CD4 and CD8 T-Lymphocyte Apoptosis Can Predict Radiation-Induced Late Toxicity: A Prospective Study in 399 Patients

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Abstract

Purpose: Predicting late effects in patients treated with radiation therapy by assessing *in vitro* radiation-induced CD4 and CD8 T-lymphocyte apoptosis can be useful in individualizing treatment.

Experimental Design: In a prospective study, 399 curatively irradiated patients were tested using a rapid assay where fresh blood samples were *in vitro* irradiated with 8 Gy X-rays. Lymphocytes were collected and prepared for flow cytometric analysis. Apoptosis was assessed by associated condensation of DNA. The incidences of late toxicities were compared for CD4 and CD8 T-lymphocyte apoptoses using receiver-operating characteristic curves and cumulative incidence.

Results: No association was found between early toxicity and T-lymphocyte apoptosis. Grade 2 and 3 late toxicities were observed in 31% and 7% of patients, respectively. More radiation-induced T-lymphocyte apoptosis was significantly associated with less grade 2 and 3 late toxicity (Gray's test, $P < 0.0001$). CD8 (area under the curve = 0.83) was more sensitive and specific than CD4. No grade 3 late toxicity was observed for patients with CD4 and CD8 values greater than 15% and 24%, respectively. The 2-year cumulative incidence for grade 2 or 3 late toxicity was 70%, 32%, and 12% for patients with absolute change in CD8 T-lymphocyte apoptosis of ≤16, 16 to 24, and >24, respectively.

Conclusions: Radiation-induced T-lymphocyte apoptosis can significantly predict differences in late toxicity between individuals. It could be used as a rapid screen for hypersensitive patients to radiotherapy. In future dose escalation studies, patients could be selected using the apoptosis assay.

The success of radiation therapy depends mainly on total radiation dose homogeneously delivered to the tumor. However, this dose is limited by the tolerance of normal tissues in the irradiated volume (1). Patients display a diverse spectrum of toxicity for any given radiation therapy schedule (2). Whereas toxicity risks for populations of patients are known, the determination of an individual's normal-tissue radiosensitivity is seldom possible before treatment. Therefore, current practice standards commonly prescribe radiation dose according to clinical scenarios without regard to the genotype or phenotype of the individual being irradiated (3).

There is wide variation among patients in normal-tissue tolerance and, hence, in reaction to the same curative dose (4). About 5% of cancer patients receiving radiation therapy display "elevated" normal-tissue reactions (1, 4). Patients with genetic disorders, such as ataxia telangiectasia or Nijmegen breakage syndrome, and a subset of patients that show severe reactions to radiation therapy but no obvious congenital defects display enhanced radiosensitivity (5, 6). Identification of radiosensitive patients may permit the dose to be increased in nonsensitive patients, which in turn should increase local control and cure (7).

Since 1996, we have developed (8, 9) and retrospectively evaluated (10, 11) a rapid (~48 hours) diagnostic assay based on flow cytometric assessment of radiation-induced T-lymphocyte apoptosis. We concluded that the assay had clear potential as a useful indicator for selecting individuals likely to display an increased probability of toxicity to radiation therapy.

In this article, we report our prospective experience with this rapid predictive test of radiation-induced late effects.

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Received 1/3/05; revised 7/8/05; accepted 7/19/05.

Grant support: Swiss Cancer League grants KFS 00539-9-1997 and SKL 00778-2-1999.

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Note: Presented at the 94th Annual Meeting of the American Association of Cancer Research, July 11-14, 2003, Washington, District of Columbia; the 12th European Cancer Conference, September 21-25, 2003, Copenhagen, Denmark; and the 45th Annual Meeting of the American Society for Therapeutic Radiology and Oncology, October 19-23, 2003, Salt Lake City, Utah.

