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Original Paper

Causes for Increased Myelosuppression with Increasing Age in Patients with Oesophageal Cancer Treated by Chemoradiotherapy

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The aim of this study was to identify why increasing myelosuppression accompanies increasing age in patients treated for oesophageal cancer by chemoradiation. Weekly neutrophil and platelet counts were obtained throughout treatment in 86 patients undergoing chemoradiation without surgery for oesophageal cancer. One or two cycles of cisplatin 80 mg/m^2/day followed by 5-fluorouracil 800 mg/m^2/day for 4–5 days were administered during the first and fourth or fifth week of radiotherapy using 2 Gy daily fractions. 44 of the patients underwent 5-fluorouracil pharmacokinetic studies. Multiple regression procedures were used to determine the strength of factors that contribute to initial and nadir neutrophil and platelet counts. The kinetics of myeloid response were evaluated from the rates of disappearance and re-appearance of neutrophils and platelets during treatment. Age, fluorouracil dose (or AUC), baseline body weight and neutrophil (or platelet) count were found to be powerfully and independently predictive of both first neutrophil and platelet nadir count. Baseline neutrophil and platelet counts were also found to correlate negatively with advancing age independently of other factors. The rate of descent of both indices, however, was independent of age, baseline count and fluorouracil dose suggesting that variations in the size of the myeloproliferative compartment prior to treatment were responsible for interpatient variations. In addition, the rate of recovery of both indices was not influenced by age amongst patients in whom data was assessable suggesting that proliferation of surviving marrow elements is not compromised by age. These data are compatible with the hypothesis that a progressive depletion of the myeloid stem cell compartment accompanies advancing age, and that this is responsible for increasing myelotoxicity. © **1999 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

IN PREVIOUS reports we have noted that patients with oesophageal cancer who are over 70 years of age tolerate chemoradiation less well than younger patients. In particular patients over the age of 70 years appear to develop more profound myelosuppression than younger patients [1]. As a consequence these patients experience more complications and require more frequent cytotoxic dose delays and reductions, which ultimately translate into inferior treatment outcomes.

The explanation for greater myelosuppression in older patients is unclear. We hypothesise that the regenerative haematopoietic response in this group of older patients is compromised by an absolute depletion of the haematopoietic stem cell compartment or by reductions in the responsiveness of the stem cell or progenitor/amplification compartments. An alternative, but not mutually exclusive hypothesis is that

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drug pharmacokinetics may be different in the elderly. Thus haematopoietic cytotoxic exposure may be greater in older patients even though the same dose had been given. To test this hypothesis we prospectively collected detailed blood count data in 86 patients and assessed the time course of changes in peripheral blood neutrophil and platelet count following commencement of therapy and the factors that influence them.

PATIENTS AND METHODS

Patients and treatment

To investigate increased myelosuppression in patients over 70 years of age treatment centres cooperating in the prospective protocols of the Trans-Tasman Radiation Oncology Group (TROG) evaluating the non-surgical management of oesophageal cancer agreed prospectively to obtain weekly blood count data in all consenting patients treated on these protocols. To be eligible for treatment on protocol, patients were required to have biopsy proven squamous cell or adenocarcinoma with disease localised to the oesophagus with or without radiological evidence of metastases in draining intrathoracic lymph nodes. In addition to staging endoscopy, barium swallow and computed tomography (CT) (chest and abdomen) patients were required to undergo a full haematological and biochemical assessment of marrow, renal and hepatic reserve, and full cardiothoracic appraisal. Patients were excluded from protocol if they had major medical or psychiatric illness, were considered unfit to undergo any component of the treatment, if a tracheo- or broncho-oesophageal fistula was present or performance status was below ECOG level 2. Informed written consent was an additional requirement for study inclusion and all treatment centres required the approval of their regional ethics committees to participate.

