# Survival and complications in patients with haemoglobin E thalassaemia in Sri Lanka: a prospective, longitudinal cohort study

*Anuja P Premawardhena, Dileepa Senajith Ediriweera, Amir Sabouhanian, Angela Allen, David Rees, Shanthimala de Silva, Windsor Perera, Nimal Katugaha, Mahinda Arambepola, Robert C Yamashita, Sachith Mettananda, Nilam Jiffry, Vikita Mehta, Refai Cader, Dayananda Bandara, Timothy St Pierre, Giulia Muraca, Christopher Fisher, Abirami Kirubarajan, Shawn Khan, Stephen Allen, Sanath P Lamabadusuriya, David J Weatherall*, Nancy F Olivieri*

## Summary

**Background** Worldwide, haemoglobin E β-thalassaemia is the most common genotype of severe β-thalassaemia. The paucity of long-term data for this form of thalassaemia makes evidence-based management challenging. We did a long-term observational study to define factors associated with survival and complications in patients with haemoglobin E thalassaemia.

**Methods** In this prospective, longitudinal cohort study, we included all patients with haemoglobin E thalassaemia who attended the National Thalassaemia Centre in Kurunegala, Sri Lanka, between Jan 1, 1997, and Dec 31, 2001. Patients were assessed up to three times a year. Approaches to blood transfusions, splenectomy, and chelation therapy shifted during this period. Survival rates between groups were evaluated using Kaplan-Meier survival function estimate curves and Cox proportional hazards models were used to identify risk factors for mortality.

**Findings** 109 patients (54 [50%] male; 55 [50%] female) were recruited and followed up for a median of 18 years (IQR 14–20). Median age at recruitment was 13 years (range 8–21). 32 (29%) patients died during follow-up. Median survival in all patients was 49 years (95% CI 45–not reached). Median survival was worse among male patients (hazard ratio [HR] 2·51, 95% CI 1·16–5·43), patients with a history of serious infections (adjusted HR 8·49, 2·90–24·84), and those with higher estimated body iron burdens as estimated by serum ferritin concentration (adjusted HR 1·03, 1·01–1·06 per 100 units). Splenectomy, while not associated with statistically significant increases in the risks of death or serious infections, ultimately did not eliminate a requirement for scheduled transfusions in 42 (58%) of 73 patients. Haemoglobin concentration less than or equal to 4·5 g/dL (*vs* concentration >4·5 g/dL), serum ferritin concentration more than 1300 µg/L (*vs* concentration ≤1300 µg/L), and liver iron concentration more than 5 mg/g dry weight of liver (*vs* concentration ≤5 mg/g) were associated with poorer survival.

**Interpretation** Patients with haemoglobin E thalassaemia often had complications and shortened survival compared with that reported in high-resource countries for thalassaemia major and for thalassaemia intermedia not involving an allele for haemoglobin E. Approaches to management in this disorder remain uncertain and prospective studies should evaluate if altered transfusion regimens, with improved control of body iron, can improve survival.

**Funding** Wellcome Trust, Medical Research Council, US March of Dimes, Anthony Cerami and Ann Dunne Foundation for World Health, and Hemoglobal.



## Introduction

The major forms of thalassaemia are recognised as a severe public health problem throughout Asia, where during the past 25 years, improvements in public health have resulted in declines in mortality in children, including those with serious genetic diseases who would, formerly, have died in infancy. As a result, there has been increasing recognition of the burden of thalassaemia.[1] Worldwide, 40 000 new births of children with thalassaemia are recorded annually: half in South-East Asia, and the rest throughout the Eastern Mediterranean region, Europe, and the Americas.[2] Although prevalence remains uneven in the USA, severe thalassaemia is now identified in one in 55 000 newborn babies in the state of California, which is home to a large Asian population.[3]

Worldwide, nearly half of the disease burden of thalassaemia is accounted for by haemoglobin E thalassaemia, a form of thalassaemia most commonly identified throughout Thailand, Cambodia, Indonesia, and China.[4] Arising from compound heterozygosity for a β-thalassaemia allele and the abnormal haemoglobin E, haemoglobin E thalassaemia has a complex patho-physiology, derived from globin chain imbalance,