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doi:10.1158/1078-0432.CCR-04-2634

Materials and Methods

Patients. Between April 1998 and October 2001, a total of 399 patients with miscellaneous cancers were included in the Swiss Cancer Research/Swiss Cancer League (KFS 00539-9-1997/SKL 00778-2-1999) prospective study evaluating the predictive value of CD4 and CD8 T-lymphocyte apoptosis on the development of radiation-induced late side effects. The Lausanne University Ethical Committee approved this study. The eligibility criteria included adult patients (age ≥ 18 years) with pathologically confirmed cancer to be treated using a curative dose of pre- or postoperative or definitive radiation therapy; no evidence of distant metastases; no previous radiation therapy; no disease known to interfere with radiosensitivity; no concomitant chemotherapy; and written informed consent before enrollment. Six patients (2%) who refused radiation therapy after the assay were excluded from subsequent data analysis.

Radiation-induced apoptosis. Details of the assay including reproducibility and intra- and interdonor variations have been reported elsewhere (8–11). Briefly, heparinized whole blood collected before starting radiation therapy was diluted 1:10 in RPMI 1640 medium containing 20% fetal bovine serum. This was irradiated at 0 and 8 Gy and incubated for 48 hours. The cells were then labeled with FITC-conjugated anti-CD4 or anti-CD8 monoclonal antibodies, RBCs were lysed, and the DNA of the remaining cells was stained with propidium iodide. Samples were measured using a FACScan flow cytometer and data analysis was done using CellQuest software (Becton Dickinson, San Jose, CA). Apoptotic CD4 and CD8 T lymphocytes were defined as those cells staining positively for their cell type–specific antibodies and displaying reduced propidium iodide fluorescence and cell size. These cells were previously shown to be apoptotic using the terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling assay (9). Data from at least 10,000 cells per sample were acquired.

Radiation treatment. Radiation therapy was implemented using 6 to 18 MV X-rays or ^{60}Co photons. Planning treatment volumes were determined according to the initial cancer site. All patients were examined clinically every week; treatment verification was ensured using port films; and shielding was customized to minimize exposed normal-tissue volume.

Toxicity assessment. During radiation therapy, including a time period of 6 weeks following treatment, early toxicities were evaluated according to the Common Toxicity Criteria of the National Cancer Institute version 2.0 (12). During the trimonthly follow-up visits, late side effects were graded according to the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer system in all patients by the same physician without knowledge of the apoptosis test results so as not to bias the evaluation (13). The timing of late side effects from 6 weeks post–radiation therapy up to 2 years corresponds to the time of observation of the worst late toxicity grade. Thus, patients are counted only once for late side effects. The exposures to concomitant chemotherapy (head and neck cancer patients) and post–radiation therapy chemotherapy (mainly breast cancer patients) were also analyzed to evaluate the contribution of chemotherapy on late side effects.

Statistical methods. Data were summarized by frequency and percentage for categorical variables and by means, SDs, median, and range for continuous variables. Three categories of absolute change in the percentages of CD4 and CD8 cells in apoptosis before and after exposure to 8-Gy irradiation were constructed around the 33% quantiles: ≤10, 10 to 15, and >15 for CD4 and ≤16, 16 to 24, and >24 for CD8. The correlation between radiation-induced CD4 and CD8 T-lymphocyte apoptosis was evaluated with the Spearman correlation coefficient.

All survival times were calculated from the date at start of radiotherapy. Overall survival, relapse-free survival, and complication-free survival rates were estimated by the Kaplan-Meier method using the following first-event definitions: death for overall survival, local or distant recurrence or death for relapse-free survival, and grade 2 or 3 side effects for complication-free survival (14). For overall survival, patients alive at the last follow-up visit were censored. For relapse-free survival, patients alive and never relapsed were censored at the last follow-up visit. For complication-free survival, patients alive who never experienced a grade ≥2 side effect were censored at the last follow-up visit. Patients who relapsed before a grade ≥2 side effect were censored at the time of relapse. Confidence intervals (95% CI) were also determined.

The log-rank test was used to identify significant categorical variables for each of the three survival curves (14). The Cox proportional hazard-regression-model was used for multivariate analysis (15). Absolute changes in CD4 and CD8 counts were evaluated as continuous and categorical variables. Their independent effects were evaluated from the likelihood ratio statistics. The cumulative incidences for side effects and relapse or death were estimated from a competing risks model using the cause-specific hazard functions and the composite relapse-free survival and complication-free survival distribution (16) and were compared using Gray's test (17). The competing risks method was preferred to take into account an eventual difference in the survival distribution of relapse as a first event. Stata was used for all statistical analyses and the cmprsk R package was used for Gray's test.