The protocols, which have been described previously, included two cycles of chemotherapy during a 6 week course of radiation [1, 3]. Chemotherapy comprised cisplatin 80 mg/m^2 on day 1 by intravenous (i.v.) infusion over 2 h after hydration with 2 l of saline and 200 mls 20% mannitol, followed by 5-fluorouracil 800 mg/m^2/day by continuous i.v. infusion over a period of 4–5 days. The first cycle was delivered during the first week of radiation. The second was delivered in the fourth week of radiation or delayed until the absolute neutrophil count was above 1.0×10^9/l level. Radiation was delivered during the first 3 weeks using opposing megavoltage AP/PA portals covering the primary tumour and all draining regional lymph nodes to a dose of 30 Gy comprising 15×2 Gy incremental daily fractions. During the final 3 weeks a three or four field arrangement employing reduced field sizes covering macroscopic tumour with a minimum 3 cm margin was used to deliver an additional 30 Gy in 2 Gy daily fractions. Patients with disease considered incurable, due to the presence of lymph nodes outside the limited version of the thorax or blood borne metastases at any site, were eligible for treatment on a protocol. These patients received one cycle of chemotherapy using the same protocol. In these patients, 3 weeks of radiation using opposing megavoltage portals to cover the primary plus involved regional nodal groups to 30 Gy in 15 daily fractions was administered.

Pharmacokinetic studies

A subgroup of patients treated at one institution (Newcastle) where facilities were available underwent pharmacokinetic studies of 5-fluorouracil. Blood samples were analysed by high-performance liquid chromatography as described previously [4].

These were collected prior to chemotherapy and at 7.00 am daily throughout each fluorouracil infusion. On collection days 2 and 3, two additional samples were collected at 11.00 am and 3.00 pm to assess diurnal variation. Fluorouracil non-compartment pharmacokinetic parameters were estimated from the concentration-time data for each patient using MKMODEL version 5.0 (Biosoft PLC, Cambridge, U.K.). Steady state plasma level (CpSS) was calculated as the mean plasma concentration on collection days 3, 4 and 5. Area under the plasma concentration-time curve (AUC) expressed as μM.hr was calculated by the log-trapezoidal method for the duration of the infusion. Plasma clearance of fluorouracil (l/h) was calculated as the infusion rate (d) divided by the CpSS.

Measures during treatment

All patients had full blood count profiles including haemoglobin, white cell differential and platelet counts and biochemistry including electrolyte, liver function tests and magnesium at least weekly throughout treatment. Most patients had blood profiles collected twice weekly or more frequently during anticipated nadir count weeks to improve the precision of the depth and timing of the nadir data.

Analytical methodology

Data were stored on a temporal database (Medlog, Information Analysis Corporation, Incline Village, Nevada, U.S.A.) which enables a range of statistical manoeuvres to be performed on the data. Grouped data were compared using the Fisher's Exact or Mann–Whitney U tests. Stepwise multiple linear regression techniques were used to assess the influence of a range of covariates (including age, gender, performance status etc.) on dependent variables associated with cytotoxic dose (including total fluorouracil dose, and fluorouracil AUC). These techniques were also used to quantify the magnitude of effect of potential covariates on baseline and first nadir neutrophil and platelet counts. Full details of the covariates used in the various models derived are described under Results.

The kinetics of peripheral blood neutrophil and platelet depletion and recovery following chemotherapy were deduced from the sequential blood count data. Grouped patient neutrophil and platelet time course data were defined on semi-logarithmic plots to permit an appreciation of the rate at which these indices disappear and reappear in the peripheral blood following chemotherapy in different patient sub-groups. A linear fit to the data implies that the phenomenon observed (e.g. disappearance of neutrophils from the peripheral blood) occurs at a constant rate. A parallel displacement in linear fits between two groups of observations implies that the phenomenon observed is occurring at the same rate in both groups even though there are absolute differences in counts between the two groups at each study point. A parallel decrease in counts following chemotherapy indicates a specific proportion of the haemopoeitic proliferating 'target' cell population is depleted when a given dose of cytotoxic is administered, regardless of the initial size of the target cell population. A parallel reappearance in counts after the nadir indicates that the remaining haemopoeitic processors repopulate after at an identical rate regardless of the level of depletion reached after chemotherapy.

