

Prediction of Hematologic Toxicity After Radioimmunotherapy with ^{131}I -Labeled Anticarcinoembryonic Antigen Monoclonal Antibodies

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This study was undertaken to determine the factors affecting myelotoxicity after radioimmunotherapy (RAIT) with ^{131}I -labeled anticarcinoembryonic antigen (anti-CEA) monoclonal antibodies (MAbs). **Methods:** Ninety-nine patients who received ^{131}I -labeled MN-14 or NP-4 anti-CEA MAbs for the treatment of CEA-producing cancers were assessed for platelet and white blood cell (WBC) toxicity based on the common Radiation Therapy Oncology Group (RTOG) criteria. Univariate and multivariate regression analyses were used to identify the statistically significant factors affecting toxicity among the following variables: red marrow dose, baseline platelet and WBC counts, bone or marrow (or both) metastases, prior chemo- or radiotherapy, timing of prior chemo- or radiotherapy in relation to RAIT, type and number of prior chemotherapeutic regimens, age, sex, antibody form and cancer type. **Results:** Red marrow dose, baseline platelet or WBC counts and multiple bone or marrow (or both) metastases were the only significant factors affecting hematologic toxicity according to both univariate and multivariate analyses, whereas chemotherapy, 3–6 mo before RAIT, was significant according to multivariate analysis. In this retrospective study, the multivariate regression equations using these four variables provided an exact fit for postRAIT platelet toxicity grade (PltGr) and WBC toxicity grade (WBCGr) in 40% and 46%, respectively, of the 99 patients included in the analysis. Moreover, severe (grade 3 or 4) PltGr and WBCGr could be classified accurately in all cases, whereas nonsevere (grade 0, 1, or 2) PltGr and WBCGr could be classified accurately in all but 6 of 13 cases of grade 2 toxicity, in which a severe toxicity grade was estimated using the regression equations. **Conclusion:** Red marrow dose, baseline blood counts, multiple bone or marrow (or both) metastases and recent chemotherapy are the most important factors related to hematologic toxicity after RAIT. This study provides a simple model for predicting myelotoxicity with reasonable accuracy in most patients. In addition, the identification of bone or marrow (or both) metastases and recent chemotherapy as significant factors for myelotoxicity may be important in the future design of clinical trials.

Key Words: radioimmunotherapy; hematologic toxicity; red marrow dosimetry; monoclonal antibodies; carcinoembryonic antigen; cancer

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Hematologic toxicity, particularly of the platelet and white blood cells (WBCs), is the primary and usually only dose-limiting toxicity after radioimmunotherapy (RAIT) with monoclonal antibodies (MAbs) without hematopoietic stem cell support (1–11). However, several investigators have reported a considerable variability in observed platelet or WBC toxicity after RAIT in relatively homogeneous populations of patients who received similar amounts of radioactivity based on body surface area or similar radiation absorbed doses to red marrow (3–11). These reports have also provided some general indications for the importance of one or more parameters thought to affect hematologic toxicity after RAIT (3–11). However, one major problem of these mostly descriptive studies is the relatively small number of patients included, often not large enough for meaningful statistical analysis of the various parameters. There is a need for a thorough statistical analysis of the factors potentially affecting hematologic toxicity in a larger number of patients. Analysis of a larger population of patients could allow assessment of the relative contribution of several factors potentially affecting hematologic toxicity and their interactions using the appropriate statistical methods. These methods will help distinguish the statistically significant factors from those perceived only to contribute substantially to postRAIT toxicity and also may provide simple and practical models for predicting hematologic toxicity after RAIT.

Previous studies with ^{131}I -labeled MAbs against carcinoembryonic antigen (CEA), corroborated by the findings of other investigators, have shown that the pharmacokinetic parameters, most notably the blood clearance rates, may vary considerably among patients. This finding is based on plasma CEA level, tumor bulk and possibly cancer type, resulting in a large variation in the red marrow dose (RMD) and hematologic toxicity for patients who received similar amounts of radioactivity based on their body surface area (12–15). It appeared, therefore, logical to examine the influence of the RMD on hematologic toxicity rather than the influence of radioactivity based on the body surface area.

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The RMD is estimated easily by means of the blood and whole-body clearance data and would automatically correct for differences in hematologic toxicity that are due merely to known variations in the pharmacokinetic parameters of radiolabeled MABs in patients (12–15). The use of the RMD, rather than radioactivity based on the body surface area, facilitated the investigation of other less-examined factors potentially affecting hematologic toxicity, the inclusion of a larger number of patients in the analysis regardless of tumor bulk or type and the inclusion of patients who received the intact or bivalent forms of radiolabeled antiCEA MABs despite known differences in their blood clearance rates (12). Moreover, this approach allowed us to determine whether parameters such as the cancer type or antibody form may have any independent influence on hematologic toxicity (i.e., independent of differences in pharmacokinetic parameters).

