

CORRESPONDENCE

‘Phenoconversion’ in adult patients with β -thalassemia

To the Editor:

Patients with clinically significant forms of β -thalassemia have been historically classified as having a β -thalassemia major or β -thalassemia intermedia phenotype, with the first primarily referring to patients who present with severe anemia during early childhood and require lifelong transfusion therapy and the latter relating to patients who present later in childhood or adolescence with mild–moderate anemia that does not necessitate immediate commitment to regular transfusion therapy.¹ In more recent years, the terms transfusion-dependent β -thalassemia (TDT) and non-transfusion-dependent β -thalassemia (NTDT) gradually replaced the two conventional phenotypes, respectively, to highlight the importance of transfusion-dependence in determining the dominant pathophysiology and treatment needs.² International management guidelines and clinical trial eligibility criteria are now clearly differentiated for TDT and NTDT.^{3,4} Based on clinical observation, NTDT patients may still require occasional transfusion therapy in cases of infection, pregnancy, or surgery, while some go on to receive more frequent transfusions or become transfusion-dependent for various clinical reasons or as their disease progresses. The rate and determinants of such ‘phenoconversion’ from NTDT to TDT remain poorly understood and have never been previously evaluated. Such data remain essential to fully understand the natural course of β -thalassemia as a disease spectrum.

We conducted a retrospective cohort study of β -thalassemia patients attending treatment centers in Italy that use Webthal[®], a computerized medical record software with standardized clinical, laboratory, and imaging data recording. An Ethics Committee approval was obtained, and written informed consents for data collection and use were retrieved from patients at each center. For this study, we retrieved data for all adult (≥ 18 years) patients with NTDT who had been originally diagnosed by treating physicians as having β -thalassemia intermedia and who had received ≤ 10 red blood cell (RBC) units during the 12 months prior to start of observation. The latter was based on recent definitions used in clinical trials evaluating novel agents targeting anemia in NTDT (≤ 5 RBC units during the 6 months prior to randomization—NCT03342404, NCT04770753).⁵ Patients were followed from January 1, 2010 until December 31, 2019, death, or loss to follow-up to reflect a 10-year observation period prior to the Covid-19 pandemic when management, including use of transfusion therapy, may have been impacted by resource limitations and mobility restrictions.

For each patient, we retrieved data on age at baseline, sex, splenectomy status, transfusion status, iron chelation status (yes vs. no, year of initiation), serum ferritin level (annual average for each year during follow-up), hemoglobin level (annual average for each year

during follow-up, pretransfusion in transfused patients), and comorbidities (yes vs. no, year of development). For transfusion status, we retrieved the total number of RBC units received for each year during follow-up. Patients were considered to have phenoconversion to TDT when their annual number of RBC units received was >10 in any individual year. The median number of records available per individual year for hemoglobin level ranged between 3 and 4 and for serum ferritin level, they ranged between 2 and 4. Evaluated comorbidities included heart failure, pulmonary hypertension, liver fibrosis/cirrhosis, hepatocellular carcinoma, diabetes, hypothyroidism, hypoparathyroidism, and osteoporosis – all diagnosed per local standards. None of the patients were receiving hydroxycarbamide or any other agent with effects on erythropoiesis.

Descriptive statistics are represented as median (interquartile range [IQR], min, max) or percentages. Bivariate correlations were done using the Mann–Whitney *U* *t*-test (independent continuous), Fisher's exact test (independent categorical), Wilcoxon signed-rank test (paired continuous), and McNemar's test (paired categorical). Receiver operating characteristic (ROC) curves were used to identify the best thresholds to predict phenoconversion, using the highest Youden Index (sensitivity + specificity – 1), and areas under the curve (AUC) were estimated. Kaplan–Meier survival curves were constructed to estimate cumulative survival. Cox regression analyses were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) of phenoconversion. Multivariate forward-stepwise models were used to adjust for confounding effects. All *p*-values were two-sided with the level of significance set at $< .05$.