*Lancet Glob Health* 2021

Published Online November 26, 2021 https://doi.org/10.1016/S2214-109X(21)00446-0

See Online/Comment https://doi.org/10.1016/S2214-109X(21)00507-6

**Department of Medicine** (Prof A P Premawardhena MD), **Health Data Science Unit** (D S Ediriweera PhD), **and Department of Paediatrics** (Prof S Mettananda DPhil), **Faculty of Medicine, University of Kelaniya, Sri Lanka; Faculty of Medicine** (A Sabouhanian MSc, A Kirubarajan MD, S Khan MD) **and Pediatrics, Medicine, and Public Health Sciences** (Prof N F Olivieri MD), **University of Toronto, Toronto, ON, Canada; Department of Molecular Haematology, MRC Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, UK** (A Allen PhD, C Fisher PhD, Prof D J Weatherall FRS); **Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK** (A Allen, Prof S Allen MD); **Department of Paediatric Haematology, King's College London, London, UK** (Prof D Rees MBBS); **Asiri Hospital, Colombo, Sri Lanka** (S de Silva MD); **Teaching Hospital Kurunegala, Kurunegala, Sri Lanka** (W Perera MD, N Katugaha MD, D Bandara MD); **Department of Paediatrics, National Teaching Hospital, Kandy, Sri Lanka** (M Arambepola MD); **Department of Hematology, University of California, San Francisco, CA, USA** (R C Yamashita PhD); **Department of Liberal Studies, California State University, San Marcos, CA, USA**

(R C Yamashita); **Sirimavo Bandaranayake Specialized Children's Hospital, Peradeniya, Sri Lanka** (N Jiffry MD); **McMaster University, Hamilton, ON, Canada** (V Mehta); **Policy Analysis and Development Ministry of Health Sri Lanka, Colombo, Sri Lanka** (R Cader MD); **Department of Physics, School of Physics, Mathematics, and Computing, University of Western Australia, Crawley, WA, Australia** (Prof T St Pierre PhD); **Clinical Epidemiology Unit, Department of Medicine, Solna, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden** (G Muraca PhD); **Faculty of Medicine, University of Colombo, Colombo, Sri Lanka** (Prof S P Lamabadusuriya PhD)

*Prof Weatherall died in December, 2018

Correspondence to: Prof Nancy F Olivieri, Pediatrics, Medicine, and Public Health Sciences, University of Toronto, Toronto, ON, M6R 2Y5, Canada
**nancy@hemoglobal.org**

## Research in context

### Evidence before this study

We searched MEDLINE and PubMed databases using the terms "haemoglobin E thalassemia" and "haemoglobin E thalassaemia" for articles published in English between Jan 1, 1960, and Dec 31, 2017. We also reviewed the references of all articles identified by this search. We found no long-term (>5 years) studies of survival, or the factors influencing survival, in people with haemoglobin E thalassaemia.

### Added value of this study

Our study provides information about survival, and factors influencing survival, in patients with haemoglobin E thalassaemia, neither of which were previously reported in the literature. We report that survival in patients with haemoglobin E thalassaemia is poor compared with that reported in high-resource countries for thalassaemia major and for thalassaemia intermedia not involving an allele for haemoglobin E, but superior to that reported in patients with thalassaemia in Sri Lanka cared for outside a main treatment centre. Our analysis defined a group of patients who, over many years, required only on-demand transfusions, and had distinct clinical characteristics, indicating a milder phenotype, compared with those requiring scheduled transfusions. We also identified, beginning in the third decade of life, a striking shift in phenotype demonstrated by the development of intolerance to anaemia in patients aged 27–39 years, which has not been reported previously in haemoglobin E thalasssaemia. Our data show that splenectomy, while not associated with increased risk of death or a statistically significant increase in serious infections, did not eliminate the requirement for scheduled transfusions in most patients. Haemoglobin concentrations of 4·5 g/dL or less, concentrations of serum ferritin exceeding 1300 µg/L, and liver iron concentrations exceeding 5 mg/g dry weight of liver were found to be associated with poor survival. These thresholds of risks have not been previously defined in haemoglobin E thalassaemia; they differ from those previously calculated for thalassaemia major and thalassaemia intermedia not involving an allele for haemoglobin E.

