

# Elevated liver iron concentration is a marker of increased morbidity in patients with $\beta$ thalassemia intermedia

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Online Supplementary Table S1. Bivariate correlations between study parameters and morbidities (Part 1).

Variable	Extramedullary hematopoiesis		Leg ulcers		Morbidity Thrombosis		Pulmonary hypertension		Heart failure	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
LIC (mg Fe/g dw) <sup>a</sup>	8.6 (6.7)	7.8 (6.6)	7.7 (6.3)	10.4 (7.5)*	7.3 (6)	11.5 (7.7)**	7 (6.1)	11.1 (7.1)***	6.1 (8.8)	12.3 (15.8)
Age (years) <sup>a</sup>	34.9 (12.2)	36.1 (13.7)	34.6 (12.3)	37 (13.3)	34.2 (12.6)†	38 (12.1)	33 (13.1)	39.7 (10.2)***	35 (18)	45 (9)*
Gender <sup>b</sup>										
Female (n=96)	71 (74)	25 (26)	70 (72.9)	26 (27.1)	64 (66.7)	32 (33.3)	63 (65.6)	33 (34.4)	90 (93.7)	6 (6.3)
Male (n=72)	54 (75)	18 (25)	57 (73.7)	15 (26.3)	60 (83.3)	12 (16.7)*	49 (68.1)	23 (31.9)	69 (95.8)	3 (4.2)
Splenectomized <sup>b</sup>										
No (n=47)	38 (80.9)	9 (19.1)	39 (83)	8 (17)	43 (91.5)	4 (8.5)	39 (83)	8 (17)	44 (93.6)	3 (6.4)
Yes (n=121)	87 (71.9)	34 (28.1)	88 (72.7)	33 (27.3)	81 (66.9)	40 (33.1)**	73 (60.3)	48 (39.7)**	115 (95)	6 (5)
Transfusion <sup>b</sup>										
None (n=44)	37 (84.1)	7 (15.9)	42 (95.5)	2 (4.5)	39 (88.6)	5 (11.4)	35 (79.5)	9 (20.5)	43 (97.7)	1 (2.3)
Occasional (n=80)	57 (71.2)	23 (28.8)	55 (68.7)	25 (31.3)	55 (68.7)	25 (31.3)	53 (66.2)	27 (33.8)	76 (95)	4 (5)
Regular (n=44)	31 (70.5)	13 (29.5)	30 (31.8)	14 (31.8)**	30 (68.2)	14 (31.8)*	24 (54.5)	20 (45.5)*	40 (90.9)	4 (9.1)
Total hemoglobin (g/dL) <sup>a</sup>	8.8 (1.6)	8.8 (1.6)	8.9 (1.6)	8.4 (1.5)†	8.9 (1.7)	8.3 (1.4)*	8.8 (1.8)	8.7 (1.3)	8.6 (1.9)	9.2 (2.4)
Fetal hemoglobin (%) <sup>a</sup>	42.7 (31.7)	49.4 (29.1)	42.8 (32.5)	50.1 (25.7)	43.3 (30.9)	51.6 (30.9)	40.6 (31.4)	44.8 (28.6)*	37.1 (57.9)	55.5 (37)
Platelet count (x10 <sup>9</sup> /L) <sup>a</sup>	591.9 (341.4)	657.8 (358)	589.8 (341.9)	667.1 (355.9)	582.2 (362.5)	684.5 (286.1)	594.1 (361.3)	641.5 (312.4)	613 (520)	351 (267)*
NRBC count (x10 <sup>9</sup> /L) <sup>c</sup>	349.5 (4745)	865 (16385)	359 (4751)	857 (16380)	325 (7947)	900 (18832)	353 (907)	6680 (25087)**	411 (12300)	567 (8860)
Serum ferritin (ng/mL) <sup>c</sup>	807.5 (919)	746.5 (1104)*	747 (751)	1095 (1142)†	740.3 (754.3)	1106.3 (916.8)	641.3 (777)	1006.5 (902.3)**	765 (855)	1403 (1297)†

LIC: liver iron concentration; dw: dry weight; NRBC: nucleated red blood cell. Data presented as "mean (SD) [except for heart failure and diabetes mellitus for which the median (IQR) was used], %n (%), or "median (IQR). All correlations evaluated by the independent samples t-test and the  $\chi^2$  test except for heart failure and diabetes mellitus for which correlations were evaluated by the Mann-Whitney U test and the Fisher's exact test. †P<0.1; \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

Online Supplementary Table S1. Bivariate correlations between study parameters and morbidities (Part 2).

