



Response to erythroid stimulating agents (ESA) and FLOW CYTOMETRIC characteristics of bone marrow erythroid precursors in IPSS-R lower risk myelodysplastic syndromes patients.

M.G.Raddi¹; E.Attardi¹; A.Consagra¹; C.Amato¹; A.Brogi²; E.Masala²; B.Peruzzi³; S.Bencini³; R.Caporale³; A.Sanna⁴; V.Santini¹.

¹MDS Unit, Division of Hematology, AOU-Careggi-University of Florence, Florence, Italy.

² Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy.

³ Department of Flow Cytometry Diagnostic Center and Immunotherapy, AOU Careggi-University of Florence, Florence, Italy.

⁴ Hematology Department, Careggi Hospital, Florence, Italy.



Abstract

Standard treatment of anemia in lower-risk myelodysplastic syndromes (LR-MDS) is based on erythropoiesis stimulating agents (ESAs), but some patients are primary refractory (PR) or lose response (secondary refractory, SR). High serum erythropoietin (sEPO) is the main negative predictor and treatment of ESA resistant anemia is challenging, so more predictors are needed. EPO promotes differentiation of committed erythroid cells (COMe) into erythroid precursors (EP), constituted by early (EEP) and late precursors (LEP). Flow cytometry (FC) can characterize COMe and EP. RED score is a FC parameter assessing dyserythropoiesis (1).

