Optimized pro-active management of anemia by Epoetin α in pre-operative chemotherapy for primary breast cancer

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Abstract. This prospective study quantifies the response of hemoglobin and other blood factors to Epoetin α (EPO) administration in the course of pre-operative chemotherapy in breast cancer. Blood count time series were analyzed in 38 primary breast cancer patients with/without EPO during the course of pre-operative chemotherapy with epirubicin and paclitaxel. EPO injections improved blood counts in 'anemic' patients (≤12.0 g/dl) receiving chemotherapy, but had little effect when administered to patients with higher hemoglobin concentrations. On the average, without chemotherapy, hemoglobin concentrations drifted toward about 11.1 g/dl without EPO but could be maintained at near 12.0 g/dl with EPO. In conclusion, there is potential for improved anemia management using EPO during pre-operative chemotherapy, which not only benefits quality of life but could also influence long-term survival in breast cancer through improved tumor oxygenation.

Introduction

There is considerable interest in the relative benefits and risks of pre-operative chemotherapy vs. adjuvant chemotherapy in breast cancer. By downstaging the primary tumor, pre-operative therapy increases the proportion of patients eligible for breast conserving therapy. 'Chemo-responsiveness' of the primary tumor represents a potential marker to improve and individualize subsequent clinical therapy recommendations (1-3). However, evidence at present does not support a long-term survival advantage when pre-operative therapy regimes are compared with the same regimes in the adjuvant setting (4-6) (Fisher B, *et al*, Annual meeting ASCO: abs. 449, 1997).

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The presence of primary tumor during pre-operative therapy could allow unfavorable 'feedback' processes to be activated (caused, for example, by interactions between chemotherapy and tumor biology) that might not have occurred in the adjuvant setting. Hence, much attention is focused on possible improvements in pre-operative therapy management that could reduce such unfavorable processes and ultimately improve long-term survival.

Anemia is a potential dose-limiting side effect of chemotherapy that can thus have an impact on its efficacy. Moreover, low hemoglobin concentrations are often associated with reduced tumor oxygenation, which may lead (7,8) to selection of tumor cells that are resistant to certain types of chemotherapy. *In vitro* experiments by Graeber *et al* (9) demonstrated selection of mutations with defective p53 genes in tumor cell populations with repeated hypoxia. Hence, hypoxia can accelerate malignant transformations. Hypoxia also stimulates formation of vascular endothelial growth factor (VEGF), leading to increased tumor angiogenesis (10,11). Moreover, Young *et al* found that hypoxia increases metastatic potential due to DNA over-replication (12). All of these disease processes could have implications for long-term survival.

Treatment modalities for fatigue syndrome include blood transfusions and erythrocyte concentrates, but these lead to only a short-term substitution and are subject to infection risk. Moreover, experimental studies have demonstrated that whole blood transfusions have an immunosuppressive effect (13,14). Anemia can be influenced by Dexamethason or oral androgen treatments. However, androgen treatments can cause side effects such as liver toxicity or virilization in female patients, and response may be slower than required.

The activity of erythropoietic agents in raising hemoglobin concentrations and hematocrit fractions and in improving oxygenation is well established. For example, Kelleher *et al* confirmed that EPO not only raised hemoglobin concentrations, but also improved tumor oxygenation (15). In animal models, reduction of anemia by EPO improved efficacy of chemotherapy.

However, although erythropoietic agents are generally well tolerated, there are potential side effects, including pain at the injection site, skeletal pain, low white blood cell counts and blood clots (16). Hence, the policy for administration of these agents during chemotherapy should appropriately address the

risk of side effects compared to the probability of a benefit. Current evidence-based guidelines recommend epoetin for chemotherapy-associated anemia with a hemoglobin concentration <10 g/dl (17,18). Use of epoetin for patients with less severe anemia (hemoglobin concentration <12 g/dl but never below 10 g/dl) should be determined by 'clinical circumstances'. In the absence of response, continuing epoetin beyond 6-8 weeks does not appear to be beneficial. According to guidelines, Epoetin should be 'titrated' once the hemoglobin concentration reaches 12 g/dl.

