Second malignancies after treatment of diffuse large B-cell non-Hodgkin's lymphoma: a GISL cohort study

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ABSTRACT

Background
Improved treatment has increased the life expectancy of patients with non-Hodgkin's lymphoma, but few studies have addressed the issue of second cancer in patients treated for diffuse large B-cell lymphoma. The aims of this study were to determine the incidence and time free of second cancers in this subset of patients.

Design and Methods
We evaluated a cohort of 1280 patients with diffuse large B-cell lymphoma who were first treated between 1988 and 2003. We utilized the central database of the *Gruppo Italiano Studio Linfomi*, which includes data on demographics, clinical characteristics, laboratory parameters, treatment and follow-up of all patients with non-Hodgkin's lymphoma enrolled in clinical trials.

Results
After a median follow-up of 51 months, 48 patients had developed a second cancer: 13 hematologic malignancies and 35 solid tumors. The overall standardized incidence ratio in our cohort (with a median age of 58 years) matched that of the general Italian population. The incidence ratio of second tumors was age related, and the age groups 20-39 and 40-59 years showed an increased risk. Overall, the cumulative incidence of second cancer was 8.2% at 15 years. A multivariate analysis showed that older age at the time of diagnosis of lymphoma had a negative influence on the time free of second tumors.

Conclusions
In our cohort, only young patients showed an increased incidence ratio of second malignancies, while the incidence ratio in patients aged over 59 years matched the incidence in the Italian general population. Demographics, baseline characteristics, laboratory parameters and treatment modalities did not have any significant impact on the incidence ratio of a second cancer.

Key words: second malignancies, non-Hodgkin's lymphoma, diffuse large B-cell lymphoma, DLBCL, risk factors, treatment.

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Introduction

The incidence of non-Hodgkin's lymphoma has been increasing in most western countries.[1] In 2007, an estimated 8,000 people will have been newly diagnosed with non-Hodgkin's lymphoma in Italy. Recent advances have improved the prognosis of this disease, and the introduction of monoclonal antibodies will further ameliorate patients' overall survival.[2-6] Nowadays, many patients with non-Hodgkin's lymphoma become long-term survivors, thus the risk of developing a second cancer is becoming an important concern.

Several studies,[7-13] but not all,[14-16] have reported an increased overall risk of second cancer after treatment for non-Hodgkin's lymphoma. However, in a large majority of the research, all the categories of non-Hodgkin's lymphoma were combined for analysis. Furthermore, nearly all the studies were population-based. Although the investigations analyzed thousands of patients, they utilized cancer registry databases that usually contain limited information on patients' clinical characteristics and radiotherapy or chemotherapy regimens. In the present study, we analyzed a large cohort of patients with diffuse large B-cell lymphoma who entered clinical trials organized by the *Gruppo Italiano Studio Linfomi* (GISL). The aims of this long term follow-up study were to determine the incidence of and risk factors for developing second cancer in a homogeneous group of patients for whom clinical characteristics as well as first line and subsequent treatments were recorded at the times of diagnosis and recurrence.

Design and Methods

Patients

The GISL maintains a central database located in Modena that includes information on the treatment and follow-up of all non-Hodgkin's lymphoma patients enrolled in clinical trials. Data managers at participating centers routinely update the central GISL database every 3-6 months throughout the trials and every 6-12 months during follow-up. Data managers fill out standardized forms that collect information on patient's characteristics, laboratory parameters, treatments, outcome, late toxicity, and the occurrence of second cancers. Data managers describe the observed second cancers, which are subsequently codified in Modena. When necessary, additional information is requested from the investigators concerned. Eligibility criteria for inclusion in this study were the following: (i) a histologically confirmed diagnosis of diffuse large B-cell lymphoma that was previously untreated, (ii) data (as reported in Table 1) on clinical characteristics, laboratory parameters, treatments, outcome, and the occurrence of second cancers available in the database, and (iii) available information in the database for follow-ups of more than 6 months after diagnosis.