In this study, a total of 99 patients with CEA-producing cancer, who received RAIT with ^{131}I -labeled anti-CEA MABs, were assessed for postRAIT platelet and WBC toxicity. In addition to the RMD, several parameters, such as baseline platelet and WBC counts, bone or marrow (or both) metastases, prior chemo- or radiotherapy, timing of prior chemo- or radiotherapy in relation to RAIT, type and number of prior chemotherapeutic regimens, age, sex, antibody form and cancer type, were examined using univariate and multivariate regression analyses.

MATERIALS AND METHODS

Patients

Patients with histologically proven, CEA-producing cancer (including colorectal, pancreas, lung, breast, ovarian and medullary thyroid), who received ^{131}I -labeled anti-CEA MABs for RAIT from 1985 to 1995, were included in the analysis. To be eligible for RAIT, the patients must have been at least 4 wk beyond any major surgery, radiation or chemotherapy. Patients with external beam radiation to >25% of the bone marrow were not eligible. The patients had a performance status of ≥ 70 on the Karnofsky scale (Eastern Cooperative Oncology Group 0–2) and a minimal life expectancy of 3 mo, no severe anorexia, nausea or vomiting, normal hepatic and renal function, WBC count $\geq 3000/\mu\text{L}$ or a granulocyte count $\geq 1500/\mu\text{L}$ and a platelet count $\geq 100,000/\mu\text{L}$. Pregnant patients were excluded from treatment. Patients were mentally responsible and signed an informed consent. All protocols were approved by the governing review boards.

One-hundred-eleven eligible patients received at least one treatment during this period. However, complete clinical records enabling a more comprehensive analysis of the data were available for only 99 patients.

Antibodies

The MABs used for RAIT were the murine NP-4 (Immu-4; Immunomedics, Inc., Morris Plains, NJ) and MN-14 (Immu-14; Immunomedics, Inc.) and their bivalent fragments directed against the class-III, CEA-specific epitope according to the classification of Primus et al. (16). These MABs were selected for their excellent tumor-targeting properties and, most important, the lack of binding to crossreactive antigens, such as nonspecific-crossreacting antigen

(NCA), or to normal tissues, particularly lack of binding to the red marrow (17–19). The F(ab')_2 fragment of NP-4 was prepared by pepsin digestion, whereas the F(ab)_2 fragment of MN-14 was prepared by papain digestion. Removal of undigested IgG was by protein A followed by repeated ultrafiltration (Amicon YM-30; Amicon, Beverly, MA). All MABs were labeled with ^{131}I Na (New England Nuclear, Billerica, MA) by the iodogen method to a specific activity of 444–592 MBq/mg, as described (20). Every labeled product was analyzed by size-exclusion high-performance liquid chromatography (HPLC) and instant thin-layer chromatography (ITLC) to determine aggregation and unbound radioiodine and by affinity chromatography for immunoreactivity. More than 70% binding to a CEA immunoabsorbent was found for the radiolabeled antibody. Less than 2% unbound isotope and <7% aggregation were shown by HPLC for all agents. HPLC analysis of plasma indicated that radiolabeled NP-4 and MN-14 bivalent fragments were stable *in vivo*, with less than 10% or 15% Fab at 1 and 24 h, respectively.

Antibody Infusions

The patients were treated under various phase I or II trials that were active at the time of their admission. The radioactive amount given was based either on the patient's body surface area or on a prescribed RMD determined by a pretherapy tracer study performed 1–2 wk before treatment.

All infusions were given intravenously, proceeding slowly over the first 5 min and then at a more rapid rate to complete the infusion within 15–30 min. All patients were premedicated with Lugol's or supersaturated potassium iodine (SSKI; Upsher Smith, Minneapolis, MN) solution (5 drops orally, three times per day) and potassium perchlorate (Mallinckrodt Medical, St. Louis, MO) (200 mg orally, twice per day) to decrease thyroid and gastric uptake of radioiodine.

Blood and Whole-Body Pharmacokinetics

Blood clearance rates were determined by counting samples of whole blood at various time points after the end of the infusion. Three to five blood samples were taken over the first 24 h, and then daily sampling was performed over the next 2–6 d. Estimates of the slopes of the distribution (α) and elimination (β) phases and their respective intercepts were then used in a nonlinear, least-squares, curve-fitting program to generate both monophasic and biphasic clearance curves. If the biphasic result significantly improved the sum of the squares, then it was selected as the best fit; otherwise, the monophasic curve was used to define the blood clearance. Whole-body clearance rates were determined by a handheld rate meter, with measurements initiated immediately at the end of the infusion, followed by twice daily readings until the end of the study.

Red Marrow Dosimetry

The RMD was estimated in all patients from the cumulated activity in the blood, based on the blood clearance data, and taking into account the contribution from the remainder of the whole-body activity. In earlier trials, the RMD was calculated by assuming equal activity concentrations of the red marrow and blood as suggested by Bigler et al. (21). Most recently, however, use of a marrow-to-blood activity concentration ratio of 0.36 was instituted and is more consistent with the recommendations of the Dosimetry Task Group of the American Association of Physicists in Medicine, suggesting a marrow-to-blood activity concentration ratio of 0.2–0.4 (22–24). All reported RMDs were calculated based on this ratio

(0.36). The corrected blood activity concentration was always multiplied by 1500, the weight in grams of the marrow in an average adult. The red marrow self-dose (RMSD) in centigray (rad) was obtained by multiplying the marrow residence time (τ) (based on blood data) by the S factor for the target red marrow, according to *MIRD Pamphlet No. 11* (25,26). The total RMD was then obtained taking into account the remainder of body dose (RBD) calculated from the whole-body clearance data (25,26).