In a general context, 'titration' refers to the administration of small incremental doses of a drug until a desired clinical effect is observed. In medicine, titration is a simple concept to apply if the response to a drug is rapid compared to the transition time from a desired to an undesired clinical state. However, in chemotherapy induced anemia, the magnitude of the difference between normal and mildly anemic states or between mildly and severely anemic states is relatively small compared to the effects of chemotherapy. Consequently, transitions between these states can occur within one chemotherapy cycle (~3 weeks), which is quite comparable to the typical delays of several weeks for response to erythropoietic agents. Hence, 'titration' represents a nontrivial problem of control and regulation in a complex, interacting, time-dependent system with feedback processes. In view of these relationships, there is a clear need for proactive management of anemia and fatigue in pre-operative breast cancer chemotherapy. Given the possible complexity of the interaction between a particular chemotherapy regimen and biological processes involved in hemoglobin production, we can not even be sure that the same guidelines should apply to all chemotherapy regimens, let alone to all diseases and settings.

To this end, the present study focuses on improved understanding and quantification of the response of hemoglobin and other blood factors to Epoetin α (EPO) administration in the course of pre-operative chemotherapy with epirubicin and paclitaxel in breast cancer, with the aim of improved anemia management, optimal response to therapy, and ultimately improved survival. Our study reflects the effectiveness of EPO therapy for control of anemia in this context under realistic clinical conditions.

Materials and methods

Patients and study context. This observational study was conducted by the department of OB/GYN department at Cologne University on initially 41 consecutive primary breast cancer patients (M0) between 2000 and 2003, who were selected for treatment by combination pre-operative chemotherapy using epirubicin (Farmorubicin® Pharmacia, Erlangen, Germany) and paclitaxel (Taxol® Bristol-Meyers Squibb, Munich, Germany). All patients signed informed consent for this IRB approved protocol. The diagnostic work-up of primary breast cancer included mammography, mammasonography, MRT, PET, as well as core biopsy for histological verification.

Three of these patients were lost to follow-up for reasons unrelated to their disease stage or treatment, leaving a total of 38 for the endpoint analysis. Median age was 49.7 years (30-69). Of the 33 patients reporting menopausal status, 17 were pre-menopausal and 7 had received hysterectomies. The study protocol specified that patients should receive up to six cycles of chemotherapy with epirubicin (intravenous 1 h infusion, dose 90 mg/m^2) and paclitaxel (dose 175 mg/m^2). Blood counts were taken every week. At each chemo-cycle, patients received a check-up, including measurements of vital functions, differential blood count, Karnofsky index, and determination of chemotherapy-dependent toxicities. As reported elsewhere, pre-operative chemotherapy with epirubicin and paclitaxel at the dosage given here did not lead to severe side effects (aside from anemia).

The effectiveness of Epoetin α therapy was studied under typical clinical conditions: According to the study protocol, supporting Epoetin α therapy (Erypo® dosage 150 I.E./kg body weight per week) was recommended if a patient's hemoglobin concentration decreased below 12 g/dl, with the objective of maintaining the level between 12 and 14 g/dl. However, individual physicians had discretion on dosage and timing, taking individual factors into account. In the following, we refer to this supporting therapy generically as 'EPO'. Fig. 1 illustrates the frequency of EPO administration as a function of hemoglobin concentration. Patients with very low levels had a higher probability of receiving the medication. However, there were also cases in which patients received EPO at hemoglobin concentrations beyond the recommendations. Some patients did not receive EPO at all, whether they developed anemia or not, and their hemoglobin time series serve as reference or baseline estimates, though not as 'controls' in the strict sense.

Statistical methods. Initial blood counts are subject to random individual variation; the influence of EPO on blood count over the course of chemotherapy was modeled as an effect on the change in blood counts over the subsequent chemotherapy cycle, i.e., change in total hemoglobin concentration (grams per dl), erythrocyte count (million cells per μl), and hematocrit fraction (as a percent) in the three-week period between chemotherapy cycles. Blood count changes during a cycle were of course also affected by chemotherapy itself. The sample of measurements (regarded as independent statistical units) consisted of chemotherapy cycle differences of patients (one less than the number of chemotherapy cycles). Significance of the mean differences between changes in blood count with and without EPO were tested by a T-test with level of significance p=0.05.

