

Endogenous thrombopoietin serum levels during multicycle chemotherapy

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Summary. Little is known about the behaviour of endogenous thrombopoietin (TPO) serum levels during rapid sequences of dose-intensified chemotherapy. To characterize the relationship between TPO levels and platelet counts in this setting we serially measured both parameters over the entire treatment period of patients receiving multicycle polychemotherapy. We found TPO and platelet responses to be generally antagonistic through all cycles. However, a cross-correlation analysis indicated that TPO responses preceded platelet responses by approximately one day in all patients. The cumulative severity of thrombocytopenia observed over successive cycles was accompanied by an

increasing TPO response which tended to grow overproportionally in relation to the degree of peripheral thrombocytopenia. These findings are consistent with a model suggesting that both platelet and megakaryocyte mass contribute to a receptor-dependent consumption process regulating the endogenous TPO level. In order to develop optimal schedules for exogenous TPO administration it might be important to consider endogenous TPO response characteristics.

Keywords: thrombopoietin, chemotherapy, thrombocytopenia, regulation, human.

With the introduction of dose-intensified conventional chemotherapy regimens the frequency of severe thrombocytopenia has risen substantially. In particular, those regimens consisting of multiple and rapidly successive cycles can show considerable cumulative toxicities. In an ongoing randomized phase III multicentre trial of the German Hodgkin's Study Group the newly developed multicycle polychemotherapy regimen BEACOPP for treatment of advanced Hodgkin's disease is being tested in a standard-dose variant against a dose-intensified variant with G-CSF support (Tesch *et al.*, 1998; Diehl *et al.*, 1998a). A recent interim analysis showed an increased efficacy of the intensified variant compared to the standard variant (Diehl *et al.*, 1998b). However, chemotherapy intensification was accompanied by substantially increased frequencies of W.H.O. grade IV thrombocytopenia and platelet transfusion rates (Engel *et al.*, 1998). Similar observations have been made in a current large multicentre trial of the German High-Grade NHL Study Group comparing a standard 3-weekly CHOP regimen against intensified variants (Truemper *et al.*, 1996, 1998). An analysis of the haemotoxicity during these regimens showed that dose intensification and later cycles

are factors related to higher incidences of thrombocytopenia (Kloess *et al.*, 1998).

Patients developing a severe thrombocytopenia during intensified regimen treatments are not only exposed to the risk of bleeding or the risks following from allogeneic platelet transfusions. Planned dose intensities may not be maintained in patients with severe thrombocytopenia since this event may enforce reduction of chemotherapy dosage or delays in administration. One relevant question arising from these observations is whether the administration of recombinant forms of thrombopoietin (TPO) will be clinically useful in this setting (Kaushansky, 1998; Lok & Foster, 1994). TPO is considered to be a major physiological regulator of thrombocytopoiesis *in vivo* (Broudy & Kaushansky, 1995). Several clinical studies showed that pharmacological doses of recombinant TPO derivatives, such as the pegylated human megakaryocyte growth and development factor (PEG-rHuMGDF) or full-length TPO (rHuTPO), effectively reduced the severity of chemotherapy-induced thrombocytopenia (Basser *et al.*, 1997; Fanucchi *et al.*, 1997; O'Malley *et al.*, 1996; Vadhan-Raj *et al.*, 1997).

To define an optimal timing strategy for recombinant TPO administration during multicycle dose-intensified chemotherapy, information detailing the response characteristics of the endogenous system might be useful. To date no detailed

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data are available in the literature about endogenous TPO level profiles during those treatments. We therefore serially measured endogenous TPO levels and platelet counts in patients receiving multicycle dose-intensified chemotherapy within the framework of the above-mentioned lymphoma trials. To obtain detailed response profiles and to get adequate information on the effect of multiple cycles we measured patients at short intervals over the entire period of their chemotherapy.

MATERIALS AND METHODS

Patients. Three patients treated with intensified chemotherapy on an outpatient basis for primary Hodgkin's or aggressive non-Hodgkin lymphomas were selected. Selection criteria were: (1) no bone marrow involvement as judged by marrow aspiration and biopsy of the iliacal crest; (2) no pretreatment with any cytostatics; (3) informed consent and availability of patients for close-meshed blood sampling over the entire period of chemotherapy.