Between 1988 and 2003, 1387 patients with diffuse large B-cell lymphoma were enrolled in a number of GISL trials. Of these patients, 107 (7.7%) were excluded, as they did not meet the eligibility criteria. Thus for the purposes of this study, we included a total of 1280 patients with diffuse large B-cell lymphoma. The treatment regimens utilized and the number of patients who entered the clinical trials and received specific treatments are as follows: LA00[17]: methylprednisolone + cyclophosphamide + doxorubicin + etoposide + cytarabine + bleomycin + vincristine + methotrexate (ProMaCE-CytaBOM) (number of patients = 24); LA01[18]: methylprednisolone + cyclophosphamide + epidoxorubicin + etoposide + cytarabine + bleomycin + vincristine + methotrexate (ProMECE-CytaBOM) versus methotrexate + leucovorin + doxorubicin + cyclophosphamide + vincristine + bleomycin + prednisone (MACOP-B) (number of patients = 176); LA02[19]: ProMECE-CytaBOM versus methylprednisolone + cyclophosphamide + idarubicin + etoposide + cytarabine + bleomycin + vincristine + methotrexate (ProMICE-CytaBOM) (number of patients = 201); LA03[20]: fixed ProMECE-CytaBOM versus fixed ProMICE-CytaBOM versus flexible ProMECE-CytaBOM versus flexible ProMICE-CytaBOM (number of patients = 281); LA04[21] pilot: sequential ProMECE-CytaBOM (number of patients=23); LA04[22]: sequential ProMECE-CytaBOM versus cyclic ProMECE-CytaBOM (number of patients = 98); LA05[23]: ProMECE-CytaBOM + rituximab (ProMECE-CytaBOM +R) (number of patients = 142); GASTRO[24]: ProMECE-CytaBOM or cyclophosphamide + mitoxantrone + vincristine + prednisone (CNOP) (number of patients = 58); PELOC (ongoing trial): ProMECE-CytaBOM + radiotherapy (number of patients = 150); ANZINTER[25]: cyclophosphamide + epidoxorubicin + vinblastine + prednisone (mini-CEOP) versus epidoxorubicin + cyclophosphamide + etoposide + vinblastine + bleomycin + prednisone (P-VEBEC) (number of patients = 75); ANZINTER2[26]: cyclophosphamide +idarubicin + etoposide + prednisone (CIEP) (number of patients = 25); ANZINTER3[27]: rituximab + cyclophosphamide + epidoxorubicin + vincristine + prednisone (R-CHOP) versus rituximab + mini-CEOP (R-mini-CEOP) (number of patients =27). Upon completion of chemotherapy, involved-field radiotherapy was permitted at the treating physician's discretion to irradiate residual masses or the sites of previous bulky or extranodal disease. According to study protocols, radiotherapy consisted of 30-38 Gy. The patients' information, grouped by treatment modality, is reported in Table 1.

All the GISL trials complied with the requirements of the Declaration of Helsinki and its amendments and were conducted in accordance with Good Clinical Practice guidelines. The protocols were approved by the institutional review board at each participating center. Written informed consent was obtained from all patients. Approval for the present study was obtained from the review board of the GISL.

Statistical methods

Follow-up began at the end of the first treatment for non-Hodgkin's lymphoma and ended at the date of death, the date of last follow-up evaluation, the date of diagnosis of second cancer, or the end of the study

Table 1. Descriptive characteristics and treatments utilized in the 1280 patients with aggressive lymphoma.