In particular, the analysis features a sample of measurements taken in patients (N=13) receiving any EPO at all, as a possibly more homogeneous group than the sample of 38 patients as a whole. We refer to this group of 13 patients as the 'supported collective'; of course any particular patient in the supported collective could contribute measurements with and without EPO. The remaining 25 patients are referred to below as the 'unsupported collective'.

Since the administration of EPO was not random, but significantly depended on hemoglobin levels (as illustrated in Fig. 1), the association of EPO with blood count changes could be confounded by selection effects with respect to anemia. In essence, 'receiving therapy' is a partial surrogate for 'anemic'. Hence, in order to obtain a less confounded

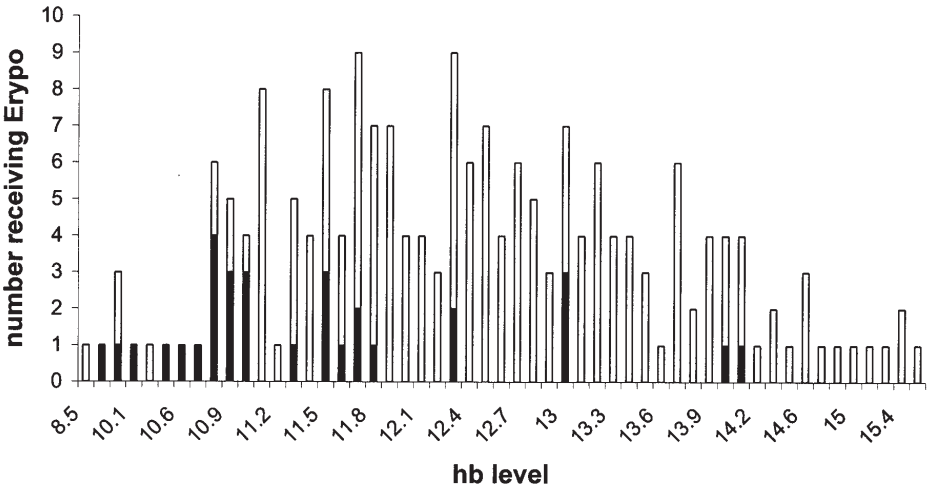

Figure 1. Frequency of EPO administration. Dark bars indicate number of chemotherapy cycles in which EPO was given compared to total number of cycles with corresponding hemoglobin measurement (light bars).

Table I. Mean blood parameters just prior to first chemotherapy cycle (week 1).

Parameter	All patients			Never received EPO			Supported collective		
	N	Mean	Standard deviation	N	Mean	Standard deviation	N	Mean	Standard deviation
Hemoglobin	38	13.43	1.22	25	13.65	1.29	13	12.99	0.98
Erythrocytes	37	4.40	0.41	24	4.45	0.46	13	4.32	0.30
Hematocrit	37	39.92	3.37	24	40.58	3.55	13	38.69	2.72

measure of EPO efficacy, we also use a propensity scoring approach (19). To this end, we separately analyze measurement sub-samples referred to in the following as 'anemic' and 'non-anemic', corresponding to hemoglobin counts below/above a cutoff value equal to 12.0 g/dl hemoglobin, respectively. Within each of these sub-samples, it turns out that the propensity for receiving EPO was fairly uniform, i.e., no longer depended significantly on hemoglobin (nor on time, erythrocytes, or hematocrit). Within the 'anemic' sub-sample of the supported collective, administration of therapy was nearly a random draw with probability 60%; within the 'non-anemic' sub-sample of the same collective, the administration of therapy was also nearly a random draw, but with probability 25%. Hence, according to the propensity scoring method, the efficacy within these sub-samples (mean difference between changes in blood count with and without EPO) more closely reflects the results that would have been obtained in a randomized study.