In all patients included in the current analysis, the RMD was derived from data collected with the therapeutic administration of radiolabeled MAb, whether the patients had a pretherapy tracer study.

Toxicity Assessment

Toxicity was graded according to the Radiation Therapy Oncology Group (RTOG) criteria. All patients given therapeutic administration of ^{131}I -MAbs were followed for hematologic toxicity by monitoring complete peripheral blood cell counts weekly. When grade 2 thrombocytopenia or leukopenia developed, biweekly measurements were taken, and, in the case of grade 3 or grade 4 thrombocytopenia or leukopenia, measurements were taken three or four times a week until the nadir had been determined. The patient's blood counts were also followed until complete hematologic recovery was established.

Statistical Analysis

Univariate regression analysis (simple linear regression) was used to assess the correlation between the platelet and WBC toxicity grades (PltGr and WBCGr, respectively) and various independent variables potentially affecting hematologic toxicity (27). Because the platelet and WBC toxicity grades are the clinically important parameters with respect to the degree of hematologic toxicity after RAIT, they were treated as the more relevant dependent variables for hematologic toxicity in the current analysis. However, because some investigators have used the postRAIT platelet or WBC count percent decrease or loss (PltPL and WBCPL, respectively) as measures of postRAIT hematologic toxicity, univariate analysis also was used to study the correlation between these parameters and the independent variables. Because univariate analysis assessed the contribution of only one factor to hematologic toxicity, without consideration of several other potentially more important factors, less stringent criteria often are used in this statistical analysis. $P < 0.10$ is considered significant.

Multivariate regression models also were used to fit the postRAIT toxicity grades with the independent variables (28). Analysis of variance was used in a stepwise regression procedure to select the statistically significant independent variables ($P < 0.05$) to be included in the multivariate regression models. The "goodness" of fit was judged by the r^2 and percent fit. The percent fit provided the proportions of patients for whom the fitted toxicity variable, in terms of rounded grade, fit the actual grade exactly, and by one, two and three grades. The percent fit by one grade provides a confidence level for the prediction equation at comparable values of the independent variables. Here again, the PLTGr and WBCGr were the clinically relevant dependent variables in the statistical analysis, whereas PltPL and WBCPL were considered of secondary importance.

The following independent variables were examined by both univariate and multivariate regression: RMD; the baseline platelet and WBC counts (BsPlt and BsWBC); bone or marrow (or both) metastases (BMet); multiple bone or marrow (or both) metastases (MBMet); external beam radiation (XRT); external beam radiation

to $>10\%$ of the marrow (XRT1); timing of prior external beam radiation in relation to RAIT (XRTT); external beam radiation within 12 mo before RAIT (XRTT12); prior chemotherapy (Ch); type and number of prior chemotherapeutic regimens (ChType and ChNmb, respectively); timing of prior chemotherapy in relation to RAIT (ChT); chemotherapy within 3, 6 and 12 mo before RAIT (ChT3, ChT6 and ChT12, respectively); antibody type (AbType); antibody form (AbF); tumor type (TType); patient age (age) and sex (sex). In addition to the RMD, we also examined, by univariate analysis only, the correlation between both RMSD and RBD, as the two components of the RMD and hematologic toxicity.

To assess the effect of the various types of chemotherapeutic or other antiproliferative agents given before RAIT, each agent was assigned a number (arabic numbers from 1 to 20 for the 20 different agents used in the various chemotherapeutic regimens) and was then classified into one of three subcategories according to the degree of hematologic toxicity caused by this agent when given in usual doses and schedules (29). These subcategories included mildly to moderately toxic agents (5-flourouracil with or without leucovorin, cisplatin, vincristine, streptozotocin, tumor necrosis factor and VP-16), moderately to markedly toxic agents (adriamycin, hydroxyurea, methotrexate, cyclophosphamide, ifosfamide, interferon, dacarbazine, carboplatin, taxol, thiotepa and velban) and markedly toxic agents (mitomycin, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea and topotecan). The overall degree of toxicity of several agents given in combination was expressed as the maximum, average or sum of the degrees of each agent. With regard to dose intensity of chemotherapy, it is important to note that patients who received chemotherapy had one or more conventional or standard chemotherapeutic regimens administered at their commonly used mass amounts and schedule (e.g., 425 mg/m² of 5-flourouracil with or without leucovorin every 4–5 wk for colorectal cancer) and not dose-intense high-dose chemotherapy. Hence, the issue of dose intensity, which applies to severalfold dose intensifications of chemotherapeutic agent(s) or giving the standard chemotherapy mass amount in much shorter intervals than usual, may be of little or no relevance here.

