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Safety and efficacy of human apotransferrin infusion in patients with β-thalassemia intermedia: the AIM study

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Data-sharing statement

Data available on request due to privacy/ethical restrictions. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Authorship contributions

KK: data-analysis, interpretation of the data, and writing the manuscript; DWS: interpretation of the data and manuscript revision; IKB: study conception and design, data acquisition, analysis, interpretation of the data, writing the manuscript; EN: study conception and design, interpretation of the data and manuscript revision; BB: study conception and design, data analysis, interpretation of the data and manuscript revision.

Disclosures

IKB works at Prothya B.V. The Netherlands.

EN is a member of an entity's Board of Directors or advisory committee of Novartis and received research Funding. BB received honorees for ad hoc advisory committees, invited lectures or pod-casts of CSL Behring, Novo Nordisk, Celgene/BMS, Pfizer, Novartis and Sanofi and has received research funding from Novartis, BMS, GBT/Pfizer, and Sanquin. DSW and KK have no disclosures to report.

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Beta-thalassemia intermedia comprises a diverse group of patients with various mutations. (1, 2) These patients mostly suffer from transfusion or non-transfusion dependent anaemia and iron overload, an important factor in morbidity related to complications of beta-thalassemia.(1) Circulatory iron levels in thalassemia intermedia exceed transferrin iron binding capacity resulting in elevated levels of free circulating non-transferrin bound iron (NTBI), responsible for oxidative stress.(3, 4) In a mouse model of beta-thalassemia intermedia, homozygous for a deletion of the gene encoding β–major globin ($Hbb^{th1/th1}$), induction of supranormal transferrin levels by repeated human apotransferrin administration normalized NTBI levels, and reduced haemolysis, increased Hb levels and reduction of splenomegaly.(5) These results were also demonstrated in another mouse model ($Hbb^{th3/+}$) with heterozygous β1/β2 globin gene deletion.(6) However, apotransferrin administration has not been studied in patients with beta-thalassemia intermedia. The aim of our study was to investigate the safety and efficacy of repeated human apotransferrin administration on markers of erythropoiesis, iron metabolism and spleen size in patients with non-transfusion dependent beta-thalassemia Intermedia (NTDTI) and transfusion dependent beta-thalassemia Intermedia (TDTI). In the current study, an effect of repeated human apotransferrin infusions on markers of erythropoiesis or iron metabolism was not observed, except for a temporary decrease in NTBI levels.

The AIM-study is a phase 2, single centre, open-label, feasibility trial conducted in a teaching hospital in Amsterdam, the Netherlands. Patients aged ≥18 years with NTDTI and TDTI were included. NTDTI is defined as ≤5 red blood cell (RBC) units during the 24-week period and no RBC transfusions within weeks prior to the start of the study. TDTI is defined as 6 to 20 RBC units transfused during a 24-week period and a transfusion-free period of ≤6-week before start of the study. Other inclusion criteria were: normal renal function, normal hepatic function, WHO performance score<3, and written informed consent. Exclusion criteria were: a history of allergic reaction on human plasma(products), a concurrent severe or uncontrolled medical condition, cardiac dysfunction (myocardial infarction <6 months of study entry, unstable angina or arrhythmias), pregnant or lactating females or known IgA deficiency. Human apotransferrin (Prothya Biosolutions B.V., Amsterdam, The Netherlands) was initially given at an intravenous dose of 170mg/kg every 2 weeks after a loading dose of 170 mg/kg at day -1, based on simulation of the single dose PK profile of transferrin in adults who received haematopoietic stem-cell transplantation.(7) Due to insufficient increases (<2g/L) in plasma transferrin concentration in the first three patients, the dose was increased to 340 mg/kg every 2 weeks without a loading dose, based on simulation data of the first three patients. Treatment duration was 14 weeks in NTDTI patients and 18 weeks in TDTI patients. Apotransferrin was administered in TDTI patients directly pre-transfusion during transfusion days.

