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Iron overload impairs proliferation of erythroid progenitors cells (BFU-E) from patients with myelodysplastic syndromes☆

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ABSTRACT

In patients with myelodysplastic syndromes (MDS) iron overload caused by long-term red blood cell transfusions is an important factor for comorbidity especially in low-risk MDS. In this report we present the results of a comparative study based on colony formation assays of hematopoietic cells in MDS patients with and without iron overload. We demonstrate that iron overload suppresses the proliferation of erythroid progenitors cells (BFU-E), while the myeloid compartment (CFU-GM) was not found to be affected. Even patients with slightly elevated ferritin values show an impaired proliferation capacity in comparison to patients with normal ferritin levels. Furthermore, we show that this negative impact is reversible by sufficient iron chelation therapy.

1. Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell disorders leading to ineffective hematopoesis with peripheral cytopenias [1–4]. The patients present with anemia and consecutive fatigue, dyspnea as well as an increased risk for cardiac events. Long-term red blood cell (RBC) transfusion therapy can be expected in 40–80% of patients [5,6]. One RBC unit contains approximately 200 mg iron, implicating a significant parenteral iron input with each transfusion [7]. The threshold for clinically relevant secondary iron overload is defined by a serum ferritin of 1000 μg/l [8], which is reached after a median number of 21 RBC units and a median period of 10.8 months [9].

In MDS transfusional iron overload is associated with increased morbidity and mortality due to cardiac and/or hepatic damage [9]. Malcovati et al. [10] described transfusion dependency in MDS as an independent marker with negative prognostic impact. Iron overload can effectively be treated by chelation therapy. Thus, Leitch [11], Rose et al. [12] and Fox et al. [13] collected data indicating a survival benefit in low-risk MDS patients who received adequate chelation therapy.

In 1996, Jensen et al. [14] for the first time demonstrated that chelation therapy can improve the transfusion need of patients with MDS. These findings were confirmed by Messa et al. [15] 2003 and Gattermann et al. [16] 2010 who observed improved hemoglobin levels, decreased transfusion requirement and reduced iron burden under sufficient chelation therapy.

As iron also accumulates in the bone marrow, the improvement of transfusion dependence by adequate iron chelation therapy suggests that iron overload might not only damage hepatocytes and cardiomyocytes, but also bone marrow progenitor cells. While the proliferative capacity of the hematopoietic progenitor cells is impaired by the disease itself [17], toxic iron overload might additionally deteriorate erythropoiesis in the MDS marrow as a "second hit".

To evaluate the proliferative capacity of hematopoietic progenitor cells and a putative impact of iron we performed 475 in vitro colony formation assays from peripheral blood samples of 101 MDS patients with and without iron overload to assess the colony forming capacity [18]. Abnormal or dysplastic colony formation, as

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Table 1
Patient characteristics ($n = 52$).

Age (years) [range]	69 [39–86]
Male/female	30/22
WHO	
5q-Syndrome	4 (7.5%)
RA/-RS	18 (35.0%)
RCMD/-RS	16 (30.5%)
RAEB I/II	11 (21.0%)
Unclassified	3 (6.0%)
IPSS	
Low	15 (29%)
Int-I	25 (48%)
Int-II	9 (17%)
High	3 (6%)
Cytogenetics	
Low	42 (81%)
Intermediate	3 (6%)
High	7 (13%)
Blood counts [range]	
Median hemoglobin (g/dl)	9.1 [5.8–13.2]
Median leucocytes ($10^3/\mu l$)	2.9 [0.5–30]
Median neutrophils (%)	43 [<2–72]
Median platelets ($10^3/\mu l$)	78 [7–529]
Median ferritin (μg/l)	674 [11–6267]

well as decreased numbers of burst forming units-erythroid (BFU-E) are typical findings in MDS patients [19,20]. In colony assays, BFU-E represent early progenitors of red blood cells, and colony forming units granulocyte-macrophage (CFU-GM) originate from myeloid progenitors [19]. This study was performed to investigate whether the proliferative capacity of bone marrow progenitor cells is influenced by iron overload in MDS. Furthermore, repeated intraindividual analyses were employed to follow the development of proliferative capacity of hematopoietic progenitors of MDS patients receiving chelation therapy.

2. Materials and methods

2.1. Patients

In 101 MDS patients a total number of 475 colony assays were performed. All patients with hepatic diseases, active infections or elevated values of C-reactive protein (CRP) or procalcitonin (PCT) at the time of sampling, chemotherapy or epigenetic therapy during the last 6 months, or receiving cytokine and/or corticoid therapy within the last 3 months were excluded from the study. Using these criteria for final evaluation, samples from 52 MDS patients with or without transfusional iron overload determined by serum ferritin levels were available for analysis (Table A suppl. data), as characterized according to age, sex, World Health Organization (WHO) criteria [20], International Prognostic Scoring System (IPSS) [21], cytogenetics [22], median peripheral blood counts and ferritin (Table 1). Using serum ferritin as a marker for body iron storage is an easy, cheap and well-established method for clinical monitoring [7]. At our institution, the normal ferritin values were defined by 20–250 μg/l.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.leukres.2012.11.005.

