

Erythropoietin and G-CSF Treatment Associated with Improved Survival in Myelodysplastic Syndrome

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Abstract

Purpose

The aims of this study were to assess the effect of erythropoietin (EPO) + Granulocyte-CSF (G) treatment on survival and leukemic transformation in myelodysplastic syndrome (MDS).

Patients and Methods

We compared the long-term outcome of EPO-G treated (n=121) with untreated (n=237) MDS patients using multivariate Cox regression with delayed entry, for the first time adjusting for all major prognostic variables (World Health Organization classification, karyotype, cytopenias, level of transfusion-need, age, and sex).

Results

The erythroid response rate to EPO-G was 39%, and the median response duration 23 months (range 3-116+). In the multivariate analysis, treatment was associated with improved overall survival (hazard ratio 0.61, 95% confidence interval 0.44-0.83, $P=0.002$). Interestingly, this positive association was primarily observed in patients requiring less than 2 units of red blood cells per month. Treatment was not linked to the rate of AML in any defined subgroup, including patients with an increase of marrow blasts or an unfavorable karyotype.

Conclusions

The inherent risk of leukemic evolution in MDS makes the current investigation highly relevant, in light of the recent reports of potential negative effects of EPO treatment on outcome in patients with cancer. We conclude that treatment of anemia in MDS with

EPO-G may have a positive impact on outcome in patients with no or low transfusion-need, while not affecting the risk of leukemic transformation.

Introduction

The bone marrow malignancy myelodysplastic syndrome (MDS) affects 15,000 Americans each year.¹ Ninety percent of patients develop a transfusion-need during the course of their disease, severely impairing their quality of life, and 30-40% transform into acute myeloid leukemia (AML).² Although Erythropoietin (EPO) is not licensed for MDS in any country, most treatment-guidelines recommend EPO with or without G-CSF (G), acting in synergy with EPO,³⁻⁵ as first-line therapy for anemia in low-risk MDS.^{6,7} EPO-G treatment gives a 38-80% erythroid response rate, depending on patient selection, with a median response duration of around 2 years.^{3-5,8,9} Treatment response is associated with an improved quality of life.¹⁰⁻¹² A predictive model based on serum (S) EPO level and transfusion-need identifies patients with reasonable chances of response.¹⁰

It is currently debated whether treatment with hematopoietic growth factors negatively affects outcome in patients with cancer. Some reports suggest that EPO increases the risk of relapse in head-and-neck carcinoma,¹³ and decreases overall survival in cervical, breast, and non-small-cell lung cancer.¹⁴⁻¹⁶ Other studies indicate that G-CSF may increase the risk of AML evolution in aplastic anemia and severe congenital neutropenia.^{17,18}

To date, no studies have indicated an increased risk associated with EPO-G treatment in MDS.⁹ On the contrary, a recent study suggests a positive impact of erythroid growth factors on survival in MDS, although not adjusting for all currently used prognostic factors such as multilineage dysplasia and level of transfusion-need, in the multivariate analysis.¹⁹ Due to the rising concern of growth factor therapy in cancer, we performed a comprehensive study – for the first time taking into account all major

prognostic variables - in order to identify any potential increased risk of EPO-G treatment in MDS.

Methods

Patients

The EPO-G treated cohort (n=129) included all patients from three Nordic multicenter phase II trials performed 1990-1999.^{4,10,20} Inclusion criteria were refractory anemia (RA), RA with ringed sideroblasts (RARS), or RA with excess blasts (RAEB), according to the French-American-British classification,²¹ in combination with hemoglobin <10 g/dL (31%) or regular red blood cell (RBC) transfusion-need (69%). Exclusion criteria were ongoing bleeding, transfusion-dependent thrombocytopenia, or eligibility for allogeneic transplantation. The poor predictive group for erythroid response (S-EPO>500 U/L and requirement of ≥ 2 units of RBC per month),¹⁰ was ineligible for the third study. Six of the 129 patients did not complete the first six weeks of treatment and were non-evaluable for response, and 7 of 48 responders did not receive maintenance treatment and were non-evaluable for response duration.⁹ All patients were reclassified according to the World Health Organization (WHO) classification²² as previously described.²³