Chemotherapy. All patients received different moderately dose-intensified multicycle polychemotherapy. Table I gives basic information on the patients enrolled and a detailed description of the chemotherapy regimen used.

Sample collection and preparation. Blood samples (EDTA-K and serum gel monovettes; Sarstedt, Nuembrecht, Germany) were obtained from each patient by venepuncture between 8.00 and 10.00 a.m. at 2–3 d intervals. Thrombocyte counts were determined within 3 h after collection. Serum was centrifuged immediately after collection at 4000 rpm for 10 min, separated into polypropylene tubes (Nunc, Wiesbaden, Germany) and stored at -80°C until needed for assay procedure.

Thrombopoietin assay. Serum TPO concentrations were measured using a commercially available sandwich ELISA kit specific for human TPO (Quantikine; R&D Systems, Minneapolis, Min., U.S.A.). The assay procedure was performed according to the manufacturer's instructions. Optical density (O.D.) values were determined using a microplate reader (SLT Labinstruments, Crailsheim, Germany). Each sample was measured in duplicate and the mean O.D. was taken as result. Standard curves were constructed by best linear curve fit.

Statistical analysis. SPSS 6.1.3 (SPSS Inc., Chicago, Ill., U.S.A.) was used for all statistical data analyses. To analyse the temporal relationship between the time series of TPO levels and platelet counts a cross-correlation analysis was performed considering varying days of temporal displacement. This method requires data at evenly spaced time intervals. Since patients were not measured daily, values on missing days were generated by linear interpolation of the two adjacent non-missing values to create time series with daily spaced intervals. To analyse the quantitative relationship between TPO levels and platelet counts a linear regression was performed using the model

$$y = \alpha x + \beta + \epsilon$$

where y is the log of the TPO concentration and x is the platelet count. To additionally account for an effect of the cycle number the following extended model was fitted to the data:

$$y = \alpha x + \sum_{k=1}^{k=n} \beta_k z_k + \epsilon$$

where y and x have the same meaning as in the above

Table I. Patients and chemotherapy.

	Patient 1	Patient 2	Patient 3
Patient characteristics*			
Age and sex	57, male	39, male	32, female
Diagnosis and clinical stage (according to Ann Arbor classification)	Non-Hodgkin lymphoma, I AE	Hodgkin's lymphoma, IV BE	Hodgkin's lymphoma, III B
Chemotherapy regimen: drug, dose, route and days of administration per cycle	CHOEP-21	BEACOPP-21 escalated	BEACOPP-14
Cyclophosphamide (mg/m ² /d) i.v.	750, day 1	1250, day 1	650, day 1
Adriamycine (mg/m ² /d) i.v.	50, day 1	35, day 1	25, day 1
Vincristine (mg/d) i.v.	2, day 1	2, day 8	2, day 8
Etoposide (mg/m ² /d) i.v.	100, days 1–3	200, days 1–3	100, days 1–3
Prednisone (mg/d) p.o.	100, days 1–5	40, days 1–14	80, days 1–7
Procarbazine (mg/m ² /d) p.o.	–	100, days 1–7	100, days 1–7
Bleomycine (mg/m ² /d) i.v.	–	10, day 8	10, day 8
G-CSF s.c.	–	5 $\mu\text{g}/\text{kg}/\text{d}$, from day 8 to days 14–15	5 $\mu\text{g}/\text{kg}/\text{d}$, from day 8 to day 13
Intended cycle duration	21 d	21 d	14 d

*Patients were enrolled in the following clinical trials: patient 1: NHL-B phase III trial of the German High-Grade NHL Study Group (Truemper *et al.*, 1996); patient 2: HD-9 phase III trial of the German Hodgkin's Study Group (Tesch *et al.*, 1998; Diehl *et al.*, 1998a); patient 3: current pilot trial of the German Hodgkin's Study Group.

model and n is the number of chemotherapy cycles given. In this model the cycle number was assumed to have an effect restricted to the intercept β . In order to yield individual intercepts β_k for each cycle k , n dichotomized indicator variables were defined. Thus the regression curves for each cycle k had a common slope α and individual intercepts β_k . Both models were

compared for goodness of fit using the coefficient of determination R^2 .

