DR. LAURA CESINI (Orcid ID : 0000-0003-4805-9928)

DR. MARCO CERRANO (Orcid ID : 0000-0003-1666-3100)

DR. ISABELLA CAPODANNO (Orcid ID : 0000-0002-6091-1899)

DR. ROBERTO LATAGLIATA (Orcid ID : 0000-0002-7741-862X)

Article type : Original Article

Corresponding author email id: rob.lati@libero.it

Erythropoietin Treatment in Chronic Phase Chronic Myeloid Leukemia Patients Treated with Frontline Imatinib who Developed Late Anemia

Laura Cesini[1], Camilla Frieri[2], Claudia Baratè[3], Federica Sorà[4], Massimiliano Bonifacio[5], Marco Cerrano[6], Antonia Cagnetta[7], Chiara Elena[8], Lara Aprile[9], Nicola Sgherza[10], Malgorzata Trawinska[11], Antonella Gozzini[12], Isabella Capodanno[13], Monica Crugnola[14], Ida Carmosino[1], Emilia Scalzulli[1], Federica Ricci[3], Monica Bocchia[9], Micaela Bergamaschi[7], Chiara Aguzzi[6], Simona Sica[4], Sara Galimberti[3], Massimo Breccia[1], Luigiana Luciano[2], Roberto Latagliata[1].

[1]Department of Translational and Precision Medicine, University "La Sapienza" of Rome [2]Hematology, University "Federico II" of Neaples [3]Department of Clinical and Experimental Medicine – Hematology, University of Pisa [4]Hematology, Catholic University of Sacred Heart of Rome [5]Department of Medicine, Section of Hematology, University of Verona [6]Department of Oncology and Hematology, University of Turin [7]Clinical Hematology, Policlinico San Martino, Genua [8]Hematology, San Matteo Hospital, Pavia [9]Division of Hematology, University of

Siena [10]Hematology, IRCCS, "Casa Sollievo della Sofferenza", San Giovanni Rotondo [11]Hematology, Sant'Eugenio Hospital, Rome [12]Hematology, University of Florence [13]Hematology, Reggio Emilia, [14]Hematology, University of Parma

Running title: EPO in late anemia of CML patients

Corresponding Author
Dr Roberto Latagliata
Hematology, Department of Precision and Translational Medicine, Policlinico Umberto I, "Sapienza" University of Rome
Via Benevento 6 – 00161 Rome (Italy)
Fax: 39.06.44241984

Abstract word count: 199
Manuscript word count: 2189
Number of references: 15
Number of figures: 2
Number of tables: 1

Keywords: chronic myeloid leukemia, imatinib, late anemia, erythropoietin

Summary statements

1) Late anemia in CML patients is still an unmeet clinical need and a specific evaluation of EPO in this subset is lacking
2) EPO is effective in resolving late anemia with no relevant toxicity
3) EPO could improve quality of life in this subset of elderly patients in the current clinical practice

ABSTRACT

Background Role of erythropoietin (EPO) in the treatment of late anemia in patients with Chronic Myeloid Leukemia (CML) is still undefined.

Methods Fifty CML patients treated at 14 Institutions with frontline imatinib for at least 12 months and in stable complete cytogenetic response who developed a late chronic anemia treated with EPO were retrospectively evaluated.

Results Median time from imatinib start to EPO treatment was 42.2 months [interquartile range (IQR) 20.8 – 91.9]. Median Hb value at EPO starting time was 9.9 g/dl (IQR 8.9 – 10.3): eleven patients (22.0%) were transfusion dependent. Alpha-EPO (40,000 UI weekly) was employed in 37 patients, beta-EPO (30,000 UI weekly) in 9 patients, zeta-EPO (40,000 UI weekly) in 2 patients and darbopoietin (150 mcg/weekly) in the remaining 2 patients. On the whole, 41 patients (82.0%) achieved an erythroid response, defined as a stable (> 3 months) improvement > 1.5 g/dl of Hb level, and 9 patients (18.0%) indeed resulted resistant. Among responding patients, 10 relapsed after a median time from EPO start of 20.7 months (IQR 10.8 – 63.7). No EPO-related toxicity was observed.

Conclusions Results of EPO treatment for late chronic anemia during long-lasting imatinib therapy are encouraging, with a high rate of response.

INTRODUCTION

The introduction of imatinib in the treatment of patients with Chronic Myeloid Leukemia (CML) has drastically changed the outcome and the overall survival has been actually estimated similar to that of general population [1-2]. In addition, treatment discontinuation in subjects achieving deep molecular response is now possible and leads to "operational" cure of CML in many cases [3].