To complement the analysis, univariate and multivariate receiver-operator characteristic curve analyses for CD4 and CD8 were done to identify patients who experienced at least a grade 2 late side effect within 2 years (18). The empirical area under the receiver-operating characteristic curves and the respective 95% CIs were compared for CD8 and CD4 in determining their respective contributions to sensitivity, specificity, and positive and negative predicted values.

Results

Patient characteristics and treatment modalities. Patient characteristics of the 393 patients including treatment modalities are presented in Table 1. Median age was 60 years (range, 18-85 years). Breast cancer represented 38% of the patients, head and neck 19%, genitourinary 11%, and gastrointestinal 10%. The other sites (22%) represented lymphoma, uterine cancer, lung cancer, soft-tissue sarcoma, pituitary gland adenoma, chordoma, glioblastoma, myeloma, meningioma, plasmacytoma, and thymoma.

In the majority of patients (n = 362, 92%), fractionation consisted of 1.8 to 2 Gy per day. Median total dose was 66 Gy (range, 20-83.7 Gy) and 335 (85%) patients received a total dose of ≥50 Gy. Median treatment duration was 45 days (range, 1-87 days).

Radiation-induced apoptosis. Radiation-induced apoptosis characteristics according to CD4 and CD8 T-lymphocyte subpopulations were measured for all patients. Median radiation-induced apoptosis was 12.5 (mean, 13.6; SD, 7.0; range, −3.6 to 45.5) and 20.7 (mean, 22.3; SD, 11.3; range, 3.4-70.8) for CD4 and CD8, respectively. CD4 and CD8 apoptoses were significantly correlated (r = 0.55, P < 0.0001).

Toxicity. Grade 1, 2, or 3 early toxicity (Common Toxicity Criteria of the National Cancer Institute version 2.0) was observed in 143 (36%), 161 (41%), and 69 (18%) patients, respectively. No significant association was found between early side effects and radiation-induced T-lymphocyte apoptosis (Table 2).

Late side effects were not evaluated for seven patients, six of whom had early relapses and one died. Among the remaining 386 patients, 164 (42%), 121 (31%), and 28 (7%) experienced grade 1, 2, and 3 side effects, respectively (Table 3). These latter patients experienced 32 grade 3 late side effects:

Table 1. Patients' characteristics

Characteristic			
Sex			
Male/female (%)	166/227 (42.2/57.8)		
Median age, y (range)	60 (18-85)		
Cancer site (%)	Male	Female	Total
Breast	2 (1.2)	147 (64.8)	149 (37.9)
Head and neck	60 (36.1)	15 (6.6)	75 (19.1)
Genitourinary	42 (25.3)	0 (0.0)	42 (10.7)
Prostate	36 (85.7)	—	
Testis	5 (11.9)	—	
Bladder	1 (2.4)	—	
Gastrointestinal	22 (13.3)	17 (7.5)	39 (9.9)
Anal canal	1 (4.6)	7 (41.2)	8 (20.5)
Colon	1 (4.6)	—	1 (4.6)
Pancreas	1 (4.6)	1 (5.9)	2 (5.1)
Rectum	16 (72.7)	9 (52.9)	25 (64.1)
Esophagus	3 (13.6)	—	3 (7.7)
Lymphoma	18 (10.8)	8 (3.5)	26 (6.6)
Hodgkin	6 (33.3)	2 (25.0)	8 (30.8)
Non-Hodgkin	12 (66.7)	6 (75.0)	18 (69.3)
Gynecology	0 (0.0)	22 (9.7)	22 (5.6)
Cervix uteri	—	2 (9.1)	
Corpus uteri	—	19 (86.4)	
Vulva	—	1 (4.6)	
Lung	10 (6.0)	9 (4.0)	19 (4.8)
Skin	3 (1.8)	3 (1.3)	6 (1.5)
Soft tissue	1 (0.6)	2 (0.9)	3 (0.8)
Other*	8 (4.8)	4 (1.8)	12 (3.1)

14 subcutaneous, 5 skin, 9 salivary gland, 1 brain, 1 pharynx, 1 ear, and 1 mucous membrane. Four patients had simultaneous subcutaneous late side effects associated with skin (2 patients), salivary gland (1 patient), and mucous membrane (1 patient). For 16 patients, these grade 3 late side effects occurred before 6 months and for 12 patients, they occurred between 6 and 12 months after radiotherapy.