RESULTS

Patient Characteristics

Table 1 provides a breakdown of the 99 patients included in the analysis, according to sex, tumor type, administered MAb form, metastatic bone or marrow (or both) involvement and therapy received before RAIT. Of the 99 patients, 67 (68%) previously received at least one regimen of chemotherapy. The mean number of prior chemotherapeutic regimens was 1.19 (SD = 1.1), with a range of one to five prior regimens. Thirty-one patients (31%) received external beam radiation (either alone or combined with chemotherapy), affecting 3%–20% of active bone marrow (median ~15%), and 18 patients (18%) had bony metastases (usually vertebral or pelvic metastases as seen by bone scan, MRI or both). Nineteen of the 99 patients (19%) had no prior chemo- or radiotherapy and no bony metastases.

The patients received radioactivity ranging from 1790.8 to 9620 MBq (4599.1 ± 2020.2 MBq [mean \pm SD]), resulting in RMDs of 34.2–311 cGy (mean 144.0 ± 71.4 cGy). In all patients included in the current analysis, RMD was derived from data collected with the therapeutic admin-

TABLE 1
Patient Characteristics

Characteristic	Total
Total number of patients	99
Sex	
Male	54
Female	45
Type of cancer	
Colorectal	48
Medullary thyroid	18
Lung	7
Pancreatic	7
Ovarian	7
Breast	5
Other	7
MN-14 and NP-4 IgG	73
Bivalent fragments	26
Prior chemotherapy	67
Prior radiotherapy	31
Patients with bony metastases	18
Patients with no prior chemo- or radiotherapy or bony metastases	19

istration of radiolabeled MAb, whether or not the patient had a pretherapy tracer study. However, in the 46 patients who had tracer studies before the therapy infusion, a good correlation ($r = 0.82$) was found between the RMD (in centigrays per megabecquerels) determined by both studies. When the 9 patients who apparently had human-antimouse antibodies at the time of their therapy infusions were excluded, the correlation coefficient was similar ($r = 0.83$). For these patients, the mean RMD for the intact IgG ($n = 28$) determined by the tracer infusions was 0.046 ± 0.025 cGy/MBq compared with 0.035 ± 0.020 cGy/MBq determined by the therapy studies ($P = \text{not significant}$). The mean RMD for the bivalent fragment ($n = 9$) determined by the tracer infusions was 0.036 ± 0.016 cGy/MBq compared with 0.033 ± 0.007 cGy/MBq determined by the therapy studies ($P = \text{not significant}$).

The patients' BsPlt and BsWbC ranged from 124,000 to 585,000/ μL (mean $277,414 \pm 107,247/\mu\text{L}$) and from 3,100 to 16,500/ μL (mean $7,615 \pm 2,766/\mu\text{L}$), respectively. However, all but 2 patients, with BsWbC of 3,100 and 3,800/ μL , had BsWbC counts of $>4,000/\mu\text{L}$. PostRAIT, 58 patients had a grade 0, 8 a grade 1, 13 a grade 2, 11 a grade 3 and 9 a grade 4 thrombocytopenia. Forty-nine patients had a grade 0, 23 a grade 1, 13 a grade 2, 10 a grade 3 and 4 a grade 4 leukopenia.

Univariate Analysis of Independent Toxicity Variables

The linear correlations between PLtGr or WBCGr and individual independent variables were examined in the univariate analysis to study their relationships. The same analysis was also performed for the PltPL or WBCPL. For both PltGr and WBCGr, univariate analysis showed that the RMD ($r = 0.5$, $P < 0.001$ for PltGr; $r = 0.59$, $P < 0.001$ for WBCGr), BsPlt ($r = -0.35$, $P < 0.001$ for PLTGr), BsWbC ($r = -0.38$, $P < 0.001$ for WBCGr) and MBMet, defined as

greater than or equal to two metastases seen by bone scan or MRI (or both) ($r = 0.26$, $P < 0.01$ for PltGr; $r = 0.19$, $P < 0.065$ for WBCGr), were the consistently significant independent toxicity variables. In addition, both components of the RMD (RMSD and RBD) correlated significantly with hematologic toxicity. For PltGr and WBCGr, the correlation coefficients with the RMSD were 0.42 and 0.48, respectively ($P < 0.001$), whereas the correlation coefficients with the RBD were 0.39 and 0.46, respectively ($P < 0.001$). Because the correlation coefficients for both parameters were somewhat lower than those for the RMD, the latter parameter was considered for multivariate analysis.

Analysis of the correlation between PltPL and WBCPL and the various independent variables also showed the consistent effects of the RMD ($r = 0.56$, $P < 0.001$ for PltPL; $r = 0.6$, $P < 0.001$ for WBCPL) and MBMet ($r = 0.20$, $P < 0.05$ for PltPL; $r = 0.23$, $P < 0.02$ for WBCPL). However, when the percent loss of baseline blood cell counts was used instead of the absolute grade, the effect of baseline cell counts became insignificant ($P > 0.97$ for BsPlt; $P > 0.56$ for BsWbC), suggesting that the use of percent loss of cell counts "corrects" for existing differences in the pre-RAIT baseline cell counts between patients. Similar to findings with PltGr and WBCGr, the RMSD and RBD correlated significantly with PltPL and WBCPL. The correlation coefficients for the RMSD were 0.46 and 0.45 for PltPL and WBCPL, respectively ($P < 0.001$), and the correlation coefficients for the RBD were 0.44 and 0.52 for PltPL and WBCPL, respectively ($P < 0.001$). Here again, the correlation coefficients for both parameters were somewhat lower than those for the RMD.