However, more than two-thirds of CP-CML patients have to receive imatinib indefinitely to maintain a treatment response [4]. In this setting, long-lasting toxicities related to imatinib may severely affect the quality of life (QoL) and the compliance to treatment.

Among other specific chronic toxicities related to the drug, late occurrence of moderate/severe anemia during long-lasting treatment with imatinib has been reported in a moderate rate of patients and seems to be more common in elderly [5]. It has been recently shown that bone marrow fibrosis is very common in CML patients at diagnosis (65% have grade 1 and 27% grade 2) [6], and that patients with two or more chromosomal abnormalities in Ph'-negative cells (including those that are related to clonal hematopoiesis or myelodysplasia) showed sometimes worse response to TKIs[7]. Thus, several factors could be responsible for developing anemia during imatinib, but its pathogenesis is not fully understood.

Treatment of such late toxicity is still subjective without specific recommendations about the possible role of erythropoietin (EPO). Recently, ASCO and ASH updated their practical guidelines on the management of cancer-associated anemia with erythropoiesis-stimulating agents [8]: they suggested that EPO "may be offered to patients with chemotherapy-associated anemia whose cancer treatment is not curative in intent and whose hemoglobin has declined to < 10 g/l…". In this context, CML could represent a "border-line" situation: we know that CD26+/BCR-ABL1-positive on genomic DNA leukemic stem cells persist even in patients achieving the deepest possible response (so, probably CML is an "incurable" disease) [9], but we also know that survival of CML patients is comparable to that of general population (so, CML,

from an operational point of view, can be considered as "curable") [1-2]. As a matter of fact, the use of EPO in the CML setting has not been explored.

We report a retrospective cohort of patients treated at 14 Italian Institutions with frontline imatinib for more than 12 months at least in stable complete cytogenetic response (CCyR) who developed a late chronic anemia and received concomitant EPO therapy, with the aim to describe the management and the outcome of patients with this possible complication.

MATERIALS AND METHODS

Patients with newly diagnosed CML were considered eligible for this retrospective analysis in the presence of all the following criteria:

- chronic phase at diagnosis
- no prior treatment, apart from hydroxyurea given for less than three months to reduce white blood cell (WBC) count
- imatinib as frontline treatment for more than 12 months, with achievement of at least stable CCyR
- onset of late chronic anemia during imatinib which was treated with EPO

Hematologic and cytogenetic responses to imatinib were categorized according to standard criteria. As to molecular responses, defined according to International Scale (IS), Major Molecular Response (MMR) was defined as BCR-ABL/ABL ratio < 0.1%; indeed, deep Molecular Response (DMR) was defined as a BCR-ABL1/ABL ratio <0.01/ (MR4) or <0.0032 (MR4.5) IS. Those patients who were evaluated only with molecular analysis with a persistent BCR-ABL1/ABL1 ratio < 1.0 were considered as in CCyR.

Late chronic anemia was defined as the presence of stable (> 3 months) and otherwise unexplained (creatinine level < 2 mg/dl, normal iron balance, bilirubin level < 2 mg/dl, folate and vitamin B12 in the normal range) Hb level < 11 g/dl which occurred or persisted for more than 6 months from imatinib start. We defined as “late”, the anemia who occurred in patients with normal Hb levels at 6th and/or 12th month of treatment; indeed, was considered as “persistent” the anemia who occurred in patients with Hb levels < 11 g/ dl at either 6th and 12th month of treatment.

Evaluation of response to EPO therapy was made according to the criteria commonly used in patients with Myelodysplastic Syndromes (MDS) treated with EPO: in particular, erythroid response was considered as a stable (> 3 months) improvementt > 1.5 g/dl of Hb level or disappearance of previous transfusion need.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) (normally distributed data), median and interquartile range (IR) (non-normally distributed data), or as percentage frequencies, and within-patient comparisons were made by unpaired t-test and χ^2 test, as appropriate, at significance levels of $p<0.05$.

Overall survival (OS) was calculated from the date of EPO start to death due to any cause or to the last follow-up. Survival probabilities were calculated using the Kaplan–Meier method. Survival comparisons were made by the log-rank test.

All calculations were made using a standard statistical package (SPSS for Windows Version 15.0; Chicago, IL).