Variable	Abnormal liver function		Diabetes mellitus		Morbidity Hypothyroidism		Osteoporosis		Hypogonadism	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
LIC (mg Fe/g dw) <sup>a</sup>	6.7 (5.2)	11.9 (8)***	6.1 (9.5)	6.5 (7.4)	7.8 (6.2)	11.1 (8.1)*	6.4 (6)	10.8 (6.7)***	7.6 (6.1)	12.3 (8)***
Age (years) <sup>a</sup>	32 (12.6)	42 (9.6)***	35 (19)	42 (9)	35.1 (13.1)	35.6 (10.1)	32.2 (13)	38.8 (11)***	35.5 (13.1)	33.8 (9.8)
Gender <sup>b</sup>										
Female (n=96)	67 (69.8)	29 (30.2)	91 (94.8)	5 (5.2)	78 (81.2)	18 (18.8)	51 (53.1)	45 (46.9)	77 (80.2)	19 (19.8)
Male (n=72)	47 (65.3)	25 (34.7)	71 (98.6)	1 (1.4)	60 (83.3)	12 (16.7)	40 (55.6)	32 (44.4)	63 (87.5)	9 (12.5)
Splenectomized <sup>b</sup>										
No (n=47)	39 (83)	8 (17)	46 (97.9)	1 (2.1)	43 (91.5)	4 (8.5)	36 (76.6)	11 (23.4)	45 (95.7)	2 (4.3)
Yes (n=121)	75 (62)	46 (38)**	116 (95.9)	5 (4.1)	95 (78.5)	26 (21.5)*	55 (45.5)	66 (54.5)***	95 (78.5)	26 (21.5)**
Transfusion <sup>b</sup>										
None (n=44)	39 (88.6)	5 (11.4)	44 (100)	0 (0)	43 (97.7)	1 (2.3)	36 (81.8)	8 (18.2)	42 (95.5)	2 (4.5)
Occasional (n=80)	52 (65)	28 (35)	78 (97.5)	2 (2.5)	64 (80)	16 (20)	37 (46.2)	43 (53.8)	67 (83.7)	13 (16.3)
Regular (n=44)	23 (52.3)	21 (47.7)**	40 (90.9)	4 (9.1)†	31 (70.5)	13 (29.5)**	18 (40.9)	26 (59.1)***	31 (70.5)	13 (29.5)**
Total hemoglobin (g/dL) <sup>a</sup>	8.8 (1.7)	8.7 (1.3)	8.7 (2)	8.7 (1.3)	8.8 (1.7)	8.6 (1.3)	8.9 (1.7)	8.6 (1.5)	8.8 (1.6)	8.8 (1.5)
Fetal hemoglobin (%) <sup>a</sup>	38.3 (31.3)	35.4 (31)	40 (58.9)	35 (44.2)	42.8 (31.2)	53.5 (29.5)	40 (30.1)	50.1 (30.9)†	42.9 (31.5)	54.5 (26.7)
Platelet count (x10 <sup>9</sup> /L) <sup>a</sup>	602.8 (370.2)	625 (283.9)	608 (542.5)	520 (295)	611.5 (360.2)	600 (279.4)	571.1 (369.4)	652.9 (314.1)	588 (348.9)	713.3 (317.1)†
NRBC count (x10 <sup>9</sup> /L) <sup>c</sup>	325 (817)	11130 (25004)**	395 (9170)	9030 (16830)	342 (9073)	900 (13786)	310 (1668)	548.5 (14295)	400 (12300)	570 (8980)
Serum ferritin (ng/mL) <sup>c</sup>	617.5 (670)	1465 (1265)***	773 (982)	831 (548)	749.3 (961)	999.5 (850)	596.5 (724)	1019 (973.5)*	747.8 (889)	978 (1004)

LIC: liver iron concentration; dw: dry weight; Hb; NRBC, nucleated red blood cell. Data presented as <sup>a</sup>mean (SD) [except for heart failure and diabetes mellitus for which the median (IQR) was used], <sup>b</sup>n (%), or <sup>c</sup>median (IQR). All correlations evaluated by the independent samples *t*-test and the  $\chi^2$  test except for heart failure and diabetes mellitus where correlations were evaluated by the Mann-Whitney *U* test and the Fisher's exact test. †, *P*<0.1; \*, *P*<0.05; \*\**P*<0.01; \*\*\**P*<0.001.