Characteristics	*N. of patients 1280*	*% of patients 100*	*Person-years at risk 6316*
Age at diagnosis (years)			
14-44	301	24	1671
45-59	424	33	2275
60-69	382	30	1835
70-86	173	14	534
Gender			
Male	688	54	3436
IPI score[a]			
0-1	604	50	3591
2	329	27	1542
3-5	281	23	899
Bulky disease			
Yes	318	25	1390
B-Symptoms			
Yes	352	28	1510
Extranodal sites			
> 1	267	21	1043
Chemotherapy regimens			
PCB-epidoxorubicin	724	57	3760
PCB-idarubicin	222	17	1126
PCB-sequential	150	12	842
CHOP or CHOP-like	184	14	588
Radiotherapy - Involved Field			
Yes	367	29	1972
More than one line of treatment			
>1	306	24	1179
>2	116	9	456
Follow-up			
5 years or less	751		1318
more than 5 years	529		4998
more than 10 years	206		2586

[a]*Missing for 66 of 1280 (5.2%). Because of rounding, percentages may not total 100. PCB-epidoxorubicin: ProMECE-CytaBOM: (methylprednisolone, cyclophosphamide, epidoxorubicin -or doxorubicin-, etoposide, cytarabine, bleomycin, vincristine, methotrexate); PCB-idarubicin: ProMICE-CytaBOM (methylprednisolone, cyclophosphamide, idarubicin, etoposide, cytarabine, bleomycin, vincristine, methotrexate); PCB-Sequential: sequential ProMECE instead of the classical cycling regimen; CHOP: cyclophosphamide, doxorubicin vincristine, prednisolone.*

(July 31, 2007), whichever occurred first. Follow-ups were updated every 3-12 months. Observed cancers were classified by site in accordance with the oncology section of the International Classification of Disease.[28] Individuals who developed malignancies within 6 months of the diagnosis of their diffuse large B-cell lymphoma, who developed synchronous cancer during chemotherapy, or whose non-Hodgkin's lymphoma was not their primary cancer were excluded from this analysis.

The primary aims of this research were to determine the crude rate of second neoplasm, the standardized incidence ratio (SIR), the cumulative incidence, the time free to second tumor (TF2T) and the risk factors for the development of second cancer. The crude rate is the ratio between the observed number of second malignancies and overall person-years of follow-up, expressed per 1000 person-years. The incidence numbers of second neoplasia by person-years under analysis of second cancers in the study population were compared to the incidence of malignancies in the Italian population, utilizing age-, sex- and calendar period-specific incidence rates, derived from the Italian Institute of Health database.[29] The SIR was calculated from the ratio between observed and expected numbers of cancers; 95% confidence intervals (95% CI) were based on the assumption that the observed numbers of second cancer were distributed as the Poisson variable. Every SIR was reported with its own 95% confidence interval.[30] Absolute excess risk (AER) of second cancer was calculated by subtracting the expected from the observed cases and dividing by the person-years at risk. The AER is expressed per 10,000 person-years. Heterogeneity and trend in SIR according to demographics, baseline characteristics and treatment factors were checked by the χ^2 test for unequal SIR, according to Breslow and Day.[31] Cumulative incidences were estimated in the competing risk model with death from any cause considered a competing event.[32,33] The TF2T was measured from the end of the first treatment to the last follow-up or date of diagnosis of the second tumor and was calculated with a Kaplan-Meier estimate.

A proportional hazards model that adjusted for competing causes of mortality, Fine and Gray's regression,[34] was used to determine the clinical factors associated with the TF2T in univariate and multivariate analyses. Examination of the Schoenfeld residuals and TF2T for each covariate was performed to assess the subdistribution proportional hazards assumption in the regressions for all models considered. For all of the fitted models, the Schoenfeld residuals were between -1.0 and 1.0. No apparent abnormal patterns were found against the assumptions of Fine and Gray's regression.

R version 2.3.1[35] was used for all calculations pertaining to the Fine and Gray's regression and cumulative incidence probability estimates of TF2T. The Stata 8.2/SE package[36] was used for all remaining statistical analyses.

As the present study is a retrospective analysis, we did not plan a sample size. For all tests, a two-sided p value <0.05 was considered to demonstrate a moderate strength of evidence against the null hypothesis. This level of probability is helpful for providing clinically useful advice.