A total of 305 adults with NTDT were included in this analysis, with 169 (55.4%) being female (Table 1). The median age at baseline was 39.8 years (IQR: 30.9–50.3, min: 18.5, max: 86.7). Patients were followed for a median of 10 years (IQR: 10–10, min: 0.02, max: 10). Ten (3.3%) patients died and 26 (8.6%) were lost to follow-up, with the remaining 269 (88.2%) patients followed for the full period of observation. A total of 86 (28.2%) patients were splenectomized (82 at baseline and 4 performed during follow-up), 81 (26.6%) received iron chelation (25 at baseline and 46 initiated during follow-up), and 54 (17.7%) had a comorbidity (38 at baseline and 21 developed during follow-up). The most common comorbidities were osteoporosis ($n = 23$ at baseline and $n = 11$ developed during follow-up), hypothyroidism ($n = 8$ and $n = 2$), diabetes ($n = 3$ and $n = 2$) heart failure ($n = 5$ and $n = 0$), liver fibrosis/cirrhosis ($n = 0$ and $n = 3$), pulmonary hypertension ($n = 0$ and $n = 1$), hepatocellular carcinoma ($n = 0$ and $n = 1$), and hypoparathyroidism ($n = 1$ and $n = 0$). The median hemoglobin level (period average) was 9.6 g/dL (IQR: 8.7–10.4, min: 6.4, max: 14.7) and the median serum ferritin

TABLE 1 Study parameters overall and in patients who had phenoconversion compared with those who did not.

Parameter	All patients (n = 305)	Phenoconversion		p-Value
		Yes (n = 42)	No (n = 263)	
Age at baseline in years, median (IQR)	39.8 (30.9–50.3)	40 (31.5–47.1)	39.8 (30.9–50.8)	.671
Female, n (%)	169 (55.4)	24 (57.1)	145 (55.1)	.868
Splenectomy, n (%)	86 (28.2)	19 (45.2)	66 (25.1)	.009
At baseline	82 (26.9)	18 (42.9)	64 (24.3)	.015
Performed during follow-up ^a	4 (1.3)	1 (2.4)	2 (0.8)	.360
Iron chelation, n (%)	81 (26.6)	15 (35.7)	51 (19.4)	.025
At baseline	35 (11.5)	9 (21.4)	26 (9.9)	.038
Initiated during follow-up ^a	46 (15.1)	6 (14.3)	25 (9.5)	.406
Hemoglobin level in g/dL, median (IQR)				
Annual average year 1	9.1 (8.2–10.2)	7.8 (7.1–8.6)	9.5 (8.6–10.5)	<.001
Period average throughout follow-up ^a	9.6 (8.7–10.4)	8.4 (7.6–9.3)	9.7 (8.8–10.5)	<.001
Serum ferritin level in ng/mL, median (IQR)				
Annual average year 1	454.3 (175.7–835.6)	454.3 (175.7–835.6)	492.5 (179.9–841.5)	.325
Period average throughout follow-up ^a	475.3 (241.2–938.0)	483 (209.3–1030)	461 (209.8–894.4)	.723
Comorbidity, n (%)	54 (17.7)	11 (26.2)	41 (15.6)	.119
At baseline	38 (12.5)	8 (19)	30 (11.4)	.205
Developed during follow-up ^a	21 (6.9)	6 (14.3)	13 (4.9)	.032

Note: Bold values indicate $p < .05$.

Abbreviation: IQR, interquartile range.

^aValues relate to the period prior to phenoconversion in patients with phenoconversion.

level (period average) was 475.3 ng/mL (IQR: 241.2–938, min: 12.4, max: 16 400) (Table 1).

A total of 42 NTD patients had phenoconversion to TDT for at least 1 year according to the prespecified criteria, corresponding to a crude phenoconversion rate of 13.8% (95% CI: 10.1–18.2) during the 10-year observation period. The median time to phenoconversion was 5.5 years (IQR: 3.5–8.5, min: 1.5, max: 9.5) and the cumulative 3-, 5-, and 10-year phenoconversion-free survival rates were 95%, 92%, and 85%. Figure 1 illustrates the annual amount of RBC received during the 10-year observation period in patients who had at least 1 year of phenoconversion to TDT. Among the 42 patients, 24 had sustained phenoconversion for ≥ 3 consecutive years, while the remaining 18 patients had phenoconversion for < 3 years and/or returned to an NTD status after temporary phenoconversion.