Table 1. *Characteristics of the study group and a subgroup of patients who underwent pharmacokinetic studies*

	Whole group ($n = 86$)	5-FU pharmacokinetic sub-group ($n = 44$)
Gender—M:F	57:29	34:10
Median age (range)	70.5 (42–91) years	72 (42–91) years
Tumour length	5(1–11) cm	5(1.5–10) cm
Tumour site		
Upper	16	9
Middle	25	8
Lower	45	27
Tumour histology		
Squamous	58	26
Adenocarcinoma	28	18
Median percentage weight loss (range)	6.6 (0–35.1) %	6.5 (0–31.8) %
Median baseline neutrophil count (range)	5.7 (2.5–14.6)$\times 10^9$/l	5.5 (2.5–14.6)$\times 10^9$/l
Median baseline platelet count (range)	271 (114–626)$\times 10^9$/l	256 (114–626)$\times 10^9$/l
Two cycles chemotherapy (n)	71	35
Delays to second cycle (n)	44	28
Median radiation dose in two cycle subgroup (range)	60 (30–66) Gy	60 (30–66) Gy
Single cycle chemotherapy only (n)	15	9
Median radiation dose in single cycle subgroup (range)	46 (20–60) Gy	30 (20–60) Gy

5-FU, 5-fluorouracil.

Curve fitting for the time course plots by least squares methodology was carried out after segmentation of the data at various time intervals (including median first nadir and recovery times after starting treatment).

RESULTS

Study Group Composition

71 patients were treated with curative intent using two cycles of chemotherapy during radiation (details are provided in Table 1). Dose delays with or without dose reductions to the second cycle of chemotherapy occurred in 44 (62%) of these patients due to myelosuppression. A single cycle of chemotherapy was given to an additional 15 patients whose cancer was considered incurable or who were medically unfit to receive a second cycle of chemotherapy. 44 patients underwent fluorouracil pharmacokinetic studies and the characteristics of this subgroup are provided beside summary data for the whole study group in Table 1. No significant differences between the characteristics of this subgroup and the entire study group were detected.

Factors influencing first nadir counts

As expected there were inverse linear relationships between age and both neutrophil and platelet nadirs after the first cycle of chemotherapy (Figure 1) correlation coefficients -0.36, $P < 0.001$ and -0.37, $P < 0.001$, respectively. Neutrophil and platelet nadirs after the first cycle also correlated with one another (correlation coefficient 0.63, $P < 0.001$) with platelet nadir developing 3–6 days earlier depending on neutrophil nadir depth (data not shown). Stepwise multiple linear regression modelling procedures confirmed that the relationships between lower first nadir and increasing age for both neutrophil and platelets were independent of all other covariates including cytotoxic dose (Table 2). These models also indicated that fluorouracil dose administered, baseline body weight and neutrophil of platelet counts were additional strong predictors of nadir levels of both indices. Factors which had limited (statistically insignificant) or no impact on either first neutrophil or platelet nadirs in these stepwise procedures were: gender, tumour site, length, percentage weight loss, performance status, cisplatin dose, radiation dose in the first 3 weeks of treatment, radiation field size, serum albumin and creatinine clearance.

In the 44 patients who underwent pharmacokinetic studies, substitution of area under the plasma fluorouracil concentration curve (AUC) during the first cycle of chemotherapy for total fluorouracil dose administered produced qualitatively similar results in multiple regression models (Table 3). In these models first neutrophil and platelet nadir levels correlated independently and negatively both with

Table 2. *Results from stepwise multiple linear modeling procedures addressing factors which influence first nadir levels in the whole study group. (Fluorouracil dose = total milligrams administered during first chemotherapy cycle.)*

(a) *Dependent variable: first neutrophil nadir*
Multiple correlation coefficient = 0.66, $R^2 = 0.43$, $P < 0.0001$

Covariate	Coefficient	S.D.	P
Age	−0.02	0.009	0.02
Gender	−0.2	0.23	0.39
Fluorouracil dose	−0.00	0.00	< 0.0001
Baseline neutrophils	0.11	0.03	0.001
Baseline weight	0.02	0.007	0.001
(Intercept value	3.01	0.92	0.002)

(b) *Dependent variable: first platelet nadir*
Multiple correlation coefficient = 0.78, $R^2 = 0.62$, $P < 0.0001$.