The International Prognostic Index (IPI) score, which takes into account elevated lactate dehydrogenase levels, clinical stage III-IV disease, age >60 years, more than one extranodal site of disease, and Eastern Cooperative Oncology Group (ECOG) score ≥ 2, was calculated for 95% of the patients in the cohort.[37]

Results

Patients' characteristics

Between 1988 and 2003, 1387 patients with diffuse large B-cell lymphoma were enrolled in a number of GISL trials. Of these patients, 107 (7.7%) were exclud-

ed: 57 did not complete the planned chemotherapy and were lost immediately during the follow-up period, 10 were misdiagnosed, and 40 lacked sufficient data.

A total of 1280 patients with diffuse large B-cell lymphoma met the defined eligibility criteria and were included in this analysis. The overall quality of the case record forms was good; only for 5% of the forms was the IPI score unable to be calculated. The overall survival rates at 5, 10 and 15 years were 60%, 52% and 46%, respectively, with a median follow-up of 51 months (range 2-213 months) for all patients and 89 months for living patients. The number of patients and follow-up times comprised 6316 person-years of risk for a second tumor. The median age at diagnosis was 58 years (range, 14-86), and 54% (n=688) of the patients were male. All patients were treated with chemotherapy, either alone (71%) or in combination with radiotherapy (29%). The patients' characteristics and treatments are summarized in Table 1.

Second malignancies

During the follow-up, 48 patients (3.8%, crude rate 7.6 per 1000 person-years) developed a second cancer. A total of 568 patients died, 48 were lost during the follow-up period, and the remaining 664 patients (18 with a second cancer) survived to the end of the study period. The most common causes of death were progressive disease (75%), infections (5%), second cancer (4%), treatment-related toxicity (4%) and cardiopathy (1%). No patients developed a third cancer during the follow-up period. Eight of the 48 patients with second cancers developed myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) (5 and 3 cases, respectively), 5 developed other hematologic malignancies, and 35 developed solid tumors, including colorectal carcinoma (n=8), lung cancer (n=8), and other types of cancer (n=19) (Table 2).

We did not observe any cases of second non-Hodgkin's lymphoma. However, biopsies with histological examination were usually not performed in relapsed cases, and further occurrences were commonly considered relapses. Thus, the incidence of Hodgkin's lymphoma and new non-Hodgkin's lymphoma could be underestimated. Fourteen of the 48 second malignancies occurred after additional treatments for progressive or recurrent disease. The median time from diagnosis of diffuse large B-cell lymphoma to diagnosis of a solid tumor was 71 months (range, 13-176 months), and that to diagnosis of MDS/AML was 43 months (range, 30-127 months).

Incidence of second malignancies

The overall risk of second cancer in patients with diffuse large B-cell lymphoma was similar to that in the general Italian population (SIR: 1.1; 95% CI: 0.8-1.5). The analysis of SIR by cancer type showed that incidences were increased for lung cancer (SIR: 1.6; 95% CI: 0.7-3.2), colorectal cancer (SIR: 1.3; 95% CI: 0.6-2.5) and prostate cancer (SIR: 1.6; 95% CI: 0.4-4.1), and decreased for breast cancer (SIR: 0.5; 95% CI: 0.1-1.6). However, differences compared to the Italian general population were not statistically significant. We did not calculate the SIR of MDS/AML and bladder cancer, as the incidence rates of these malignancies are not reported by the Italian Institute of Health. Furthermore, we did not evaluate the SIR for cancers for which fewer than three cases were diagnosed in our cohort. Gender, IPI score, chemotherapy regimens, radiotherapy, number of chemotherapy lines and time of first treatment did not have any significant impact on the SIR of developing a second cancer (Table 3). The risk of second malignancies in relation to age at diagnosis of second cancer is shown in Table 4. An increased and statistically significant risk of second cancer was observed in the cohort groups 20-39 and 40-59 years of age.