As an alternative method of capturing and controlling for the effects of the two factors (degree of current anemia and EPO administration) on blood count, multiple regression of blood count parameters was carried out. To this end, the regression variables *Hb12* (continuous), *EPO* (binary), and *EPO and anemia* (binary) were defined as follows:
Hb12 ≡ Hb-12.0 (in units of g/dl); if EPO is administered then EPO ≡ 1, else EPO ≡ 0.
if Hb≤12 g/dl and EPO is administered then EPO and anemia ≡ 1, else EPO and anemia ≡ 0. Correlations are reported as Spearman correlations.

Results

Chemotherapy and anemia. The distribution of blood counts (hemoglobin concentration, erythrocyte count and hematocrit fraction) prior to first chemotherapy cycle is summarized in the Tables.

Differences at this time between the 'supported collective' and the 'unsupported collective' were not significant (Table I). Spearman correlations of hemoglobin concentration, erythrocyte count and hematocrit fraction throughout the course of chemotherapy as well as their changes per chemotherapy cycle are summarized in Table II for all patients and separately within the 'anemic' and 'non-anemic' sub-samples. With or without EPO, correlations of absolute levels are very strong in all patients and somewhat lower but still strong in the sub-samples. Of greater interest are the changes in these quantities per chemotherapy cycle, which are also highly correlated.

The cumulative effects of chemotherapy cycles on blood count distributions are summarized in Table III. In the unsupported collective (III,A), the trend is a general decline of levels towards anemia, although some patients exhibited

Table II. Spearman correlations among blood parameters and their changes.

	All patients		Anemic		Non-anemic	
	R (levels)	R (changes)	R (levels)	R (changes)	R (levels)	R (changes)
Hemoglobin/erythrocytes	0.83	0.78	0.57	0.75	0.65	0.77
Hemoglobin/hematocrit	0.93	0.80	0.68	0.73	0.86	0.80
Erythrocytes/hematocrit	0.88	0.87	0.78	0.83	0.73	0.89

Table III. Evolution of blood parameters during chemotherapy cycles (weeks 1-16).

A, Unsupported collective

Unsupported	Hemoglobin				Erythrocytes				Hematocrit			
week	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max	N
1	13.65	10.00	15.50	25	4.45	3.50	5.20	24	40.58	31	47	24
4	12.85	10.20	15.40	23	4.24	3.50	5.30	23	38.36	31	45	22
7	12.60	10.00	14.60	24	4.20	3.60	5.01	24	37.79	32	43	24
10	12.30	11.10	13.90	23	4.09	3.50	4.70	21	36.95	33	42	22
13	12.24	10.90	14.10	16	4.01	3.50	4.50	16	35.94	31	40	16
16	11.85	10.80	13.00	14	3.98	3.40	4.70	13	35.77	31	42	13

B, Supported collective

Supported	Hemoglobin				Erythrocytes				Hematocrit			
week	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max	N
1	12.99	11.50	14.60	13	4.32	4.60	4.60	13	38.69	34	44	13
4	11.92	10.80	13.40	13	3.97	3.90	4.80	13	35.38	31	39	13
7	11.84	8.50	14.10	13	4.01	3.40	4.70	12	35.97	25	44	12
10	11.44	9.70	14.00	13	4.00	2.80	4.70	13	35.31	30	43	13
13	11.80	10.10	14.00	11	4.00	3.20	4.80	11	35.82	30	41	11
16	11.32	10.00	12.50	9	4.00	3.50	4.70	9	35.66	33	38	9

mild anemia at an early stage. Due to different numbers of cycles in different patients, the averages from one cycle to the next are not directly comparable, but a rough idea of the typical decline during the course of unsupported chemotherapy in this collective is obtained by comparing week 16 with week 1. By the 16th week, all parameter averages are below the normal ranges, with a drop of more than 1.3 g/dl in hemoglobin concentration during the first 3 cycles.

Table III,B shows the time-dependence of the blood parameter distributions for the supported collective. Keeping in mind that anemic patients are more likely to be in the treated collective in the first place, it is remarkable that there is a basic tendency toward stabilization between 11 and 12 g/dl hemoglobin, although some patients exhibited severe anemia before they were given EPO.