### Implications of all the available evidence

As the disease burden of thalassaemia continues to increase worldwide, resources for most of the world's patients are scarce and fragmented, and survival is poor. Studies to generate evidence for the most cost-effective approach to management of haemoglobin E thalassaemia, the most common form of severe β-thalassaemia worldwide, are crucially needed. The implications of this study are important for haemoglobin E thalassaemia in Asia, where in many countries, children with this disease are placed on a lifetime of possibly unnecessary transfusion, on the basis of anaemia alone. To develop an evidence-based approach to transfusions in haemoglobin E thalassaemia, we propose that a controlled clinical trial should evaluate differing intensities of transfusion.

ineffective erythropoiesis, and shortened red blood cell survival.[5] The clinical course of many patients appears indistinguishable from that of severe thalassaemia major in which, beginning in infancy, regular transfusions are required to prevent anaemia, hepatosplenomegaly, extramedullary marrow expansion, and impaired growth and development. By contrast, even in the absence of regular blood transfusions, many patients with haemoglobin E thalassaemia do not have these complications[6,7] and, even when irregularly transfused, have better survival than do patients with thalassaemia major.[8,9] Because of this broad clinical variability and because no long-term studies have reported survival or complications in people with haemoglobin E thalassaemia, optimal treatment for the disease remains uncertain.

See Online for appendix

This study was initiated in Sri Lanka, following a request from a local paediatrician in one centre in Sri Lanka for advice on the management of haemoglobin E thalassaemia, which, as we ultimately reported, accounts for a third of patients with severe β-thalassaemia in the country.[10] Subsequently, we documented the clinical features, approaches to transfusion, complications, and deaths in patients with haemoglobin E thalassaemia, in an effort to elucidate the factors associated with survival and complications in this under-studied form of thalassaemia. To our knowledge, this is the first longitudinal analysis of survival, factors affecting survival, and complications in patients with haemoglobin E thalassaemia, as observed over 20 years in a single Asian centre.

## Methods

### Study design and participants

In this prospective, longitudinal cohort study, we included all patients with haemoglobin E thalassaemia who attended the National Thalassaemia Centre, Kurunegala, Sri Lanka, between Jan 1, 1997, and Dec 31, 2001. There were no exclusion criteria. No patients were lost to follow-up. A Strengthening the Reporting of Observational Studies in Epidemiology flowchart is shown in the appendix (p 1). Ethical permission for the study was obtained from the University of Colombo and the Central Oxford Research Ethics Committee (first obtained in 1996 and extended in 2006, 2013, and 2018). Written informed consent was not sought from individual patients because no experimental intervention was implemented.

### Procedures and outcomes

Haemoglobin E thalassaemia was confirmed by DNA analysis, which also confirmed co-inheritance of genetic modifiers (α-thalassaemia; the rs7482144 [*Xmn1*] polymorphism [G→A] 5′ to *HBG2*; and the rs4671393

polymorphism [G→A] in intron 2 of the *BCL11A* gene associated with increased fetal haemoglobin).[11] We reviewed patient history and performed physical, height, and weight examinations and full blood counts up to three times a year, with a minimum of one examination per year. If patients did not return more frequently they were assessed only annually. Serum ferritin concentration was assessed every 6 months; serum thyroid stimulating hormone, fasting glucose, alanine aminotransferase, creatinine (and after 2011, calcium and phosphate) were measured annually. Abdominal ultrasound, and 2D echocardiogram to estimate left ventricular ejection fraction and right atrial pressure, were performed annually after 2002. Liver iron concentrations were assessed over two periods, by chemical analysis of liver tissue obtained by percutaneous biopsy[12] up to 2002, and through evaluation by FerriScan (Resonance Health, Burswood, WA, Australia)[13] after 2009.

