SUPPLEMENTARY APPENDIX

Labile plasma iron levels predict survival in patients with lower-risk myelodysplastic syndromes

Louise de Swart,[1] Chloé Reiniers,[2] Timothy Bagguley,[3] Corine van Marrewijk,[1] David Bowen,[4] Eva Hellström-Lindberg,[5] Aurelia Tatic,[6] Argiris Symeonidis,[7] Gerwin Huls,[2] Jaroslav Cermak,[8] Arjan A. van de Loosdrecht,[9] Hege Garelius,[10] Dominic Culligan,[11] Mac Macheta,[12] Michail Spanoudakis,[13] Panagiotis Panagiotidis,[14] Marta Krejci,[15] Nicole Blijlevens,[1] Saskia Langemeijer,[1] Jackie Droste,[1] Dorine W. Swinkels,[16] Alex Smith[2] and Theo de Witte[17] on behalf of the EUMDS Steering Committee

[1]Department of Hematology, Radboud university medical center, Nijmegen, the Netherlands; [2]Department of Hematology, University Medical Centre, Groningen, the Netherlands; [3]Epidemiology and Cancer Statistics Group, University of York, UK; [4]St. James's Institute of Oncology, Leeds Teaching Hospitals, UK; [5]Department of Medicine, Division of Hematology, Karolinska Institutet, Stockholm, Sweden; [6]Center of Hematology and Bone Marrow Transplantation, Fundeni Clinical Institute, Bucharest, Romania; [7]Department of Medicine, Division of Hematology, University of Patras Medical School, Greece; [8]Department of Clinical Hematology, Institute of Hematology & Blood Transfusion, Prague, Czech Republic; [9]Department of Hematology – Cancer Center Amsterdam VU University Medical Center, The Netherlands; [10]Department of Medicine, Section of Hematology and Coagulation, Sahlgrenska University Hospital, Göteborg, Sweden; [11]Department of Haematology, Aberdeen Royal Infirmary, UK; [12]Department of Haematology, Blackpool Victoria Hospital, Lancashire, UK; [13]Department of Haematology, Airedale NHS trust, UK; [14]Department of Hematology, Laikon General Hospital, National and Kapodistrian University of Athens, Greece; [15]Department of Internal Medicine, Hematology and Oncology, University Hospital Brno and Masaryk University, Czech Republic; [16]Department of Laboratory Medicine, Hepcidinanalysis.com, and Radboudumc expertise center for iron disorders, Radboud university medical center, Nijmegen, the Netherlands and [17]Nijmegen Center for Molecular Life Sciences, Department of Tumor Immunology, Radboud university medical center, the Netherlands

 doi:10.3324/haematol.2017.171884

Received: May 26, 2017.

Accepted: October 27, 2017.

Pre-published: November 9, 2017.

Correspondence: theo.dewitte@radboudumc.nl

Online supplemental information

Methods

Study design and participants

Serum samples were collected just prior to transfusion in transfusion dependent patients, stored at -80C and shipped on dry ice to the central Laboratory in Nijmegen, the Netherlands. The overall analyses had to be restricted to 100 patients, since samples from three centers showed consistently elevated NTBI/LPI levels (even in samples with low transferrin saturation), due to technical issues. Clinical Information was collected at registration and 6-monthly intervals thereafter via a bespoke web-based database on: concomitant diseases, detailed red cell transfusion history, other treatment modalities, peripheral blood values, bone marrow pathology, progression of MDS or acute myeloid leukemia (AML), and loss to follow-up. Additionally analyzed parameters included the conventional iron parameters ferritin, serum iron, TSAT, the less standard iron parameters: hepcidin, GDF15, sTfR, NTBI, LPI and the inflammation parameter CRP.

Biochemical assays

Serum ferritin, iron, transferrin, and CRP were measured with routine methodologies. The serum hepcidin-25 assay was based on a combination of weak cation exchange chromatography and time-of-flight mass spectrometry using an stable hepcidin-25 isotope for quantification at nM level as previously reported[40] (www.hepcidinanalysis.com). The lower limit of detection of this method was 0.5 nM. The median reference value of serum hepcidin-25 (Dutch population) is 4.5 nM for men, 2.0 nM for premenopausal women, and 4.9 nM for postmenopausal women (www.hepcidinanalysis.com).[41]

GDF15 levels were measured with DuoSet (R&D Systems, Minneapolis, MN) enzyme-linked immunosorbent assay for human GDF15 following the manufacturer's protocol. Serum concentration of sTfR was measured immunonephelometrically with the use of polystyrene particles coated with monoclonal antibody specific to human sTfR on a BN II System (Dade Behring Marburg GmbH, Marburg, Germany).