RESULTS

TPO levels and platelet counts during multicycle chemotherapy

Fig 1 shows the observed time courses of platelet counts and

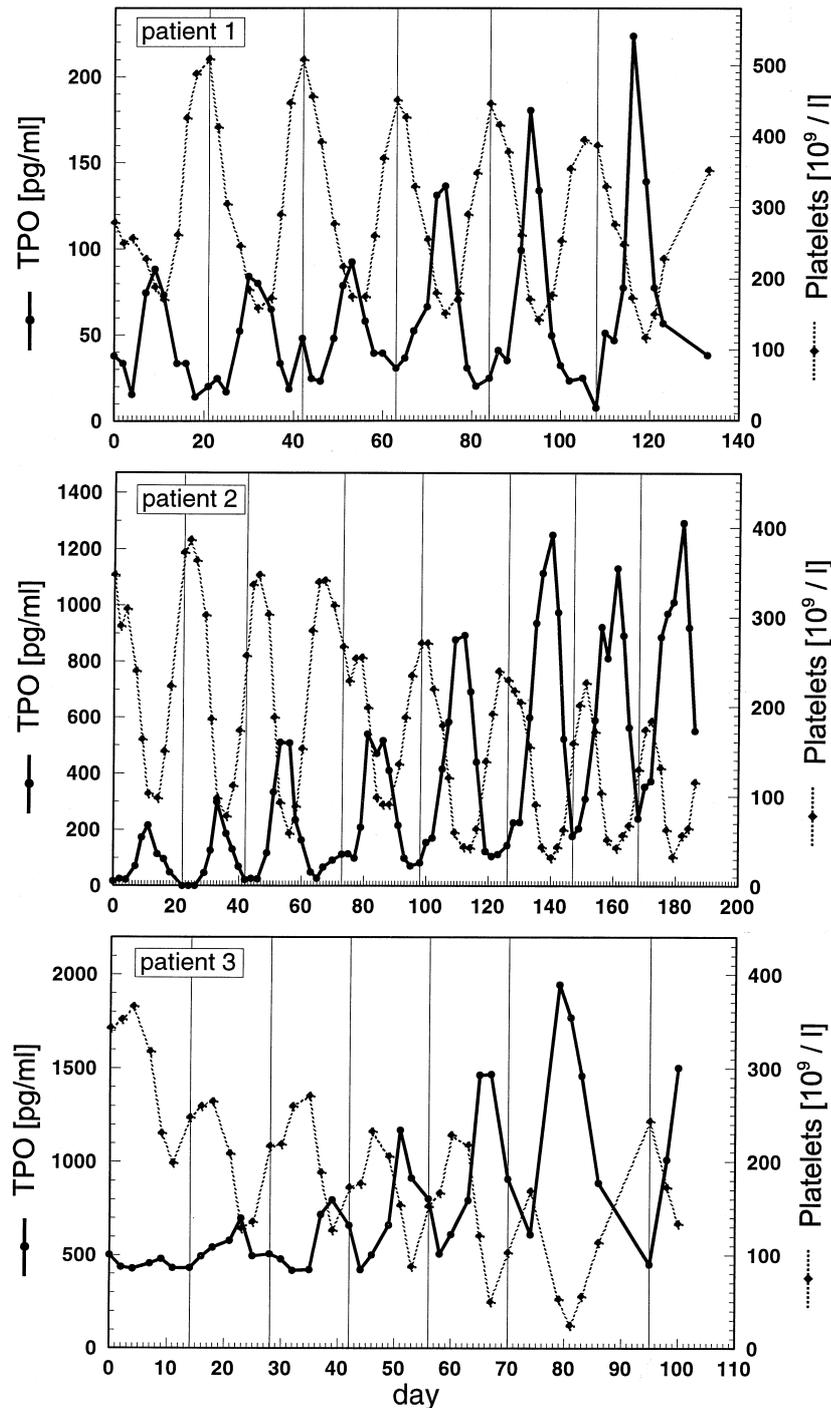


Fig 1. Time course of endogenous TPO serum levels and platelet counts during multiple cycles of chemotherapy. The first day of each therapy cycle is indicated by a vertical line. TPO concentrations of patient 2 on days 22, 24 and 26 were below the detection level (drawn as zero).

