylating agents in combination, we also examined interaction between the agents most commonly given together (ie, mechlorethamine and procarbazine, cyclophosphamide and procarbazine, cyclophosphamide and teniposide). More than 95% of all patients who received mechlorethamine were also treated with procarbazine, and vice versa. However, since the cumulative doses of these agents were not highly correlated in persons who used both drugs (Pearson's $r = .44$), we were able to assess independently dose effects of mechlorethamine and procarbazine. For mechlorethamine, we also determined the dose-intensity, which was defined as the cumulative dose divided by months of treatment with mechlorethamine. The final model for the risk estimation of individual cytostatic agents included all CT variables with a P value less than .10 on entry in the full model, and all variables (with a higher P value) that on entry in the model changed the regression coefficients of one of the other variables by greater than 20%.

The relation between the risk of leukemia and indicators of bone marrow toxicity was evaluated in a model that adjusted for the type and amount of all cytostatic drugs with leukemogenic potential. First, we calculated for all subjects the number of half-year periods (from diagnosis of HD up to one-half year (or 1 year) before diagnosis of leukemia or myelodysplastic syndrome, whichever came first), during which the WBC and platelet counts had fallen below $1.5 \times 10^6/\text{mL}$ and $75 \times 10^6/\text{mL}$, respectively. These cutoff points were chosen on the basis of the distribution in the patient group, and also since these levels are often used in the clinic as an indicator of severe acute bone marrow toxicity, necessitating dose modification of CT. The same analyses were performed with cutoff points of $2.5 \times 10^6/\text{mL}$ and $125 \times 10^6/\text{mL}$ for WBCs and platelets, respectively. Second, we calculated the relative decrease of WBC and platelet counts during initial treatment for HD. For this analysis, the period of initial treatment was defined as the period from start of the very first treatment (with splenectomy, RT or CT), until treatment was interrupted for at least 1 month.

RESULTS

Table 1 lists general characteristics of the study group. Most cases of leukemia occurred between 3 and 10 years

after the diagnosis of HD. The average age at diagnosis of HD was 36.5 years for the leukemia cases, and 36.8 years for their matched controls. Overall, 79% of the controls were matched within 1 year of the case patient according to age at diagnosis of HD, and 93% within 2 years. Most of the cases of leukemia (77%) occurred in patients who had been diagnosed with HD between 1970 and 1980. Sixty-two percent of controls were matched within 1 calendar-year of the case patient's year of diagnosis of HD, and 82% were matched within 3 calendar-years.

Table 2 lists the distribution of leukemia cases and controls according to overall treatment modalities received. Only one case (2.2%) was treated with RT alone, as compared with 30% of the controls. In patients who received CT alone, the relative risk of developing leuke-

Table 1. Characteristics of Case Patients With Leukemia and Their Matched Controls

Characteristic	Case Patients (n = 44)		Controls (n = 124)	
	No.	%	No.	%
Calendar-years of diagnosis of HD				
< 1970	6	13.6	11	8.9
1970-1973	12	27.3	29	23.4
1974-1977	11	25.0	41	33.1
1978-1981	11	25.0	35	28.2
≥ 1982	4	9.1	8	6.5
Age at diagnosis of HD (years)				
< 25	10	22.7	28	22.6
25-34	11	25.0	30	24.2
35-44	11	25.0	30	24.2
≥ 45	12	27.3	36	29.0
Interval between diagnosis of HD and leukemic condition (years)				
1-3	11	25.0	NA	
4-6	13	29.5	NA	
7-9	13	29.5	NA	
≥ 10	7	15.9	NA	
Institute				
Netherlands Cancer Institute	29	65.9	79	63.7
Dr Daniel den Hoed Cancer Center	15	34.1	45	36.3
Sex				
Male	26	59.1	74	59.7
Female	18	40.9	50	40.3
Stage of HD				
I	3	6.8	47	37.9
II	16	36.4	46	37.1
III	12	27.3	23	18.5
IV	13	29.5	7	5.6
Unknown			1	0.8
Morphology of HD				
Nodular sclerosing	29	65.9	74	59.7
Mixed cellularity	9	20.5	41	33.1
Lymphocyte predominant	3	6.8	5	4.0
Lymphocyte depleted	1	2.3	0	0
Unknown	2	4.5	4	3.2

Abbreviation: NA, not applicable.

mia was more than 40-fold greater than in patients treated with RT alone. Patients treated with both CT and RT had an approximately 20-fold excess risk (also with the RT-only category as baseline). The relative risk of leukemia among patients who received combined modality treatment was not significantly different from the relative risk in patients treated with CT alone ($P = .16$).