Kaplan-Meir estimates of cumulative incidence of second cancer including MDS/AML, calculated as 1.0 minus Kaplan-Meier estimate of TF2T, were 3.4%, 7.6%, and 14% at 5, 10, and 15 years, respectively. After correction in a competing-risk model by Grays' method, the cumulative incidences were reduced to 2.3, 4.7, and 8.2, respectively (Figure 1A). Considered separately, the cumulative incidence for solid tumors was 1.5, 3.3, and 6.8, and for hematologic malignancies 0.8, 1.4, and 1.4 at 5, 10, and 15 years, respectively (Figure 1B). We did not observe any plateau in the solid tumor curve, while the curve of hematologic malignancies stopped increasing after 10 years.

Risk factors for developing second malignancies

In a univariate Gray-Fine regression analysis, the only factor that had a significant negative impact on TF2T was age >60 years at first treatment. This result was confirmed in a multivariate analysis. Factors that did not significantly influence TF2T included gender, IPI score, type of chemotherapy, radiotherapy and number of

Table 2. Cases and sites of second primary cancer.

Second cancer	*ICD-9 code*	*N. of patients*	*% of patients*	*Person-years at risk*
MDS/AML	238.72-238.73-205-208	8	17	40
Lung	162	8	17	43
Digestive traet	153-154	8	17	42
Prostate	185	4	8	30
Breast	174	3	6	18
Bladder	188	3	6	22
Stomach	151	2	2	9
Skin	173	2	4	9
Vulva	184	2	4	18
Hodgkin's lymphoma	201	2	4	3
Acute lymphoblastic leukemia	204.1	2	4	3
Multiple myeloma	203	1	2	2
Brain	191-192	1	2	3
Ovary	183	1	2	3
Testicle	186	1	2	10
Total n. of tumors	–	48	100	6316
No second tumors	–	1232	96.2	6061

ICD-9: International Classification of Diseases 9th Edition. http://www.cdc.gov/nchs/icd9.htm. Because of rounding, percentages may not total 100.

chemotherapy lines (Table 5). Having received more than two lines of chemotherapy plus radiotherapy did not have a negative impact on TF2T. However, this conclusion was drawn from the observation of a cohort of only 80 patients (*data not shown*). In a separate analysis, no variables, including age, sex and advanced Ann Arbor stage disease, appeared to be associated with the development of MDS/AML. Lung cancer was most frequent in males, but the association was not statistically significant.

Discussion

Because of successful treatment, a large number of patients with Hodgkin's lymphoma become long-term survivors, and thus remain at life-long risk of late sequelae. Better treatments[2-6] have improved the life expectancy of patients with non-Hodgkin's lymphoma, and the risk of late treatment effects is now becoming an important concern. Despite a few reports to the contrary,[14-16] the majority of published studies have shown that non-Hodgkin's lymphoma patients are at a greater risk of second malignancies.[7-13]

Table 3. Standardized incidence risk (SIR) and absolute excess risk (AER per 10,000 person-years) of second cancer analyzed according to demographics, baseline characteristics and treatments (41 cases out of 48).*