The control cohort (n=272) was selected from an Italian cohort of consecutive untreated MDS patients based on the same criteria as for the EPO-G studies. All patients were reclassified according to the WHO classification by two independent cytologists.²⁴ Except for supportive care, the patients remained untreated during the follow-up period in line with the current practice in Italy at the time.

The patients were followed up at the centers of the Nordic MDS Group and at the study center in Pavia. Bone marrow sampling was performed regularly and in case of clinical signs of progression in both cohorts, and the diagnosis of AML was always based on written morphology reports. Thus, the risk of differential bias regarding outcome measures was low.

The two cohorts were considered suitable for a valid comparison since the patients were enrolled during the same time-period in Western Europe, with detailed recording of important prognostic factors, and only a few patients received iron-chelation therapy. Eight of the 129 EPO-G patients and 35 of the 272 untreated patients lacked information about ≥ 1 variables included in the multivariate analysis, and therefore 121 EPO-G treated and 237 untreated patients were included in the final analysis (Table 1). The clinical trials and register studies followed the regulations of the National authorities, as previously reported.^{9,24}

Treatment and response criteria

Induction treatment with EPO and G-CSF was given for 12 to 18 weeks, and followed by maintenance treatment at the lowest effective dose in case of a response.^{4,9,10,20}

The definition of complete erythroid response was an increase in hemoglobin level to at least 11.5 g/dL without transfusion-need, while a partial response required an increase in hemoglobin level of 1.5 g/dL for patients with non-transfused anemia, or an abolished transfusion-need. Both response-criteria fulfilled the revised International Working Group criteria for erythroid response.²⁵ The date of relapse was defined as the date of first transfusion.

Statistical analysis

We used an intention-to-treat approach, including also EPO-G treated patients having discontinued treatment prematurely (n=6). Overall survival, in months, was measured from time of diagnosis to death, end of follow-up, or time of allogeneic bone marrow transplantation (n=7; in the untreated cohort only). A complete follow-up of the EPO-G cohort was performed by December 1, 2002, 45 months after last inclusion, and in the untreated cohort by December 31, 2005.

To adjust for the variable time between diagnosis and start of EPO-G treatment (in median 6 months [interquartile range 2.0-16.3]), a multivariate Cox model with delayed entry, or left truncation, was used. This allowed measurement of the survival-time from the time of MDS diagnosis also in the treated patients. However, at each observed event only those patients who had entered the study by that specific time point affected the analysis.²⁶ Though this is a potential source of bias, its effect can be considered negligible under the reasonable assumption that the patients entering the EPO-G studies were representative of untreated MDS patients with similar survival. Adjustment was also made for all major prognostic variables, and they were modeled as continuous (age, number of RBC units per month, absolute neutrophil count, S-EPO, S-LDH, and platelet count) or as indicator (EPO-G treatment, WHO-group, karyotype risk-group, and sex) covariates. Log-transformation and interval division into categorical measures modeled as binary dummy-variables were explored for skewed measures (S-EPO, S-LDH, and platelet count), and exponential-transformation was explored for age.

We investigated the proportional hazard assumption by testing for a non-zero slope in a generalized linear regression of the scaled Schoenfeld residuals on functions

of time. In both survival and AML the test was non-significant, thus indicating no major deviations from this assumption.