RESULTS

Overall, 50 patients were collected because all the eligibility criteria refereed before were met. Patients were followed in 14 hematologic Institutions in Italy. The main clinical features of these patients at diagnosis are reported in the Table 1. Starting dose of imatinib was 400 mg daily in 46 patients (92.0%) and 300 mg daily in 4 patients (8.0%).

In 14 patients, late chronic anemia occurred without a previous episode of early anemia (< 6 months from imatinib start), after a median period from imatinib start of 47.1 months (IQR 29.6 – 88.2). Fifteen patients had indeed a previous episode of early anemia completely resolved, and then presented late chronic anemia, after a median period from imatinib initiation of 16.1 months (IQR 7.5 – 108.6). The remaining 21 patients had an episode of early anemia, which never completely resolved, and became definitely affected by chronic anemia after 6 months from imatinib initiation.

Median time from imatinib start to EPO treatment was 42.2 months (IQR 20.8 – 91.9): median age at baseline of EPO treatment was 75.1 years (IQR 68.0 – 79.2), all patients were in stable CCyR and 40/50 (80.0%) were also in stable molecular response [MMR in 18 patients (36.0%) and DMR in 22 patients (44.0%)]. Median Hb value at baseline was 9.9 g/dl (IQR 8.9 – 10.3): according to the mean corpuscular volume (MCV) at baseline, late anemia was microcytic with MCV < 80 fl in 2 patients (4.0%), normocytic with MCV ≥ 80 and < 100 fl in 29 patients (58.0%) and macrocytic with MCV ≥ 100 fl in the remaining 19 patients (38.0%). Eleven out 50 patients (22.0%) received periodic packed red cell transfusions before the starting of EPO treatment.

According to the definition of late anemia in this analysis, total bilirubin values, folate and cyanocobalamin levels and iron balance at baseline were in the normal range in all patients. Median creatinine level at baseline was 1.20 mg/ml (IQR 0.90 – 1.37): four patients had a creatinine level > 1.5 but < 2.0 mg/ml. Endogenous EPO levels at baseline were available in 28 patients, with a median value of 22.0 mU/ml (IQR 15.1 – 30.6).

At baseline of EPO treatment, imatinib daily dose was 400 mg in 22 patients (44.0%), 300 mg in 21 patients (42.0%) and 200 mg in the remaining 7 patients (14.0%). In particular, among the 46 patients who had started imatinib 400 mg daily at diagnosis, 24 (52.1%) reduced the dosage before EPO starting time (300 mg daily in 19 patients and 200 mg daily in 5 patients): among the 4 patients who had started imatinib 300 mg daily at diagnosis, 2 reduced the dosage at 200 mg daily before EPO starting time.

Alpha-EPO (40,000 UI weekly) was employed in 37 patients, beta-EPO (30,000 UI weekly) in 9 patients, zeta-EPO (40,000 UI weekly) in 2 patients and darbepoietin (150 mcg/weekly) in the remaining 2 patients.

On the whole, 41 patients (82.0%) achieved an erythroid response, with a stable (> 3 months) improvement > 1.5 g/dl of Hb level, whereas 9 patients (18.0%) were resistant to treatment: median Hb value at different time-points during EPO treatment for the entire cohort of patients are reported in the Figure 1. All the 11 patients with transfusion requirement achieved transfusion independence during EPO treatment.

Among responding patients, 10 (24.3%) had a relapse after a median time from EPO start of 20.7 months (IQR 10.8 – 63.7) and stopped EPO treatment: four of them needed transfusions after relapse. In addition, 5 patients died while in response from CML unrelated causes and 2 stopped EPO while in response: the remaining 24 responding patients are still alive and in treatment with EPO after a median time from EPO start of 28.0 months (IQR 14.5 – 52.3). No thrombotic event was observed during EPO treatment.

Five-year overall survival from EPO treatment of the entire cohort was 81.1% (95%CI 67.0 – 95.2) (Figure 2): there was no difference in the OS between patients responding or resistant to EPO treatment.

DISCUSSION

While early anemia is a well-recognized complication of initial treatment with imatinib [10], there are very few data on the incidence, prognostic features for onset, management, and clinical significance of late/persistent chronic anemia.

In a previous study, the occurrence of late chronic anemia was reported in about 30% of patients treated frontline with imatinib at a single Center, with a higher incidence in elderly patients, in female and in patients with high Sokal risk at diagnosis [5]. This complication is often associated with patient discomfort and a reduced QoL, particularly in aged subjects due to their baseline frailty and the usual presence of concomitant severe diseases: in addition, a reduced event-free survival was recently reported in elderly CML patients with untreated late chronic anemia compared to elderly without chronic anemia [11]. As a matter of fact, late chronic anemia during long-lasting treatment for CML still represents an unmet need.