A total of 330 patients (85%) were evaluated for late toxicity at 2 years; 56 patients relapsed or died before 2 years. At the 2-year visit, 24 patients maintained the same grade 3 late toxicity; 4 patients relapsed before 2 years.

Grade 3 late toxicities were mainly observed for patients with breast (9%) and head and neck (16%) cancer. There was an inverse relationship between the incidence of late side effects and percent CD4 or CD8 apoptosis. A decreased percentage of grade $\geq$2 late toxicity was observed for increasing values of CD4 and CD8. No grade 3 side effects were observed for patients with CD4 apoptosis >15% and CD8 apoptosis >24%.

A multivariate receiver-operating characteristic analysis for CD4 and CD8 was done among the 348 patients (89%) who were evaluated at 2 years (330 patients) or who experienced a grade $\geq$2 late side effect before 2 years (18 patients). The area under the receiver-operating characteristic curve was greater for CD8 (0.827; 95% CI, 0.78-0.87) than for CD4 (0.714; 95% CI, 0.66-0.77). The addition of CD4 into the model did not contribute significantly in separating the two groups (area under the receiver-operating characteristic curve = 0.84). Sensitivity and specificity were near 80% for cutoff values of CD4 >15% and $\leq$10%, respectively, and they were near 90% for cutoff values of CD8 >24% and $\leq$16%, respectively. The positive predicted value was 83% for CD8 $\leq$16% and the negative predicted value was 86% for CD8 >24% in this population of patients, where the overall prevalence of grade $\geq$2 late toxicity was estimated at 43%. Similar area under the receiver-operating characteristic curve results were observed when considering only grade 3 late toxicity with values of 0.89, 0.84, and 0.92 for CD4, CD8, and both, respectively.

The positive predicted value for grade 3 toxicity was 20% for CD8 $\leq$16% and the negative predicted value was 98.5% for CD8 >24% in this population of patients, where the overall prevalence of grade 3 late side effects was estimated at 8%.

Patterns of failure. Median follow-up was 30 months and all patients had a follow-up of at least 22 months. There were 98 relapses (25%) and 71 patients died (18%). As a first event, there were 143 side effects (36%), 71 relapses (18%), and 5 deaths (1%). Fifteen patients had a local recurrence after a Radiation Therapy Oncology Group grade 2 side effect, six patients had a distant relapse afterwards, and one patient died. At 3 years, 39 patients were event-free. No side effects, one relapse, and two deaths occurred as a first event after 3 years.

The 3-year overall survival and relapse-free survival rates were 80% (95% CI, 75-85) and 73% (95% CI, 67-77), respectively

Table 1. Patients' characteristics (Cont'd)

Characteristic	No adjuvant treatment	Hormone therapy	Chemotherapy	Total
Cancer site (%)	231 (58.8)	73 (18.6)	89 (22.6)	
Breast	31 (20.8)	61 (40.9)	57 (38.3)	149
Head and neck	67 (89.3)	0 (0.0)	8 (10.7)	75
Genitourinary	26 (61.9)	12 (28.6)	4 (9.5)	42
Prostate	20	12	4	36
Testis	5	—	—	5
Bladder	1	—	—	1
Gastrointestinal	23 (58.9)	0 (0.0)	16 (41.1)	39
Anal canal	2	—	6	8
Colon	—	—	1	1
Pancreas	2	—	—	2
Rectum	16	—	9	25
Esophagus	3	—	—	3
Lymphoma	26 (100)	0 (0.0)	0 (0.0)	26
Hodgkin	8	—	—	8
Non-Hodgkin	18	—	—	18
Gynecology	21 (95.5)	0 (0.0)	1 (4.5)	22
Cervix uteri	2	—	—	2
Corpus uteri	19	—	—	19
Vulva	—	—	1	1
Lung	18 (94.7)	0 (0.0)	1 (5.3)	19
Skin	6 (100)	0 (0.0)	0 (0.0)	6
Soft tissue	2 (66.7)	0 (0.0)	1 (33.3)	3
Other*	11 (91.7)	0 (0.0)	1 (8.3)	12
Brachytherapy †				
No	369 (94.0)			
Yes	24 (6.0)			