Factor	*Observed*	*Expected*	*SIR (95%CI)*	*AER*	*p value***
Gender					
Female	16	16.0	1.00 (0.57-1.62)	0.00	0.618
Male	25	21.4	1.17 (0.76-1.73)	+10.1	
IPI score					
0-1	20	17.6	1.13 (0.69-1.75)	+6.60	0.959
2-5	21	18.2	1.15 (0.71-1.76)	+10.8	
Chemotherapy					
PCB-epidoxorubicin	24	19.7	1.22 (0.78-1.81)	+11.1	0.610
PCB-idarubicin	7	6.2	1.12 (0.45-2.31)	+6.87	
PCB-sequential	6	4.8	1.24 (0.46-2.70)	+13.8	
CHOP or CHOP-like	4	6.6	0.60 (0.16-1.55)	-42.1	
RT-IF					
No	32	27.6	1.16 (0.79-1.63)	+9.72	0.549
Yes	9	9.7	0.92 (0.42-1.75)	-3.52	
N. of chemotherapies lines					
1	29	27.2	1.06 (0.71-1.53)	+3.73	0.804
2	7	6.7	1.04 (0.42-2.15)	+2.46	
3	5	3.4	1.45 (0.47-3.39)	+34.2	
Years of first treatment					
1988-91	10	11.6	0.86 (0.41-1.59)	-7.93	0.678
1992-95	17	12.5	1.36 (0.79-2.18)	+20.2	
1996-99	9	7.9	1.14 (0.52-2.15)	+8.35	
2000-03	5	5.4	0.93 (0.30-2.16)	-4.18	

**Five MDS and two skin cancers cases were excluded. **Chi-square test for unequal SIR 315. RT-IF: involved field radiotherapy; IPI: International Prognostic Index for aggressive lymphoma; PCB-epidoxorubicin: ProMECE-CytaBOM: (methylprednisolone, cyclophosphamide, epidoxorubicin -or doxorubicin-, etoposide, cytarabine, bleomycin, vincristine, methotrexate); PCB-idarubicin: ProMICE-CytaBOM (methylprednisolone, cyclophosphamide, idarubicin, etoposide, cytarabine, bleomycin, vincristine, methotrexate); PCB-Sequential: sequential ProMECE instead of the classical cycling regimen; CHOP: cyclophosphamide, doxorubicin vincristine, prednisolone.*

These conflicting results could be explained in part by the different epidemiological and statistical methods utilized for evaluating the incidence of second cancers.[38] Two commonly used epidemiological designs are cohort and case-control studies. Among cohort studies, sources of information include population-based cancer registries and clinical trial databases. The major disadvantage of population-based studies is that treatment data are limited, when present. However, these studies usually include a large number of patients, and thus even a small risk can be detected. In contrast, clinical trial databases often include a relatively low number of patients thus not enabling the assessment of small risks. However, these databases contain precise information

Table 4. Standardized incidence risk (SIR) and absolute excess risk (AER per 10,000 person-years) of second cancer (41 cases out of 48)* related to age-, and calendar period- specific incidence derived from the ISS** database, by ICD-9 code from 140 to 208.

Cohort Age	*Observed failures*	*Expected failures*	*SIR*	*95% C.I.*	*AER*
20–39	2	0.09	23.0	5.76-92	+20.6
40–59	6	1.37	4.39	1.97-9.78	+18.6
60–64	5	5.69	0.88	0.37-2.11	-7.49
65–69	11	9.51	1.16	0.64-2.09	+15.2
70–74	11	10.4	1.06	0.58-1.91	+8.16
75–79	3	6.84	0.44	0.14-1.36	-102
80+	3	3.42	0.88	0.28-2.72	-25.6
Total	41	37.3	1.10	0.81-1.49	+5.70

**Five MDS and two skin cancers cases were excluded; **ISS: Istituto Superiore di Sanità (Italian National Institute of Health).*

Table 5. Gray-Fine univariate regression, accounting for competing risk of death, for demographics, baseline characteristics and treatments of the patients at diagnosis of DLBCL.

Covariate	*Coef.*	*SE*	*HR*	*p*
Age >60 years old	0.783	0.295	2.19	0.008
Gender, Male	0.260	0.294	1.30	0.38
LDH > UNL	-0.007	0.300	0.99	0.98
Stage III-IV	-0.218	0.285	0.44	0.80
PS 2-4	0.061	0.521	1.06	0.91
Extra nodal sites >1	-0.196	0.339	0.82	0.56
IPI 3-5	0.070	0.287	1.07	0.81
RT-IF Yes	-0.314	0.354	0.73	0.37
Chemotherapy				
PCB epidoxorubicin	1.00			
PCB idarubicin	-0.293	0.408	0.75	0.47
PCB sequential	-0.080	0.432	0.92	0.85
CHOP or CHOP-like	0.095	0.434	1.10	0.83
N. of lines of chemotherapy				
1 line	1.00			
2 lines	-0.455	0.384	0.63	0.24
3 lines	0.035	0.463	1.04	0.94

