

Low serum haptoglobin and blood films suggest intravascular hemolysis contributes to severe anemia in hereditary hemorrhagic telangiectasia

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Supplementary Table 1:
Demographic indices of the 27 HHT patients with serum haptoglobin measurements

	N (%)	minimum	maximum	p25	p75	Median
Sex (% female)	22 (81.5)					
Oral iron (mg/day) ‡	15 (55.5)	14	195	14	130	50
Intravenous iron (freq) †	13 (48.1)	once	weekly	twice	4-6 weekly	8-12 weekly
Red cell transfusion (freq) †	6 (22.2)	once	weekly	twice	4-6 weekly	8-12 weekly
	N	minimum	maximum	p25	p75	Median
Age (ys)	27	19	81	50	64	58
Haptoglobin (g/L)	27	0	3.47	0.49	1.99	1.16
Epistaxis severity score (0-10)	27	0	8.59	3.41	7.97	6.54
SaO ₂ (%)	27	93.75	99.5	96	97.5	96.5
Haemoglobin (g/L)	26	64	147	88	132	118
Haematocrit (%)	26	0.22	0.443	0.29	0.41	0.373
MCH (pg)	26	71.7	95.8	83.2	91.4	87.8
MCHC (g/L)	26	22.3	30.7	24.7	29.2	28
MCV (fl)	26	279	340	304	323	311.5
Red blood cells (x 10 ⁹ /L)	26	2.43	5.83	3.28	4.75	4.455
RDW (fl)	26	12.6	22.7	14.7	19.3	17.65
White blood cells (x10 ⁹ /L)	26	3.5	8.6	5.9	7.5	6.65
Platelet count (x10 ⁹ /L)	26	80	627	262	368	290.5
Mean platelet volume (fl)	26	9.1	12.8	9.8	11	10.65
Neutrophil count (x10 ⁹ /L)	26	1.8	6.8	3.5	5	4.3
Lymphocyte count (x10 ⁹ /L)	26	0.4	3	1	1.7	1.5
Monocyte count (x10 ⁹ /L)	26	0.3	1.4	0.5	0.6	0.5
Eosinophil (x10 ⁹ /L)	26	0	0.7	0.1	0.2	0.2
Basophil count (x10 ⁹ /L)	26	0	0.1	0	0	0
Reticulocyte count (x10 ⁹ /L)	17	0	301.2	38.7	152.7	78.3
Reticulocytes (%)	17	0	11.9	1.1	3.7	1.7
Prothrombin time (s)	20	10.2	12.7	10.65	11.45	11.15
APTT (s)	20	20.3	30.4	22	28.6	24.4
TfSI (%)	21	2	35	6	15	10
Serum iron (µmol/l)	21	2	24	4	11	8
Serum ferritin (µg/l)	21	4	154	17	81	25
Alanine transaminase (iu/L)	21	6	42	15	27	17
Alkaline phosphatase (iu/L)	22	37	259	58	110	84
Albumin (g/L)	22	27	46	35	42	37.5
Bilirubin (µmol/L)	21	4	68	5	11	6
Fibrinogen (g/L)	20	1.86	5.4	2.77	3.545	3.14
C-reactive protein (mg/L)	21	0.3	14.3	0.7	4.4	2.5

All measurements made on the same day as serum haptoglobin, between June 2017-March 2018. ‡ elemental iron content. † For numeric calculations of infusion/ transfusion frequency, never=0, once=1, twice=2, 3-4 times in life=3, 2-3 monthly=4, 4-6 weekly=5, alternate weekly=6, weekly=7. MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, MCV: mean corpuscular volume, APTT: activated partial thromboplastin time, RDW: red cell distribution width, SaO₂:% haemoglobin binding sites occupied by oxygen, TfSI: transferrin saturation index.

Supplementary Table 2:
Extended relationships of same-day patient variables with ln(haptoglobin)