Covariate	Coefficient	S.D.	P
Age	−1.15	0.48	0.02
Gender	5.64	12.5	0.65
Fluorouracil dose	−0.02	0.004	< 0.0001
Baseline platelets	0.34	0.05	< 0.0001
Baseline weight	1.31	0.38	0.001

Covariates non-significantly correlated and discarded: performance status, percentage weight loss, tumour size and position, radiation dose during first 3 weeks, cisplatin dose, albumin and creatinine clearance. S.D., standard deviation.

increasing age and with increasing area under the fluorouracil concentration curve.

Factors influencing variables that predict first nadir levels

In the investigation of factors that contribute to marrow fluorouracil exposure, only those variables that impact on body surface area calculation (height, weight and gender indirectly) were seen to correlate with the total number of milligrams of fluorouracil delivered. However, increasing age had a separate independent influence on measured fluorouracil AUC over and above the factors that influence dose itself. In stepwise multiple regression procedures a correlation between fluorouracil AUC and increasing age was observed which was independent of fluorouracil dose administered (Table 4). However, the effect was weak and it should be noted from the table that the modelling process left over 60% of the variation in AUC unexplained.

Another factor to predict first nadir levels was the baseline counts of both indices. These counts were also observed to correlate inversely with increasing age. In both univariate regression analyses (correlation coefficient -0.29, $P = 0.007$ for neutrophils and -0.28, $P = 0.04$ for platelets) and in stepwise multiple regression models independently of other variables (not shown; multiple correlation coefficient -0.09, $P = 0.002$ for neutrophils; -2.45, $P = 0.03$ for platelets) increasing age was observed to be associated with decreasing baseline neutrophil and platelet counts. Further investigation of this phenomenon suggested that baseline neutrophil and platelet levels were within the normal range in 37 of 44 patients (84%) over the age of 70 years (allowing that 7.25×10^9/l and 440×10^9/l are at the upper 95% confidence intervals (CI) for neutrophils and platelets, respectively, for the adult population). However, in patients under 70 years of age, levels of baseline neutrophil and platelet counts were significantly higher ($P = 0.002$ and 0.005, respectively) and 17 of 42 individual values (40%) were outside the normal range. Outlying baseline neutrophil counts were observed more commonly than outlying platelet counts in patients under the age of 70 years ($P = 0.01$ and $P = 0.11$, respectively).