Taking the minimum hemoglobin level over all chemotherapy cycles as a measure of anemia, we found that of the 25 patients in the unsupported group, 10 maintained hemoglobin levels of at least 12.0 g/dl, 21 at least 11.0 g/dl, and all 25 at least 10.0 g/dl. Of the 13 patients in the supported group, none maintained hemoglobin of at least 12.0 g/dl (i.e., all 13 had drops below this level), but 3 maintained at least 11.0 g/dl, and all but one at least 10 g/dl. One patient had a minimum measured level of 8.5 (before receiving EPO). Nonetheless, taking into account potential dose limiting effects of anemia on chemotherapy, it is noteworthy that anemia did not lead to deviations from the planned chemotherapy schedule in this study.

Effectiveness of EPO in supported collective. The effectiveness of EPO within the supported collective is summarized in Table IV. Without EPO in the entire supported collective (Table IV,A), all three indicators decreased on the average, reflecting the effects of chemotherapy. Significant improvements in all three indicators are associated with administration of EPO, i.e., not only higher blood counts relative to chemotherapy without EPO, but increases on an absolute basis. The improvements per chemotherapy cycle were about 1.2 g/dl in

Table IV. Effects of EPO on blood parameters in supported collective.

A, Anemic and non-anemic

All supported	EPO given	N	Mean	Standard deviation	Difference of means	Upper 95% C.L.	Lower 95% C.L.	2-sided p-value
ΔHb	0	30	-0.82	0.82				
(g/dl)	1	25	0.38	1.12	**1.21**	1.74	0.68	**<0.001**
ΔEryth.	0	28	-0.21	0.32				
(10^6 cells/μl)	1	25	0.15	0.34	**0.36**	0.54	0.18	**<0.001**
Δhematocrit	0	28	-1.90	2.99				
(percent)	1	25	1.17	3.41	**3.08**	4.84	1.31	**0.001**

B, Anemic

Anemic	EPO given	N	Mean	Standard deviation	Difference of means	Upper 95% C.L.	Lower 95% C.L.	2-sided p-value
ΔHb	0	9	-0.73	1.23				
(g/dl)	1	20	0.69	0.97	**1.42**	2.29	0.56	**0.002**
ΔEryth.	0	9	-0.17	0.48				
(10^6 cells/μl)	1	20	0.23	0.32	**0.41**	0.72	0.10	**0.012**
Δhematocrit	0	9	-1.27	4.12				
(percent)	1	20	1.97	3.24	**3.23**	6.13	0.33	**0.030**

C, Non-anemic

Non-anemic	EPO given	N	Mean	Standard deviation	Difference of means	Upper 95% C.L.	Lower 95% C.L.	2-sided p-value
ΔHb	0	21	-0.86	0.62				
(g/dl)	1	5	-0.84	0.87	0.02	0.71	-0.67	0.95
ΔEryth.	0	19	-0.22	0.23				
(10^6 cells/μl)	1	5	-0.14	0.24	0.08	0.32	-0.16	0.50
Δhematocrit	0	19	-2.21	2.37				
(percent)	1	5	-2.00	2.00	0.21	2.61	-2.20	0.85

Bold = significant difference.

hemoglobin concentration (0.38 vs. 0.82), 0.36 million cells/μl in erythrocyte count (0.15 vs. 0.21), and 3.1% in hematocrit fraction (1.17 vs. 1.90).

On the other hand, this association alone does not prove that EPO administration is the reason for the improvement, since EPO administration was not randomized. In order to explore this important issue, Table IV,B summarizes the subset of measurements with hemoglobin below a cutoff value of 12.0 g/dl. Now, as explained above under statistical methods, within this 'anemic' sub-sample, administration of therapy was nearly a random draw with a probability of 60%. Table IV,B shows that, similarly to the supported collective as a whole, all three indicators decreased on the average without EPO but improved with administration of EPO. The improvements per chemotherapy cycle were ~1.42 g/dl in hemoglobin concentration (0.69 vs. -0.73), 0.41 million cells/μl in erythrocyte count (0.23 vs. -0.17), and 3.2% in hematocrit fraction (1.97 vs. -1.27). Thus, the relative improvements were slightly stronger than in the supported collective as a whole. To the extent that EPO administration within this group was close to a random draw, the results more nearly reflect the effects of EPO than those in the whole collective.