Table S1 Treatment with iron chelation and corresponding LPI and follow-up

Patient nr	Nr Visits	Max LPI levels	First visit LPI>LLOD	Max TSAT	Max Ferritin	Chelation/months	Survival (years)/AML
1	5	0.38	1	96	1954	Deferasirox/7.5	5.2+
2	4	-	-	>100	3560	Deferoxamine/6.5	1.8
3	7	0.24	1	>100	1237	Deferasirox/26	6.1+
4	7	0.92	3	100	2563	Deferasirox/10+	3.5/AML
5	6	0.81	1	>100	1840	Deferasirox/36	5.0+
6	10	1.25	8	98	4857	Deferoxamine/14	4.7/AML

1. Good response; LPI low all the time with the exception of visit 1 before start of chelation.
2. No LPI levels available
3. LPI levels low all the time with the exception of one determination
4. Only first LPI positive
5. Only visit 6 LPI positive, the starting date of chelation. No LPI levels measured after this date.
6. LPI levels became positive during treatment (last visit).

Table S2 Frequency, median and percentiles of parameters by transfusion status at registration, 1-year follow up and 2-years follow up

	At registration		1-yr follow-up(3)		2-yr follow-up(5)	
	N	**Median (p10-p90)**	**N**	**Median (p10-p90)**	**N**	**Median (p10-p90)**
Serum Iron (µmol/L)	100	20.0 (12.0 - 37.5)	78	18.5 (8.0 - 41.0)	64	21.5 (11.1 - 43.0)
0 units	88	20.0 (12.0 - 36.0)	52	17.0 (11.0 - 32.0)	40	17.0 (11.1 - 40.0)
<=10 units	11	23.0 (4.0 - 45.0)	12	22.0 (7.0 - 43.0)	15	22.0 (11.0 - 47.0)
>10 units	1	16.0 (16.0 - 16.0)	14	38.0 (5.0 - 47.0)	9	38.0 (18.0 - 47.5)
Ferritin (µg/L)	100	287 (48 - 982)	78	285 (57 - 1573)	64	341 (59 - 2387)
0 units	88	272 (49 - 819)	52	207 (56 - 662)	40	237 (51 - 777)
<=10 units	11	408 (20 - 1897)	12	593 (192 - 901)	15	590 (61 - 870)
>10 units	1	1885 (1885 - 1885)	14	1528 (829 - 2217)	9	2085 (591 - 7904)
Transferrin saturation (%)	100	35.6 (19.0 - 87.4)	78	34.4 (16.4 - 92.9)	64	37.5 (22.2 - 94.3)
0 units	88	36.1 (19.0 - 85.7)	52	31.8 (17.4 - 73.3)	40	31.6 (21.0 - 93.0)
<=10 units	11	29.8 (12.9 - 90.0)	12	37.4 (14.0 - 93.5)	15	59.5 (22.2 - 92.9)
>10 units	1	32.0 (32.0 - 32.0)	14	87.6 (11.1 - 102.3)	9	94.1 (31.6 - 102.2)
Hepcidin (nmol/L)	99	4.5 (1.1 - 21.7)	78	5.6 (1.2 - 19.6)	65	5.2 (1.0 - 19.6)
0 units	87	4.2 (1.2 - 13.8)	52	4.3 (1.2 - 10.4)	41	3.9 (0.9 - 13.6)
<=10 units	11	5.1 (0.5 - 53.7)	12	9.3 (3.8 - 19.6)	15	5.2 (1.0 - 19.6)
>10 units	1	39.1 (39.1 - 39.1)	14	15.9 (4.9 - 39.4)	9	10.5 (2.9 - 48.5)