The use of EPO in CML has been already reported as effective in some studies. Cortes et al, investigating the prognostic significance of anemia during therapy with imatinib: 102 patients received EPO resulting in an increase of Hb level >2 g/dL in 68% of cases [12]. In a more recent study, a total of 608 patients with CML patients treated with imatinib were evaluated. Anemia was detected in 502 patients and 217 of these (36%) received a treatment with EPO, achieving erythroid response in 80% of cases [13]: in this experience, an excess of thrombotic events as a consequence of EPO treatment, has been reported.

However, the vast majority of patients treated with EPO in those studies, were treated for anemia of early onset (< 6 months from imatinib start), which has been described as early toxicity and therefore with a different pathogenesis from late chronic anemia: this type of anemia very often resolves spontaneously or with a temporary reduction of imatinib dosage.

On the contrary, only sporadic data are reported since now in literature about the EPO treatment in patients with late chronic anemia. Our analysis tried to overcome this lack of knowledge, based on a relatively high number of patients with similar clinical

features collected in the current clinical practice from different Centers with expertise in the CML management.

As expected, median age of our cohort was higher if compared to general CML population, either at diagnosis than at EPO start. Due to the real-life nature of our experience, different EPO molecules were employed, but all patients received the standard dose recommended for the treatment of anemia during antineoplastic therapies (40,000 UI weekly of EPO alpha or equivalent doses for other EPO molecules) [14].

This approach seemed very effective, and more than 80% of patients achieved erythroid response, with a stable rise in Hb level of > 1.5g/dl from baseline value: all patients with a baseline transfusion requirement became transfusion-independent. In addition, as shown in Figure 1, Hb rise was generally faster (behind the first 2 months of treatment) and remains stable over time. A relapse of anemia occurred in about 25% of responding patients, but after a median period of response exceeding 18 months: in our experience, all relapsed patients stopped EPO treatment, thus an eventual increase in the dosage, as usually done in MDS patients resistant/relapsed during standard EPO doses, was not explored.

Two further questions should be addressed. First of all, is EPO treatment safe in this setting? In our experience EPO treatment was safe and no adverse event was reported: in particular no episode of uncontrolled hypertension or thrombotic event occurred, differently from the already mentioned study of Santos et al [13], even if our cohort included very elderly patients. It is worth of note, however, that 42 patients (84.0%) were already on antiaggregating or anticoagulant treatment for concomitant diseases before EPO start.

Moreover, is EPO treatment able to improve OS in patients with late chronic anemia? The OS of our cohort from the start of EPO treatment was impressive considering the median age > 75 years at baseline, even if no difference was observed according to EPO response. An effect of TKIs on the long-term OS cannot be excluded as already reported from other groups [15]. A comparison with both patients without late anemia

or with untreated late anemia of matched age was not possible with present study, thus this second question remains still open at present.

In conclusion, the occurrence of late chronic anemia in CML patients receiving long-lasting imatinib therapy is quite common but its pathogenesis and treatment are still unclear: further evaluations, as for example analysis of kayotypic MDS-related abnormalities in the bone marrow of patients at late anemia onset, would be useful to highlight possible causes of this side effect. Results with EPO are encouraging, with a high rate of response and an excellent safety profile: however, larger prospective studies are warranted to define more precisely the management (when to start EPO?, at what dosage?, for how long?) of such approach in the treatment of this common late complication of prolonged imatinib therapy.