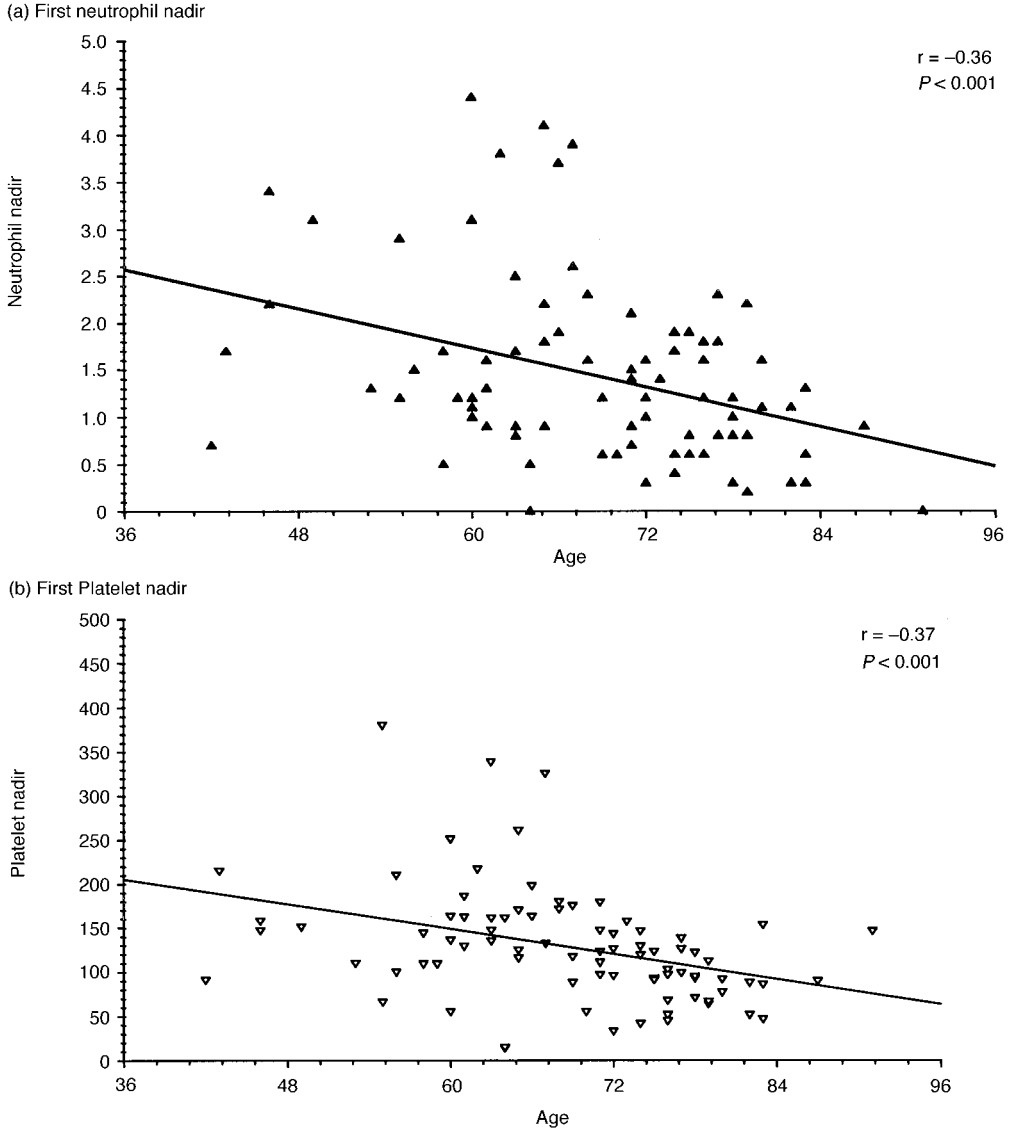

Figure 1. The dependence of first nadir level on increasing age.

Table 3. Factors that influence first nadir values in patients undergoing pharmacokinetic studies

(a) *Dependent variable: first neutrophil nadir*
Multiple correlation coefficient = 0.71, R^2 = 0.5, P < 0.0006.

Covariate	Coefficient	S.D.	P
Age	− 0.036	0.01	0.01
Gender	0.1	0.29	0.74
AUC	− 0.002	0.001	0.03
Baseline neutrophils	0.04	0.04	0.3
Baseline albumin	0.05	0.03	0.09
(Intercept value	1.29	2.04	0.53)

(b) *Dependent variable: first platelet nadir*
Multiple correlation coefficient = 0.8, R^2 = 0.65, P < 0.0001

Covariate	Coefficient	S.D.	P
Age	− 1.09	0.54	0.05
Gender	11.88	13.4	0.38
AUC	− 0.14	0.05	0.01
Baseline platelets	0.19	0.04	0.0002
Baseline weight	− 0.12	0.36	0.73
Baseline albumin	3.59	1.46	0.02
(Intercept value	38.16	82.74	0.65)

Covariates non-significantly correlated and discarded: performance status, percentage weight loss, baseline weight, tumour size and position, radiation dose during the first 3 weeks, cisplatin dose and creatinine clearance. AUC, area under plasma fluorouracil concentration curve. S.D., standard deviation.

Kinetics of neutrophil and platelet disappearance and reappearance during treatment

In Figures 2 and 3 grouped neutrophil and platelet levels have been plotted at regular intervals after starting treatment on a logarithmic scale to permit an appreciation of the rate at which these indices change in the peripheral blood. As described in Patients and Methods, this enables the underlying haematopoietic kinetics to be deduced. The figures indicate that there was an increase in neutrophil counts but not platelets in most patients in the first few days following the start of treatment which was likely to have been related to the use of dexamethasone as an anti-emetic on days 1–4. This increase in levels was greater in patients over the age of 70 years (Figure 2a) and in those with lower baseline levels (Figure 2b). Following this, both indices fell linearly over the next 14–24 days at a rate that was observed to be independent of age (Figures 2a and 3a), baseline index level (Figure 2b and Figure 3b) or fluorouracil dose/AUC (dose effect not shown in figures) suggesting that the initial size of the stem cell/precursor compartment was the key determinant of ultimate nadir level. As noted earlier nadir times were earlier for platelets than neutrophils (10–16 and 17–22 days respectively). Unfortunately, rate of recovery could not be estimated reliably in the whole study group for either parameter because the treatment protocol determined that the second cycle of chemotherapy should commence on day 21. However, in patients whose second cycle of chemotherapy was delayed due to myelosuppression the rate of recovery of both indices in the peripheral blood was seen to be no less rapid for