In order to address possible differences in age-specific mortality between the countries, the directly standardized mortality rates of Italy and Sweden were calculated by applying the calendar-year, age and sex specific mortality rates of each country (as provided by the respective national institutes of statistics) to a reference population with uniform age and sex distribution.²⁷

Results

Response characteristics

Forty-eight of the 123 evaluable patients (39%) had an erythroid response. Twenty-five of 85 transfusion-dependent patients (29 %) became transfusion-independent, and all patients responding to treatment stabilized their S-ferritin-levels during the course of the response. The median response duration was 23 months (3-116+), and 20% of responses lasted more than 4 years.

Optimization of the multivariate analysis

We explored different ways of modeling the covariates. The continuous covariates S-EPO level and S-LDH were not associated with outcome in the multivariate analysis ($P=0.88$ and 0.44 respectively), and log-transformation of S-EPO or entering either S-EPO or S-LDH as categorical measures modeled as dummy-variables did not lead to qualitatively different results. To prevent redundancy of covariates and to maximize the number of patients in the analysis, S-EPO and S-LDH were excluded. Log-transformation of platelet

count did not alter the result, and neither did modeling age as age-squared, and thus both were kept as continuous covariates.

Treatment associated with better overall survival

The EPO-G treated patients were significantly older and more frequently transfused than the untreated patients (Table 1). In a multivariate analysis (adjusted for EPO-G treatment, WHO-group, karyotype risk-group, number of RBC units per month, age, sex, and platelet and absolute neutrophil counts), EPO-G treatment was associated with better overall survival (hazard ratio [HR] 0.61, 95% confidence interval [CI] 0.44-0.83, $P=0.002$; Figure 1 and Table 2), and also decreased risk of non-leukemic death (HR 0.66, 95% CI 0.44-0.99, $P=0.042$). There was no association with the risk of AML evolution (HR 0.89, 95% CI 0.52-1.52, $P=0.66$; Figure 1 and Table 2). In addition, when selecting high-risk patients with inherent increased risk of AML evolution (International Prognostic Scoring System Intermediate-2 or High),²⁸ treatment was not linked to an increased rate of AML (HR 0.64, 95% CI 0.25-1.61, $P=0.34$).

Next, we investigated the potential effect of the exclusion of 8 of 129 treated and 35 of 272 untreated patients due to missing variables included in the overall multivariate analysis. When only adjusting for age, WHO-group, sex, EPO-G treatment, and transfusion-dependency, all but 6 patients could be kept in the analysis. A similar association of EPO-G treatment with improved survival was seen both among the unselected and selected patients (HR 0.66, 95% CI 0.49-0.89, $P=0.006$ and 0.59, 0.44-0.81, $P=0.001$, respectively).

Finally, when using separate Cox models for males and females, and patients below and above 70 years of age, the association of treatment with improved survival was highly similar in all subsets (data not shown).

Association with better survival limited to patients with low transfusion-need

Patients requiring <2 units of RBC per month have a higher probability of response to EPO-G according to the predictive model.¹⁰ Therefore we included an interaction term consisting of treatment and transfusion-need (<2 [$n_{\text{treated}}=75$, $n_{\text{untreated}}=196$] or ≥ 2 [$n_{\text{treated}}=46$ $n_{\text{untreated}}=41$] units of RBC per month) in the multivariate analysis, and found a significant interaction ($P=0.039$). Hence, we performed a stratified analysis, still adjusting for the same variables as in the overall Cox analysis. EPO-G treatment was associated with enhanced survival only in patients receiving <2 units per month (HR_{<2 U/month} 0.44, 95% CI 0.29-0.66, $P<0.001$, HR _{≥ 2 units/month} 1.04, 95% CI 0.57-1.89, $P=0.91$; Figure 2).

The proportions of patients with IPSS Low/Int-1 vs. Int-2/High did not differ significantly between treated and untreated patients in the two strata ($P=0.96$ and 0.21 , respectively). The response rate for the less compared to the more heavily transfused patients was higher, 56% vs. 18% ($P<0.001$), although the response duration was similar ($P=0.57$). As expected due to the higher response rate, the less transfused patients received EPO-G for a longer time period than the more heavily transfused patients (47% and 11%, respectively, were on therapy ≥ 6 months, $P<0.001$).

