Iron overload in acute myeloid leukemia patients is not related to HFE and TFR2 gene mutations

We analyzed the influence of 12 hereditary hemochromatosis (HH) gene mutations on iron status before and after treatment in 45 iron overloaded long-surviving patients with acute myeloid leukemia (AML). In 24 patients (53.3%) ferritin values and/or transferrin saturation increased during the follow-up, in the absence of further transfusions or signs of inflammation. Mean ferritin value and transferrin saturation at diagnosis, after chemotherapy and at the end of follow-up (36 months) did not significantly differ between patients positive or negative for HH gene mutations. Our study supports the evidence that the persistence of elevated ferritin values in AML patients is not associated with the most common HH gene mutations.

haematologica 2003; 88:1069-1070
(http://www.haematologica.org/2003_09/*1069.htm)*

Transfusional iron overload is a frequent finding in long-surviving acute myeloid leukemia (AML) patients.[1,2] Some authors[3-6] have investigated patients with hematologic malignancies for hereditary hemochromatosis (HH), but have failed to demonstrate an association between high ferritin values and the presence of the most frequent HH gene mutations (C282Y and H63D).

In this study, we tested 45 consecutive long-surviving adult patients with AML to check the presence of 12 HH gene mutations (C282Y, H63D, H63H, V53M, V59M, S65C, Q127H, E168Q, E168X, W169X, Q283P in the HFE gene and Y250X in the TFR2 gene) and to verify whether there was a correlation between iron status and HH mutations. All patients (26 men and 19 women; M/F ratio 1.4; median age 46 years, range 19-66) were in first complete remission after standard chemotherapy or bone marrow transplant (BMT) and with at least 1 year of disease-free survival (median 36.0 months, range 15-92). According to the French American British (FAB) criteria, 1 patient was classified as having M1 disease, 5 patients as M2, 28 patients as M3, 6 patients as M4 and 5 patients as M5. No patient had chronic viral B or C hepatitis. Patients received several red blood cell transfusions following chemotherapy and BMT (median number of transfused units: 23, range 9-38). Serum ferritin levels and transferrin saturation were determined at diagnosis, at the end of induction chemotherapy or after BMT and every four months thereafter.

Clinical characteristics and iron status of the patients are shown in Table 1. Like other investigators,[1,2] we found that transfusions administered during the induction chemotherapy significantly increased ferritin levels and transferrin saturation in these patients. However, ferritin values and/or transferrin saturation continued to increase in 24/45 patients (53.3%) during the follow-up, although none of them received further transfusions after chemotherapy completion and inflammation parameters were normal. In fact, we did not find a significant correlation between end follow-up ferritin levels and total amount of iron transfused. Fourteen of 45 patients (31.1%), with increasing serum ferritin levels (> 1,500 μg/L) and transferrin saturation (> 50%) during the follow-up, required phlebotomies or iron-chelating therapy with deferoxamine.

Patients were also divided into two groups on the basis of the HH gene pattern (presence or absence of gene mutations), and then compared for iron status (Table 1). The two groups were homogeneous as far as age, transfusional iron load and follow-up period were concerned. Seventeen patients (37.8%) were positive for HH gene mutations. Heterozygosity for C282Y and H63D mutations was observed in 4 (8.8%) and 6 patients (13.3%), respectively. Five of the 45 patients (11.1%) were homozygous for H63D and 2 patients (4.4%) were heterozygous for S65C mutation. We did not find any statistically significant difference as regards the values (before and at the end of treatment) of ferritin and transferrin saturation, between patients with or without HH gene mutations. The increases of ferritin values and transferrin saturation during the follow-up period were observed in both groups of patients. This increase, statistically not significant if compared with the value at the end of induction chemotherapy, was independent of HH gene status. Iron-depleting therapy to lower ferritin levels was equally necessary in both groups: 5/17 (29.4%) patients with HH gene mutations (3 patients with heterozygosis for H63D, 1 for C282Y and 1 for S65C gene mutations) and 9/28 (32.1%) patients with no HH gene mutations.

Only C282Y, H63D and S65C mutations were observed. The allele frequency (4.4% for C282Y, 17.7% for H63D and 2.2% for S65C mutations) was similar to that found by others investigators in normal and oncohematologic individuals.[3,7,8] Thus, while the abnormal iron status at diagnosis and during treatment can be easily explained by the evidence of inflammation and the transfusions carried out, its cause during the follow-up remains unclear, as it was not associated with any clear

Table 1. Characteristics of AML patients and analysis of HH gene mutations.