Table 3 lists the CT histories of the leukemia cases and their matched controls. This overview is restricted to the use of alkylating agents and teniposide, with additional information on whether the agent was used in combination or singly. The most extensively used form of CT was the mechlorethamine-procarbazine (MP) type. Overall, 95% of leukemia cases (v. 50% of controls) had been treated with combinations of this type, and 16 cases (36%) and 36 controls (29%) received these exclusively. Other frequently used CT regimens were cyclophosphamide-procarbazine combinations, cyclophosphamide-teniposide combinations, and various combinations including lomustine or cyclophosphamide as the only alkylating agent. The more recently used dacarbazine combinations (mostly consisting of doxorubicin, bleomycin, vinblastine, and dacarbazine [ABVD]) were given to six cases and to only one control. Cyclophosphamide and procarbazine were the single agents most commonly used; 20% of case patients and 16% of controls had received one of these singly, nearly always in addition to combination CT.

Table 4 lists relative risks of leukemia (with the RT-only category as baseline) for patients with specific CT histories, regardless of whether they also received RT. For patients who only received MP combinations, the crude relative risk was 23.9 (95% CI, 2.5 to 225). Patients who received more than six cycles of MP experienced far higher risk than patients treated with six or fewer cycles ($P = .02$). The risk of patients who had received a cyclophosphamide combination in addition to MP treatment was not much different from the risk due to MP combinations alone ($P = .62$). Strongly increased relative risk (> 100 -fold excess relative to RT alone) was associated with a history of having received both MP and lo-

Table 3. Distribution of Leukemia Cases and Controls According to Complete History of CT With Alkylating Agents and Teniposide

History of Chemotherapy*	No. of Cases	No. of Controls
MP	16	36
ChlP	1	1
CP	0	2
P-s	0	5
MP + C-s	2	9
MP + C-s + P-s	2	0
MP + C-s + CTp	2	0
MP + CTp	0	4
MP + CP + C-s + P-s	1	0
MP + Lom	0	2
MP + LomTp	1	0
MP + LomP	3	1
MP + D	2	1
MP + Chl	3	1
MP + ChlP	1	1
MP + CTp + D	1	0
MP + CTp + Lom	2	0
MP + CP + Lom	1	0
MP + CP + D	1	0
MP + MLom + C-s	1	0
MP + ChlP + LomTp	0	2
MP + D + Lom + C-s	1	0
MP + D + Lom + CTp	1	0
MP + C + LomChl	1	0
Other†	0	8

Abbreviations: M, mechlorethamine; P, procarbazine; Chl, chlorambucil; C, cyclophosphamide; D, dacarbazine; Lom, lomustine; Tp, teniposide.

* The CT categories are mutually exclusive; categories without cases and with less than 2 controls are not listed. Unless the suffix -s (= single agent) is added, all drugs were given in combination with at least one other cytostatic agent. For each combination, only the alkylating drugs and teniposide are listed here, regardless of the inclusion of other drugs in the combination. For example, MP denotes all combinations including mechlorethamine and procarbazine as the alkylating agents. Thus, MP, includes both the widely used MOPP chemotherapy (mechlorethamine, vincristine, procarbazine, and prednisone) and the less often used MVPP combination (in which vincristine is replaced by vinblastine).

† The following treatments were received by one control each: C-s, M-s, Chl, P-s + C-s, LomP + C-s, MP + CP + P-s, Chl + C-s + M-s, MP + Lom + C-s.