There was no association between treatment and risk of leukemic transformation for patients with low or high transfusion-need (HR 0.87, 95% CI 0.45-1.66, $P=0.67$ and HR 0.92, 95% CI 0.28-3.03, $P=0.89$, respectively).

Treatment response correlated with favorable outcome

Next, we modified the overall Cox analysis by splitting the treatment covariate into three categories, modeled as binary dummy-variables: untreated, responder, non-responder. Responders to EPO-G had an association with better survival compared to untreated patients (HR 0.40, 95% CI 0.26-0.62, $P<0.001$), while non-responders showed no such association (HR 0.80, 95% CI 0.56-1.14, $P=0.21$). Neither response nor non-response was significantly associated with the rate of AML evolution (HR 0.60, 95% CI 0.29-1.24, $P=0.17$, and HR 1.13, 95% CI 0.61-2.10, $P=0.69$, respectively). Also, there was a close association between treatment response and longer non-leukemic survival (HR_{responders} 0.39, 95% CI 0.22-0.67, $P=0.001$, HR_{non-responders} 0.97, 95% CI 0.62-1.52, $P=0.91$).

Similar age-specific mortality in the patients' countries of origin

The directly standardized mortality rates were calculated for Sweden and Italy for 5 year intervals during the enrollment-period 1990-2000. The rate ratios of Sweden vs. Italy were highly similar, varying between 1.03 and 1.13.

Discussion

Recent reports of potential adverse effects of EPO-treatment in patients with solid tumors have challenged the use of EPO +/- G-CSF also for the anemia in MDS and launched an active discussion in the Food and Drug Administration aiming at defining the role of EPO in patients with malignancies.^{13-16,29,30} After reviewing the existing literature, the recent American Society of Clinical Oncology/American Society of Hematology guideline still

recommends treatment with erythroid growth factors for patients with low-risk MDS, however, EPO is not indicated for other cancer patients with disease related anemia.³¹ Furthermore, they comment that there is no evidence of improved survival by EPO treatment in any type of cancer.

Recent data in MDS suggests that EPO +/- G improves overall survival without affecting the rate of progression to AML.¹⁹ In order to more thoroughly explore this issue we designed a comprehensive study comparing a well defined cohort of patients treated with EPO-G^{4,9,10,20} and a suitable cohort of untreated patients.²⁴

Despite the fact that the EPO-G patients were significantly older and more frequently transfused than the untreated patients, which *per se* would imply a worse prognosis for this group,^{24,28,32} a comparison using a multivariate Cox analysis adjusting for all major prognostic variables demonstrated that EPO-G treatment was associated with significantly enhanced overall and non-leukemic survival. In an analysis stratified by transfusion-burden, the association with improved survival was restricted to patients requiring <2 units of RBC per month, which was not unexpected since these patients responded better to EPO-G compared to the more heavily transfused.

There was no association between treatment and the estimated probability of AML evolution in the overall analysis or in the subgroup analysis of high-risk patients (IPSS Int-2 or High). Hence, neither prolonged exposure in responding patients, nor short-term exposure in high-risk patients was associated with disease progression.

A valid comparison of the two cohorts requires the assumption that the natural history of untreated MDS patients in the Nordic Countries and Italy was similar during the study period. Following the introduction of the French-American-British classification in 1982, the diagnosis and management of MDS have been uniformed and

are therefore comparable in most western countries. Furthermore, the survival according to IPSS prognostic groups has been shown to be overlapping in several countries, hence, making this assumption reasonable.^{28, 32} In addition, the standard mortality ratio of Sweden vs. Italy showed a similar mortality during the years of patient-enrollment, even somewhat higher in Sweden, leaving it unlikely that a difference in age-specific mortality confounded the analyses.