Table IV,C summarizes results in the sub-sample of the supported collective with hemoglobin above 12.0 g/dl. Within this 'non-anemic' sub-sample, the administration of therapy was also nearly a random draw, but with probability 25%. Remarkably, in this group there is no significant effect on blood counts associated with EPO. Blood counts decreased with or without EPO, and the declines per chemotherapy cycle were comparable to or somewhat larger than in the anemic group even without EPO.

Table V. Effects of EPO on blood parameters in entire collective.

A, All patients

All	EPO given	N	Mean	Standard deviation	Difference of means	Upper 95% C.L.	Lower 95% C.L.	2-sided p-value
ΔHb	0	129	-0.47	0.80				
(g/dl)	1	25	0.38	1.12	**0.85**	1.22	0.48	**<0.001**
ΔEryth.	0	123	-0.13	0.27				
(10^6 cells/μl)	1	25	0.15	0.34	**0.28**	0.40	0.16	**<0.001**
Δhematocrit	0	122	-1.19	2.56				
(percent)	1	25	1.17	3.41	**2.36**	3.54	1.18	**<0.001**

B, Anemic cycles

Anemic	EPO given	N	Mean	Standard deviation	Difference of means	Upper 95% C.L.	Lower 95% C.L.	2-sided p-value
ΔHb	0	39	-0.17	0.79				
(g/dl)	1	20	0.69	0.97	**0.86**	1.33	0.39	**0.001**
ΔEryth.	0	39	-0.05	0.28				
(10^6 cells/μl)	1	20	0.23	0.32	**0.28**	0.44	0.12	**0.001**
Δhematocrit	0	39	-0.45	2.71				
(percent)	1	20	1.97	3.24	**2.41**	4.01	0.81	**0.004**

C, Non-anemic cycles

Non-anemic	EPO given	N	Mean	Standard deviation	Difference of means	Upper 95% C.L.	Lower 95% C.L.	2-sided p-value
ΔHb	0	90	-0.60	0.77				
(g/dl)	1	5	-0.84	0.88	-0.24	0.47	-0.95	0.50
ΔEryth.	0	84	-0.16	0.26				
(10^6 cells/μl)	1	5	-0.14	0.24	0.02	0.26	-0.22	0.89
Δhematocrit	0	83	-1.54	2.43				
(percent)	1	5	-2.00	2.00	1.75	1.75	-2.66	0.68

Bold = significant difference.

Effectiveness of EPO in entire collective. Table V summarizes the effectiveness of EPO within the entire collective (supported and unsupported). As in the supported collective, without EPO, all three indicators decreased on the average (Table V,A), reflecting the effects of chemotherapy, and significant improvements are associated with administration of EPO. However, the improvements are lower on an absolute basis than in the supported collective. The improvements per chemotherapy cycle were about 0.85 g/dl in hemoglobin concentration (0.38 vs. 0.47), 0.28 million cells/μl in erythrocyte count (0.15 vs. 0.13), and 2.36% in hematocrit fraction (1.17 vs. 1.19).

Table V,B summarizes the subset of measurements corresponding to anemia (hemoglobin 12.0 g/dl or lower). Declines were considerably smaller without EPO than in the entire collective, but significantly improved with administration of EPO by similar amounts per chemotherapy cycle: 0.86 g/dl in hemoglobin concentration (0.69 vs. 0.17), 0.28 million cells/μl in erythrocyte count (0.23 vs. 0.05), and 2.41% in hematocrit fraction (1.97 vs. 0.45).