Before 1993 in Sri Lanka, most patients with haemoglobin E thalassaemia were not generally scheduled for blood transfusions. Instead, most were treated with on-demand transfusions during episodes of acute infection, before surgery, and during pregnancy. At the beginning of the study, we learned that, throughout 1993 and 1994 in most patients, prescheduled regular blood transfusions had been initiated; in most patients, these had supported steady-state pretransfusion haemoglobin concentrations of approximately 6 g/dL. In many patients, transfusions had been administered in the absence of clear indications, and without provision of adequate iron-chelation therapy, access to which was rare throughout the country before 1995. As a result, in several patients, there were indications, later confirmed by quantitation of liver iron concentrations, of iron burdens exceeding established thresholds of risk for premature death.[14] Consequently, the first priorities in these patients were to reduce or discontinue transfusions and to intensify iron-chelation therapy, despite ongoing shortages of the infusion pumps required for deferoxamine infusions. After discussions with patients and the clinic paediatricians regarding potential risks and benefits, prescheduled transfusions were discontinued in 85 patients, because of uncertainty about indications, increasing concentrations of body iron, or both. Other patients continued to receive scheduled (regular [eight or more per year] or irregular [fewer than eight per year] based on clinician discretion) transfusions. In patients with increasing spleen size, poor growth, failure to thrive, and other complications, attempts were made to maintain steady-state haemoglobin concentrations exceeding 8 g/dL. Further details are provided in the appendix (p 2).

Up to 2011, 73 patients underwent splenectomy, the indications for which were not consistently clear. Pneumococcal vaccination was administered before splenectomy. Daily folic acid was prescribed in all patients. Penicillin was prescribed in splenectomised patients. Up to 2010, access to chelation with

| | All (n=109) | Survivors (n=77) | Non-survivors (n=32) | p value |
|---|---|---|---|---|
| Age at recruitment (years; n=109) | 13 (8–21) | 12 (7–19) | 16 (8–36) | 0·068* |
| Range | 1–50 | 1–50 | 3–49 | .. |
| Sex | | | | |
| Male | 54/109 (50%) | 32/77 (42%) | 22/32 (69%) | 0·012† |
| Female | 55/109 (50%) | 45/77 (58%) | 10/32 (31%) | .. |
| Ethnicity | | | | |
| Sinhalese | 97/109 (89%) | 67/77 (87%) | 30/32 (94%) | 0·50‡ |
| Moors | 12/109 (11%) | 10/77 (13%) | 2/32 (6%) | .. |
| Employed or in education | 73/94 (78%) | 57/74 (77%) | 16/20 (80%) | 1·0‡ |
| Married | 38/95 (40%) | 31/72 (43%) | 7/23 (30%) | 0·42† |
| Having living children | 28/95 (29%) | 22/72 (31%) | 6/23 (26%) | 0·88† |
| Genetic modifiers | | | | |
| Single gene deletional α-thalassaemia | 7/109 (6%) | 4/77 (5%) | 3/32 (9%) | 0·42‡ |
| rs7482144 (*Xmn1*) polymorphism (G→A) 5′ to *HBG2* | 14/109 (13%) | 10/77 (13%) | 4/32 (13%) | 1·0‡ |
| rs4671393 polymorphism (G→A) in intron 2 of the *BCL11A* gene | 30/105 (29%) | 24/77 (31%) | 6/28 (21%) | 0·46† |
| Number of genetic modifiers | .. | .. | .. | 0·88‡ |
| 0 | 63/105 (60%) | 45/77 (58%) | 18/28 (64%) | .. |
| 1 | 34/105 (32%) | 26/77 (34%) | 8/28 (29%) | .. |
| 2 | 8/105 (8%) | 6/77 (8%) | 2/28 (7%) | .. |
| Splenectomy | 73/109 (67%) | 49/77 (64%) | 24/32 (75%) | 0·36† |
| Complications | | | | |
| Gallstones | 53/100 (53%) | 43/75 (57%) | 10/25 (40%) | 0·20† |
| Pulmonary artery pressure >30 mm Hg | 35/88 (40%) | 25/69 (36%) | 10/19 (53%) | 0·30† |
| Leg ulcers | 35/103 (34%) | 25/77 (32%) | 10/26 (38%) | 0·75† |
| Fractures | 32/103 (31%) | 24/77 (31%) | 8/26 (31%) | 1·0† |
| Ejection fraction <60% | 31/101 (31%) | 21/77 (27%) | 10/24 (42%) | 0·28† |
| Hypothyroidism | 30/99 (30%) | 22/77 (29%) | 8/22 (36%) | 0·66† |
| Delayed menarche | 9/16 (56%) | 9/14 (64%) | 0/2 | 0·18‡ |
| Short stature (less than the third percentile) | 28/89 (31%) | 24/73 (33%) | 4/16 (25%) | 0·77‡ |
| Serious infections | 20/99 (20%) | 7/77 (9%) | 13/22 (59%) | <0·0001‡ |
| Diabetes | 13/106 (12%) | 5/77 (6%) | 8/29 (28%) | 0·0062‡ |
| Transfusion requirement§ (at end of follow-up or death) | .. | .. | .. | 0·44† |
| Regular | 32/109 (29%) | 25/77 (32%) | 7/32 (22%) | .. |
| Irregular | 22/109 (20%) | 16/77 (21%) | 6/32 (19%) | .. |
| On demand | 55/109 (50%) | 36/77 (47%) | 19/32 (59%) | .. |
| Total number of transfusions given (n=107) | 50·0 (27·5–94·5) | 50·0 (22·5–90·0) | 49·5 (30·0–94·2) | 0·41* |
| Number of transfusions per year from birth (n=107) | 1·6 (0·9–3·3) | 1·6 (0·9–3·1) | 1·8 (0·9–3·9) | 0·92* |
| Number of transfusions per year from recruitment (n=107) | 3·9 (1·8–7·6) | 2·7 (1·6–5·2) | 6·0 (3·8–9·5) | 0·0022* |