The primary outcomes were defined as change from baseline of Hb level in NTDTI and change from baseline of number of RBC units transfused per week in TDTI patients. Secondary outcomes were defined as: number of patients with an increase of >1.5 g/dL in Hb levels for both NTDTI and TDTI patients (before transfusion) as compared to baseline, as well as a reduction in transfusion dependency (number of RBC units/week) by at least 50% compared to baseline for TDTI patients. Baseline transfusion dependency was defined as the number of RBC units transfused in the 20 weeks prior to inclusion. Reduction in iron overload was reflected by changes in levels of serum iron, transferrin, transferrin saturation, ferritin, NTBI levels, hepcidin-25, and soluble transferrin receptor (sTfR). The effect on erythropoiesis was determined by measuring pre-dose Hb levels, reticulocyte count, red cell indices (in the TDTI group only prior to RBC transfusion), pre-transfusion Hb levels (in the TDTI patients only) and spleen size. Serum hepcidin was measured using the enzyme immunoassay (ELISA) kit (Hepcidin 25 (bioactive), EIA-5782, DRG Diagnostics, Marburg, Germany). NTBI was measured using a nitrolotriacetic chelation-ultrafiltration-detection approach. Iron was measured using a colorimetric assay and transferrin by immunochemical turbidimetry. Iron was released from transferrin by acidifying the serum, reduced from Fe^{3+} to Fe^{2+}, complexed with a chromogen. For calculation of transferrin saturation (%) from the measured transferrin (g/L) and iron (umol/L) levels a conversion factor of 25.2 was used. Levels of sTfR (Ramco Laboratories, TX, USA) and ferritin (Cobas, Roche diagnostics B.V., Almere, the Netherlands) were measured using immunochemical assays. Blood samples were analysed after collection or stored at -80°C. Spleen size was measured by ultrasound in patients receiving 170mg/kg dose and by MRI in patients receiving the 340mg/kg dose. Pharmacokinetic parameters were evaluated during steady state after blood sampling in week 4 or 6. Samples were taken before infusion of apotransferrin, and 5 minutes, 2 hours, 1 day, 7 days and 14 days thereafter to determine the elimination half-life ($T_{1/2term}$), pre-dose serum transferrin concentration, the average steady state concentration, the maximal observed concentration (C_{max}), time to reach the maximal observed concentration (T_{max}), and the area under the curve (AUC). In case of transfusions, samples were obtained pre-tranfusion. Adverse events (AEs) and serious adverse events (SAEs) were monitored. The cut-off data point for the lab results was after 14 weeks for NTDTI patients and prior to the last RBC transfusion on the date most closely to week 18 in TDTI patients (in order to have a longer follow-up to assess transfusion burden). This trial was approved by the institutional review board of the Amsterdam UMC, the Central Committee on Research Involving Human Subjects (CCMO) and was registered in the EudraCT database (2014-001936-12).

Parametric data were described by mean and standard deviation (SD), non-parametric data by median and interquartile range (IQR) or range in case the dataset was too small to use IQR. For the pharmacokinetic (PK) analysis Phoenix™ WinNonlin® v8.1.1. was used. Non-compartmental analysis (NCA; Model Type: Plasma (200-202), Dose Type: IV Infusion) was applied. The linear trapezoidal method was used in order to calculate AUC values.

Five patients with beta-thalassemia intermedia (NTDTI: HbE/ß□-thalassemia; HbE/$ß^+$-thalassemia; ß□-thalassemia and α-triplication) ; TDTI: δβ/$ß^0$-thalassemia; ß□/$β^+$-thalassemia and homozygous -$α^{3.7}$) received either human apotransferrin at a dose of 170 mg/kg or at a dose of 340mg/kg (for study flow see Figure S1 and baseline demographics Table S1). Two subjects participated in both dosing groups. Blood transfusions in TDTI patients started during their life because of increasing tiredness and repeating hemoglobin levels below 8 g/dL without any other explanation. The transfusion burden 20 weeks prior to inclusion was 0.6 units/week for the TDTI patient in the 170 mg/kg group and 0.4 and 0.75 units/week respectively for the two patients in the 340mg/kg group.