serum TPO concentrations in three patients during consecutive cycles of chemotherapy. No major complications such as severe infections or bleeding were observed. Some deviations from the intended cycle durations (see Table I) occurred. Cycle 6 of patient 3 was delayed due to insufficient haemopoietic recovery. In this cycle a platelet transfusion was required on day 81 due to severe thrombopenia. In patient 2 the doses of cyclophosphamide and etoposide were reduced by 12% according to the study protocol for the last three cycles due to prolonged severe neutropenia.

Both platelets and TPO showed remarkable antagonistic periodical fluctuations in response to the recurrent administration of haemotoxic drugs. Within each cycle, platelet counts of all patients decreased until a nadir was reached (cycle days 11–14) and increased subsequently. In some cycles (patient 2 and particularly patient 3) the increase of platelet count was not completed within the cycle period, with the peak values of platelet recovery being found somewhat after the initiation of the next cycle. The concomitant fluctuations of the endogenous serum TPO concentrations showed a qualitatively reversed pattern within each cycle, with an initial increase until a peak value was reached followed by a subsequent decrease. In patient 3 the response of TPO was not very pronounced in the first cycle despite a clear decrease of platelets. In addition, the endogenous TPO level before beginning of chemotherapy was considerably higher in this patient (501 pg/ml) than in patients 1 (38 pg/ml) or 2 (17 pg/ml).

Temporal relationship between TPO levels and platelet counts

To characterize the temporal relationship between the response pattern of TPO and platelets in greater detail, we performed a longitudinal cross-correlation analysis. In this analysis the correlation between both time courses of TPO and platelets was calculated dependent on a certain temporal displacement. The correlations can be drawn as a function of the temporal displacement and therefore displacements yielding a maximum of correlation can easily be identified. Fig 2 shows the results of this analysis. Assuming no displacement, a strong negative correlation can be found as expected. However, the diagram shows consistently for all patients that the correlation was maximally negative if the platelet time course was displaced by 1 d to the left (as indicated by the negative displacement in Fig 2). This indicated that the response of TPO apparently preceded the response of the platelets.

Effect of multiple cycles on TPO and platelet responses

Besides the cyclic fluctuations, both the platelet and TPO response showed a characteristic quantitative change with successive cycles. Whereas platelet nadirs decreased with each cycle, TPO response peaks increased concomitantly. There was no evidence that TPO response could be exhausted over multiple cycles. In contrast, in relation to the degree of thrombopenia there seemed to be an inadequate increase of TPO levels with later cycles. This is highlighted in Fig 3 which shows a scatter plot of platelet counts and the corresponding TPO levels of patient 2 grouped by the cycle number. To allow for the above