(Table 1 continues on next page)

| | All (n=109) | Survivors (n=77) | Non-survivors (n=32) | p value |
|---|---|---|---|---|
| (Continued from previous page) | | | | |
| Steady-state haemoglobin (g/dL; n=107) | 5·8 (5·2–6·6) | 6·1 (5·3–6·9) | 5·3 (4·0–6·1) | 0·0015* |
| Haemoglobin F (%; n=20) | 33·9 (30·5–44·7) | 33·9 (30·5–44·7) | NA | .. |
| Most recent serum ferritin concentration (µg/L; n=105) | 949 (603–1658) | 854 (563–1259) | 1377 (917–3100) | 0·00080* |
| Range | 33–9081 | 33–3395 | 334–9081 | .. |
| >2500 µg/L | 15/105 (14%) | 5/76 (7%) | 10/29 (34%) | 0·00083‡ |
| Most recent liver iron concentration (mg/g dry weight; n=103) | 8·8 (4·7–13·4) | 8·1 (4·1–12·7) | 10·5 (6·7–14·6) | 0·063* |
| Range | 0·7–65·0 | 0·7–65·0 | 2·0–52·6 | .. |
| >15 mg/g | 16/103 (16%) | 10/74 (14%) | 6/29 (21%) | 0·38‡ |
| Age as of December, 2017, or at death (years; n=109) | 30 (24–39) | 31 (27–38) | 27 (19–41) | 0·11* |

Data are median (IQR) or n/N (%), unless otherwise stated. The number available varies, in each case, because the data were either not available in the records or could not be provided by the patient. p values compare survivors versus non-survivors. NA=not available. *Mann-Whitney *U* test. †Pearson's $\chi^2$ test. ‡Fisher's exact test. §Classified as regular (≥8 per year), irregular (<8 per year), or on demand.

***Table 1:*** **Patient demographic and clinical characteristics, stratified by outcome**

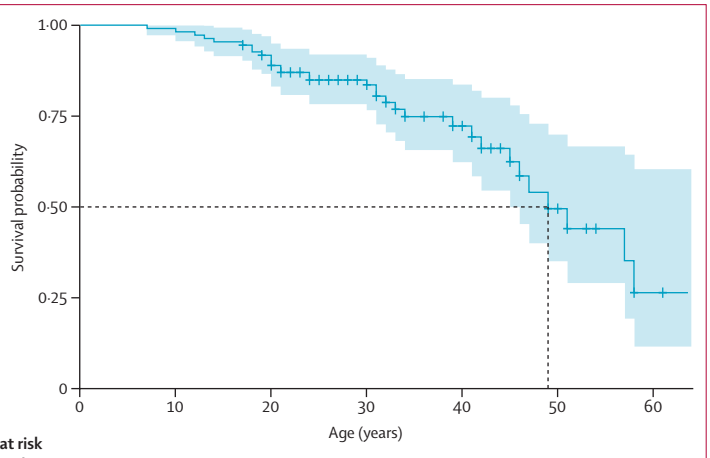


***Figure:*** **Survival according to age in patients with haemoglobin E thalassaemia**
Blue line is Kaplan-Meier survival function estimate curve with 95% confidence band. Censoring is indicated by vertical marks. Black dashed line is median survival (49 years, 95% CI 45–not reached).

deferoxamine was irregular. After 2010, most patients who required chelation therapy were switched to orally active deferasirox.