NOTE: Data are presented as *n* (%).
*Other locations (M/F): pituitary gland adenoma (1/0), chordoma (1/0), glioblastoma (2/1), multiple myeloma (1/0), meningioma (1/2), solitary plasmocytoma (0/1), porocarcinoma (1/0), and thymoma (1/0).
† Brachytherapy in 20 patients with gynecologic, 2 with head and neck, 1 with gastrointestinal, and 1 with soft-tissue tumor.

(Table 4). No significant differences were observed for the percent apoptoses of CD4 and CD8 T-lymphocytes as far as overall survival and relapse-free survival are concerned.

The 2-year overall complication-free survival rate was 59% (95% CI, 54-64). When analyzing separately the two major disease groups (i.e., breast cancer and head and neck cancer), CD8 T-lymphocyte apoptosis significantly predicted the 2-year complication-free survival either in all patients or in patients who did not receive adjuvant or concomitant chemotherapy. However, CD4 T-lymphocyte apoptosis was not found to be significant in either all head and neck cancer patients ($n = 75$) or those not receiving chemotherapy ($n = 67$).

The 2-year cumulative incidence rates for grade ≥2 side effects were 70%, 32%, and 12% for patients with change in the three categories of percent CD8 T-lymphocyte apoptosis, ≤16, 16 to 24, and >24, respectively ($P < 0.0001$; Table 5; Fig. 1A). The relapse component of the composite relapse-free survival and complication-free survival rates was similarly distributed between the three categories of CD8 with estimated cumulative relapse incidences of 17%, 24%, and 22%, respectively (Table 5; Fig. 1B).

The 3-year cumulative incidence rates for grade ≥2 side effects were 59%, 30%, and 19% for patients with change in the three categories of percent CD4 T-lymphocyte apoptosis, ≤10, 10 to 15, and >15, respectively ($P < 0.0001$; Table 5). The relapse component of the composite relapse-free survival and complication-free survival rates was similarly distributed between the three categories of CD4 with estimated 3-year cumulative relapse incidences of 14%, 23%, and 26%, respectively.

These estimates are compatible with the ones obtained for complication-free survival, which treat relapses and death as censored observations rather than as competing risks. Because relapses and deaths as a first event were not significantly different between the categories of CD4 and CD8, it is safe to conclude that these variables have a significant prognostic effect on the incidence of grade 2 and 3 late side effects.

As far as the complication-free survival rates are concerned, CD8 T-lymphocyte apoptosis has a greater effect than CD4. CD8 T-lymphocyte apoptosis retains statistical significance when adjusted for CD4 ($P < 0.0001$) whereas CD4 T-lymphocyte apoptosis is at the limit of statistical significance when adjusted for CD8 ($P = 0.053$).

Table 2. Early side effects according to radiation-induced CD4 and CD8 T-lymphocyte apoptosis (Common Toxicity Criteria of the National Cancer Institute version 2.0)

	Grade 0	Grade 1	Grade 2	Grade 3
Number of patients	20 (5%)	143 (36%)	161 (41%)	69 (18%)
Percent CD4 apoptosis				
≤10	5 (3.6%)	56 (40.6%)	55 (39.9%)	22 (15.9%)
10-15	10 (8.7%)	38 (33.0%)	42 (36.5%)	25 (21.7%)
>15	5 (3.6%)	49 (35.0%)	64 (45.7%)	22 (15.7%)
Percent CD8 apoptosis				
≤16	5 (4.1%)	44 (36.4%)	47 (38.8%)	25 (20.7%)
16-24	6 (4.8%)	47 (37.3%)	48 (38.1%)	25 (19.8%)
>24	9 (6.2%)	52 (35.6%)	66 (45.2%)	19 (13.0%)
CD4 ≤10% and CD8 ≤16%	2 (2.8%)	31 (43.1%)	27 (37.5%)	12 (16.7%)
Intermediate	15 (6.3%)	81 (34.2%)	94 (39.7%)	47 (19.8%)
CD4 >15% or CD8 >24%	3 (3.6%)	31 (36.9%)	40 (47.6%)	10 (11.9%)