Parameters	*All patients (n =45)*	*HH Present (n = 17)*	*Gene mutation Absent (n = 28)*	*p*
M/F ratio	1.4	0.6	2.5	0.03
Age, years	46.4±11.3	45.6±11.1	47.0±11.6	NS
Initial ferritin level* (μg/L)	843.6±872.5	770.7±1043.0	891.1±763.0	NS
Initial TS† (%)	44.1±20.4	38.9±12.6	47.2±23.6	NS
TIL‡ (mg/kg)	84.4±35.6	78.9±28.9	87.9±39.3	NS
ECT ferritin value (μg/L)§	1539.9±780.8	1371.9±581.1	1785.1±811.8	NS
ECT TS (%)§	51.0±15.3	47.5±14.9	54.1±15.6	NS
Last ferritin value (μg/L)	1728.0±1038.9	1480.4±1151.6	1866.8±966.9	NS
Last TS (%)	51.8±19.9	49.9±17.4	55.9±19.9	NS
Follow-up time (months)	36.0±23.0	38.3±25.0	32.1±19.2	NS

*TS: transferrin saturation; TIL: total iron load; ECT: end of chemotherapy. *Normal range of serum ferritin concentration (15-250 μg/L). †Normal range of transferrin saturation (16-44%). ‡TIL is expressed as the total amount of iron transfused per kilogram of body weight. §ECT ferritin value and ECT TS vs. the latest ferritin value and the latest TS, p = NS.*

genetic or secondary cause of iron accumulation or with relapse of leukemia. A possible explanation for this phenomenon could be increased intestinal iron absorption caused by chemotherapy-induced mucosal damage.

Our data confirm that there is not a clear-cut association between HH gene mutations and iron overload, in AML patients. However, as a significant percentage of long-surviving AML patients develop iron overload which then persists for several years after transfusions, the follow-up of these patients should include iron status measurement in order to intervene to prevent the development of complications.

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Key words: iron overload, hemochromatosis, acute leukemia, HFE and TRF2 genes.

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Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Clara Camaschella, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Professor Camaschella and the Editors. Manuscript received July 22, 2003; accepted August 7, 2003.

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Antioxidant enzyme expression in myelodysplastic and acute myeloid leukemia bone marrow: further evidence of a pathogenetic role for oxidative stress?

We studied the expression of four antioxidant enzymes in normal, myelodysplastic (MDS) and acute myeloid leukemia (AML) bone marrow cells. Enzyme expression in normal marrow differed with lineage and cellular maturation. Expression was most frequently increased in MDS/AML granulocytes, but less so in CD34+ cells, suggesting that oxidative stress may select surviving progenitor cells for augmented antioxidant defence.

haematologica 2003; 88:1070-1072
(http://www.haematologica.org/2003_09/*1069.htm)*

Indirect evidence suggests a role for oxidant DNA damage in the pathogenesis of myelodysplasia.[1] An elevated plasma concentration of the lipid peroxidation product malondialdehyde[2] and the *in vitro* stimulation of MDS progenitor cells by thiol antioxidants such as amifostine,[3] thioredoxin and N-acetyl cysteine[4] provide some support for this.

We quantified expression of the antioxidant enzymes glutathione peroxidase 1 (GPX1), manganese superoxide dismutase (MnSOD), catalase (CAT) and the rate-limiting enzyme for glutathione synthesis, γ-glutamyl cysteine synthetase heavy sub unit (GCS), in bone marrow from healthy individuals (n=12), and from patients with MDS and AML [RA/RARS (n=21), RAEB (n=14), CMML (n=9), and AML/RAEBt (n=18)].

Bone marrow mononuclear cells (MNC) were isolated by density gradient centrifugation. Granulocytes were obtained as a high-density pellet, followed by ammonium chloride red cell lysis (apoptosis >2% in 3/25 and granulocyte/band form >70% in 20/25). $CD34^{+}/^{-}$, $CD8^{+}/^{-}$ and glycophorin A $(GPA)^{+}/^{-}$ cells were separated from MNC by magnetic labeling. Enzyme expression was quantified by real-time polymerase chain reaction (ABI 7700). TaqMan™ primer and probe sequences and locations are listed in Table 1. 18S rRNA was used as reference RNA for quantification of target gene expression. Expression Ct value was first normalized for RNA concentration against an 18S ribosomal RNA internal control. This normalized expression ratio was then standardized for each plate relative to the normalized expression of an internal standard (K562 cell line for MnSOD, catalase, GCS, and HEL cell line for GPX1) and expressed as a standardized expression ratio (normalized

Table 1. Primer and probe sequences/genomic location.

Enzyme	*Primer/probe*	*Location*	*Sequence*
GPX1	Forward	Exon 1-2	5'-gcttcccgtgcaaccagtt -3'
	Reverse		5'-gcacttctcgaagagcatgaagt -3'
	Probe		5'-aatctcttcgttcttggcgttctcctgatg -3'
MnSOD	Forward	Exon 2-3	5'-tgaacgtcaccgaggagaagtac -3'
	Reverse		5'-tgatatgaccaccaccattgaac -3'
	Probe		5'-tgggctgtaacatctcccttggcca-3'
CAT	Forward	Exon 1-2	5'-cagcgaccagatgcagca -3'
	Reverse		5'-agtgaaaaccacatcctgaacaag -3'
	Probe		5'-cgcagaaagctgatgtcctgaccactg -3'
GCS	Forward	Exon 1-2	5'-ttgcaggaaggcattgatca -3'
	Reverse		5'-agcatcatccaggtgtattttctctt -3'
	Probe		5'-ctggcccagcatgttgctcatctctt -3'