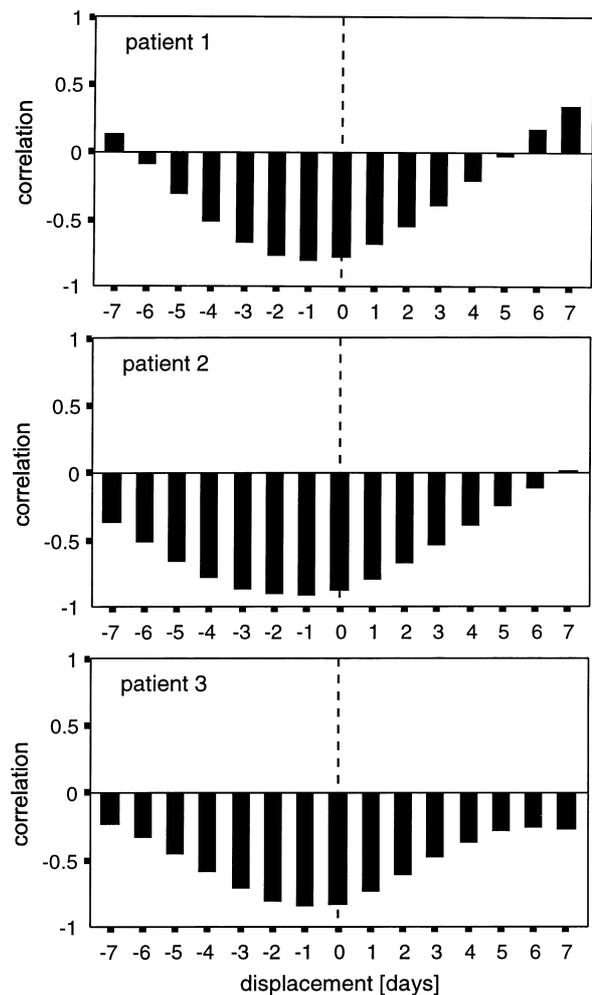


Fig 2. Analysis of the temporal relationship between TPO and platelet response profiles. The correlation between the profiles (correlation coefficient on the *y*-axis) is drawn for various relative temporal displacements of both profiles against each other (by the number of days shown on the *x*-axis). A negative number of days indicates a displacement of the platelet profile to the left, a positive number to the right, respectively.

mentioned phase displacement the platelet counts were drawn against the TPO level for the preceding day. In a first step, a simple univariate linear regression model was fitted to the data explaining the log of TPO concentration by the platelet counts (regression curve not shown). The coefficient of determination (R^2) of this model was 0.80 for patient 2. In a second model the cycle number was then additionally taken into account as a further explanatory variable (see Material and Methods section for detailed description of the model). Regression curves of this model are drawn in Fig 3 separately for each cycle. They show a trend of upward shift from the first to the last cycle. In this model, R^2 improved to 0.95. Similar results were obtained for patients 1 and 3, although these were less pronounced ($R^2 = 0.67$ and 0.77 for the first model, 0.71 and 0.86 for the second model, respectively).

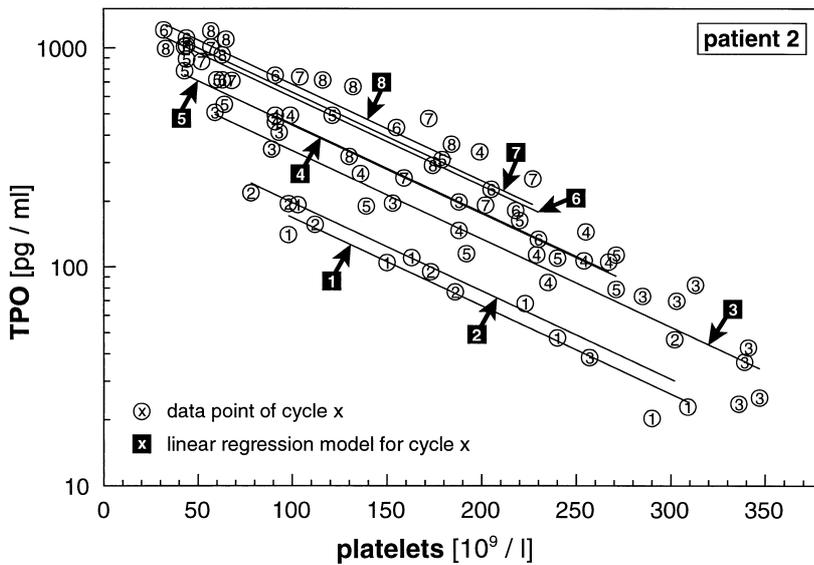


Fig 3. The effect of cycle number on the relationship between platelet count and TPO level (patient 2). Circles represent measured data, lines show estimates from a regression model fitted to the data. Numbers indicate to which chemotherapy cycle the experimental data and the regression lines belong.

DISCUSSION

In this study we analysed the dynamic responses of circulating TPO levels and platelet counts in three individual patients during treatment with multiple cycles of conventional dose-intensified chemotherapy. We deliberately focused our interest on a detailed evaluation of the response profiles in individual patients by continuous sampling at short intervals over the entire period of chemotherapy. Due to the low number of patients enrolled and the heterogeneity of the chemotherapy regimens no comparisons between patients could be made. Data were therefore analysed individually for each patient.