Table 2. Distribution and Relative Risk of Leukemia According to Overall Treatment Category

Treatment	No. of Case Patients	No. of Controls	Relative Risk	95% CI	P
Radiotherapy only	1	45	1.0		
Chemotherapy only	8	8	44.6	4.8-414	< .001
Radiotherapy and chemotherapy	35	71	20.9	2.8-158	.003
Total	44	124			

mustine combinations, with or without a cyclophosphamide combination. Patients in this treatment group had significantly higher risk of leukemia than patients who received MP combinations alone ($P = .02$). The crude relative risk associated with a specific type of CT is likely to be determined by the alkylating agents in the combination, as well as the total amount of CT administered. To obtain a more valid estimate of the leukemogenicity of specific treatments, we therefore adjusted the crude relative risks in Table 4 for the total number of cycles of combination CT given. In this analysis, the total number of cycles with alkylating CT administered to a patient proved to be a strong determinant of leukemia risk. Risk increased linearly up to 12 cycles (P trend = .001), but

Table 4. Relative Risk of Leukemia for Patients With Specific CT Histories

Treatment*	No. of Case Patients	No. of Controls	Crude Relative Risk	95% CI	Mean No. of Cycles		Adjusted† Relative Risk	95% CI
					Case Patients	Controls		
Radiotherapy only	1	45	1.0	(ref)				
MP only								
≤ 6 cycles	5	23	8.5	0.9-78.4	6	6	—	
> 6 cycles	11	13	44.2	4.8-406§	11	11	—	
Any	16	36	23.9	2.5-225¶	9	8	33.7	3.4-338¶
MP + C(P)	7	14	33.4	2.9-381¶	5	7	39.1	3.3-460¶
MP + lom(P) ± C(P)	8	4	138	10.6-1,809§	16	12	104	6.6-1627§
MP + other	11	11	55.0	4.7-639¶	14	9	49.0	3.9-608¶
Total no. of cycles with alkylating CT								
1-6	13	37	12.9	1.6-102			—	
7-12	19	17	57.1	6.7-491§			—	
> 12	11	10	51.9	5.5-489§			—	
No. of alkylating agents ever received								
2	17	40	14.6	1.9-114			18.7	2.3-154¶
3	17	22	35.8	4.2-303§			32.1	3.7-280¶
4 or 5	9	3	109	9.9-1,204§			63.7	5.6-726§
No. of CT episodes‡								
1	26	71	12.9	1.7-97.2			17.4	2.2-135¶
≥ 2	17	8	91.5	9.4-890§			91.5	8.6-973§

*MP denotes combinations including mechlorethamine and procarbazine and no other alkylating agents; C(P) denotes the use of cyclophosphamide, either singly or in combinations, with or without procarbazine, but without any other alkylating agent; lom(P) denotes combinations including lomustine, with or without procarbazine, but without any other alkylating agent; "other" denotes all other combinations with at least one alkylating agent. The use of an agent both singly and in combination was not considered separately in this analysis.

†Risks were estimated after adjustment for splenectomy and for the total number of cycles with alkylating CT.

‡By definition, a new episode of CT started when the previous one had ended a year ago or longer.

§ $P < .001$.

¶ $P < .01$.

|| $P < .05$.

no further increase of leukemia risk was observed for more than 12 cycles. Adjustment for the total number of cycles resulted in somewhat less difference in risk of leukemia between the selected CT histories. The total number of alkylating agents ever received was also strongly related to leukemia risk. Patients who had been treated with four or five alkylating agents had a greater than 60-fold increased risk as compared with patients who had received RT alone, whereas for patients treated with only two alkylating agents, the relative risk was 18.7 (adjusted for total number of cycles). The number of episodes of CT appeared to be another strong determinant of leukemia risk, with a relative risk of approximately 6.0 for two or more periods of CT as compared with only one such period.

Table 5 lists the results of an analysis in which the risk associated with individual cytostatic agents was estimated, while adjusting by multiple logistic regression for the effects of all other agents. Since splenectomy and the number of treatment episodes with CT also affected leukemia risk, we adjusted all risk estimates in Table 5 for these factors as well. The cumulative dose of mechlor-

ethamine was the strongest determinant of leukemia risk ($P < .001$ on entry of the log-transformed dose, as a continuous variable, in the full model). A high increase in risk was particularly seen for patients who received greater than 110 mg, which was about the average dose given to our patient group during six cycles. The dose-intensity of mechlorethamine was not significantly related to risk of leukemia in a model accounting for cumulative dose. Treatment with lomustine and dacarbazine, regardless of dose, also increased risk of leukemia, but the effect of dacarbazine was only marginally statistically significant ($P = .07$). None of the alkylating agents, procarbazine, chlorambucil, or cyclophosphamide (administered as a single agent or in combination), was associated with significantly increased risk of leukemia, and the addition of these agents to the model did not substantially alter the risk estimates for the other agents. No significant interaction, in the sense of a synergistic action, was found to operate between mechlorethamine and procarbazine, or between cyclophosphamide and procarbazine ($P > .50$). However, the combination of cyclophosphamide and teniposide, used in six leukemia cases and four controls,