Because univariate analysis examines only the contribution of one individual variable without adjustment or consideration of other potentially more important variables or factors, this analysis may not be fully reliable in depicting the true effect of a particular variable. Hence, more attention was focused on multivariate analysis in this study.

Multivariate Analysis of Independent Toxicity Variables

The results of multivariate analysis for PltGr and WBCGr are given in Table 2. The RMD ($P < 0.001$), BsPlt ($P < 0.001$), MBMet ($P < 0.001$) and chemotherapy within 6 mo before RAIT ($P < 0.001$) were the only significant ($P < 0.05$) independent variables affecting the postRAIT PltGr. For postRAIT WBCGr, RMD ($P < 0.001$), BsWbC ($P < 0.001$), MBMet ($P < 0.014$) and chemotherapy within the last 3 mo before RAIT ($P < 0.039$) were the only significant ($P < 0.05$) independent toxicity variables. The r^2 for the multivariate regression equations for PltGr and WBCGr were 0.48 and 0.47, respectively.

Table 3 lists the significance values for independent variables not included in the regression models in Table 2. P values for adding these variables were all >0.12 and >0.087 for PltGr and WBCGr, respectively. Most important, adding more variables to the regression models in Table 2 did not result in any significant improvement in r^2 or percent fit. The

TABLE 2
Predicting Regression Models in Multivariate Analysis

PltGr = 0.356 + 0.914 RMD + 1.184 MBMet
– 0.426 BsPlt + 0.756 ChT6
P: 0.001 for RMD, 0.001 for MBMet, 0.001 for BsPlt and 0.001 for ChT6
$r^2 = 0.481$
Percent fit: 40 (exact), 85 (by ± 1), 100 (by ± 2), 100 (by ± 3)
WBCGr = 0.387 + 0.920 RMD + 0.650 MBMet
– 0.126 BsWBC + 0.437 ChT3
P: 0.001 for RMD, 0.014 for MBMet, 0.001 for BsWBC and 0.039 ChT3
$r^2 = 0.469$
Percent fit: 46 (exact), 89 (by ± 1), 100 (by ± 2), 100 (by ± 3)

PltGr = platelet toxicity grade; RMD = red marrow dose; MBMet = multiple bone or bone marrow (or both) metastases; BsPlt = baseline platelet count; ChT6 = chemotherapy within 6 mo before radioimmunotherapy; WBCGr = white blood cell (WBC) toxicity grade; BsWBC = baseline WBC count; ChT3 = chemotherapy within 3 mo before radioimmunotherapy.

multivariate regression equations using the four significant variables provided an exact fit for postRAIT PltGr in 40% of patients included in the analysis. For postRAIT WBCGr, the regression equations provided an exact fit in 46% of patients. Figures 1 and 2 show the distribution of estimated PltGr and WBCGr (using the regression model) compared with actual toxicity grades in all 99 patients. If toxicity grades are classified as severe (grade 3 or grade 4) and nonsevere (grade 0, grade 1 or grade 2), severe PltGr or WBCGr could be accurately classified in all cases because the change from grade 3 to grade 4 or vice versa does not affect the classification of severe toxicity. On the other hand, nonsevere PltGr could be accurately classified in all but 5 of 13 cases of grade 2 Plt toxicity, whereas nonsevere WBCGr was accurately classified all but 6 of 13 cases of grade 2 WBC toxicity. In these cases, grade 3 or grade 4 was estimated using the regression equations, resulting in a change of toxicity classification from nonsevere to severe.

The examination of the PltPL and WBCPL in the multivariate analysis revealed the RMD and MBMet as the only significant ($P < 0.05$) independent variables in the regression models, although chemotherapy within the last 6 mo or chemotherapy within the last 3 mo was marginally significant ($P = 0.10$) for PltPL and WBCPL, respectively. However, the regression equations for PltPL and WBCPL yielded lower r^2 values (0.36 and 0.41 for PltPL and WBCPL, respectively) and percent fit compared with those for PLtGr and WBCGr and therefore were considered less reliable in the multivariate analysis.

DISCUSSION

To the best of our knowledge, this is the first extensive analysis of a considerable number of factors potentially affecting hematologic toxicity in a relatively large number of patients treated with ^{131}I -labeled anti-CEA MABs. However,

this analysis may also have general applications for other radiolabeled MABs used for the RAIT of various cancer types.