LDH: lactate dehydrogenase; UNL :institutional upper normal limit; Stage: Ann Arbor staging; PS: performance status; IPI: International Prognostic Index for aggressive lymphoma; RT-IF: involved field radiotherapy. PCB-epidoxorubicin: ProMECE-CytaBOM: (methylprednisolone, cyclophosphamide, epidoxorubicin -or doxorubicin-, etoposide, cytarabine, bleomycin, vincristine, methotrexate); PCB-idarubicin: ProMICE-CytaBOM (methylprednisolone, cyclophosphamide, idarubicin, etoposide, cytarabine, bleomycin, vincristine, methotrexate); PCB-Sequential: sequential ProMECE instead of the classical cycling regimen; CHOP: cyclophosphamide, doxorubicin vincristine, prednisolone.

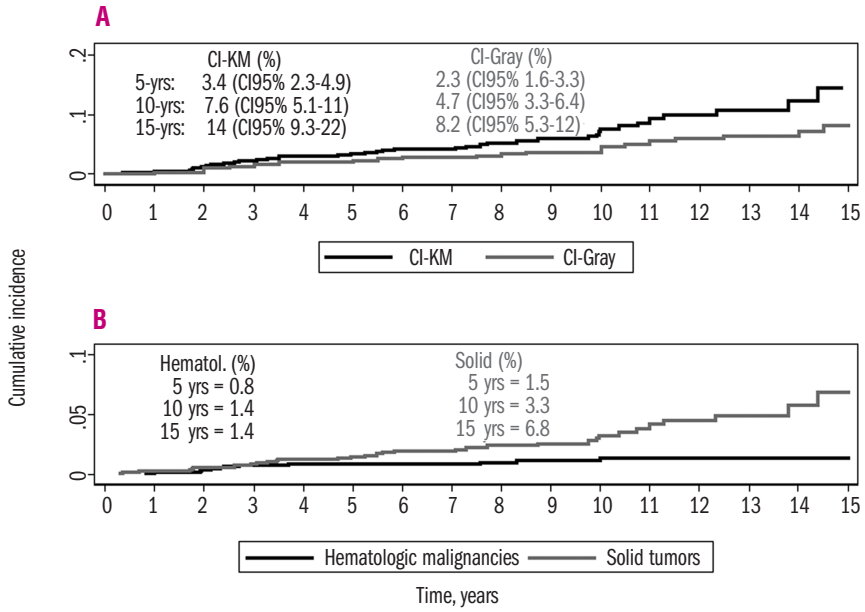

Figure 1. (A) Cumulative incidence of second cancer in the Kaplan-Meier (CI - KM) estimation compared to the cumulative incidence according to Gray (CI - Gray). (B) Cumulative incidence of second cancer according to Gray, divided into solid and hematologic malignancies.

on clinical characteristics, treatments and occasionally data on long-term follow-up. The methodology and statistical approach utilized for estimation of second malignancies is also important. For a disease such as diffuse large B-cell lymphoma, in which early deaths are frequent due to recurrent or refractory disease, any cumulative incidence evaluated by the Kaplan-Meier method will result in an overestimated percentage of second malignancies. Thus, correction by a competing risk model is necessary. Furthermore, as time, sex, environmental and genetic factors influence and modify the incidence of cancer, person-years analysis per geographical region, age-, sex- and calendar period-specific incidence ratios are needed for optimal evaluation of the SIR and AER. In our study, the incidence of second malignancy was analyzed in a cohort of 1280 patients treated for diffuse large B-cell lymphoma. The source of the data was GISL, which maintains a database on treatment and follow-up of all patients with non-Hodgkin's lymphoma enrolled in clinical trials. Although performed in a homogeneous and relatively large cohort of patient with a long-term follow-up, the results of this research should be interpreted cautiously due to the retrospective design of the study. Furthermore, as data managers at participating centers were not urged to specifically report second cancers, our results could slightly underestimate the risk of such cancer. Our research, exclusively examining patients treated for diffuse large B-cell lymphoma could only be conclusively compared with a small number of other studies[15,16] that explored the same topic, and reports of those studies revealed partially similar findings.