Nor Hb levels in NTDTI patients neither the number of RBC units transfused per week in TDTI patients changed following apotransferrin administration. A significant increase of > 1.5g/dL in Hb levels was not observed in the NTDTI patients nor did we observe a 50% reduction in transfusion burden in the TDTI patients. Repeated human apotransferrin infusions did not result in any significant effect on markers of erythropoiesis, red cell indices (Table 1) and spleen size (data not shown). Despite increased transferrin levels, no significant changes in levels of serum iron, ferritin, and transferrin saturation were observed (Table 1). Neither in levels of NTBI, hepcidin-25 levels, and STfR (data not shown). A temporary effect was observed on NTBI levels, as their levels became undetectable (≤0.47 µM) five minutes after human apotransferrin infusion in all eight participants, including two cases with already undetectable levels at baseline, and became detectable after 24 hours in one patient with the highest baseline NTBI levels, four days after apotransferrin infusion in four cases, and after seven days and 14 days respectively in two cases. (Figure 1). PK data are presented in Table 2. Apotransferrin administration appeared safe (Table S2).

Our findings are in contrast to previous observations in mice models of beta-thalassemia intermedia which showed efficacy of repeated human apotransferrin infusions.(5, 6) A possible explanation could be that in the mouse model more stable elevated transferrin level were reached by daily intraperitoneal injections instead of the biweekly intravenously administrations in current study. An alternative explanation might be that human apotransferrin in mice may not deliver iron as effectively to mouse erythroid precursors due to lower affinity of human apotransferrin to the mouse apotransferrin receptor, as Huebers et al. described the possible difference of transferrin receptors across species (8, 9). Together this might have led to competition between mouse transferrin and human transferrin, limiting iron delivery in mouse models resulting in the favourable response that was not observed in this cohort. Similar to our findings, two other human studies, performed in patients receiving haematopoietic stem cell transplantations,(7, 10) showed no persistent effect of human apotransferrin infusions on markers of iron overload.

In conclusion, despite promising effects of apotransferrin infusion in mice models of thalassemia intermedia, no effect of repeated intravenous human apotransferrin administration was observed on erythropoiesis and markers of iron metabolism in patients with beta-thalassemia intermedia. Only a temporary reduction in NTBI levels was observed. Future studies have to demonstrate whether more frequent or higher doses of human apotransferrin may improve erythropoiesis and iron metabolism in patients with beta-thalassemia intermedia.