Discussion

For assays of normal-tissue radiation response, blood is considered the tissue of choice because of the ease of collection in a standardized, patient-convenient manner. Following exposure to ionizing radiation, lymphocytes die via apoptosis, which can be readily assessed using various methods (19, 20). CD4 and CD8 T-lymphocytes were chosen in this study because of their better flow cytometrical separation compared with other types of lymphocytes (8, 9). Lymphocyte-based predictive tests using methods other than apoptosis were also described (21, 22) and studies using nonblood cells such as skin fibroblasts were proposed (23, 24). Several methods were developed with other biomarkers for the prediction of intrinsic radiosensitivity (24, 25). The micronucleus assay can be used as an indicator of normal-tissue or tumor radiosensitivity (22, 26). Lee et al. (27) reported that radiation-induced micronuclei in lymphocytes of prostate cancer patients can be used to predict gastrointestinal or genitourinary toxicity. Recently, in a retrospective study, the number of lethal chromosomal aberrations in *in vitro* irradiated peripheral blood lymphocytes correlated well with radiation-induced fibrosis in patients with breast cancer (28).

The relationship between cellular radiosensitivity and radiation-induced apoptosis is not certain. In several cell lines, high apoptotic frequency is correlated with increased radiosensitivity (20, 29, 30). Our previous data showed that lymphocytes from patients displaying increased radiation toxicity showed low, and not high, levels of radiation-induced apoptosis (10). Survival fraction at 2 Gy from the skin fibroblast clonogenic assays has been shown to be effective in predicting intrinsic radiosensitivity; however, it is a time-consuming (ca. 2-3 months) method for use as a diagnostic assay in routine practice (23, 24). Lymphocyte assays are rapid and reproducible between donors (9) and between healthy donors and cancer patients (11, 31). The nonapoptotic methods require more time whereas results of micronucleus assay in peripheral blood lymphocytes are available within 2 weeks.

In the present study, we confirmed prospectively that radiation-induced T-lymphocyte apoptosis significantly predicted grade 2 and 3 late effects ($P < 0.0001$). Patients with grade 3 late effects ($n = 25$) showed CD4 or CD8 radiation-induced apoptosis below

Table 3. Late side effects according to radiation-induced CD4 and CD8 T-lymphocyte apoptosis

	Maximal Radiation Therapy Oncology Group grade			
	0	1	2	3
Number of patients	73 (19%)	164 (43%)	121 (31%)	28 (7%)
Percent CD4 apoptosis				
≤10	18 (13.2%)	33 (24.3%)	61 (44.9%)	24 (17.6%)
10-15	24 (21.4%)	52 (46.4%)	32 (28.6%)	4 (3.6%)
>15	31 (22.5%)	79 (57.3%)	28 (20.3%)	–
Percent CD8 apoptosis				
≤16	10 (8.6%)	18 (15.4%)	65 (55.6%)	24 (20.5%)
16-24	26 (21.1%)	56 (45.5%)	37 (30.1%)	4 (3.2%)
>24	37 (25.4%)	90 (61.6%)	19 (13.0%)	–
CD4 ≤10% and CD8 ≤16%	4 (5.7%)	5 (7.1%)	40 (57.1%)	21 (30.0%)
Intermediate	51 (22.0%)	107 (46.1%)	67 (28.9%)	7 (3.0%)
CD4 >15% or CD8 >24%	18 (21.4%)	52 (61.9%)	14 (16.7%)	–