We found that the TPO response preceded platelet response by about one day. Furthermore, we observed that the quantitative relationship between TPO level and platelet count changed over successive cycles showing an 'inadequate' increase in TPO response.

Several investigators have characterized the time course of TPO levels and platelet counts in patients receiving high-dose chemotherapy (Chang *et al.*, 1996; Ishida *et al.*, 1996; Meng *et al.*, 1996; Nichol *et al.*, 1995; Hamaguchi *et al.*, 1996; Shimazaki *et al.*, 1997) and found an antagonistic behaviour between both parameters. Our data confirmed these observations for the conventional dose-intensified multicycle setting. However, the results of our analysis provided two new details on the mutual relationship between TPO level and platelet number which might be explained within the following context.

It has been suggested that the endogenous TPO level is regulated mainly by a consumption process in which TPO binds to the c-Mpl receptor and is then removed from circulation. The strong negative relationship between TPO levels and platelet counts observed during various thrombocytopenic states has led to the first hypothesis that this consumption process is directly dependent upon platelet mass (Kuter & Rosenberg, 1995; Stoffel *et al.*, 1996; Fielder *et al.*, 1996, 1997). A preceding TPO response would not be

explained by an exclusive dependency of TPO consumption on platelets. However, it has been concluded from observations on patients with immune thrombocytopenic purpura (ITP) and aplastic anaemia (AA) that also megakaryocytes may considerably consume TPO by this process. Despite a similar degree of thrombocytopenia in both groups, TPO levels are higher when thrombocytopenia is caused by megakaryocyte deficiency (as in AA) than by increased peripheral platelet destruction (as in ITP) (Emmons *et al.*, 1996; Mukai *et al.*, 1996; Ichikawa *et al.*, 1996; Hou *et al.*, 1998).

As this conclusion was drawn from observations on chronic thrombocytopenic states, our data on a dynamic disturbance of thrombopoiesis support this concept. Changes in platelet number during chemotherapy may simply be regarded as an image of the changes in megakaryocyte number appearing, however, after a certain delay. If the TPO consumption process is assumed to depend also on megakaryocyte mass the TPO response will appear before platelet response. The observed time lag may be dependent on various parameters, e.g. the receptor numbers in both compartments, differences in receptor binding kinetics, and the time of platelets to emerge from megakaryocytes. Data from other reports show that this typical pattern can be observed also during other dynamic disturbances which are not caused by chemotherapy. Mukai *et al.* (1996) measured TPO levels, platelet counts and megakaryocyte counts in a patient with amegakaryocytic thrombocytopenia during steroid treatment. They found that elevated TPO levels started to decrease according to the increase of megakaryocyte counts which also preceded the increase of platelet counts. Oh *et al.* (1995) present data for a patient with cyclic thrombocytopenia showing that TPO levels decrease slightly before platelets begin to rise.

The inadequate increase in TPO response over cycles also clearly indicates that TPO levels are not exclusively dependent on the platelet number. However, a clear interpretation for this phenomenon cannot be drawn from

our data. One possible explanation within the concept of a receptor-dependent TPO consumption could be a loss in the number of receptors being present on platelets and megakaryocytes over successive cycles due to a cumulative damage to thrombocytopoiesis. The cumulative damage could possibly result from an insufficient recovery due to the short cycle intervals, which might be improved by exogenous administration of recombinant forms of TPO (PEG-rHuMGDF or full-length rHuTPO). However, the possible risks of platelet excess following overstimulation of thrombocytopoiesis requires careful planning of recombinant TPO administration. According to this, a small gain in platelet recovery in each cycle by an additional exogenous stimulation might be sufficient to avoid cumulative toxicity. It can be speculated that exogenous TPO administration is most effective during a 'sensitive window' in the first days after chemotherapy administration when the endogenous system is not yet stimulated by thrombocytopenia, i.e. endogenous TPO levels are low and target cell numbers are still high.

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