Table 4. Factors influencing area under the plasma fluorouracil concentration curve (fluorouracil dose = milligrams administered during first chemotherapy cycle)

Dependent variable: first AUC
Multiple correlation coefficient = 0.56, R^2 = 0.32, P < 0.004

Covariate	Coefficient	S.D.	P
Age	3.04	1.29	0.02
Gender	58.9	37.6	0.12
Fluorouracil dose	0.07	0.02	0.0007
(Intercept value	− 321	158	0.05)

Covariates non-significantly correlated and discarded: performance status, baseline weight, percentage weight loss, creatinine clearance and albumin. AUC, area under fluorouracil concentration curve; S.D., standard deviation.

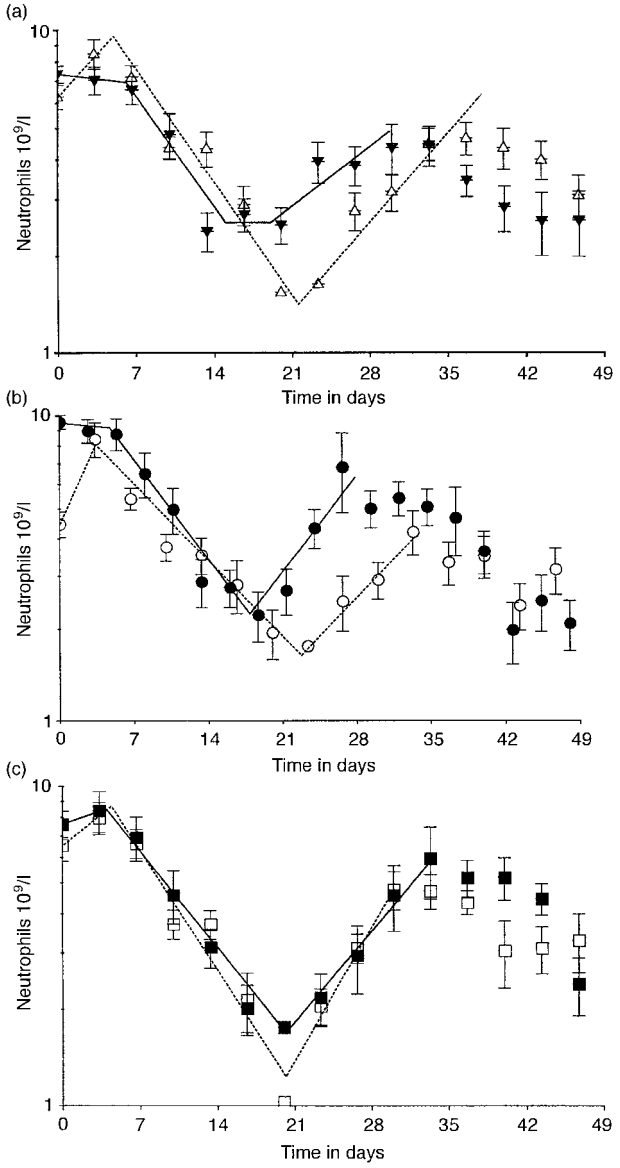

Figure 2. Time course of neutrophil levels during treatment. (a) For all patients aged < 70 years (–▼–, n = 42) and all patients aged 70 years or more (–△–, n = 44). (b) For patients with baseline neutrophil counts in the upper tertile (–●–, n = 28) and lower tertile (–○–, n = 30). (c) For patients whose second cycle of chemotherapy was delayed. Patients aged < 70 years (–■–, n = 17), patients aged 70 years or more (–□–, n = 27).

patients over the age of 70 years than for younger patients (Figures 2c and 3c) suggesting that the regenerative potential of surviving myeloid precursors is no greater in patients under 70 years.