Table V,C shows that in the 'non-anemic' sub-sample of the entire collective (supported and unsupported), there is no significant effect on blood counts associated with EPO. Blood counts decreased with or without EPO, and the declines per chemotherapy cycle were larger than in the anemic group even without EPO.

Regression model for effects of EPO. As an alternative method of capturing the effects of the two factors (degree of current anemia and EPO administration) on blood count, multiple regression of blood count parameters was carried out using the regression variables *Hb12* (continuous), *EPO* (binary), and

Table VI. Regression analysis of EPO and anemia on changes in blood parameters in entire collective.

A, Hemoglobin

Hemoglobin	ß	Upper 95% C.L.	Lower 95% C.L	P-value
Constant	-0.27	-0.12	-0.43	0.001
Hb12	-0.29	-0.18	-0.40	<0.001
EPO + anemia	0.67	1.08	0.26	0.001

B, Erythrocytes

Erythrocytes	ß	Upper 95% C.L.	Lower 95% C.L.	P-value
Constant	-0.07	-0.01	-0.12	0.016
Hb12	-0.09	-0.05	-0.12	<0.001
EPO + anemia	0.21	0.35	0.07	0.004

C, Hematocrit

Hematocrit	ß	Upper 95% C.L	Lower 95% C.L	P-value
Constant	-0.59	-0.09	-1.08	0.021
Hb12	-0.90	-0.55	-1.26	<0.001
EPO + anemia	1.63	2.94	0.31	0.01

EPO and anemia (binary) defined as explained under statistical methods above. The estimates including uncertainties and p-values are given in Table VI; the model predicts the expected change in hemoglobin level during a cycle as follows (Table VI,A):

$$\Delta Hb = [-0.27-0.29 * Hb12+0.67 * EPO\ and\ anemia]\ g/dl. \quad [1]$$

Consistent with the previous results, it is noteworthy that the variable entering the model is not EPO itself, but rather *EPO and anemia*, which is 1 only if the patient received EPO and had anemia (Hb≤12 g/dl). According to this model, a patient with Hb=12.0 g/dl not receiving EPO would lose ~0.27 g/dl hemoglobin in a chemotherapy cycle.

Similar effects are seen with respect to other blood count variables. The change in erythrocyte count in the three-week period between chemotherapy cycles is modeled (Table VI,B) by:

$$\Delta Eryth. = [-0.07-0.09 * Hb12+0.21 * EPO\ and\ anemia]\ million\ cells\ per\ \mu l. \quad [2]$$

For the change in hematocrit fraction in the three-week period between chemotherapy cycles, we obtain the model of Table VI,C:

$$\Delta hematocrit = [-0.59-0.90 * hb12+1.63 * EPO\ and\ anemia]\ percent. \quad [3]$$

Discussion

In breast cancer, maintaining adequate hemoglobin concentrations contributes to improved quality of life and is associated with better response to therapy and long-term survival (20). In particular, in primary breast cancer therapy such as described in this report, the curative therapy approach demands that alterations in the intended chemotherapy regimen should be kept to a minimum. Thus, optimal control of hemoglobin levels is one of the prerequisites for therapy success in this setting. Low hemoglobin concentrations before and during radiation therapy have also been associated with poor prognosis in small-cell bronchial carcinoma, cervical cancer and head and neck tumors (21-24).

Moreover, many chemotherapy patients suffer from a chronic state of exhaustion, known as fatigue syndrome. This syndrome occurs frequently in combined radiation, endocrine, and chemotherapy regimens (25). It leads to reduced quality of life and may be associated with cognitive impairment. In particular, anemia, an important cause of fatigue, is one of the most common side effects of chemotherapy in cancer. In 15 of 18 reviewed studies, cancer patients with anemia had poor survival and local tumor control than non-anemic patients (26).

One possible explanation for an association of anemia and fatigue with poorer survival in cancer is that anemia is a potential dose-limiting side effect of chemotherapy and can thus have an indirect impact on its efficacy.