Table 5. Relative Risk of Leukemia According to Type and Cumulative Dose of Cytostatic Drugs, Number of Episodes of CT, and Extent of RT

Treatment	No. of Case Patients	No. of Controls	Median Dose (mg)	Relative Risk*	95% CI	P
Mechlorethamine dose (mg)						
≤ 75.5	10	16	59	7.5	0.50-112	.14
> 75.5-110	5	22	100	9.5	0.55-163	.12
111-172	12	13	122	63.4	4.2-964	.003
≥ 173	15	11	220	84.2	4.1-1,740	.004
Dacarbazine	6	1	4,350	22.6	0.76-672	.07
Procarbazine dose (mg)						
≤ 11,950	20	36	8,125	2.4	0.13-44.7	.56
> 11,950	23	32	16,800	7.6	0.27-216	.23
Teniposide	7	6	300	0.9	0.12-7.0	.92
Cyclophosphamide (in combinations)	10	7	3,000	1.9	0.55-66.1	.72
Teniposide combined with cyclophosphamide	6	4	—	12.5 × 10 ⁴	3.4-45.6 × 10 ⁸	.03
Chlorambucil	6	7	893	5.2	0.27-99.5	.25
Lomustine	11	7	8,070	10.1	1.1-98.2	.046
Cyclophosphamide alone (mg)			34,150			
≤ 34,150	5	7	16,440	0.88	0.09-8.6	.91
> 34,150	4	7	51,200	0.30	0.01-6.2	.44
No. of periods with chemotherapy						
0 or 1†	27	116		1.0		
≥ 2	17	8		38.0	2.6-547	.008
Extent of radiotherapy‡						
None	8	8		1.0		
Mantle only, inverted Y only, or local only	13	32		1.1	0.15-7.8	.94
Subtotal nodal irradiation	7	13		0.67	0.08-6.0	.72
Total nodal irradiation	15	26		2.5	0.32-18.8	.39

*The reference group consisted of patients never exposed to a specific drug (relative risk, 1.0). All relative risks were adjusted for mechlorethamine (log-transformed cumulative dose as a continuous variable), lomustine (yes/no), dacarbazine (yes/no), cyclophosphamide given in combinations (yes/no), teniposide (yes/no), interaction between cyclophosphamide and teniposide, splenectomy, and number of episodes of CT.

†After adjustment for type and amount of CT, patients who had only 1 episode of CT had a relative risk of 1.0 as compared with patients who received no CT; therefore, the groups of patients with only 1 episode, or none, were combined in this analysis to provide a larger reference group for the estimation of relative risk.

‡Analysis restricted to patients treated with CT.

was associated with strongly increased risk ($P = .03$). Most patients who received this combination were treated with alternating MOPP and cyclophosphamide, doxorubicin, teniposide, and prednisone (CHVP), according to European Organization for Research and Treatment of Cancer protocols.²⁷ The potential leukemogenic risk from CHVP cycles came to our attention when we noted that this regimen had been administered to five leukemia patients who received only very low doses of mechlorethamine or other alkylating drugs. The interaction between teniposide and cyclophosphamide was found to explain the unexpectedly high crude relative risk for the lowest quartile of the cumulative mechlorethamine dose (crude relative risks for the lowest quartile [≤ 75 mg] and the subsequent one [75 to 110 mg] were 33 and 13, respectively). Besides the influence of cytostatic agents, the number of episodes of CT showed a strong and independent effect on risk of leukemia in this model. Patients who had received CT during two or more separate periods

in time were subject to a nearly 40-fold higher risk of leukemia than patients who experienced only one CT episode ($P = .008$), while adjusting for all relevant CT variables. The number of CT episodes remained a significant factor when the analysis was restricted to patients who received more than six cycles.