DISCUSSION

The difficulty in a study like this is to separate the varying contributions of a range of factors that may each influence myelotoxicity. Fluorouracil is well known to by myelotoxic but the two other anticancer agents used in this protocol (cisplatin and radiotherapy) may also have contributed. Although the multivariate modelling process discounted significant additional contributions, real additive effects can not be fully discounted. Cisplatin dose correlated closely with fluorouracil dose making a myelotoxic effect that was independent of the powerful fluorouracil effect difficult for the modelling process to discern. It is not surprising that radiation dose up to the time of the first nadir was not shown to contribute because it varied little in the study population. However, a factor that could have been expected to be more important was radiation field size because this would have correlated with the volume of marrow irradiated. The modelling process did not discern an independent effect and it might reasonably be concluded that a field size effect could have been present in the study group but its magnitude was limited. The finding from this study that is important, however, is the confirmation of earlier observations that increasing age inversely correlated with first neutrophil and platelet nadir levels in this group of patients. In addition, this study indicated that the relationship was independent of the powerful, and expected, effect of the dose of myelotoxic agents used in this protocol (in particular increasing fluorouracil dose). The 'age effect' is, therefore, a potent one and its origins deserve further investigation.

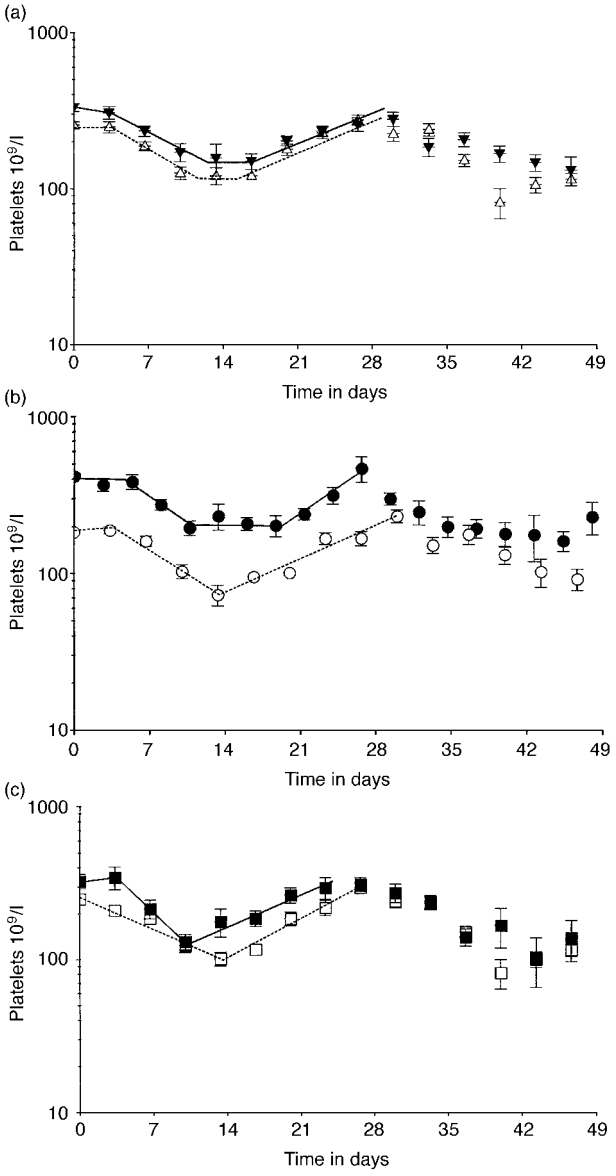

Figure 3. Time course of platelet levels during treatment. (a) For all patients aged < 70 years (–▼–, $n = 42$) and all patients aged 70 years or more (–△–, $n = 44$). (b) For patients with baseline platelet counts in the upper tertile (–●–, $n = 28$) and lower tertile (–○–, $n = 28$). (c) For patients whose second cycle of chemotherapy was delayed. Patients aged < 70 years (–■–, $n = 17$), patients aged 70 years or more (–□–, $n = 27$).