Both univariate and multivariate analyses used in this investigation confirmed the significance of the RMD as the single most important factor affecting hematologic toxicity after RAIT. However, other important factors may be crucial in determining toxicity in the treated patients. In fact, these factors probably explain the only moderate overall correlation between RMD alone and postRAIT PLtGr or WBCGr in the 99 patients examined ($r^2 = 24\%–34\%$) and the large variability in hematologic toxicity between patients who received similar radiation doses to their red marrow. Both the moderate correlation between the RMD and hematologic toxicity and the variability in the level of toxicity between patients receiving similar red marrow radiation doses have been reported by other investigators (7–11).

The finding that the preRAIT (baseline) blood cell counts are important toxicity factors, explaining about 7%–8% of

TABLE 3
Probability Values for Adding One Variable
to Regression Models in Table 2

Variable	PltGr	WBCGr
ChT	0.820 (–)	0.477 (+)
ChT3	0.904 (–)	*
ChT6	*	0.601 (+)
ChT12	0.865 (+)	0.472 (–)
ChType	0.431 (+)	0.264 (–)
ChNmb	0.245 (–)	0.428 (–)
ChYes	0.751 (+)	0.464 (–)
BMet	0.544 (–)	0.620 (–)
XRT	0.175 (–)	0.157 (–)
XRT1	0.165 (–)	0.187 (–)
XRTT	0.216 (+)	0.136 (+)
XRTT12	0.120 (–)	0.087 (–)
AbF	0.454 (–)	0.586 (+)
AbType	0.438 (+)	0.331 (–)
TType	0.170 (+)	0.441 (–)
Sex	0.898 (+)	0.244 (+)
Age	0.733 (+)	0.812 (+)
BsPlt	*	0.055 (–)
BsWBC	0.326 (+)	*

*Variable was significant for this measure and was included in regression models in Table 2.

PltGr = platelet toxicity grade; WBCGr = white blood cell (WBC) toxicity grade; ChT = timing of prior chemotherapy in relation to radioimmunotherapy; ChT3 = chemotherapy within 3 mo before radioimmunotherapy; ChT6 = chemotherapy within 6 mo before radioimmunotherapy; ChT12 = chemotherapy within 12 mo before radioimmunotherapy; ChType = type of prior chemotherapeutic regimens; ChNmb = number of prior chemotherapeutic regimens; ChYes = prior chemotherapy; BMet = bone or marrow (or both) metastases; XRT = external beam radiation; XRT1 = external beam radiation to $>10\%$ of marrow; XRTT = timing of prior external beam radiation in relation to radioimmunotherapy; XRTT12 = external beam radiation within 12 mo before radioimmunotherapy; AbF = antibody form; AbType = antibody type; TType = tumor type; BsPlt = baseline platelet count; BsWBC = baseline WBC count.

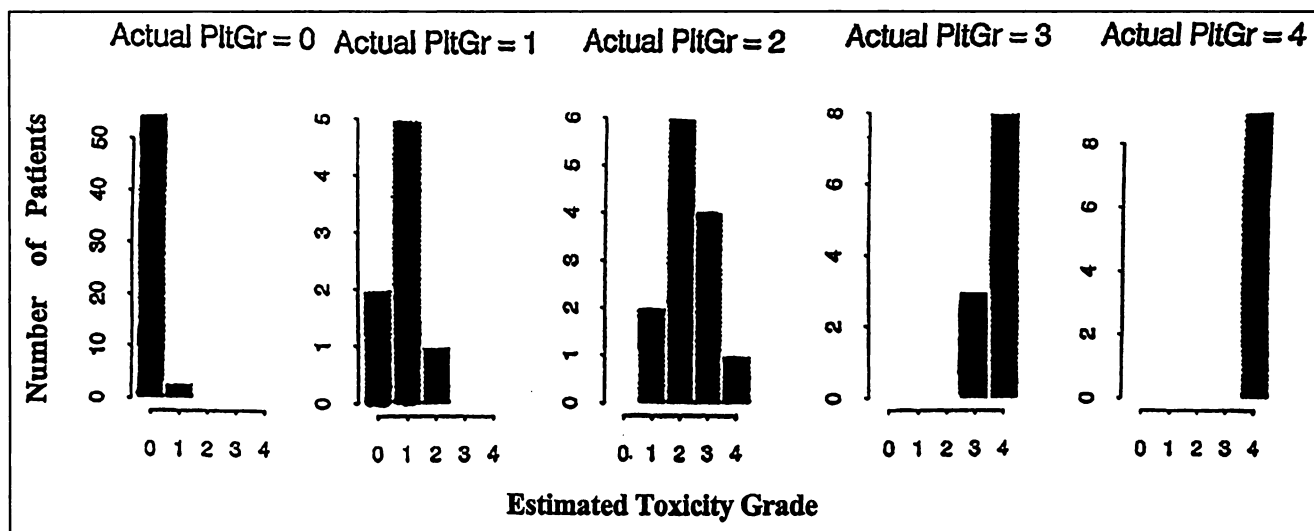


FIGURE 1. Distribution of estimated platelet toxicity grade (PltGr) compared with actual grades in 99 patients using regression equations in Table 2. The x-axis indicates estimated grades; y-axis indicates number of patients.