NTD patients who had phenoconversion to TDT were significantly more likely to have been splenectomized or receiving iron chelation therapy prior to phenoconversion than patients who did not. They were also significantly more likely to have developed a comorbidity during follow-up, prior to phenoconversion. The median hemoglobin level (period average), prior to phenoconversion, was significantly lower in patients who had phenoconversion than those who did not. The remaining variables were comparable between both groups (Table 1). On multivariate, forward-stepwise Cox regression analysis including all variables in Table 1 as independent variables, iron chelation receipt (HR: 2.503, 95% CI: 1.143–5.481, $p = .022$), development of comorbidity during follow-up (HR: 4.561, 95% CI: 1.729–12.031, $p = .022$), and

median hemoglobin level (period average) (HR: 0.492 per 1 g/dL increase, 95% CI: 0.357–0.677, $p < .001$) were significant and independent risk factors for phenoconversion. On ROC curve analysis, a median hemoglobin level (period average) of < 8.5 g/dL was the best predictor of phenoconversion (AUC: 0.762, 95% CI: 0.661–0.863, $p < .001$, 84.1% sensitivity, 59.3% specificity). Analyses restricted to patients with ‘sustained’ phenoconversion revealed similar association trends.

Patients were followed for a median of 4.5 years (IQR: 1.5–6.5, min: 0, max: 8.5) following phenoconversion. No deaths were documented following phenoconversion. Two (4.8%) patients developed new comorbidities following phenoconversion compared with 6 (14.3%) newly developing prior ($p = .289$). One (2.4%) patient underwent a new splenectomy procedure following phenoconversion compared with one (2.4%) newly performed prior ($p = 1.000$). Fifteen (35.7%) patients were newly initiated on iron chelation therapy following phenoconversion compared to 6 (14.3%) newly initiated prior ($p = .078$). The median hemoglobin level (period average) increased from 8.4 g/dL (IQR: 7.5–9.3) to 9.4 g/dL (IQR: 8.5–10) ($p = .005$), and serum ferritin level (period average) increased from 483 ng/mL (IQR: 209.3–1030) to 619.1 ng/mL (IQR: 389.3–1132.3) ($p = .088$) following phenoconversion.

Our data illustrate that NTD to TDT phenoconversion in adult patients with β -thalassemia is not uncommon. Low hemoglobin levels and new morbidity development are associated with increased phenoconversion rates. This may be reflecting clinical practice change, especially in the last decade, in view of accumulating data on the detrimental effects of untreated anemia in NTD and evidence from

Pt.	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10
1		7	23	20	21	16	22	22	21	22
2		23	9	24	34	32	33	28	30	29
3			16	68	65	69	70	57	56	57
4	8	9	10	11	12	11	13	13	13	11
5	8	11	9	12	9	11	12	16	12	13
6	2	7	6	22	16	12	29	30	16	16
7	2	6	16	9	25	26	32	24	26	24
8			7	29	26	27	33	30	26	25
9		1	5	10	26	26	25	26	26	26
10			9	8	18	28	22	23	26	26
11				8	24	19	31	30	27	25
12					11	26	29	30	34	36
13					18	58	27	24	21	24
14					12	44	46	40	40	32
15	1	1	1	1	9	21	11	13	13	14
16				29	4			22	24	28
17						15	29	36	29	25
18						29	51	45	41	46
19		2	13	9	10	6	6	8	16	17
20	4	4	8	26	27	17	12	6	4	8
21			14	15	27	13				

Pt.	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10
22							21	24	26	26
23							14	39	44	37
24								29	40	40
25								30	44	50
26						8	50	44	46	63
27								33	66	39
28						10		1	11	15
29									12	30
30									14	50
31									35	37
32			2			2	8	12	2	4
33						9	6	9	11	10
34									4	20
35										15
36		31		4						
37									10	25
38										18
39										19
40										21
41										26
42		33								

FIGURE 1 Annual amount of red blood cell units received during the 10-year observation period in patients who had at least 1 year of phenoconversion to transfusion-dependent β -thalassemia. Red squares indicate a year with >10 red blood cell units received (phenoconversion to transfusion-dependent β -thalassemia). [Color figure can be viewed at wileyonlinelibrary.com]

observational studies on the benefit of regular transfusion therapy.^{6,7} It also illustrates that patients at the severe end of the NTD spectrum are more likely to undergo phenoconversion and thus require close monitoring and timely intervention to prevent further morbidity progression. Whether similar trends will continue to be observed following the introduction of pharmacologic agents targeting anemia in NTD is yet to be determined.⁵ As observed in our study, although transfusion therapy can effectively improve anemia, it is associated with secondary iron overload and increased iron chelation use. Patient and physician preferences for regular transfusion therapy due to other reasons cannot be dismissed and could be evaluated in future prospective studies, alongside the role of other clinical and molecular modifiers not evaluated in this study.