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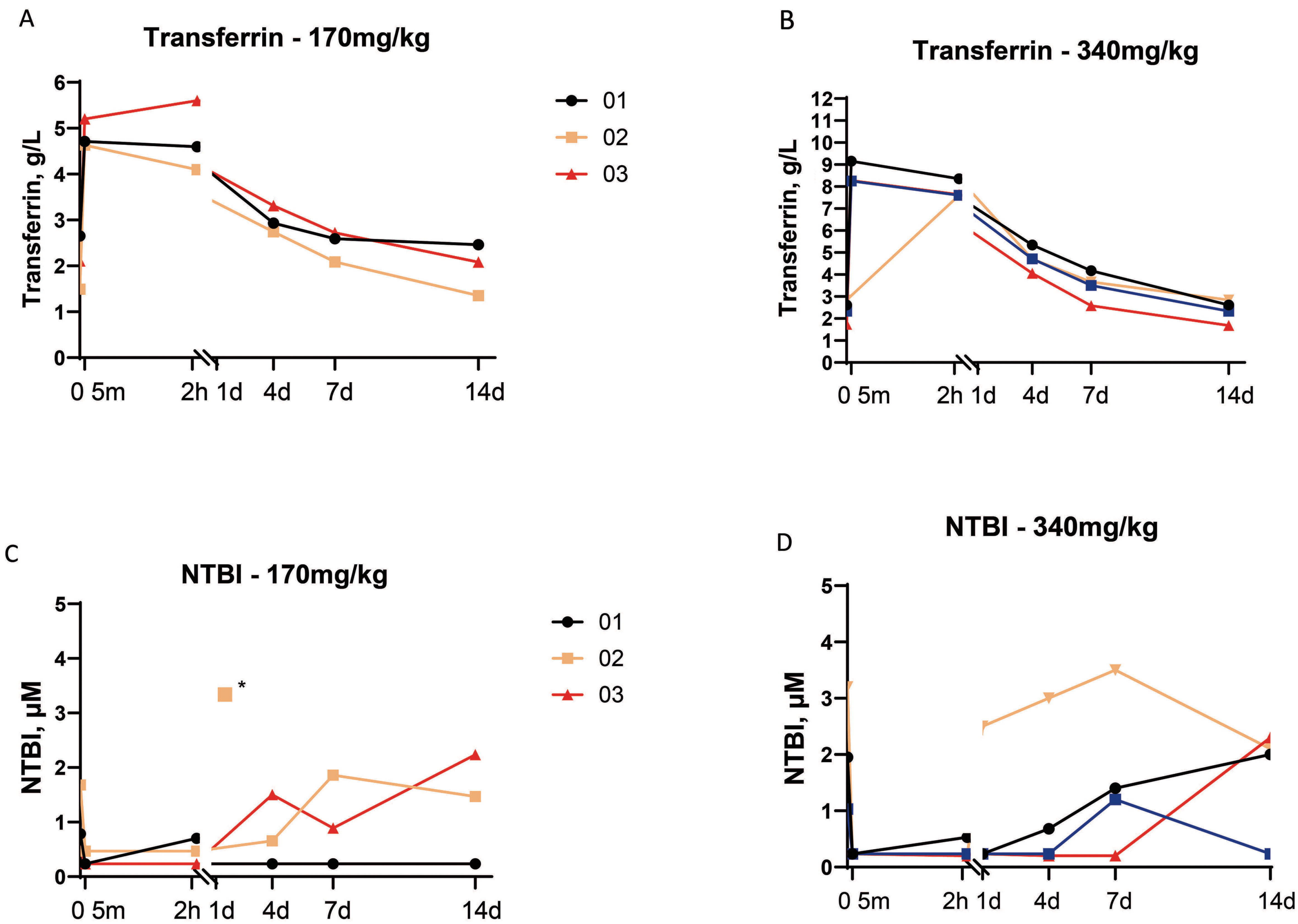
	NTDT 170 mg/kg (n=2)		TDT 340 mg/kg (n=1)	
	Baseline	Post-treatment	Baseline	Post-treatment
Hb, *g/dL*	6.8 (5.3 - 8.2)	7.2 (6.5 - 7.9)	8.9	8.5
Ht, *L/L*	0.22 (0.17 - 0.27)	0.23 (0.21 - 0.25)	0.28	0.27
MCV, *fL*	71.9 (68.7 - 75.1)	70.0 (68.2 - 71.8)	74.9	76
Reticulocytes, *%*	6.4 (4.1 - 8.7)	4.4 (-)	4.3	4.1
Reticulocyte count, $10^9/L$	181 (147.8 - 214)	150 (-)	161	144
Bilirubin, *µmol/L*	38 (30 - 45)	37 (33 - 40)	72	43
LDH, *U/L*	273 (-)	262 (212 - 311)	174	172
Transferrin*, g/L,*	2.0 (1.83 - 2.11)	2.2 (2.18 - 2.23)	1.3	1.4
Transferrin saturation, *%*	62 (47 - 77)	71 (61 - 80)	112	84
Ferritin, *µg/L*	274 (244 - 304)	150 (102 - 198)	293	263
Iron, *µmol/L*	30 (25 - 36)	39 (33 - 44)	36	30
	NTDT 170 mg/kg (n=2)		**TDT 340 mg/kg (n=2)**	
	Baseline	Post-treatment	Baseline	Post-treatment
Hb, *g/dL*	8 (7.7 - 8.2)	7.6 (7.3 - 7.9)	9.7 (9.5 - 9.8)	8.8 (7.7 - 9.8)
Ht, *L/L*	0.27 (0.26 - 0.27)	0.26 (0.25 - 0.26)	0.29 (0.28 - 0.29)	0.27 (0.25 - 0.29)
MCV, *fL*	72.4 (69.5 - 75.3)	73.9 (74.2 - 76.8)	76 (74.2 - 76.8)	74.9 (73.9 - 75.8)
Reticulocytes, *%*	5.4 (4.9 - 5.8)	3.6 (-)	8 (2 - 14)	12 (5.2 - 18)
Reticulocyte count, $10^9/L$	198 (167 - 229)	123 (-)	311 (73 - 548)	438 (171 - 705)
Bilirubin, *µmol/L*	48 (46 - 49)	51 (41 - 61)	31 (21 - 40)	38 (22 - 54)
LDH, *U/L*	386 (-)	385 (339 - 430)	209 (169 - 249)	206 (187 - 225)
Transferrin*, g/L,*	2.3 (2.0 - 2.5)	2.7 (2.48 - 2.89)	1.4 (1.2 - 1.7)	2.1 (1.53 - 2.63)
Transferrin saturation, *%*	71 (56 - 85)	71 (52 - 90)	94 (94 - 96)	98 (95 - 101)
Ferritin, *µg/L*	279 (258 - 300)	234 (224 - 243)	621 (296 - 945)	550 (175 - 924)
Iron, *µmol/L*	41 (29 - 54)	49 (32 - 65)	34 (29 - 40)	51 (39 - 63)