Table 5 also lists data on the association between risk of leukemia and extent of RT, in patients who also received CT. Since patients who received combined modality treatment received slightly less CT, and received different agents than patients whose only treatment was CT, all of these analyses were adjusted for the type and amount of leukemogenic agents (Table 5). With the group of patients who only received CT as baseline, no increased risk was observed among patients who also received subtotal nodal RT or extended-field RT to only one side of the diaphragm. Patients treated with total nodal RT and CT had slightly higher risk of leukemia than patients who received CT alone (relative risk, 2.5),

but this result did not approach statistical significance ($P = .39$). Patients with a history of splenal irradiation ($n = 41$) did not have an increased risk of leukemia; their relative risk as compared with patients who did not undergo splenal irradiation or splenectomy was 1.2 ($P = .82$). The influence of RT on the risk of leukemia was also examined in the subgroup of 16 leukemia patients and 36 controls who had received MP combinations and no other CT. After adjustment for the effect of the mechlorethamine dose, no excess risk from any type of RT emerged. Total nodal RT had been given to six cases and 11 controls in this subgroup, resulting in a relative risk of 1.0 (95% CI, 0.21 to 4.8).

In Table 6, the relative risk of leukemia is listed for various indicators of bone marrow damage, while adjusting for the type and amount of alkylating agents received, and for the number of CT episodes. Patients who experienced two or more half-year periods during which their platelets dropped below $75 \times 10^6/\text{mL}$ had an approximately fivefold increased risk of developing leukemia as compared with patients who experienced only one such period or none ($P = .03$). For patients with four or more periods with such low platelet counts, the risk was increased 16-fold ($P = .17$); this estimate was based on nine cases and two controls. These risk estimates were hardly affected by the inclusion of the number of CT episodes in the model. Somewhat lower relative risks were found when a less extreme cutoff point was chosen (platelet count $< 125 \times 10^6/\text{mL}$). No association was observed between the risk of leukemia and the number of half-year periods with WBC counts less than 1.5 or $2.5 \times 10^6/\text{mL}$.

Subsequently, we examined the association between risk of leukemia and the relative decrease of the blood counts while on initial therapy for HD. For this analysis, patients were grouped into three categories of approximately equal size, according to the relative decrease of their WBCs and platelets. Patients who showed the strongest platelet response to initial therapy ($> 70\%$ decrease from the level before treatment started) had a relative risk of 5.5 as compared with patients whose decrease of platelets was $\leq 50\%$ ($P = .06$). When the analysis was repeated with the relative decrease of the platelets as a continuous (log-transformed) variable included in the model, a clear trend emerged of increasing risk of leukemia with increasing decrease of platelets on initial therapy ($P = .04$). The picture was much less clear for the relative decrease of WBC counts. Although patients who responded to initial treatment with a relative decrease of greater than 60% had nearly fourfold increased risk of leukemia as compared with patients with a smaller decrease, this result was not statistically significant ($P =$

Table 6. Relative Risk of Leukemia According to Selected Indicators of Bone Marrow Damage

Indicator	No. of Case Patients	No. of Controls	Relative Risk*	95% CI	P
No. of half-year periods with platelets $< 75 \times 10^6/\text{mL}$					
0-1†	24	109	1.0		
≥ 2	20	15	4.9	1.1-21.5	.03
No. of half-year periods with WBC count $< 1.5 \times 10^6/\text{mL}$					
0	30	102	1.0		
1	10	15	1.7	0.42-6.6	.47
≥ 2	4	7	1.3	0.06-23.9	.89
No. of half-year periods with platelets < 75 and WBC count $< 1.5 \times 10^6/\text{mL}$					
0	34	109	1.0		
≥ 1	10	15	1.5	0.30-7.2	.64
Relative decrease of platelets on primary treatment for HD (%)					
≤ 50	3	33	1.0		
51-70	12	40	3.3	0.56-19.6	.19
> 70	15	23	5.5	0.93-32.8	.06
Relative decrease of WBCs on primary treatment for HD (%)					
≤ 60	5	34	1.0		
61-75	14	39	4.9	0.78-30.9	.09
> 75	13	27	2.7	0.42-18.0	.29

*All relative risks were adjusted for mechlorethamine (log-transformed cumulative dose as a continuous variable), lomustine (yes/no), dacarbazine (yes/no), cyclophosphamide given in combinations (yes/no), teniposide (yes/no), interaction between cyclophosphamide and teniposide, splenectomy, and number of CT episodes. The relative risks for the relative decreases of blood counts on primary treatment were unadjusted for number of CT episodes.

†Patients who had only one half-year period with platelets less than $75 \times 10^6/\text{mL}$ (7 cases and 26 controls) had a relative risk of 1.1 as compared with patients who had no such periods; therefore, the groups of patients with only 1 such period, or none, were combined in this analysis to provide a larger reference group for the estimation of relative risk.