Our results showed that the overall incidence of second malignancies is not significantly increased. However, comparing our diffuse large B-cell lymphoma cohort with the general Italian population, the SIR of cancer was found to be increased in the age groups 20-39 and 40-59 years old. The incidence in patients aged over 59 years old matched that in the Italian general population. Thus, the risk of second cancer was clearly age-related: in young patients a strongly increased risk was observed while the incidence in patients aged more than 59 years, when first treated for non-Hodgkin's lymphoma, matched the incidence in the general population. We observed an increased cancer-specific risk for lung, colorectal and prostate cancer, and a decreased risk for breast cancer, but the differences from the Italian general population were not statistically significant. The three female patients who developed breast cancer were over 58 years of age; no cases were observed in younger women, likely due to the hormonal changes induced by chemotherapy.[39] Baseline characteristics and the different treatment modalities did not have an influence on the SIR. In particular, having received radiotherapy after chemotherapy or more than one line of chemotherapy did not have a negative impact on SIR. One possible explanation for these findings could be the use of involved field radiotherapy with a maximum dose of 3.8 Gy or too short a follow-up period. Furthermore, it is possible that relapsed or refractory patients who received salvage treatments died before living long enough to develop a second cancer. Some authors observed an increased SIR for AML,[10,12] others for AML/MDS[15], while others did not find any increase for AML and did not evaluate the SIR for MDS.[16] As we could not evaluate the SIR for AML and MDS for technical reasons, we calculated the crude rate in our cohort and compared this with crude rates evaluated in other research. We observed a crude rate for MDS/AML together of 1.3 (95% CI, 0.5-2.5) which is an intermediate value between the 1.1 (95% CI, 0.6-2.5) and 2.0 (95% CI, 0.9-3.7) calculated in other reports that showed[15] or failed to show[16] an increased risk for AML/MDS or AML, respectively. The cumulative incidence of second tumors was similar to those reported by others.[15,16] However, while we did not observe any plateau in the curve of solid tumors, the cumulative incidence of hematologic malignancies stopped increasing

after 10 years. The only factor with a negative impact on the probability of remaining free of second cancers was age over 60 years old at diagnosis of diffuse large B-cell lymphoma; the other baseline characteristics and treatment did not have any significant influence.

In conclusion, data from our homogeneous GISL cohort of patients treated for diffuse large B-cell lymphoma showed that it is principally young patients who have an increased risk of second cancers, while older patients are at the same risk as the normal Italian population. Our cohort had a median age of 58 years, and this is likely one of the reasons that the overall risk did not appear elevated with respect to the entire cohort. However, the cumulative incidence of solid tumors is still increasing after 12-15 years, and a longer follow-up could be necessary to confirm that the risk in our cohort is the same as that observed in the normal population.

Authorship and Disclosures

SS: conception and design of the study, acquisition, analysis and interpretation of the data, final approval of the version to be published; LM and RM: statistical analysis, data collection, interpretation of data and creation of the tables and figures. SS and LM wrote the manuscript. SS, AB, SP, GB, FA, MB, PM and FM participated in the patients' care, data recording, and the interpretation and analysis of data. All authors contributed critically to the drafting of the article, and approved the final draft. The preliminary results of this study were presented at the American Society of Hematology annual meeting in 2007 (Atlanta, USA). The authors reported no potential conflicts of interest.

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