All clinical decisions were taken in consultation with the Kurunegala Hospital's (rotating) paediatricians.

### Statistical analysis

Descriptive statistics of patients were presented as numbers with percentages, or median with IQR. Non-parametric analyses were performed for continuous data, and Pearson's $\chi^2$ test or Fisher's exact test for categorical data. To calculate steady-state haemoglobin values, a summary statistic was calculated from all measurements excluding those during acute infections or less than 3 months following a blood transfusion. Survival analysis was done with right-censored data. Time to event was defined as the time between birth and death. Survival rates between groups were evaluated using Kaplan-Meier survival function estimate curves. Cox proportional hazards (CPH) models were used to identify risk factors of mortality. Stepwise variable selection procedure was adopted to identify risk factors of the multiple CPH models. Separate CPH models were fitted to identify the baseline characteristics, biochemical investigation, and complications that were associated with survival to control the confounding effects. Further, effects of the later two models were obtained with and without adjustment for the factors in the model with baseline characteristics. The effect of risk factors on mortality was presented with hazard ratios with 95% CIs. We evaluated the thresholds (cutoffs) beyond which patients showed poor survival. We serially assessed survival of patients for different thresholds of steady-state haemoglobin and liver iron and serum ferritin concentrations, and identified the levels associated with poor survival. Analyses were done with R version 3.6.6.

### Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## Results

Patients were recruited from Jan 1, 1997, to Dec 31, 2001, and followed up until Dec 31, 2017, for a median follow-up of 18 years (IQR 14–20). The original cohort included 54 (50%) male patients and 55 (50%) female patients (appendix p 1).

Table 1 shows characteristics of the full cohort and in surviving and deceased patients. All patients had severe β-thalassaemia mutations.[6] Genetic modifiers were identified in 42 (40%) of 105 patients (appendix p 3).

32 (29%) of 109 patients died during follow-up, of whom 22 (69%) were male. 77 (71%) patients were still alive as of 2017. Median survival was 49 years (95% CI 45–not reached; figure). Compared with survivors, patients who died during the study period had a greater incidence of diabetes and of serious infections, many of which were fatal (table 1), including cholangitis, subdural empyema, pyogenic meningitis, schistosomiasis, skin abscesses, dental abscess (all in one patient each); meningoencephalitis (two patients); pneumonia (two patients); sepsis (two patients); and liver abscess (five patients). Deceased patients had received more transfusions per year from recruitment and had higher body iron burdens as estimated by serum ferritin concentration. Causes of death were infection (in 11 [34%] of 32 patients), heart failure (eight [25%] patients), cirrhosis (four [13%] patients),

and cancer (two [6%] patients). The cause of death was unknown in seven (22%) patients. Causes of death according to age are shown in the appendix (p 4).

Survival was poorer in male patients than in female patients (HR 2·51, 95% CI 1·16–5·43), and poorer in patients with elevated estimated body iron burdens as estimated by serum ferritin concentration than in patients without elevated serum ferritin concentration (adjusted HR 1·03, 1·01–1·06 per 100 units; table 2). Survival was better in patients who had presented late (ie, at older than younger ages) to medical attention. Survival was also better in those homozygous for the rs7482144 (*Xmn1*) polymorphism 5′ to *HBG2* than in those without this polymorphism (HR 0·27, 95% CI 0·08–0·92). Patients who were homozygous for the rs7482144 (*Xmn1*) polymorphism 5′ to *HBG2* also had higher steady-state haemoglobin concentrations (6·4 g/dL, IQR 5·1–7·8) than those without this polymorphism (5·9 g/dL, 5·2–6·4; p=0·029). Gallstones were associated with better survival (adjusted HR 0·28, 95% CI 0·10–0·74), and serious infections with poorer survival (adjusted HR 8·49, 2·90–24·84). Steady-state haemoglobin concentrations of 4·5 g/dL or lower (*vs* patients with concentration >4·5 g/dL; p=0·0036), and concentrations of serum ferritin exceeding 1300 µg/L (*vs* patients with concentration ≤1300 µg/L; p=0·015) and liver iron exceeding 5 mg/g dry weight of liver tissue (*vs* patients with concentration ≤5 mg/g; p=0·035) were associated with poorer survival (table 2, appendix p 5).