.15) and no relationship between increasing risk of leukemia with a greater decrease of WBC counts was observed ($P = .72$).

When we restricted the previous analyses to the 17 leukemia cases and 81 controls who had received RT alone or for whom MP combinations were the only type of CT administered, even stronger associations between risk of leukemia and low platelet counts were seen. The relative risk for two or more half-year periods with platelet counts falling below $75 \times 10^6/\text{mL}$ was 13.6 as com-

pared with the group with only one such period or none ($P = .04$, adjusted for dose of mechlorethamine and splenectomy). A relative decrease of platelet counts of $\geq 60\%$ on initial therapy was associated with a 3.5 times higher risk than a relative decrease of $\leq 35\%$ ($P = .20$). Although the number of CT episodes did not seem to confound the relation between risk of leukemia and multiple episodes with low platelet counts, we also did an analysis restricted to the 13 cases and 80 controls who had only received initial treatment for HD. In this subgroup, a history of two or more half-year periods with platelet counts less than $75 \times 10^6/\text{mL}$ was associated with a relative risk of 10.6 ($P = .04$, adjusted for dose of mechlorethamine and splenectomy).

Patients who underwent a splenectomy had significantly increased risk of leukemia (relative risk, 3.1; 95% CI, 1.1 to 8.8; adjusted for type and amount of CT). Ninety-one percent of patients received a splenectomy as part of their staging laparotomy; splenic involvement was present in 50% of the leukemia cases and 23% of the controls. After adjustment for type and amount of alkylating agents received, splenic involvement did not affect subsequent risk of leukemia, nor did a short interval (< 3 weeks $v \geq 3$ weeks) between splenectomy and start of therapy for HD. Furthermore, no significant associations emerged between the risk of leukemia and the hematologic changes surrounding the event of a splenectomy, although leukemia risk tended to be somewhat higher among patients whose platelet and WBC counts showed a stronger response to splenectomy.

Patients with all stages of HD beyond stage I appeared to have significantly increased risk of leukemia in a univariate analysis (Table 7; P trend $< .001$). Since patients with advanced disease tend to receive more intensive CT, assessment of the independent effect of stage is only possible after taking into account the amount of CT. After

allowance for the type and amount of alkylating agents received, the effect of stage became less pronounced, although patients with stage IV disease still had a greater than 10-fold increased risk of leukemia as compared with patients diagnosed with stage I disease ($P = .04$). When we also adjusted for the number of CT episodes, the association between stage and leukemia risk weakened even further and was no longer statistically significant (Table 7). The effect of stage was also examined in the subgroup of patients who received no other CT than MP combinations. After adjustment for the effects of splenectomy and the dose of mechlorethamine, no risk increase with higher stage was observed. The estimate for stage III and IV disease (relative risk, 0.50; $P = .08$) was based on five cases and 10 controls.

The morphology of HD appeared unrelated to the subsequent risk of leukemia (data not shown).

DISCUSSION

Several new findings have emerged from this case-control study of risk factors for leukemia after treatment for HD. A major new observation is the significantly increased risk of leukemia in patients with low platelet counts, both in response to initial therapy and during follow-up. Patients who experienced two or more half-year periods with platelet counts less than $75 \times 10^6/\text{mL}$ had an approximately fivefold risk of leukemia, and a similar risk increase was found for patients who responded to initial treatment with a decrease of their platelets of $\geq 70\%$ (as compared with patients who had a $\leq 50\%$ decrease). Other important results of our study include the leukemogenicity of lomustine and teniposide-cyclophosphamide combinations in HD patients, and the large and independent effect of the number of episodes with alkylating CT on the risk of leukemia.