Background

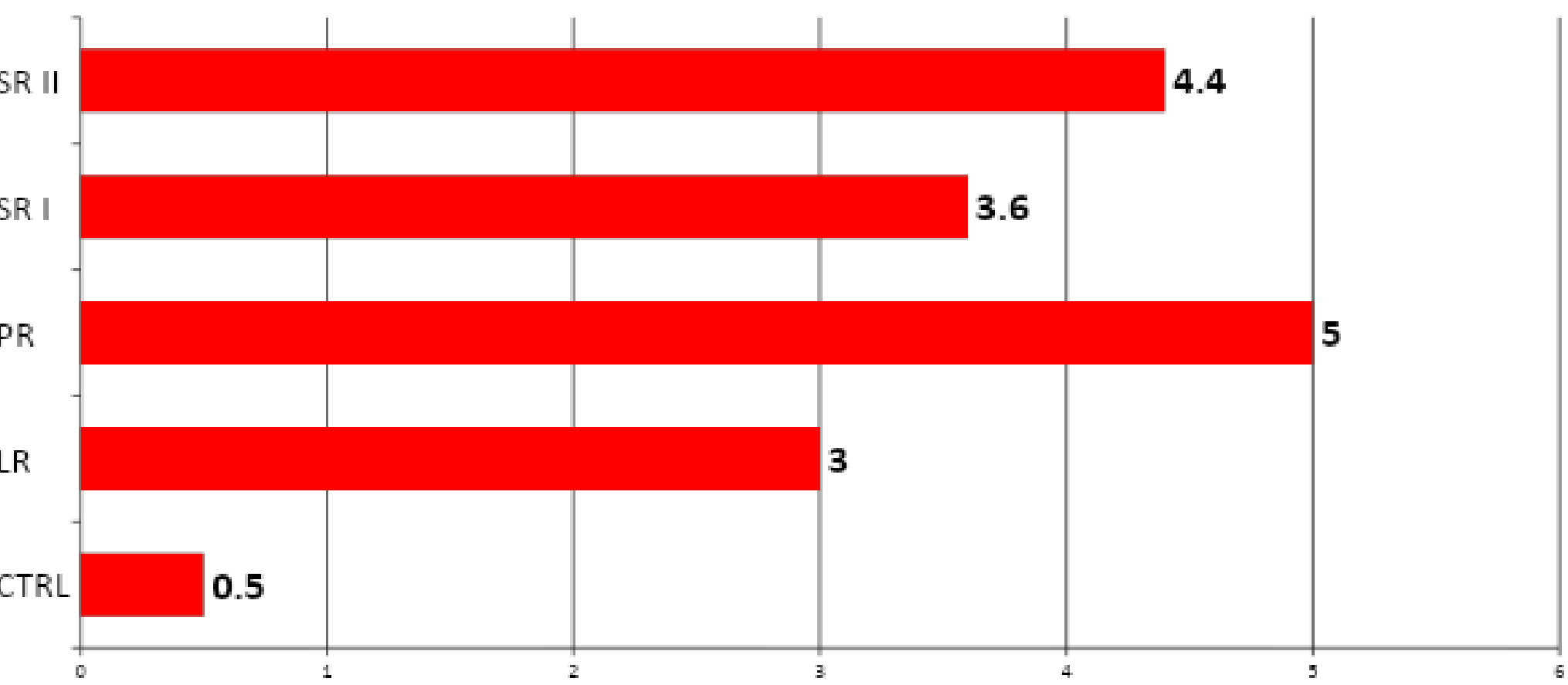
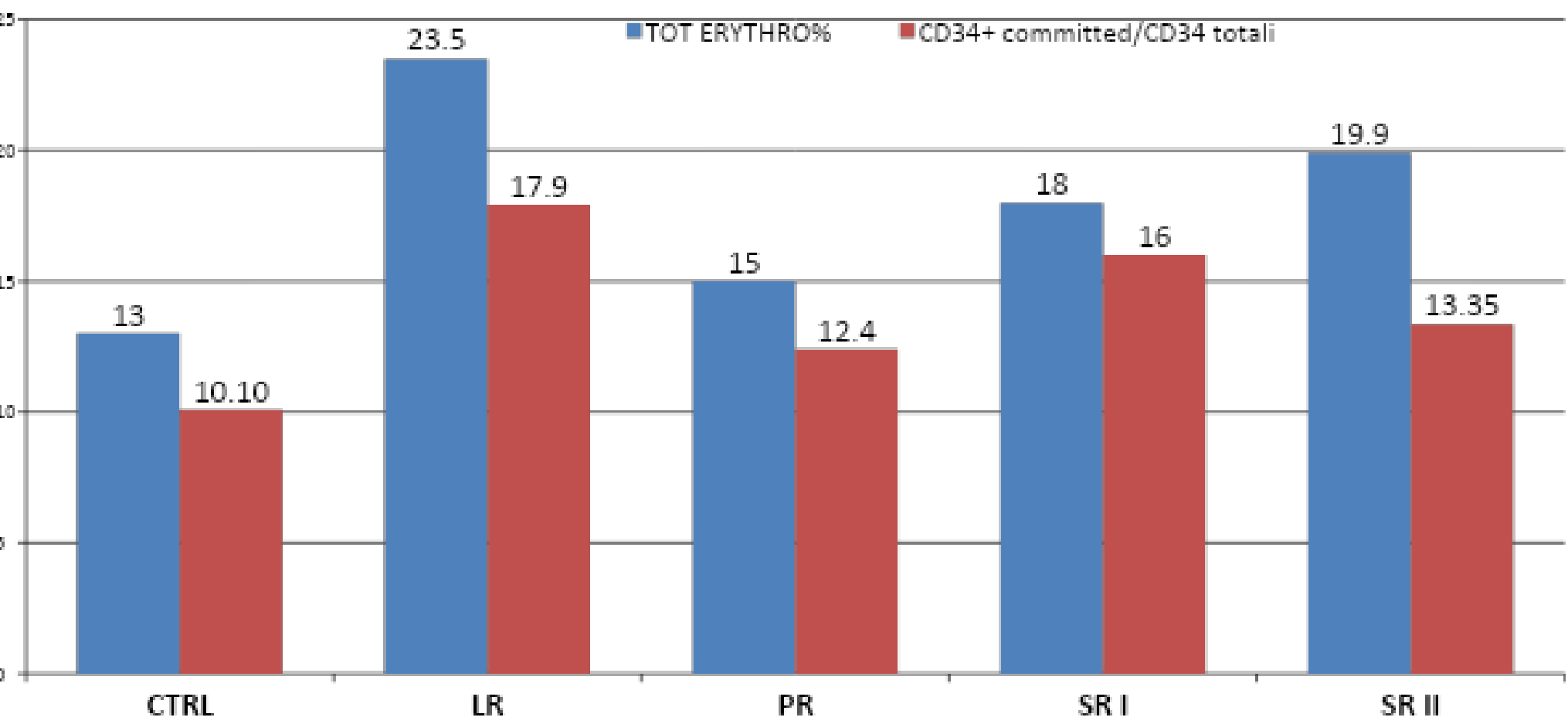
Early erythropoiesis is strictly EPO dependent and promotes the differentiation of COMe into EPP, identified by pro-erythroblasts and basophilic erythroblasts (2). On the contrary, late erythropoiesis is iron dependent and allows erythroblast hemoglobinization of polychromatophilic and orthochromatophilic erythroblasts (3). Anemia in LR-MDS may derive from a block in early or in late erythroid differentiation, both leading to ineffective erythropoiesis. Nowadays, erythropoietic stimulating agents (ESA) represents the mainstay of treatment of anemia in LR-MDS and it is anyhow well ascertained that MDS patients with serum EPO (sEPO) levels < 200mU/mL have best clinical responses to these agents (4).