REFERENCES

1. Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, Deininger MW, Silver RT, Goldman JM, Stone RM, Cervantes F, Hochhaus A, Powell BL, Gabrilove JL, Rousselot P, Reiffers J, Cornelissen JJ, Hughes T, Agis H, Fischer T, Verhoef G, Shepherd J, Saglio G, Gratwohl A, Nielsen JL, Radich JP, Simonsson B, Taylor K, Baccarani M, So C, Letvak L, Larson RA; IRIS Investigators. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med 2006; 355(23):2408-17.
2. Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, Baccarani M, Deininger MW, Cervantes F, Fujihara S, Ortmann CE, Menssen HD, Kantarjian H, O'Brien SG, Druker BJ; IRIS Investigators. Long-Term Outcomes of Imatinib Treatment for Chronic Myeloid Leukemia. N Engl J Med 2017; 376(10):917-927.
3. Fava C, Rege-Cambrin G, Dogliotti I, Cerrano M, Berchialla P, Dragani M, Rosti G, Castagnetti F, Gugliotta G, Martino B, Gambacorti-Passerini C, Abruzzese E, Elena C, Pregno P, Gozzini A, Capodanno I, Bergamaschi M, Crugnola M, Bocchia M, Galimberti S, Rapezzi D, Iurlo A, Cattaneo D, Latagliata R, Breccia B, Cedrone M, Santoro M, Annunziata M, Levato L, Stagno F, Cavazzini F, Sgherza N, Giai V, Luciano L, Russo S, Musto P, Caocci G, Sorà F, Iuliano F, Lunghi F, Specchia G, Pane F, Ferrero D, Baccarani M, G Saglio. Observational study of chronic myeloid leukemia Italian patients who discontinued tyrosine kinase inhibitors in clinical practice. Haematologica 2019;104(8):1589–1596
4. Molica M, Colafigli G, Scalzulli E, Alunni Fegatelli D, Chiatamone Ranieri S, Rizzo L, Diverio D, Efficace F, Latagliata R, Foà R, Breccia M. Ten-year outcome of chronic-phase chronic myeloid leukemia patients treated with imatinib in real life. Ann Hematol 2019;98(8):1891-1904.
5. Latagliata R, Volpicelli P, Breccia M, Vozella F, Romano A, Montagna C, Molica M, Finsinger P, Carmosino I, Serrao A, Zacheo I, Santopietro M, Salaroli A, Alimena G. Incidence of persistent/late chronic anemia in newly

diagnosed patients with chronic myeloid leukemia responsive to imatinib. Am J Hematol. 2015 Feb;90(2):105-8. doi: 10.1002/ajh.23879. Epub 2014 Nov 19.

6. Hamid A, Ashraf S, Qamar S, Naveed MA, Hameed A, Farooq MA. Myelofibrosis in Patients of Chronic Myeloid Leukemia in Chronic Phase at Presentation. J Coll Phy Surg Pak 2019;29(11):1096-1100.
7. Ni H, Sun X, Xu Y, Lyle D, Petersen P, Zhao X, Drum H, You B, Liu D, Liu C, Jiang JG. Clinical implications of clonal chromosomal abnormalities in Philadelphia negative cells in CML patients after treated with tyrosine kinase inhibitors. Cancer Genet 2019;238:44-49.
8. Bohlius J, Bohlke K, Castelli R, Djulbegovic B, Lustberg MB, Martino M, Mountzios G, Peswani N, Porter L, Tanaka TN, Trifirò G, Yang H, Lazo-Langner A. Management of cancer-associated anemia with erythropoiesis-stimulating agents: ASCO/ASH clinical practice guideline update. Blood Adv 2019;3(8):1197-1210
9. Bocchia M, Sicuranza A, Abruzzese E, Iurlo A, Sirianni S, Gozzini A, Galimberti S, Aprile L, Martino B, Pregno P, Sorà F, Alunni G, Fava C, Castagnetti F, Puccetti L, Breccia M, Cattaneo D, Defina M, Mulas O, Baratè C, Caocci G, Sica S, Gozzetti A, Luciano L, Crugnola M, Annunziata M, Tiribelli M, Pacelli P, Ferrigno I, Usala E, Sgherza N, Rosti G, Bosi A, Raspadori D. Residual Peripheral Blood CD26+ Leukemic Stem Cells in Chronic Myeloid Leukemia Patients During TKI Therapy and During Treatment-Free Remission. Front Oncol 2018;8:194.
10. Sneed TB, Kantarjian HM, Talpaz M, O'Brien S, Rios MB, Bekele BN, Zhou X, Resta D, Wierda W, Faderl S, Giles F, Cortes JE. The significance of myelosuppression during therapy with imatinib mesylate in patients with chronic myelogenous leukemia in chronic phase. Cancer 2004;100:116–121.
11. Cesini L, Carmosino I, Breccia M, De Benedittis D, Mohamed S, De Luca ML, Colafigli G, Molica M, Scalzulli E, Massaro F, Mariggiò E, Rizzo L, Loglisci MG, Scamuffa MC, Vozella F, Diverio D, Mancini M, Alimena G, Foà R, Latagliata R. Incidence of Clinically Significant (≤10 g/dL) Late Anemia in Elderly Patients with Newly Diagnosed Chronic Myeloid Leukemia Treated with Imatinib. Oncol Res Treat 2019 Oct 8:1-5.