the variability in observed toxicity, may seem trivial. However, this factor should not be underestimated, being nearly as important as recent chemotherapy or MBMet and considerably more important than the other independent variables examined. Most important, the significance of this factor appeared to be largely independent of prior therapy because it was also observed for the 19 chemotherapy-naïve patients (data not shown) and because no significant differences were found in the mean baseline blood cell counts between therapy-naïve and previously treated patients (data not shown). This finding is best explained by the relatively wide range of “normal” numbers of platelets or WBCs (130,000–400,000/ μ L and 4,000–10,000/ μ L, respectively) even in the blood of healthy subjects. Because patients entered into the RAIT trials are also likely to exhibit such pronounced variations in normal blood cell counts, it is conceivable that preRAIT baseline blood cell counts will

affect the toxicity grade observed. For example, a RAIT-induced 50% reduction of peripheral platelet counts in a subject with “normal” baseline platelets of only 130,000/ μ L will result in a grade 2 platelet toxicity, whereas the same percent decrease in a subject with a platelet count of 300,000/ μ L will result in a grade 0 toxicity. Although some investigators (3,7,9) generally use the percent decrease or loss of blood cell counts to correct for variability of observed postRAIT toxicity and obtain a better correlation between RMD and postRAIT toxicity, it is the toxicity grade and not the percent decrease that is actually clinically important, eventually influencing patient management.

Of particular interest in this analysis was the effect of prior chemotherapy in postRAIT hematologic toxicity, particularly because most patients entered into the RAIT trials had at least one regimen of standard or investigational chemotherapy before receiving RAIT. To our surprise, both

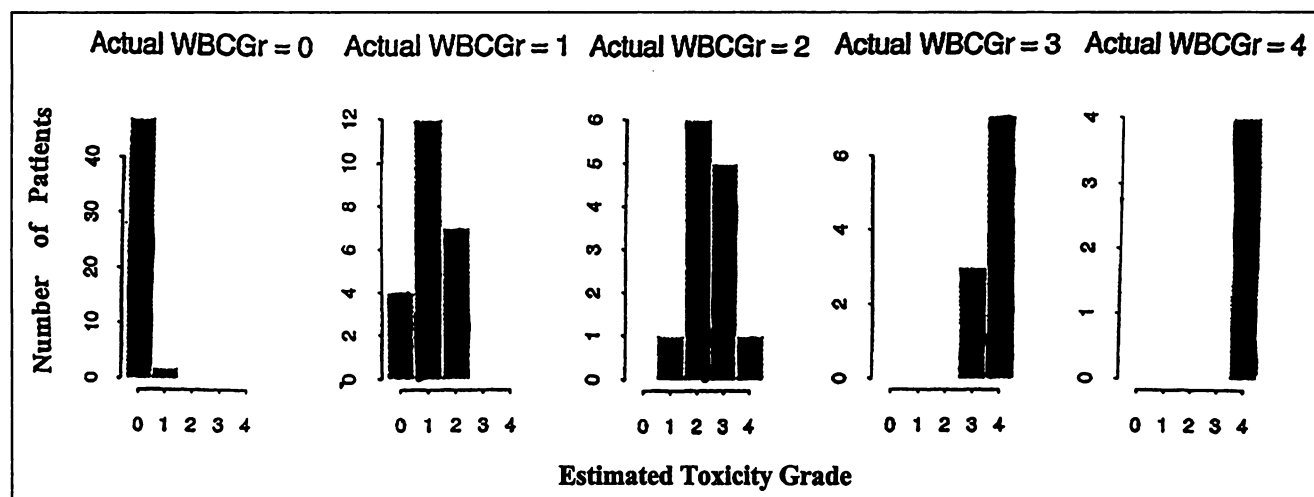


FIGURE 2. Distribution of estimated white blood cell toxicity grade (WBCGr) compared with actual grades in 99 patients using regression equations in Table 2. The x-axis indicates estimated grades; y-axis indicates number of patients.

univariate and multivariate analyses showed that prior chemotherapy per se was not a statistically significant factor affecting hematologic toxicity after RAIT. Moreover, the type and number of chemotherapeutic regimens previously administered to patients for the treatment of their advanced cancers, which included a wide range of mildly to markedly myelotoxic agents, had no statistically significant effect on postRAIT toxicity. In contrast, the timing of prior chemotherapy in relation to RAIT was a significant factor for toxicity. Chemotherapy within the last 3 or 6 mo before RAIT was a significant factor for postRAIT PLtGr and WBCGr, respectively; because all patients had their last chemotherapy at least 4 wk before RAIT, the time spans of 1–3 or 1–6 mo before RAIT were the critical periods revealed by this analysis.