Table 4. Prognostic factors for survival in 393 patients

	Percent overall survival (3-y 95% CI)	Percent relapse-free survival (3-y 95% CI)	Percent complication-free survival (2-y 95% CI)
All patients	80 (75-85)	73 (67-77)	59 (54-64)
Age, y			
‹60	81 (72-87)	71 (62-77)	56 (48-63)
≥60	80 (73-86)	75 (67-81)	62 (55-69)
P	0.884	0.498	0.333
CD8			
≤16%	79 (68-87)	71 (60-80)	21 (14-29)
16-24%	78 (66-86)	70 (60-78)	64 (54-72)
›24%	84 (75-89)	76 (66-83)	86 (79-91)
P	0.448	0.729	‹0.001
CD4			
≤10%	83 (75-89)	75 (65-82)	35 (27-43)
10-15%	78 (62-87)	72 (60-80)	65 (55-74)
›15%	80 (71-86)	71 (61-78)	78 (70-84)
P	0.910	0.597	‹0.001
Cancer site			
Breast	97 (89-99)	91 (82-96)	52 (44-60)
Head and neck	66 (50-78)	64 (51-75)	45 (33-57)
Genitourinary	95 (82-99)	86 (68-94)	80 (65-90)
Gastrointestinal	66 (47-80)	56 (38-71)	78 (60-89)
Hematologic	82 (58-93)	68 (42-84)	65 (42-81)
Gynecologic	91 (68-98)	76 (51-89)	81 (57-92)
Lung	24 (5-51)	0	59 (31-79)
Other	48 (10-79)	43 (18-67)	50 (24-71)
P	‹0.001	‹0.001	0.001

NOTE: Log-rank test was used for all *P* calculations.

the median ($P < 0.0001$). CD8 was more sensitive and more specific than CD4 T-lymphocyte apoptosis from the results of the multivariate receiver-operating characteristic analyses and the Cox model. No significant association was found either between early side effects and radiation-induced T-lymphocyte apoptosis or between early and late side effects. Others confirmed the lack of correlation between cellular radiosensitivity and early skin reactions (32–34). However, a number of authors have confirmed the correlation between cellular radiosensitivity and late effects (24, 25, 35, 36).

The mechanism behind the relationship between increased radiation toxicity and reduced apoptotic response in peripheral blood lymphocytes is still unclear. In most proliferating cells, including lymphoblastoid cells, apoptosis

Table 5. Number and cumulative incidence of first event according to radiation-induced CD4 and CD8 T-lymphocyte apoptosis

	Total number of events	Percent 2-y cumulative incidence of grade ≥2 late toxicity	*P*	Percent 3-y cumulative incidence of relapse	*P*
CD8 apoptosis					
≤16%	105	70.0	‹0.0001	16.8	NS*
16-24%	66	31.7		23.7	
›24%	48	12.3		22.0	
CD4 apoptosis					
≤10%	99	58.7	‹0.0001	14.3	‹0.0001
10-15%	59	30.4		22.9	
›15%	61	19.3		25.8	

*NS, not statistically significant.