The observation that EPO-G treatment was associated with longer survival only in patients with non-transfusion dependent anemia or a moderate transfusion-need, raises several hypotheses. The patients may benefit from correction of anemia in terms of improved cardiac function and performance status. Anemia is associated with a significant decline in physical performance in persons 70 years or older,³³ and a poorer outcome in patients with heart failure.³⁴ Anemia in MDS not only deteriorates the quality of life,^{10,35} but is also associated with poor outcome²⁸ and increased incidence of heart failure.³⁶ We recently showed that the onset of RBC transfusion-need worsens the survival of patients with MDS, in part due to a higher risk of heart failure-related death.^{24,32} This adverse outcome might be explained by the fact that transfused patients often show lower hemoglobin-levels over time compared to untransfused. Another positive effect of treatment response may be attributed to the prevention of progressive iron overload, by elimination of the transfusion-dependency. Progressive iron overload is inversely correlated to survival in transfusion-dependent MDS patients.²⁴ EPO also has an intriguing impact on the immune system. EPO has been shown to induce an anti-tumor effect in a multiple myeloma mouse model, presumably mediated by activation of CD8+ T-cells,³⁷ and recently similar effects on T-cells in MDS have been reported (Prutchi-Sagiv *et al*, *Blood* 108[11]: 756A, Nov. 16 2006). Moreover, EPO-G diminishes

the relative CLIP (class II-associated invariant chain peptide) amount on hematopoietic precursor cells, conceivably increasing their ability to present tumor antigens to the T helper cells (Chamuleau *et al*, Leukemia Research 31: S61A, May 2007).

EPO-receptors have been found in several types of primary tumor samples and tumor cell lines, although the effects of EPO on these cells *in vitro* have been somewhat conflicting and it is debated whether all EPO-receptors are functional.^{38,39} Around 60% of AML patients have EPO-receptors present on their leukemic blasts, although few are sensitive to EPO *in vitro*.⁴⁰ Cells from low-risk MDS patients also express EPO-receptors, however, the expression is lower than on normal bone marrow progenitors.⁴¹ In addition, we have shown that EPO has negligible proliferative effect on MDS stem cells carrying a 5q deletion *in vitro*, while normal stem cells respond with rapid proliferation.^{42,43} Moreover, recent data also suggests a positive impact of EPO on survival of critically ill trauma patients, conceivably by non-hematopoietic EPO-effects.⁴⁴

Regarding the effects of G-CSF in MDS, it has been shown that sub-clones of cells with a deletion of chromosome 7 have a growth advantage over diploid cells when exposed to G-CSF *in vitro*.⁴⁵ A preliminary reported randomized study of chronic G-CSF treatment vs. placebo in high risk MDS patients (n=102) showed no increased risk of AML-evolution in the treatment arm (Greenberg *et al*, *Blood* 82[10]: 196A, Nov. 15 1993).

In our treated cohort as many as half of the patients were treated within 6 months of diagnosis, however, this time-interval until start of treatment potentially gives rise to selection effects that could affect outcome. A longer disease duration before starting treatment implies a longer time of suffering from the negative effects of anemia and chronic transfusions. Also, a patient with longer disease duration may have a more stable disease, with lower risk of leukemic transformation, although the contrary is also

conceivable. However, any such effects were adjusted for using Cox regression with delayed entry (or left truncation), which is generally considered to be the preferred method since it minimizes potential bias introduced by such selection effects.²⁶ Therefore we interpret the observed increased survival in the EPO-G treated patients as a likely effect of treatment.

We stress that patients should be selected in a rational way before commencing treatment, by excluding patients in the poor predictive group for response,^{9,10}. Furthermore, that EPO-G should be administered at the lowest possible maintenance dose, and should be discontinued at the time of relapse of transfusion-dependency, in order to avoid futile treatment with undocumented long-term effects on outcome.