Table 1: Haematological parameters and markers of iron metabolism. Data are given as median and range. **,* In chelated patients we cannot exclude that iron levels and transferrin saturation may include iron bound to the chelator. *Abbreviations: non-transfusion dependent thalassemia (NTDT), transfusion dependent thalassemia (TDT), mean corposcular volume (MCV), lactate dehydrogenase (LDH).*

	170mg/kg (n=3)	**340mg/kg** (n=4)
$T_{1/2term}$, h	-	104.9 (SD, 25.9)
$C_{avg,ss}$, *g/L*	1.0 (SD, 0.1)	2.7 (SD, 1.0)
T_{max}, h,	1.6 (range, 1.6 - 3.8)	2.4 (range, 2.3 - 2.6)
C_{max}, g/L	3.3 (SD, 0.6)	7.4 (SD, 1.1)
AUC, g.h/L	332 (SD, 38.5)	907 (SD, 317.0)

Table 2: *Pharmacokinetic parameters during steady state, data was described as median (range) or mean (SD). Abbreviations: the apparent terminal half-life ($T_{1/2term}$), average steady-state analyte concentration ($C_{avg,ss}$), time to reach the maximal observed analyte concentration (T_{max}), maximal observed analyte concentration (C_{max}), area under the analyte concentration (AUC).*

Figure 1: *Transferrin and NTBI levels during pharmacokinetic sampling in week 4 or 6 during steady state. Panel A: transferrin levels in the 170mg/kg group, panel B: transferrin levels in the 340mg/kg group, panel C: NTBI levels in the 170mg/kg group, panel D: transferrin levels in the 340mg/kg group. In subfigure C a data point* (24 hrs) has been omitted from the pharmacokinetic curve due to the measurement being overtly erroneous. Abbreviations: non-transferrin bound iron (NTBI).*


A
Transferrin - 170mg/kg
Transferrin, g/L
0 5m
2h 1d 4d 7d 14d
01
02
03
B
Transferrin - 340mg/kg
Transferrin, g/L
04
05
06
07
C
NTBI - 170mg/kg
NTBI, μM
*
D
NTBI - 340mg/kg
NTBI, μM

Apotransferrin dose	170 mg/kg (n=3)	340 mg/kg (n=4)
Age, years	43 (26- 30)	44 (27-53)
Gender, F/M	3/0	2/2
Transfusion dependent (chronically), n, %	1	2
Chelation therapy, n, %	3	5
Liver iron content, mg/g	6 (4.3 - 7.3)	5.8 (4.3 - 6)

Table S1: *Baseline characteristics, data are presented as median and IQR.*

	170mg/kg study population (n=3)	340mg/kg study population (n=4)
Any TEAE at least possibly related (per patient)	**3 (100%)**	**2 (50%)**
TEAE occurring in entire group		
Fatigue	1 (33.3%)	1 (25%)
Dizziness	1 (33.3%)	1 (25%)
Cold extremities	-	1 (25%)
Pyrexia	1 (33.3%)*	-
Oral dysesthesia	1 (33.3%)	-
Muscle spasm	1 (33.3%)	-
Any serious adverse event	-	-