AUTHOR CONTRIBUTIONS

Study conception and design: Khaled M. Musallam and Gian Luca Forni. *Data collection:* Susanna Barella, Raffaella Origa, Giovanni Battista Ferrero, Roberto Lisi, Annamaria Pasanisi, Filomena Longo, Barbara Giancesin, and Gian Luca Forni. *Statistical analysis:* Khaled M. Musallam. *Review and interpretation of results:* All authors. *Manuscript drafting:* Khaled M. Musallam. *Manuscript review for important intellectual content:* All authors. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

Members of the Webthal[®] project also include Valeria Pinto (Galliera Hospital, Genoa, Italy), Roberta Sciortino (ARNAS Garibaldi, Catania,



Italy), Domenico Roberti (Università Vanvitelli, Napoli, Italy), Lucia De Franceschi (Università di Verona AOIU, Verona, Italy), and Martina Culcasi (Azienda Ospedaliero Universitaria S. Anna, Ferrara, Italy).

CONFLICT OF INTEREST STATEMENT

K.M.M. reports consultancy fees from Novartis, Celgene Corp (Bristol Myers Squibb), Agios Pharmaceuticals, CRISPR Therapeutics, Vifor Pharma, and Pharmacosmos; and research funding from Agios Pharmaceuticals and Pharmacosmos. The remaining authors have no relevant financial or nonfinancial interests to disclose.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Khaled M. Musallam¹ , Susanna Barella², Raffaella Origa³ , Giovanni Battista Ferrero⁴, Roberto Lisi⁵, Annamaria Pasanisi⁶, Filomena Longo⁷, Barbara Giancesin⁸, Gian Luca Forni⁸ on behalf of the Webthal[®] project

¹Center for Research on Rare Blood Disorders (CR-RBD), Burjeel Medical City, Abu Dhabi, United Arab Emirates

²S.C. Centro delle Microcitemie e Anemie Rare, ASL Cagliari, Cagliari, Italy

³Università di Cagliari, S.C. Centro delle Microcitemie e Anemie Rare, ASL Cagliari, Cagliari, Italy

⁴Hemoglobinopathies Reference Center, San Luigi Gonzaga Teaching Hospital, Department of Biological and Clinical Sciences, University of Turin, Turin, Italy

⁵Thalassemia Unit, ARNAS Garibaldi, Catania, Italy

⁶Centro della Microcitemia A.Quarta, Hematology Unit, A. Perrino Hospital, Brindisi, Italy

⁷Day Hospital della Talassemia e delle Emoglobinopatie, Azienda Ospedaliero Universitaria S. Anna, Ferrara, Italy

⁸ForAnemia Foundation, Genoa, Italy

Correspondence

Gian Luca Forni, ForAnemia Foundation, Via Garibaldi 7, 3C, 16124 Genoa, Italy.

Email: gianlucaforni14@gmail.com

ORCID

Khaled M. Musallam  <https://orcid.org/0000-0003-3935-903X>

Raffaella Origa  <https://orcid.org/0000-0002-2346-9616>

REFERENCES

1. Kattamis A, Kwiatkowski JL, Aydinok Y. Thalassaemia. *Lancet*. 2022; 399:2310-2324.
2. Musallam KM, Cappellini MD, Viprakasit V, Kattamis A, Rivella S, Taher AT. Revisiting the non-transfusion-dependent (NTDT) vs. transfusion-dependent (TDT) thalassaemia classification 10 years later. *Am J Hematol*. 2021;96:E54-E56.
3. Cappellini MD, Farmakis D, Porter J, Taher A. *Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT)*. 4th ed. Thalassaemia International Federation; 2021.
4. Taher A, Musallam K, Cappellini MD. *Guidelines for the Management of Non-Transfusion-Dependent β -Thalassaemia*. 3rd ed. Thalassaemia International Federation; 2023.
5. Musallam KM, Bou-Fakhredin R, Cappellini MD, Taher AT. 2021 update on clinical trials in beta-thalassaemia. *Am J Hematol*. 2021;96: 1518-1531.
6. Musallam KM, Vitrano A, Meloni A, et al. Survival and causes of death in 2,033 patients with non-transfusion-dependent beta-thalassaemia. *Haematologica*. 2021;106:2489-2492.
7. Musallam KM, Taher AT, Cappellini MD, et al. Untreated anemia in nontransfusion-dependent beta-thalassaemia: time to sound the alarm. *Hemasphere*. 2022;6:e806.