	N	Median	Range	Q1, Q3	Crude regression		CRP-adjusted regression	
					coefficient	p-value	coefficient	p-value
Haptoglobin (g/L)	18	1.1	0-3.47	0.49,2.06	-	-	-	-
C-reactive protein (mg/L)	18	2.5	0.3-14.3	0.6,4.4	0.11	0.023	-	-
Red blood cells (x 10 ⁹ /L)	18	4.61	3.27-5.83	4.42,4.9	0.74	0.005	0.84	<0.001
Haematocrit (%)	18	0.4035	0.29-0.44	0.37,0.43	7.91	0.069	10.5	0.006
MCV (fl)	18	87.8	71.7-95.8	83.2,90.4	-0.065	0.018	-0.06	0.014
Lymphocyte count (x 10 ⁹ /L)	18	1.6	1-3	1.3,2	0.59	0.13	0.84	0.015
Haemoglobin (g/L)	18	131.5	88-147	117,136	0.011	0.32	0.025	0.023
White blood cells (x 10 ⁹ /L)	18	7.15	3.5-8.6	6,7.7	0.31	0.012	0.27	0.027
APTT (s)	17	24.5	20.4-29.9	23.1,28.5	-0.13	0.048	-0.12	0.041
Epistaxis severity score (0-10)	18	6.23	0-8.59	2,7.1	-0.073	0.24	-0.113	0.045
Reticulocytes (%)	10	1.55	0-5.3	1.1,3.1	-0.089	0.57	-0.30	0.047
MCH (pg)	18	28.45	22.3-30.7	25.9,29.2	-0.16	0.015	-0.13	0.054
Basophil count (x 10 ⁹ /L)	18	0	0-0.1	0,0	-8.67	0.08	-8.3	0.07
Albumin (g/L)	18	39	27-46	36,43	0.013	0.76	0.078	0.075
Intravenous iron†	9/18	twice	Once-weekly	Twice, 2-3 monthly	-0.15	0.20	-0.17	0.10
Fibrinogen (g/L)	17	3.14	1.86-5.4	2.98,3.44	0.55	0.11	0.44	0.11
PT (s)	17	11.2	10.2-12.7	10.9,11.5	-0.43	0.22	-0.49	0.12
Reticulocyte count (x10 ⁹ /L)	10	71.1	0-174.4	52.3,139.2	-0.0002	0.97	-0.007	0.16
Neutrophil count (x 10 ⁹ /L)	18	4.3	1.8-6.8	3.7,5	0.27	0.07	0.20	0.19
Monocyte count (x 10 ⁹ /L)	18	0.55	0.3-1	0.5,0.6	-0.79	0.19	1.27	0.26
Oral iron (mg/day) ‡	10/18	67.5	14-195	15,130	-0.0017	0.64	-0.23	0.30
Eosinophil (x 10 ⁹ /L)	18	0.1	0-0.4	0.1,0.2	2.84	0.13	1.85	0.32
Alkaline phosphatase (iu/L)	18	75.5	37-212	58,100	-0.0014	0.76	-0.0038	0.37
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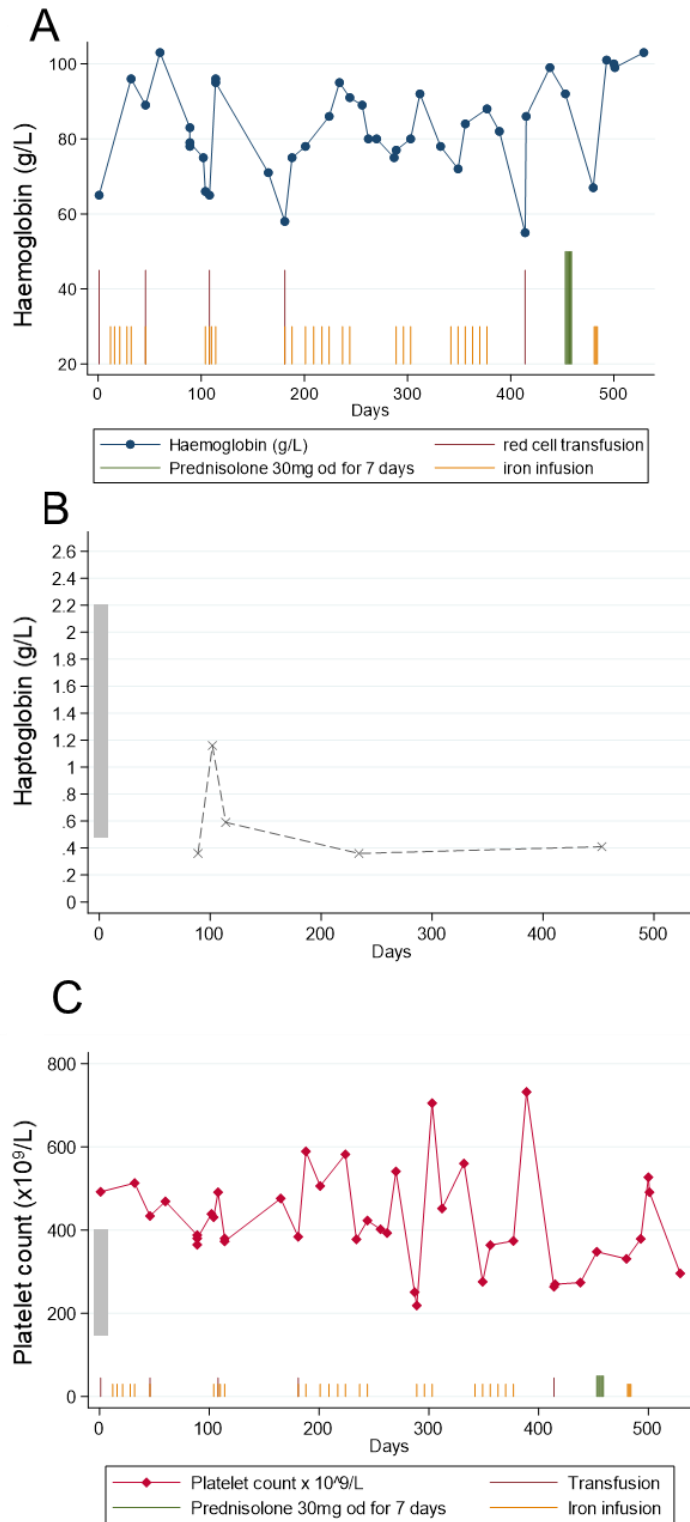
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.../	N	Median	Range	Q1, Q3	Crude regression		CRP-adjusted regression	
					coefficient	p-value	coefficient	p-value
Sex (% female)	14/18	77.7			-0.345	0.51	-0.42	0.38
SaO ₂ (%)	18	96.5	93.75-99.5	96, 97.5	-0.021	0.88	0.12	0.39
Serum ferritin (µg/l)	18	26	6-154	21,101	-0.0030	0.47	-0.003	0.41
Alanine transaminase (iu/L)	18	18	6-42	15,27	0.00023	0.99	0.015	0.45
Age (ys)	18	55.5	19-81	50,61	-0.0039	0.773	-0.009	0.47
Red cell transfusion †	2/18	twice	Once-twice	Once, twice	0.08	0.33	0.057	0.48
Bilirubin (µmol/L)	18	7	4-68	5,12	-0.0056	0.62	-0.005	0.62
Platelet count (x 10 ⁹ /L)	18	290	161-627	232,352	0.0015	0.41	0.0008	0.65
TfSI (%)	18	12.5	3-35	7,15	-0.027	0.30	-0.011	0.69
MCHC (g/L)	18	318	287-340	311,334	5.39	0.22	0.002	0.89
RDW (%)	18	15.9	12.6-22.7	14.5,18.9	0.056	0.43	0.006	0.93
Serum iron (µmol/l)	18	8.5	3-24	5,12	-0.025	0.54	0.003	0.93
Mean platelet volume (fl)	18	10.8	9.1-12.8	9.8,11.1	0.013	0.95	0.012	0.95