Online Supplementary Table S2. Multivariate logistic regression to determine independent risk factors for morbidities (Part 1).

Variable	Extramedullary hematopoiesis		Leg ulcers		Morbidity Thrombosis		Pulmonary hypertension		Heart failure	
	AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95% CI
LIC, 1 mg Fe/g dw increase	1.01	0.94-1.08	1.04	0.99-1.10	1.12	1.05-1.20	1.08	1.02-1.14	1.06	0.97-1.16
Age, 1 year increase	--	--	--	--	1.04	1.01-1.07	1.05	1.02-1.09	--	--
Gender										
Female	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Male	--	--	--	--	0.35	0.16-0.81	--	--	--	--
Splenectomized										
No	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Yes	--	--	--	--	5.82	1.77-19.19	2.99	1.20-7.44	--	--
Transfusion										
No	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Yes	1.81	1.07-3.08	2.01	1.17-3.47	--	--	--	--	--	--
Total Hb, 1 g/dL increase	--	--	--	--	--	--	--	--	--	--
Fetal Hb, 1% increase	--	--	--	--	--	--	--	--	--	--
Platelet count, x10 <sup>9</sup> /L increase	--	--	--	--	--	--	--	--	0.992	0.986-0.998
NRBC count, x10 <sup>9</sup> /L increase	--	--	--	--	--	--	--	--	--	--
Ferritin, 100 ng/mL increase	--	--	--	--	--	--	--	--	--	--

AOR: adjusted odds ratio; CI: confidence interval; LIC: liver iron concentration; dw: dry weight; Hb: hemoglobin; NRBC: nucleated red blood cell. The model was built using forward-stepwise selection. *P*≤0.1 was used as the criterion for inclusion. Multicollinearity was absent in the model as demonstrated by a variation inflation factor ≤3 (acceptable limit up to 10).

Online Supplementary Table S2. Multivariate logistic regression to determine independent risk factors for morbidities (Part 2).

Variable	Abnormal liver function		Diabetes mellitus		Morbidity Hypothyroidism		Osteoporosis		Hypogonadism	
	AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95% CI
LIC, 1 mg Fe/g dw increase	1.05	0.97-1.13	0.92	0.78-1.07	1.05	1.01-1.11	1.10	1.04-1.16	1.10	1.03-1.16
Age, 1 year increase	1.09	1.05-1.14	--	--	--	--	1.05	1.02-1.08	--	--
Gender										
Female	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Male	--	--	--	--	--	--	--	--	--	--
Splenectomized										
No	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Yes	--	--	--	--	--	--	3.67	1.57-8.55	--	--
Transfusion										
No	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Yes	--	--	5.49	1.21-24.85	2.54	1.34-4.84	--	--	2.97	1.39-6.35
Total Hb, 1 g/dL increase	--	--	--	--	--	--	--	--	--	--
Fetal Hb, 1% increase	--	--	--	--	--	--	--	--	--	--
Platelet count, x10 <sup>9</sup> /L increase	--	--	--	--	--	--	--	--	--	--
NRBC count, x10 <sup>6</sup> /L increase	--	--	--	--	--	--	--	--	--	--
Ferritin, 100 ng/mL increase	1.14	1.06-1.23	--	--	--	--	--	--	--	--

AOR: adjusted odds ratio; CI: confidence interval; LIC: liver iron concentration; dw: dry weight; Hb: hemoglobin; NRBC: nucleated red blood cell. The model was built using forward-stepwise selection.  $P \leq 0.1$  was used as the criterion for inclusion. Multicollinearity was absent in the model as demonstrated by a variation inflation factor  $\leq 3$  (acceptable limit up to 10).