The finding that factors such as performance status, percentage weight loss and various biochemical parameters (in particular renal function) were associated with no additional myelosuppression in our multiple regression procedures support the idea that it is age, *per se*, that is responsible rather than various disease related factors that accompany or are exacerbated by advancing age. Furthermore our hypothesis that increasing age acts as a surrogate, in this series of cases, for an absolute depletion of the haematopoietic proliferative compartments has gained support from the fact that, although absolute baseline and nadir neutrophil and platelet counts were lower in patients over the age of 70 years, the rate of descent of these indices was not influenced by age. This indicates that age differences are due to the size of the myeloproliferative compartment prior to therapy (ie the number of progenitor/percursor cells vulnerable to cytotoxic injury). Our finding that the rate of recovery of the peripheral blood indices after myelosuppression is independent of age is not at odds with this 'senile depletion model'. It could simply mean that amplification in the progenitor/precursor compartments is not compromised by advancing age.

The well described reduction in bone marrow cellularity that accompanies advancing age may be responsible for the increased myelosuppression observed in this study. Hartsock and colleagues [5] showed that bone marrow cellularity declined quite sharply until the age of 35 years, followed by a more gradual decline up to the age of 65 years. Past this age cellularity once again sharply declined in an apparently linear manner. Unfortunately, a paucity of data for patients in our analysis below the age of 65 years prevents any conclusions on the entire age spectrum. In fact, at present, there are no data to confirm that age-related reductions in marrow cellularity correspond to absolute reductions in any of the haematopoietic proliferative compartments [6]. Several groups have reported that circulating CFU-GM levels in the peripheral blood do not decline with age [6–9]. Thus, there is, as yet, no direct evidence to confirm the suggestion that the haematopoietic stem cell compartments diminish in absolute terms with advancing age [6]. Similarly the effect of age on the proliferative response of bone marrow stem cells is uncertain. We have shown that the rate of recovery of peripheral blood indices after myelosuppression is independent of age. In contrast, Selig and Nothdurft showed that proliferation of

CFU-GM in response to serious infection was reduced in older patients [10]. It is now well recognised that telomere length of cells from various tissues progressively reduce with age [11–13]. This includes the haematopoietic stem cell 'candidates' characterised by the CD 34+ CD45RA− CD71− phenotype [14]. In addition Lansdorp and colleagues have also shown that induced proliferation of these stem cell candidates is less successful with increasing age and also leads to telomere shortening [14–17]. However, at present evidence in the clinical setting for reduced proliferative responsiveness with increasing age (as distinct from absolute proliferative capacity) is, as in this study, lacking.

We cannot fully explain why baseline neutrophil and platelet counts correlated negatively with age in our study group. This effect may be partly due to the number of patients below the age of 70 years who had an absolute neutrophilia at presentation. Neutrophilia and thrombocytosis are not reported to be associated with oesophageal cancer, and we are left to speculate that these raised counts are a reflection of a stress response that is manifest more frequently in younger patients due to their better 'marrow reserve'. Indeed this explanation would be compatible with the time course data from this analysis which suggest that patients who have lower circulating neutrophil and platelet pools prior to chemotherapy experience the lowest nadirs.

We conclude by cautioning that although models to predict myelosuppression can be improved by factoring in patient age, the power of these models remains limited. The use of such models in clinical practice is therefore curtailed. The incorporation of currently unidentified factors are required if the predictive value of the modelling process is to be improved. We suspect that genetically based sensitivities to one or more cytotoxic agents may be important. Slagboom and colleagues concluded from mono and di-zygotic twin studies that variations in telomere length in subjects of the same age are genetically determined [18]. If telomere length is the 'biological clock' that results in haematopoietic stem cell depletion, as suggested by Vaziri and colleagues [14], then measurement of telomere length in stem cell candidates may have important additive predictive potential. Since telomeres shorten with successive mitoses in a stem cell population, and stem cells senesce when their telomeres reach a minimum limit, it might be expected that mean telomere length will be a better predictor of bone marrow proliferative capacity than age. We are currently investigating this possibility in further studies of this interesting 'age effect'.

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