However, sEPO levels are variable in MDS cases and the reason for such heterogeneity is not clear.

Correlations between FC identification of EP in bone marrow (BM) of MDS patients and type of response to ESA have not been investigated yet.

Objective

We aimed at correlating COMe, EP and RED score with response to ESAs, WHO category and sEPO in 44 anemic (Hb<10g/dL) LR-MDS patients.

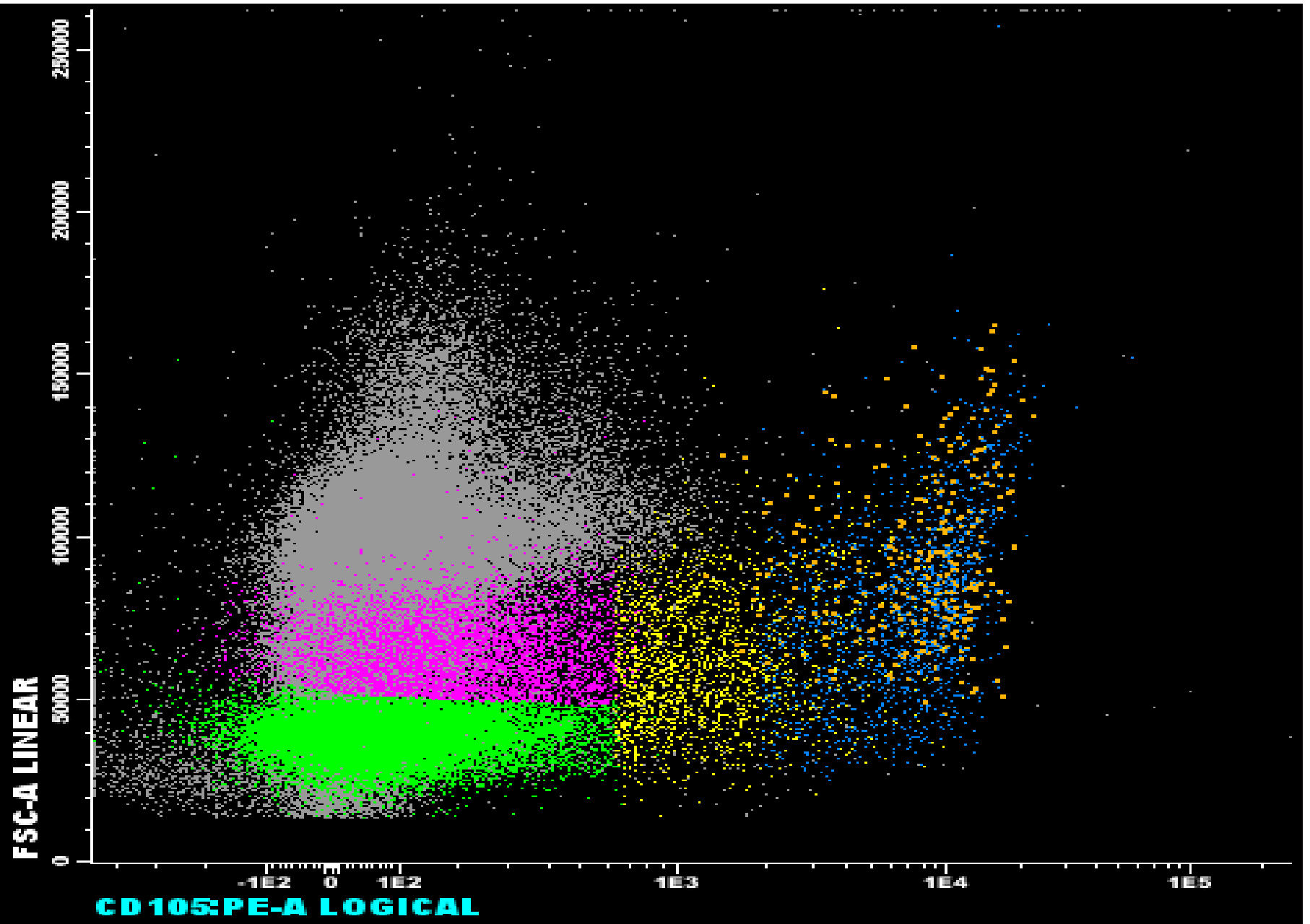


Correlations between ESA response, BM erythroid precursors (high) and RED score (low)

LR = long responders; PR = primary refractory; SR = secondary refractory

Materials & Methods

We evaluated 10 negative controls, 17 PR patients, 17 SR patients and 10 long responders (LR, response duration of > 24 months). 59% had sEPO<200mU/mL (L-EPO), 25% had 200-500mU/mL (M-EPO) and 16% had >500mU/mL (H-EPO); 23% had single lineage dysplasia (MDS-SLD), 34% multilineage dysplasia (MDS-MLD), 23% ring sideroblasts (MDS-RS) and 20% deletion of 5q (MDS-5q⁻). FC analysis was performed on bone marrow aspirates for all subgroups at diagnosis and, within SR subgroup, at loss of response to ESA (SRII). We used anti-CD34, CD36, CD105, CD117, CD33, CD71 and CD45 antibodies to identify COMe, total erythroid BM cells (TOTe) and EP. Coefficient of variance of CD71 and CD36 were used to calculate RED score.



FC analysis of BM erythroid progenitors and precursors
CD34+ Early erythroid progenitors Proerythroblast
Basophilic Erithroblast Polycromatic Erithroblasts Ortocromatic Erithroblas

Summary

TOTe and COMe were increased in LR-MDS compared to controls (p=0.013 and p=0.04 respectively), with the exception of MDS-5q⁻ cases. In PR subgroup LEP were increased and COMe reduced (see graphic) compared to LR. In the SRII group COMe were lower than diagnosis(p=0.039, see graphic). RED score was higher in PR compared to responders (p=0.011, see graphic). MDS-5q⁻ cases showed reduced TOTe (p=0.047), while MDS-RS cases had higher LEP and RED score (p=0.008 and p=0.012, respectively). Finally, LEP were increased in H-EPO group compared to L-EPO (p=0.023)

Discussion

These data suggest that COMe seem to be positively associated with ESA response, whereas higher LEP and RED score are negatively associated and also correlate with MDS-RS and H-EPO. Finally, MDS-5q⁻ cases displayed fewer TOTe and COMe, suggesting premature apoptosis of erythroid precursors. Taken together these results show potential new predictors to EPO response in LR-MDS based FC characteristics of BM erythroid precursors.

References

- 2013 Oct;27(10):1981-7. *Leukemia*
- 2005 Apr;54(1):63-75. *Elsevier*
- 2005 Jan;23(1):69-74. *Nature biotechnology.*
- 1997 Nov;99(2):344-51. *Br J Haematol.*