Table S2: *Treatment emergent adverse events at least possibly related to study treatment, split in any treatment emergent adverse event per patient and treatment emergent adverse events occurring in the entire group Presented: number of subjects (percent of subjects) (* temperature already elevated pre-infusion). Abbreviations: treatment emergent adverse event (TEAE).*

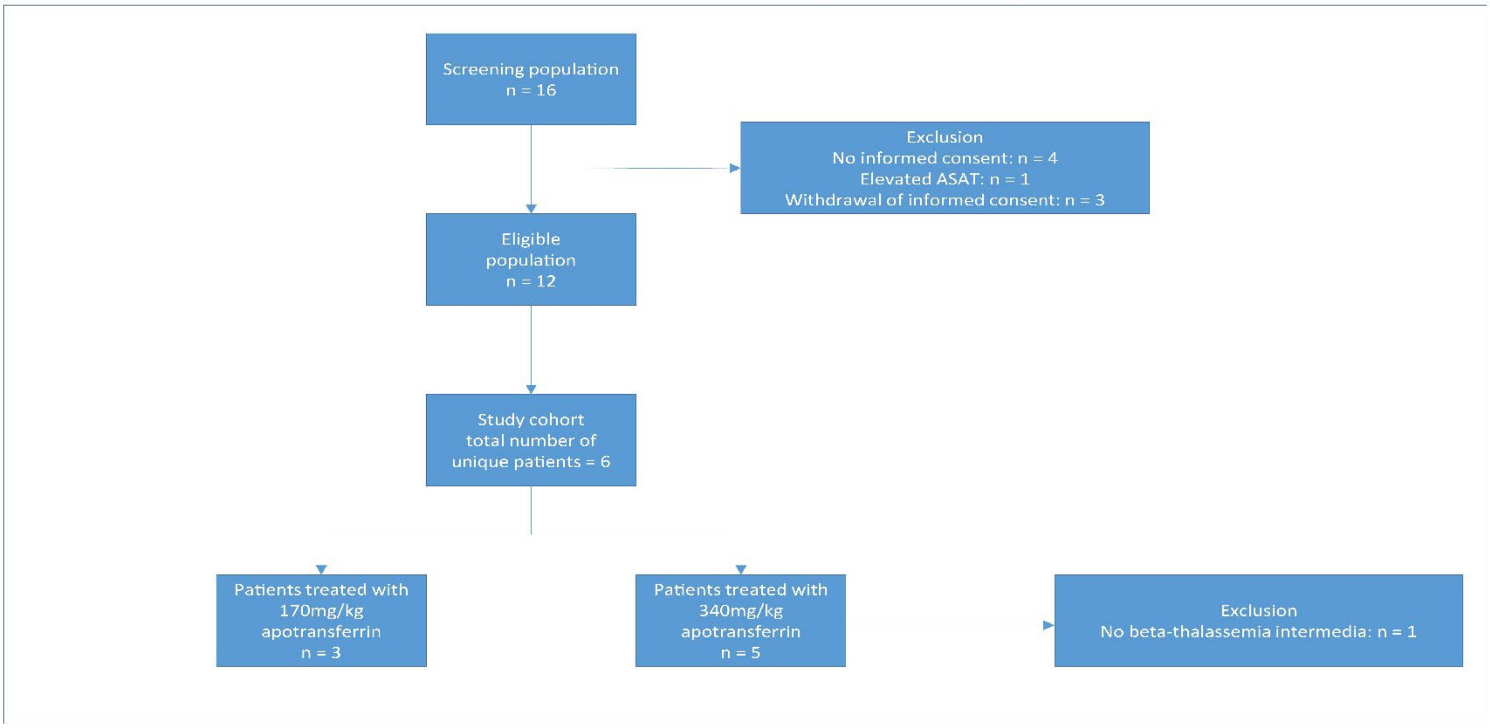


Figure S1: *Study flow. Abbreviations: aspartate-aminotransferase (ASAT).*