Because recent chemotherapy may result in a decrease of preRAIT baseline blood cell counts, we examined whether the significance of recent chemotherapy may have been merely the result of this effect. Multivariate analysis showed that the effect of timing of chemotherapy was an independent factor for postRAIT toxicity, remaining significant in the regression analysis even when differences in baseline blood cell counts were accounted for, i.e., after the inclusion of baseline blood cell count as an independent variable. Further supporting the independent role of recent chemotherapy was the lack of any correlation between the preRAIT baseline blood cell counts and prior chemotherapy per se ($r^2 < 1\%$) or the timing of chemotherapy ($r^2 < 1\%$), suggesting that the peripheral blood counts have actually recovered to their pretherapy values in most patients. This may indicate that the peripheral blood count recovery is not synonymous with myelorecovery and suggests a time delay between hematologic recovery and actual myelorecovery. Ample evidence for this phenomenon comes from experimental RAIT of mice performed in our laboratory (30). Although the peripheral blood counts have recovered to pretherapy (or even higher) values in the treated mice, retreatment of the mice was not possible until 2–3 wk later because of excessive hematologic toxicity resulting in the death of these animals when they were retreated at an earlier time point. A likely explanation for this finding, both in animals and human subjects, is that megakaryopoiesis, granulopoiesis and erythropoiesis have higher priorities for recovery after RAIT at the expense of self-renewal of the important pluripotent stem cells (31). The net result is an increased differentiation of the pluripotent stem cells to the more mature marrow and peripheral blood cells, an effect probably induced by certain feedback mechanisms (31) and, hence, a delayed stem cell recovery. Only when the peripheral blood counts and their (late) marrow precursors have fully recovered will the effect of this feedback subside, leading to a gradual recovery of these pluripotent stem cells. The increased hematologic toxicity seen in patients who had recent chemotherapy, despite normal peripheral blood cell counts, may be related to a temporary decrease in the pluripotent stem cells and, hence, incomplete myelo-

recovery. Further research is needed, however, to prove this supposition. An additional factor may also be that the proliferating stem cells are more radiosensitive than those in the quiescent phase of the cell cycle. The alternative explanation, that the effect of recent chemotherapy may have been related to a permanently “compromised” marrow simply because of this prior chemotherapy, is not supported. Neither chemotherapy per se nor the number or type of previous chemotherapeutic regimens was statistically significant in this analysis.

An interesting finding was that chemotherapy within 6 mo, but not within 3 mo, was included in the regression equation for predicting platelet toxicity, whereas only chemotherapy within 3 mo was included in the regression equation for predicting WBC toxicity. However, it is important to note that the multivariate analysis indicated that chemotherapy within 3 mo of RAIT was indeed a significant factor influencing platelet toxicity ($P = 0.047$) when chemotherapy within 6 mo of RAIT was not included in the analysis. Yet, when the latter parameter was included, the P value for adding chemotherapy within 3 mo (i.e., the difference between 3 and 6 mo) became insignificant ($P = 0.904$, see Table 3), apparently because of the high correlation between chemotherapy within 3 and 6 mo (i.e., chemotherapy within 3 mo of RAIT is also within 6 mo of RAIT). Most important, chemotherapy within 6 mo of RAIT appeared to be a stronger predictor of platelet toxicity ($P = 0.001$) compared with chemotherapy within 3 mo; because both parameters are predictors of platelet toxicity, the stronger variable was chosen in the regression equation. This was not the case for WBC toxicity, where only chemotherapy within 3 mo but not within 6 mo of RAIT was a significant variable. Although the reason for this finding is not fully clear, it is interesting to speculate that a longer period may be needed for complete recovery of platelet compared with that for WBC precursors in the marrow.

Because MBMet, particularly in patients with lung, breast and medullary thyroid cancers, are almost always associated with bone marrow metastases (as was proven in some cases by marrow biopsies, MRI studies or both) (32–34), the effect of bony metastases may be readily explained by a substantial reduction in the bone marrow reserve of the affected patients. In fact, the extent of marrow involvement in these patients may have been greater than that seen by conventional imaging modalities, such as bone scan or MRI, because of the limited ability of these methods to detect occult micrometastatic spread throughout the marrow, the existence of which could further reduce the marrow reserve.

CONCLUSION

Despite the relatively large number of factors considered in this study, multivariate analysis showed that the RMD, baseline peripheral blood cell counts, MBMet and chemotherapy within the last 3–6 mo before RAIT were the only four significant factors affecting postRAIT hematologic toxicity. Adding the other factors examined did not result in any

significant improvement in the ability to predict hematologic toxicity after RAIT. Providing a simple and reasonably accurate model for predicting postRAIT toxicity in patients using only four clinical variables may help identify patients in need of closer blood-count monitoring and allow the earlier administration of colony-stimulating factors or other interventions to correct potentially dangerous peripheral blood count abnormalities. Moreover, identification of the statistically significant toxicity factors may be useful for the design of future phase I or phase II trials that take into account the existence of some of these factors in the patient population studied, i.e., by separately studying patients with high or low risk for developing hematologic toxicity. This may actually result in the determination of different maximum tolerated doses (in centigrays to the marrow or in megabecquerels per square meter) of the same radiolabeled MAb in various subpopulations of patients, such as those with or without bony metastases or recent chemotherapy (or both). Patients with lower risks for developing excessive toxicity then may be able to receive higher doses of radiolabeled MAb and have potentially greater benefits from treatment.

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