Aside from dose limitations, anemia induced by chemotherapy could also directly affect the relative effectiveness of pre-operative vs. adjuvant chemotherapy in primary breast cancer with regard to long-term survival; any interactions between tumor biology and therapy that influence subsequent disease processes, especially treatment resistance, could ultimately be relevant to long-term survival. In particular, hypoxia is a characteristic feature of locally advanced solid tumors resulting from an imbalance between oxygen supply and consumption. Tumor hypoxia, particularly under chemotherapy, can induce selection of malignant cells with favorably altered proteome and genome, allowing them to overcome nutritive deprivation or to escape their hostile environment, thus promoting treatment resistant disease.

Several mechanisms could be involved in tumor-associated anemia. For example, the tumor could activate immunological and inflammatory responses. Moreover, there is *in vitro* evidence for suppression of erythropoiesis due to the involved cytokines interleukin-1, -6 and tumor necrosis factor (27).

Del Mastro *et al* reported average hemoglobin drops of 3.0 g/dl without EPO compared to 0.8 g/dl with EPO on the average for breast cancer patients receiving adjuvant epirubicin (60 mg/m^2), cyclophosphamide (600 mg/m^2) and 5-fluoruracil (600 mg/m^2) chemotherapy (28). About half the patients without EPO developed severe anemia (Hb<10g/dl) compared to zero patients in the EPO group. Thus, it is clear that patients who receive injections of EPO increase their erythrocyte production on the average, resulting in higher hemoglobin levels and reduced fatigue on the average. However, due to side effects, it is good practice to administer EPO only when it is required and to titrate it otherwise.

Hence, there is a need to quantify possible differences in effectiveness between patients with some degree of anemia compared to those without anemia.

Our results indicate that patient response to EPO injections is heterogeneous and dynamic, depending strongly on current blood counts. Thus, reports of average response to EPO do not necessarily apply to individuals. In the current observational study, special care was taken to reduce possible selection bias by considering anemic and non-anemic sub-groups in which the decision to administer EPO was closer to a random draw. Within the 'anemic' sub-sample, all three indicators decreased on the average due to epirubicin and paclitaxel chemotherapy without EPO, but they improved with EPO injections. The relative improvements were slightly stronger than in the supported collective as a whole. In the 'non-anemic' sub-sample, no significant difference was seen with and without EPO injections.

The absolute levels and the changes of hemoglobin concentration, erythrocyte count and hematocrit fraction per chemotherapy cycle were highly correlated throughout the course of chemotherapy, in the collective as a whole and in subgroups, indicating that the biological processes determining these levels are all closely dynamically linked even during chemotherapy and EPO treatment.

If the hemoglobin level exceeds 12.0 g/dl at the beginning of a chemotherapy cycle, the regression models [1]-[3] imply declines of hemoglobin concentration, erythrocyte count and hematocrit fraction, whether or not EPO is given. As levels decline toward anemia, then even without EPO, the models imply that, on the average, there is a stabilization of all three variables under chemotherapy for a hemoglobin concentration of ~11.1 g/dl on the average but with individual minima far below that. Hence, even without EPO there appear to be biological mechanisms acting to regulate oxygen transport. On the other hand, this level may be undesirably low even on the average, and there are severe individual variations. With EPO, hemoglobin could evidently be maintained at ~12.0 g/dl, although the model does not strictly predict stabilization because of the difference in effectiveness above vs. <12.0 g/dl. The other variables could similarly be maintained at higher levels with EPO than without it, according to the models.

Our results indicate that there is still considerable potential for improved modeling and understanding of the effects of erythropoietic agents in pre-operative breast cancer, particularly in the range of mildly anemic blood counts. In view of our results and evidence from the literature, there is a clear basis for a pro-active approach to monitoring and control of all blood components in chemotherapy of cancer, particularly in pre-operative breast cancer chemotherapy. Improvements in pre-operative therapy management such as control of anemia are generally evaluated in terms of their immediate quality of life benefits. However, in view of the proven relationship between decreased hemoglobin concentration and anemic hypoxia in tumors, better management and control of the body's ability to transport and supply oxygen during pre-operative chemotherapy could also ultimately influence long-term survival in breast cancer and conceivably even provide a survival advantage compared to adjuvant therapy.

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