Inappropriately low hepcidin levels in patients with myelodysplastic syndrome carrying a somatic mutation of *SF3B1*

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Online Supplementary Appendix
Results

Erythroid activity in MDS patients according to SF3B1 *mutation status*

A significant association was found between *SF3B1* mutation and proportion of bone marrow erythroblasts (median value 43% *vs.* 26% in patients with and without *SF3B1* mutation, respectively; *P*=0.001), and between *SF3B1* mutation and ring sideroblasts (median value 58% *vs.* 0% in patients with and without *SF3B1* mutation, respectively; *P*<0.001). In addition, a direct relationship was observed between *SF3B1* mutant allele burden and proportion of bone marrow ring sideroblasts (r=0.46, *P*<0.001).

A non-significant higher transferrin saturation was observed in patients with mutation of *SF3B1* compared with unmutated patients (median value 60% *vs.* 52%, respectively). In addition, a positive correlation was found between *SF3B1* mutant allele burden and transferrin saturation (r=0.34). Comparable results were obtained limiting the analysis to non-transfused patients (median value 58% *vs.* 47%, respectively; r=0.37).

Levels of sTfR ranged from 0.21 to 4.19 mg/L (median value 1.40 mg/L). A significant positive correlation was observed between soluble transferrin receptor (sTfR) and proportion of bone marrow erythroblasts (r=0.73, *P*<0.001) or that of ring sideroblasts (r=0.32, *P*=0.007), whereas inverse relationships were found between sTfR and serum Epo (r=-0.26, *P*=0.03), and between sTfR and serum ferritin (r=-0.26, *P*=0.026).

Variable GDF15 concentrations were detected in the patients studied (median 2,147 pg/mL, range 538-10,686 pg/mL). A significant positive relationship was found between GDF15 level and proportion of bone marrow erythroblasts (r=0.28, *P*=0.026), whereas a significant negative correlation was observed between GDF15 and hemoglobin level (r=-0.31, *P*=0.009).

Hepcidin levels in MDS patients according to SF3B1 *mutation status*

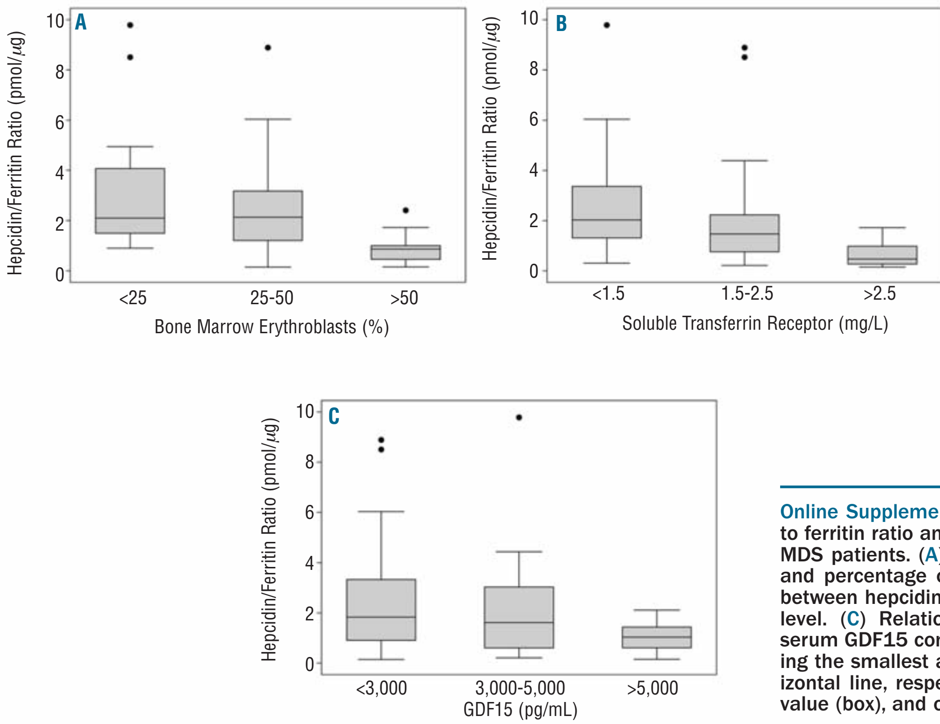
Significant linear correlations were found between hepcidin and proportion of bone marrow erythroblasts (r=-0.61, *P*<0.001), and sTfR (r=-0.61, *P*<0.001). No significant association was noticed between serum hepcidin and GDF15 (*P*=0.85).