We conclude that EPO-G treatment deserves its prominent place in the treatment of anemia in low-risk MDS since it is associated with better overall survival in patients with no or low transfusion-need, without any apparent affect on the risk of leukemic transformation.

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Tables

Table 1: Patient characteristics

Variable	EPO-G cohort (n=121)	Untreated cohort (n=237)	Cohort differences (P-values§)
Median age, years (interquart. range)	71 (65-79)	66 (58-73)	<0.0001
Sex, n (%)			0.17
Male	66 (54.6)	147 (62.0)	
Female	55 (45.4)	90 (38.0)	
WHO-group*, n (%)			0.007
RA/RARS/5q-	33 (27.3)	87 (36.7)	
RCMD/RCMD-RS	42 (34.7)	67 (28.3)	
RAEB-1	30 (24.8)	32 (13.5)	
RAEB-2	16 (13.2)	51 (21.5)	
IPSS group†, n (%)			0.003
Low	31 (25.6)	54 (22.8)	
Intermediate-1	57 (47.1)	86 (36.3)	
Intermediate-2	22 (18.2)	33 (13.9)	
High	4 (3.3)	22 (9.3)	
Missing karyotype	7 (5.8)	42 (17.7)	
Transfusion-dependent, n (%)			<0.0001
No	38 (31.4)	148 (62.5)	
Yes	83 (68.6)	89 (37.6)	
Predictive group for response‡, n (%)			<0.0001
Good	59 (48.8)	58 (24.5)	
Intermediate	43 (35.5)	28 (11.8)	
Poor	14 (11.6)	4 (1.7)	
Unknown	5 (4.1)	147 (62.0)	

*WHO-group RA: refractory anemia, RARS: RA with ringed sideroblasts, 5q-: 5q- syndrome, RCMD: refractory cytopenia with multilineage dysplasia, RCMD-RS: RCMD with ringed sideroblasts, RAEB-1: refractory anemia with excess blasts (5-9% bone marrow blasts), RAEB-2: refractory anemia with excess blasts (10-19% bone marrow blasts)

†IPSS = International Prognostic Scoring System²⁸

‡Predictive group for erythroid response to EPO-G according to a validated predictive model based on level of transfusion-need and S-EPO level.¹⁰

§P-values were calculated using the Pearson's chi² test, except for age where Wilcoxon Rank-sum (Mann-Whitney) test was used

Table 2: Multivariate analysis of overall survival and risk of AML evolution

Covariate	Mortality						AML					
	n	n _{dead}	Person-Years at Risk	Hazard Ratio	95% CI	P	n	n _{aml}	Person-Years at Risk	Hazard Ratio	95% CI	P
Cohort												
Untreated	237	140	405.9	Ref			237	61	365.1	Ref		
EPO-G treated	121	95	631.3	0.61	0.44-0.83	0.002	117	32	607.9	0.89	0.52-1.52	0.67
WHO group*												
RA/RARS/5q-RCMD/RCMD-RS	120	54	516.0	Ref			119	13	506.7	Ref		
RAEB-1	109	77	300.2	2.13	1.46-3.09	<0.001	107	19	284.4	2.46	1.18-5.13	0.017
RAEB-2	62	50	127.8	3.38	2.22-5.15	<0.001	62	30	114.1	9.83	4.87-19.86	<0.001
	67	54	93.2	5.23	3.34-8.21	<0.001	66	31	67.7	15.2	7.08-32.55	<0.001
Karyotype group†												
Good	204	117	730.4	Ref			202	49	687.9	Ref		
Intermediate	54	42	134.8	1.59	1.10-2.31	0.010	54	15	128.1	1.13	0.61-2.10	0.61
Poor	51	45	70.7	3.31	2.25-4.88	<0.001	50	21	62.3	2.53	1.43-4.47	0.001
Unknown	49	31	101.3	1.49	0.99-2.24	0.057	48	8	94.6	0.84	0.39-1.79	0.65
RBC units per month	NA	NA	NA	1.10	1.01-1.20	0.034	NA	NA	NA	0.86	0.72-1.03	0.10
Age	NA	NA	NA	1.03	1.02-1.04	<0.001	NA	NA	NA	1.00	0.98-1.02	0.93
Sex												
Male	213	147	575.4	Ref			209	61	538.9	Ref		
Female	145	88	461.8	1.16	0.87-1.54	0.30	145	32	434.1	1.36	0.85-2.16	0.20
Neutrophil count ($\Delta 1 \times 10^9/L$)	NA	NA	NA	1.03	0.98-1.08	0.27	NA	NA	NA	1.07	1.02-1.13	0.010
Platelet count ($\Delta 1 \times 10^9/L$)	NA	NA	NA	0.99	0.98-1.00	0.13	NA	NA	NA	0.98	0.97-1.00	0.075