In interpreting our results regarding bone marrow damage, we considered whether the findings might be attributed to other risk factors for leukemia. However, the increased risk of leukemia associated with low platelet counts could not be explained by the cumulative dose and dose-intensity of alkylating agents received, or by the number of CT episodes. Furthermore, the associations also held in the subgroup of patients who only received MP combinations, as well as among patients who received no further treatment after initial therapy. Since leukemic conditions may be preceded by an episode of decreasing platelet counts, we had already excluded the 6-month period immediately before the diagnosis of leukemia when we determined the number of half-year periods with low blood counts. Similar results were obtained in an analysis in which we excluded platelet counts in a 1-year period before diagnosis of leukemia. To exclude the

Table 7. Relative Risk of Leukemia According to Stage of HD

Stage of HD	No. of Case Patients	No. of Controls	Crude Relative Risk	Adjusted Relative Risk*	Adjusted Relative Risk†	95% CI	P
I	3	47	1.0	1.0	1.0		
II	16	46	4.8¶	3.8	2.3	0.38-13.5	.37
III	12	23	9.6§	1.8	0.62	0.06-6.9	.69
IV	13	7	32.1‡	12.5¶	2.0	0.15-27.8	.60

*Adjusted for mechlorethamine (log-transformed cumulative dose as a continuous variable), dacarbazine (yes/no), lomustine (yes/no), cyclophosphamide given in combinations (yes/no), teniposide (yes/no), interaction between cyclophosphamide and teniposide, and splenectomy.

†Adjusted for all variables mentioned above, and also for number of CT episodes.

‡ $P < .001$.

§ $P < .01$.

¶ $P < .05$.

possibility that the positive association between leukemia risk and the number of half-year periods with low platelet counts was due to a more prolonged period of thrombocytopenia preceding the leukemia diagnosis in the case group, we examined whether any of the platelet counts less than $75 \times 10^6/\text{mL}$ immediately preceded the half-year period before diagnosis of the leukemic condition. This turned out not to be the case. We therefore conclude that the association between low platelet counts and a subsequent increase of leukemia risk seems real. Although we have no clear explanation for this finding, it might be explained by large interpatient variability in the pharmacokinetics and metabolism of alkylating drugs. Severe thrombocytopenia may indicate greater bioavailability of these agents, which would be a likely causal factor in the development of leukemia. Alternatively, it might be that platelet counts are an indicator of a specific type of bone marrow damage, which in the long run may lead to the development of leukemia. We can only speculate why an association was observed for low platelet counts, and not for low WBC counts. It may be that the overall WBC population is too heterogeneous to exhibit an association which is only present for one of the WBC subtypes.

The effects of acute bone marrow toxicity and long-term bone marrow damage on the subsequent risk of leukemia have not been studied before, and as a consequence we cannot compare our results with those of others. However, interestingly, a recent report by Radford et al.²¹ showed impaired bone marrow function up to 9 years after completion of CT with MP combinations. The investigators speculated that the abnormalities found might result in an impaired response to periods of increased demand on the bone marrow, such as further CT, and might also be relevant to the evolution of second leukemia. More clinical and experimental studies are needed to confirm our findings and those reported by Radford et al. If these findings are reproduced, patients at high risk for developing leukemia might be identified from the strong response of their platelets to initial therapy for HD.

Multiple logistic regression analysis showed that, of all cytostatic drugs, mechlorethamine had by far the strongest effect on leukemia risk in our study. A particularly strong increase of leukemia risk was noted for patients who received more than six courses of mechlorethamine-based therapy; at very high doses, the dose-response curve became less steep. Similar results have been reported by Kaldor et al.¹⁶ The completeness of the dose information in our study enabled us, for the first time, to investigate the leukemogenicity of individual cytostatic drugs used in HD, while controlling for the effects of mechlorethamine and other agents. In this analysis,

lomustine and a combination of teniposide and cyclophosphamide emerged as significant and independent determinants of leukemia risk. The nitrosourea lomustine is a known carcinogen to animals²⁸ and was already known to be leukemogenic when used in the treatment of gastrointestinal cancers.²⁹ Evidence for its leukemogenic effect in patients with HD has, to date, only come from a recent study of the British National Lymphoma Investigation.¹⁷ Both in that study and in our series, nearly all patients received lomustine as second- or third-line treatment. The leukemogenic effect of this agent is of particular importance in the light of the increasing use of lomustine in high-dose salvage regimens.

The epipodophyllotoxins teniposide and etoposide have only recently been implicated in the development of leukemia.^{25,26} Our study is the first to show an increased risk of leukemia associated with the use of a combination of teniposide and cyclophosphamide in the treatment of lymphoma patients. Cyclophosphamide is known to be a weak leukemogen,^{30,31} and the use of cyclophosphamide in itself was not significantly associated with risk of leukemia in our data. Since only one case and two controls received teniposide without cyclophosphamide, we were unable to assess reliably the independent effect of teniposide on leukemia risk. The median dose given to our case group (300 mg) is much lower than the doses for which leukemogenic effects have been reported in patients treated for other malignancies,²⁶ but in our study, all patients on teniposide had also received alkylating CT before or after the teniposide combination. Our finding of the leukemogenicity of teniposide combination is extremely important because of the increasing use of the epipodophyllotoxins in first-line treatment of non-Hodgkin's lymphomas.