Regression analyses in the 18 patients who had not received a blood transfusion in the previous month, using natural log-transformed haptoglobin (ln(haptoglobin)) as the dependent (outcome) variable, as this was more normally distributed than haptoglobin (data not shown). Patient variables are ranked in order of strength of association in CRP-adjusted regression. ‡ elemental iron content.† For numeric calculations of intravenous iron and red blood cell transfusion frequency, a scale of 0-7 was used where never=0, once=1, twice=2, 3-4 times in life=3, 2-3monthly=4, 4-6 weekly=5, alternate weekly=6, and weekly=7. *MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, MCV: mean corpuscular volume, PT: prothrombin time, APTT: activated partial thromboplastin time, RDW: red cell distribution width. SaO₂, % of haemoglobin binding sites occupied by oxygen. TfSI transferrin saturation index.

Data on the patients who had received a recent blood transfusion in the past month are not shown. This recently transfused group had more frequent intravenous iron infusions (odds ratio [OR] 2.20 (95% confidence intervals 1.05, 4.6, p=0.036). This group also tended to have higher epistaxis severity scores (OR 3.84 [0.64, 23.2]); higher haptoglobin (OR 1.67[0.56-5.01]; lower serum iron (OR 0.16 [0.016, 1.66]); lower TfSI (OR 0.68 [0.29, 1.15]); and lower bilirubin (OR 0.41 [0.067, 2.54]), though these and other trends did not reach statistical significance.

Supplementary Figure 1. Serial haptoglobin measurements



A) Haemoglobin (blue circles) and treatment data as presented in Figure 2A). **B)** Serum haptoglobin measurements (grey crosses). Between day 89 and day 104, there were two negative haemoglobin electrophoresis tests, a negative haemoglobin gene screen, and a negative extended direct antibody test. **C)** There was a past history of idiopathic thrombocytopenia purpura but platelet counts (red diamonds) were normal to high throughout this period and did not track haemoglobin. Normal ranges are indicated by grey bars. Timings of iron infusions (yellow lines), red cell transfusions (red lines), and 1 week course of prednisolone (green line) are indicated.