12. Cortes J, O'Brien S, Quintas A, Giles F, Shan J, Rios MB, Talpaz M, Kantarjian H. Erythropoietin is effective in improving the anemia induced by imatinib mesylate therapy in patients with chronic myeloid leukemia in chronic phase. Cancer 2004;100:2396–2402.
13. Santos PS, Alvarado Y, Kanarjian H, Verma D, O'Brien S, Mattiuzzi G, Ravandi F, Borthakur G, Cortes J. Long-term prognostic impact of the use of erythropoietic-stimulating agents in patients with chronic myeloid leukemia in chronic phase treated with imatinib. Cancer 2011; 117(5): 982-991.
14. Rizzo JD, Somerfield MR, Hagerty KL, Seidenfeld J, Bohlius J, Bennett CL, Cella DF, Djulbegovic B, Goode MJ, Jakubowski AA, Rarick MU, Regan DH, Lichtin AE. Use of epoetin and darbepoetin in patients with cancer: 2007 American Society of Hematology/American Society of Clinical Oncology clinical practice guideline update. Blood 2008;111:25–41.
15. Bower H, Björkholm M, Dickman PW, Höglund M, Lambert PC, Andersson TM. Life Expectancy of Patients With Chronic Myeloid Leukemia Approaches the Life Expectancy of the General Population. J Clin Oncol 2016;34(24):2851-7.

Table 1 – Patient features at diagnosis

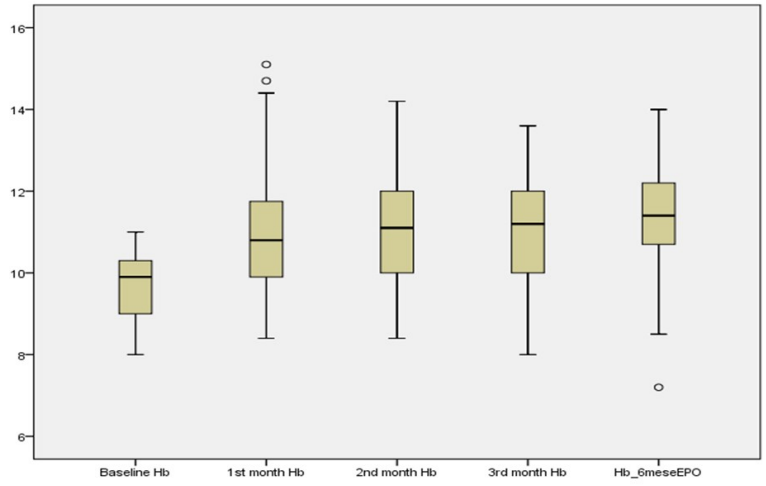
N° of patients	50
M/F, n° (%)	30/20 (60.0/40.0)
Median age, years (IQR)	69.5 (62.9 - 75.0)
Median Hb, g/dl (IQR)	12.3 (11.2 – 13.7)
Median WBC, x 10^9/l (IQR)	43.6 (24.7 – 75.8)
Median PLTS, x 10^9/l (IQR)	377 (276 – 644)
Sokal risk score, n° (%):	
Low	11 (23.4)
Intermediate	31 (66.0)
High	5 (10.6)
Not evaluable	3

Figure legends

Figure 1 – Median Hb values at different time-point during EPO treatment

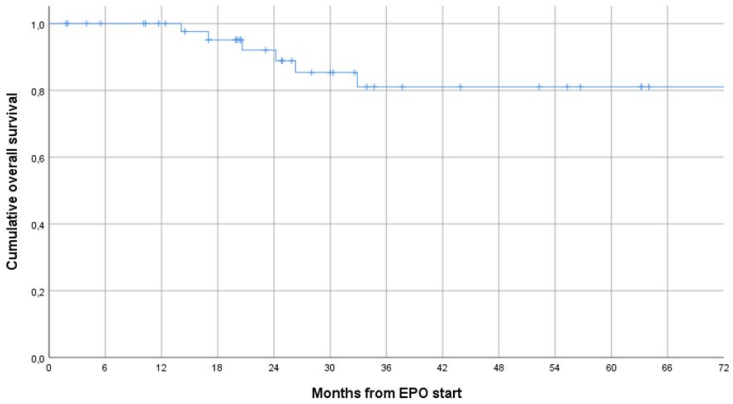
Figure 2 – Overall survival of the entire cohort from EPO start

Figure 1 – Median Hb values at different time-point during EPO treatment



ejh_13436_f1.jpg

Figure 2 – Overall survival of the entire cohort from EPO start



ejh_13436_f2.jpg