Informed consent was obtained from every patient in accordance with the modified Declaration of Helsinki.

2.2. Colony formation assays

In 101 MDS patients 475 colony assays from peripheral blood were performed according to the protocols described before [22]: Peripheral blood samples were diluted with buffer medium PBS (Biotec, Germany), mononuclear cells were isolated by density gradient centrifugation (Lymphoprep, Axis Shield, Norway). After several washing steps and RPMI dilution (Invitrogen, Germany), the mononuclear cells were counted by neubauer field (1.5×10^5 peripheral blood cells/culture dish) and cultivated in a 1%-medium of methylcellulose. To stimulate BFU-E growth 25 ng/ml SCF (R&D Systems, Germany) and 50 ng/ml erythropoetin (Roche, Germany) were applied. To stimulate CFU-GM growth 25 ng/ml SCF, 50 ng/ml IL-3 (R&D Systems, Germany), 50 ng/ml GM-CSF (R&D Systems, Germany) and 50 ng/ml G-CSF (R&D Systems, Wiesbaden, Germany) were applied. The plates were incubated at 37 °C under a fully humidified atmosphere and 5% CO_2.

The BFU-E (Fig. 1a) and CFU-GM (Fig. 1b) were analyzed at day 12–16 (median day 14) by the same experienced technician at our institution (U.S.). The normal range of BFU-E (healthy controls) at our lab was $40 \pm 25/1 \times 10^5$ cells. Cell aggregates of more than 50 cells were scored as colonies, cell aggregates of less than 50 cells were counted as clusters. Patients with a diffuse growth pattern or leukemic cluster formation were excluded from final analysis [23].

2.3. Statistical evaluation

The statistical evaluation was performed with SAS 9.1 software (SAS Institute Inc., Cary NC, USA) using Wilcoxon–Mann–Whitney tests and chi-squared tests (F.K.). Multiplicity adjustments were performed by Bonferroni correction. The results were regarded as significant if the p-value was below 5%.

3. Results

3.1. Patients characteristics

The final study cohort consisted of 52 MDS patients with and without iron overload (Table 1). The cohort could be divided into two groups according to serum ferritin levels: 14 patients had normal ferritin levels (<250 μg/l) with a median ferritin of 92 μg/l (range 11–213 μg/l), and 38 patients showed elevated ferritin values (>250 μg/l) with a median level of 869 μg/l (range 273–6267 μg/l). These two groups were characterized and statistically compared according to age, sex, WHO, IPSS, cytogenetics, transfusion dependence and peripheral blood counts (Table 2).

Comparing the patients with normal ($n = 14$) and elevated ($n = 38$) ferritin values, there were no significant differences in the WHO subtypes ($p = 0.57$) and IPSS ($p = 0.41$), in the cytogenetic risk groups ($p = 0.71$), age ($p = 0.7$) or gender ($p = 0.19$).

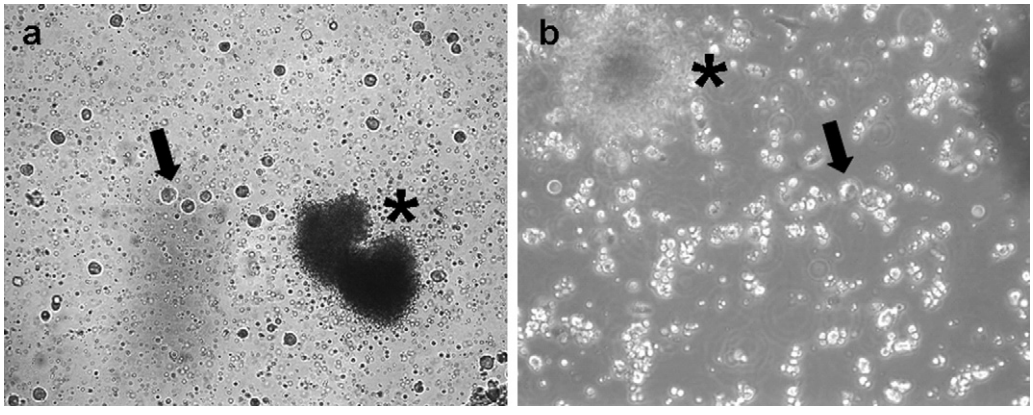

Fig. 1. (a) Burst forming units-erythroid (BFU-E): colony (*) in the lower right, parallel growth of cluster (→). (b) Colony forming units granulocyte-macrophage (CFU-GM): colony (*) in the upper left, parallel growth of cluster (→).