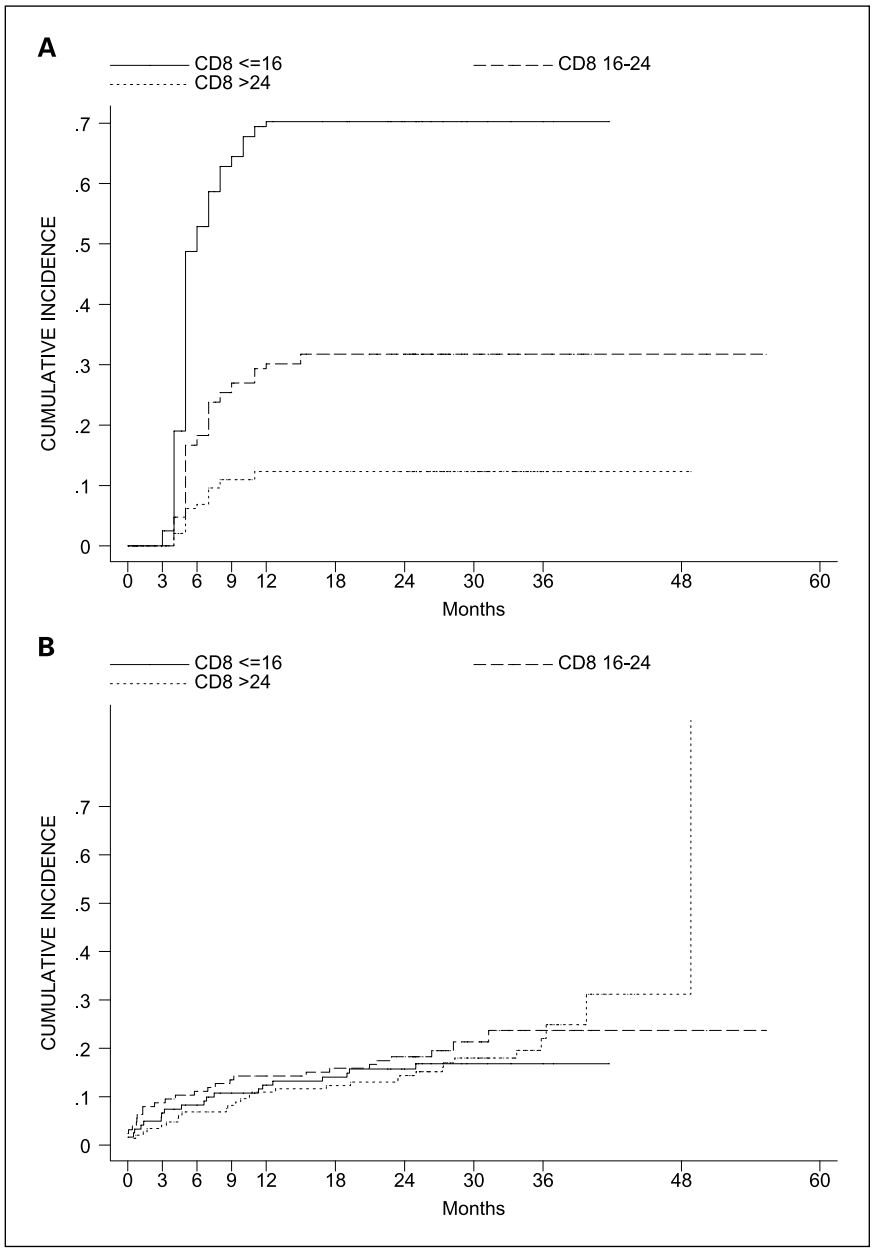

Fig. 1. Cumulative incidence of first events according to CD8 T-lymphocyte apoptosis. *A*, percent cumulative incidence of grade ≥2 late side effects. *B*, percent cumulative incidence of relapse. Three categories of CD8 T-lymphocyte apoptosis are presented: ≤16, 16 to 24, and >24.

is induced by residual DNA damage and occurs in late interphase or after one or several mitoses (29, 37). However, induction of apoptosis in quiescent lymphocytes is dominated by initial DNA damage (38). It is dose and time dependent, following a sigmoid expression curve (8). At a fixed dose and time after exposure, here 8 Gy and 48 hours, lymphocyte apoptosis reflects the rate at which physiologic response to radiation damage is mobilized. Individuals expressing high levels of T-lymphocyte apoptosis mobilize physiologic response rapidly. Individuals expressing low levels of apoptosis mobilize response more slowly. Retardation in mobilizing physiologic response to radiation injury is expected to result in compounded damage and increased late toxicity. Lymphocyte apoptosis is a representative type of programmed cellular response to ionizing radiation damage.

Exposure to adjuvant or concomitant chemotherapy was at the limit of statistical significance for the incidence of late side effects (P = 0.058). However, the effect of chemotherapy, adjusted for CD8, was not significant (P = 0.76).

The lymphocyte apoptosis assay can be used in all cancer sites and as a stratification factor in future dose escalation studies. For example, in a group of 100 cancer patients, the test can select 70 patients with a 98.5% chance of not experiencing a grade 3 late toxicity and the remaining 30 patients with a 20% risk of experiencing a grade 3 late toxicity.

Acknowledgments

We thank Frances Godson for her assistance in the preparation of this article and Michèle Ben Sta and Eliane Cottin for their technical assistance.

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Clin Cancer Res 2005;11:7426-7433.