*World Health Organization classification (WHO) group RA: refractory anemia, RARS: RA with ringed sideroblasts, 5q-: 5q- syndrome, RCMD: refractory cytopenia with multilineage dysplasia, RCMD-RS: RCMD with ringed sideroblasts, RAEB-1: refractory anemia with excess blasts (5-9% bone marrow blasts), RAEB-2: refractory anemia with excess blasts (10-19% bone marrow blasts)
†Karyotype risk-group according to the International Prognostic Scoring System.²⁸

Figures

Figure 1. EPO-G treatment associated with better overall survival

Treatment with EPO-G was significantly associated with improved overall survival, while no association with the risk of AML evolution was observed. The curves were estimated from multivariate Cox regression analyses with delayed entry, adjusted for World Health Organization classification, karyotype, cytopenias, level of transfusion-need, age, and sex.

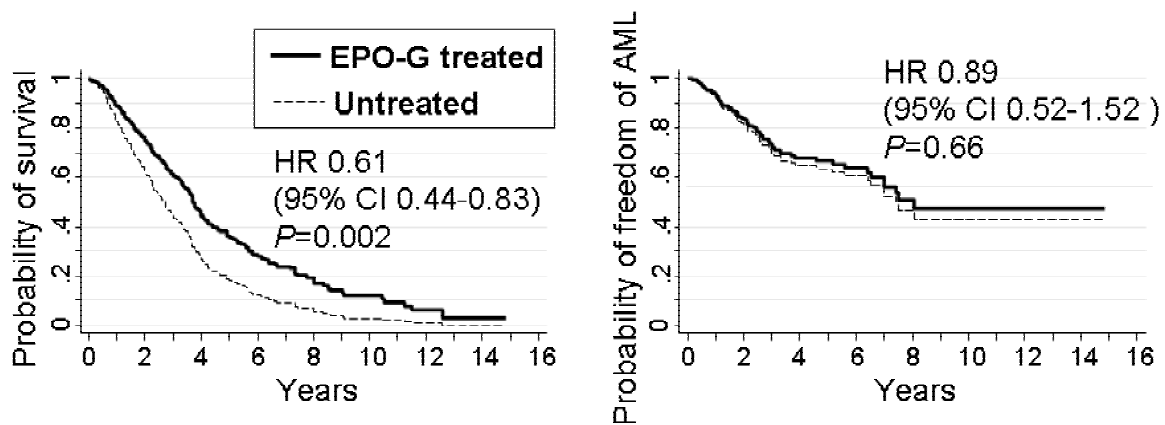


Figure 2. EPO-G treatment associated with better survival mainly in patients with low transfusion-need

The strongest association of EPO-G treatment with survival was observed in patients requiring less than 2 units of RBC per month. No association with survival was seen in more heavily transfused patients. In addition, there was no association with the risk of AML evolution in either group. The curves are estimated from multivariate Cox regression analyses with delayed entry, adjusted for adjusting for World Health Organization classification, karyotype, cytopenias, level of transfusion-need, age, and sex.