GDF15 (ng/L)	100	2193 (952 - 5663)	77	2479 (1016 - 7982)	63	2576 (1045 - 7746)
0 units	88	2165 (921 - 6084)	52	2268 (1014 - 5909)	39	1986 (664 - 6737)
<=10 units	11	2823 (1232 - 4987)	12	2417 (1923 - 11543)	16	3235 (1053 - 7488)
>10 units	1	1856 (1856 - 1856)	13	3210 (1765 - 7071)	8	3780 (1247 - 9474)
Soluble transferrin receptor (mg/L)	100	1.3 (0.7 - 2.8)	78	1.4 (0.7 - 3.0)	62	1.3 (0.8 - 2.7)
0 units	88	1.3 (0.8 - 2.8)	52	1.5 (0.9 - 3.0)	39	1.3 (0.9 - 2.8)
<=10 units	11	1.1 (0.6 - 2.6)	12	1.3 (1.0 - 2.6)	15	1.4 (0.7 - 2.3)
>10 units	1	0.6 (0.6 - 0.6)	14	0.9 (0.4 - 1.7)	8	1.1 (0.4 - 5.4)
Non transferrin bound iron (µmol/L)	100	0.65 (0.14 - 3.03)	77	0.59 (0.15 - 3.64)	65	0.64 (0.14 - 5.42)
0 units	88	0.63 (0.14 - 2.97)	51	0.54 (0.16 - 2.62)	41	0.52 (0.18 - 2.86)
<=10 units	11	0.97 (0.05 - 3.36)	12	0.71 (0.20 - 4.01)	15	1.00 (0.09 - 2.91)
>10 units	1	0.81 (0.81 - 0.81)	14	3.21 (0.08 - 5.03)	9	3.78 (0.12 - 8.42)
Labile plasma iron (µmol/L)	100	0.09 (0.02 - 0.22)	77	0.13 (0.03 - 0.38)	65	0.13 (0.02 - 0.38)
0 units	88	0.09 (0.02 - 0.21)	51	0.10 (0.02 - 0.27)	41	0.11 (0.01 - 0.30)
<=10 units	11	0.09 (0.01 - 0.32)	12	0.16 (0.06 - 1.14)	15	0.13 (0.07 - 0.20)
>10 units	1	0.02 (0.02 - 0.02)	14	0.17 (0.06 - 0.47)	9	0.18 (0.02 - 1.39)

Table S3 Frequency, median and percentiles of parameters by ring sideroblast status at registration, 1 year follow-up and 2 years follow-up

	Registration		1-yr follow-up		2-yr follow-up	
	N	**Median (p10-p90)**	**N**	**Median (p10-p90)**	**N**	**Median (p10-p90)**
Serum Iron (µmol/L)	100	20.0 (12.0 - 37.5)	78	18.5 (8.0 - 41.0)	64	21.5 (11.1 - 43.0)
Non-RS	66	17.0 (10.0 - 26.0)	53	16.0 (7.0 - 35.0)	43	18.0 (11.0 - 38.0)
RARS/RCMD-RS	34	29.5 (16.0 - 45.0)	25	30.0 (12.0 - 44.0)	21	33.0 (13.0 - 47.0)
Ferritin (µg/L)	100	287 (48 - 982)	78	285 (57 - 1573)	64	341 (59 - 2387)
Non-RS	66	246 (36 - 665)	53	279 (56 - 1367)	43	283 (54 - 1970)
RARS/RCMD-RS	34	376 (127 - 1242)	25	287 (149 - 2217)	21	590 (215 - 2560)
Transferrin saturation (%)	100	35.6 (19.0 - 87.4)	78	34.4 (16.4 - 92.9)	64	37.5 (22.2 - 94.3)
Non-RS	66	31.2 (17.1 - 61.2)	53	30.2 (14.0 - 76.1)	43	31.7 (20.4 - 92.7)
RARS/RCMD-RS	34	58.5 (25.0 - 93.0)	25	46.2 (22.2 - 95.1)	21	85.0 (26.2 - 95.5)
Hepcidin (nmol/L)	99	4.5 (1.1 - 21.7)	78	5.6 (1.2 - 19.6)	65	5.2 (1.0 - 19.6)
Non-RS	66	4.7 (1.1 - 24.2)	53	7.4 (1.2 - 23.7)	43	6.3 (1.0 - 22.4)
RARS/RCMD-RS	33	4.2 (1.2 - 10.3)	25	4.9 (1.4 - 9.8)	22	3.4 (1.0 - 14.2)
Growth differentiation factor 15 (ng/L)	100	2193 (952 - 5663)	77	2479 (1016 - 7982)	63	2576 (1045 - 7746)
Non-RS	66	1844 (921 - 4828)	52	2195 (1016 - 5909)	43	2113 (982 - 5995)
RARS/RCMD-RS	34	2888 (1026 - 10361)	25	3148 (1223 - 10303)	20	3661 (1192 - 8977)