The observation in some studies^{7,13-15} that combined modality treatment produces greater risk of leukemia than CT alone has recently been a cause of serious concern. In our study, involved-field RT or subtotal nodal RT did not add to the risk of leukemia from CT alone, while the effect of total nodal RT was small and nonsignificant. The fact that two other large and recent studies have also failed to find an association with extensive RT^{12,16} leads us to conclude that the risk of leukemia is not significantly greater after combined treatment than after CT alone.

The role of the number of episodes of therapy has only been investigated in one other study of leukemia following HD.¹⁷ Patients who had three or more attempts at treatment were found to experience about three times higher risk of leukemia than patients with only two attempts or less. In the latter study, RT, if not given concurrently with CT, also contributed to the number of treatment attempts, and no adjustment was made for amount

of CT.¹⁷ We found in our study that the number of CT episodes was a strong determinant of leukemia risk, even after the amount and type of CT had been taken into account. This would imply that, for example, uninterrupted treatment with 12 cycles of CT is significantly less leukemogenic than treatment with 12 similar cycles during two or more separate periods in time. An explanation may be that multiple insults to the bone marrow of short duration are more detrimental than one, more prolonged, period of myelosuppression.

At least three studies have recently pointed to advanced stage as a possible independent risk factor for the development of leukemia.^{16,19,20} Even after adjustment for CT, stage IV disease carried an approximately fivefold increased risk of leukemia as compared with stage I in the large case-control study reported by Kaldor et al,¹⁶ while in our own study, the risk in stage IV disease was increased by more than 10-fold. However, the effect of stage disappeared in our data after adjustment for the number of episodes of CT, a variable that was not considered in the other studies.^{16,19,20} We conclude that advanced stage is not an independent risk factor for leukemia.

In 1987, our group reported a significantly increased risk of leukemia in patients who underwent a splenectomy.¹⁸ Since then, this finding has been confirmed in at least five other studies.^{16,20,32,33} However, the present literature offers no mechanism by which the removal of the spleen could result in the development of leukemia. Our examination of splenectomy-related factors, which was limited by its retrospective character, did also not provide an explanation for the observed association. We had hypothesized that irradiation of the spleen, through its associated hyposplenia,^{34,35} might also influence leukemia risk, but no such effect was found.

The case-control method used in the present study has only recently come into use for the investigation of risk factors for second cancers. A major advantage of this method over a traditional cohort study is that it provides a valid design for the collection of detailed treatment data on only a fraction of all patients in a cohort. The only

drawback of most case-control studies of second cancer risk is that the risk associated with a specific treatment is estimated relative to the risk in patients receiving other treatment, while the absolute risk of second cancer development (as compared with the general population) cannot be determined. However, our case-control study was conducted after completion of a cohort study in which treatment-specific observed-to-expected ratios had been determined.²² By combining these observed-to-expected ratios with the relative risks estimated in the case-control study, we can therefore calculate absolute risks of second cancer development associated with specific treatments. In the cohort study, patients who were treated with RT alone had an approximately threefold (nonsignificantly) increased risk of developing leukemia as compared with the general population (observed-to-expected ratio, 1:0.32 = 3.1; 95% CI, 0.079 to 17.4).²² Thus, in the case-control study, all relative risks that were estimated relative to the treatment category involving RT alone (eg, in Table 4) may be converted to absolute risks by multiplying times 3.1.

Leukemia is considered to be one of the most serious long-term consequences of modern treatment for HD. The 20-year actuarial risk of 6% observed in CT-treated patients in our cohort study²² shows that a sizeable proportion of CT-treated patients may be confronted with this complication. Present treatment strategies for HD are already focusing on the selection of CT regimens with a lower leukemogenic potential. However, it is important that such therapies continue to yield high initial cure rates, since the number of CT episodes is also a strong determinant of leukemia risk. Further studies are warranted to investigate whether patients at high risk for developing leukemia may be identified from the response of their platelets to initial therapy for HD.

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