Twenty-five of 76 patients (33%) showed a need for RBC transfusion (median number of RBC transfusions received 20, range 5-160). Both the proportion of transfusion-dependent patients and the RBC transfusion burden were comparable between *SF3B1* mutated and unmutated groups (26% *vs.* 34%, respectively, *P*=0.594; median n. of RBC units 19 *vs.* 20). Transfusion-dependent patients showed significantly higher serum ferritin levels (*P*<0.001), and a significant positive correlation was observed between transfusion burden and serum ferritin (r=0.53, *P*=0.003).

Significantly higher hepcidin levels were observed in RBC transfusion-dependent patients compared with those without regular transfusion need (*P*<0.001). A positive correlation was also found this between transfusion burden and hepcidin lev-

Online Supplementary Table S1. Multivariable analyses considering hepcidin level and hepcidin to ferritin ratio, respectively, as dependent variable.

Variable	Coefficient	*P*
Hepcidin level (nM)		
Soluble transferrin receptor (mg/L)	-2.896	0.037
GDF15 concentration (pg/mL)	-0.0003	0.068
Serum ferritin (ng/mL)	0.006	0.000
Transfusion dependency (yes/no)	6.102	0.034
SF3B1 mutant allele burden (%)	-9.927	0.038
Bone marrow blasts (%)	-0.174	0.498
Serum erythropoietin (U/L)	-0.002	0.165
Hemoglobin (g/dL)	-1.804	0.059
Cytogenetic risk group	0.222	0.880
Hepcidin to ferritin ratio		
Soluble transferrin receptor (mg/L)	-0.003	0.442
GDF15 concentration (pg/mL)	-0.0001	0.350
SF3B1 mutant allele burden (%)	-0.038	0.013
Bone marrow blasts (%)	-0.0007	0.441
Serum erythropoietin (U/L)	-0.0001	0.311
Hemoglobin (g/dL)	0.001	0.712
Cytogenetic risk group	0.007	0.162



Online Supplementary Figure S1. **Relationship between hepcidin to ferritin ratio and measurements of erythroid marrow activity in MDS patients. (A) Relationship between hepcidin to ferritin ratio and percentage of bone marrow erythroblasts. (B) Relationship between hepcidin to ferritin ratio and soluble transferrin receptor level. (C) Relationship between hepcidin to ferritin ratio and serum GDF15 concentration. Data are shown in a box plot depicting the smallest and largest observation (lowest and highest horizontal line, respectively), lower and upper quartile with median value (box), and outliers (dots).**

els (r=0.23), but did not reach statistical significance (*P*=0.22).

A borderline association was noticed between *SF3B1* mutation status and hepcidin level, patients with mutation having lower values than those without mutation (*P*=0.07). In addition, an inverse relationship was observed between hepcidin levels and *SF3B1* mutation burden (r=-0.22, *P*=0.06).

Hepcidin to ferritin ratio

The hepcidin to ferritin ratio represents a measure of adequacy of hepcidin levels relative to body iron stores. The hepcidin to ferritin ratio ranged from 1.5 to 170 pmol/µg (median value 16 pmol/µg). Significant linear correlations were found between hepcidin/ferritin ratio and proportion of bone marrow erythroblasts (r=-0.54, *P*<0.001), sTfR (r=-0.44, *P*<0.001) or GDF15 (r=-0.036, *P*=0.001) (*Online Supplementary Figure S1*).

Multivariable analysis

A multivariable analysis including hemoglobin, Epo, sTfR, GDF15, serum ferritin, transfusion dependency, BM blasts, cytogenetic risk groups and *SF3B1* mutant allele burden showed that serum hepcidin levels were independently associated with sTfR levels (*P*=0.037), serum ferritin (*P*<0.001), transfusion-dependency (*P*=0.034) and *SF3B1* mutant allele burden (*P*=0.038) (*Online Supplementary Table S1*).