Soluble transferrin receptor (mg/L)	100	1.3 (0.7 - 2.8)	78	1.4 (0.7 - 3.0)	62	1.3 (0.8 - 2.7)
Non-RS	66	1.2 (0.7 - 2.7)	53	1.3 (0.5 - 3.0)	42	1.2 (0.8 - 2.2)
RARS/RCMD-RS	34	1.5 (0.8 - 3.1)	25	1.8 (0.9 - 2.8)	20	1.5 (0.5 - 3.2)
Non transferrin bound iron (µmol/L)	100	0.65 (0.14 - 3.03)	77	0.59 (0.15 - 3.64)	65	0.64 (0.14 - 5.42)
Non-RS	66	0.46 (0.08 - 1.75)	52	0.51 (0.08 - 2.78)	43	0.52 (0.14 - 3.31)
RARS/RCMD-RS	34	1.22 (0.30 - 3.85)	25	1.61 (0.16 - 5.20)	22	2.02 (0.16 - 6.00)
Labile plasma iron (mg/L)	100	0.09 (0.02 - 0.22)	77	0.13 (0.03 - 0.38)	65	0.13 (0.02 - 0.38)
Non-RS	66	0.09 (0.02 - 0.19)	52	0.12 (0.02 - 0.31)	43	0.12 (0.01 - 0.30)
RARS/RCMD-RS	34	0.09 (0.02 - 0.32)	25	0.16 (0.06 - 0.87)	22	0.14 (0.04 - 0.94)

Table S4 Cause of death by LPI status

	Total n (%)	LPI status N (%)	
		LPI<LLOD	LPI≥LLOD
Total	19	9	10
AML	3 (15.8)	2 (22.2)	1 (10.0)
Cardiovascular	2 (10.5)	1 (11.1)	1 (10.0)
Hemorrhage	2 (10.5)	-	2 (20.0)
Infection	5 (26.3)	2 (22.2)	3 (30.0)
Myelodysplastic	2 (10.5)	2 (22.2)	-
Not Known	2 (10.5)	-	2 (20.0)
Other	2 (10.5)	1 (11.1)	1 (10.0)
Pulmonary	1 (5.3)	1 (11.1)	-

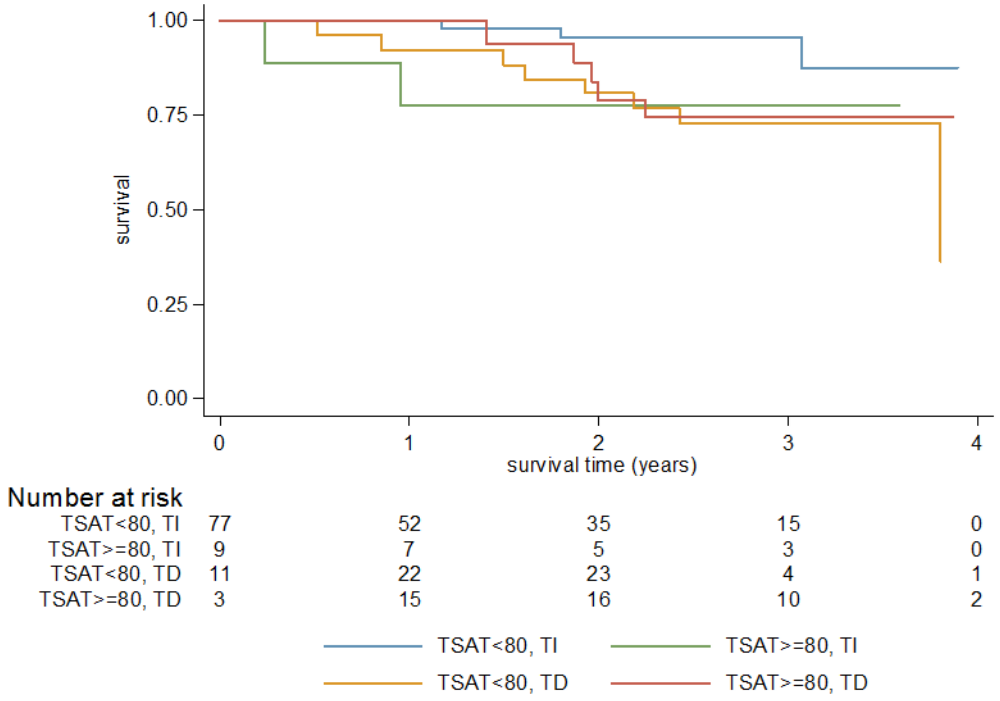

Figure S1 Survival according to transferrin saturation (TSAT) and transfusion status.