73 (67%) of 109 patients had a splenectomy. Median age at splenectomy was 11 years (IQR 8–19; range 2–50) years. Steady-state haemoglobin was lower, serum ferritin concentration higher, and diabetes and hypothyroidism more common, in patients who had splenectomy than in patients who did not (appendix p 6). Splenectomy was not associated with significant increases in the risks of death or serious infections (appendix p 6). Splenectomy did not eliminate a transfusion requirement in most patients: 42 (58%) of 73 patients who had undergone a splenectomy returned to scheduled transfusions, following a median 7·5 years (IQR 4–14) of unscheduled (on-demand) transfusions only. It was unclear in many patients whether post-splenectomy transfusion requirements were similar to those that had been recorded before splenectomy. Another 31 (43%) patients remained on unscheduled transfusions, a median 16 years (IQR 5–20) following splenectomy. Age at splenectomy did not differ significantly between patients who resumed (median age 12 years, IQR 8–17), or did not resume (median age 11 years, 8–21), scheduled transfusions (p=0·97). One in four evaluable patients who had splenectomy (17 [26%] of 66) reported a history of serious infections; in most, the time between infection and splenectomy was not recorded. Awareness of serious infections altered referrals for splenectomy, and only one patient was referred after 2011. 24 (33%) of 73 patients who had a splenectomy died, a median 17 years (IQR 13–21; range 10–57) following splenectomy; at the time of death, 12 (50%) had been receiving scheduled transfusions. Causes of death, recorded in 19 (79%) patients, included infections (seven [29%] of 24 patients), heart failure (six [25%] patients), liver disease (four [17%] patients), and cancer (two [8%] patients).

Of 85 patients who discontinued prescheduled transfusions early in the study (ie, between 1997 and 2001), 26 (31%) resumed regular transfusions at a median age of 18 years (IQR 12–26), after 7 years (5–13) of freedom from scheduled transfusions. Another 20 (24%) patients resumed irregular transfusions at a median age of 28 years (IQR 17–36), following 10 years (6–16) of freedom from scheduled transfusions. Transfusions were most commonly resumed because of worsening linear growth; other reasons included fatigue, headaches, exercise intolerance, chest pain, splenic enlargement, and the development of a leg ulcer. Two patients resumed after they developed extramedullary haematopoiesis and spinal cord compression; another resumed after developing severe bone pain related to undiagnosed hypoparathyroidism.

| | Hazard ratio | p value |
|---|---|---|
| **Baseline characteristics** | | |
| Age at diagnosis (per additional year) | 0·94 (0·90–0·98) | 0·050 |
| Male sex (*vs* female) | 2·51 (1·16–5·43) | 0·019 |
| rs7482144 (*Xmn1*) polymorphism (G→A) 5′ to *HBG2** | 0·27 (0·08–0·92) | 0·037 |
| **Biochemical investigations** | | |
| Elevated steady-state haemoglobin (per additional unit) | 0·78 (0·63–0·97) | 0·026 |
| Elevated mean serum ferritin concentration (per additional 100 units) | 1·02 (1·01–1·04) | 0·010 |
| **Complications** | | |
| Gallstones | 0·19 (0·05–0·77) | 0·020 |
| Serious infections | 11·50 (3·41–38·82) | <0·0001 |
| **After adjustment for baseline characteristics** | | |
| Biochemical investigations | | |
| Elevated steady-state haemoglobin (per additional unit) | 0·76 (0·55–1·05) | 0·099* |
| Elevated mean serum ferritin concentration (per additional 100 units) | 1·03 (1·01–1·06) | 0·0036 |
| Complications | | |
| Gallstones | 0·28 (0·10–0·74) | 0·010 |
| Serious infections | 8·49 (2·90–24·84) | <0·0001 |

Baseline characteristics, biochemical investigations, and complications that are associated with the survival of patients with haemoglobin E thalassaemia. Biochemical investigations and complications have been evaluated with and without adjustment for significant baseline characteristics (ie, age, sex, and rs7482144 [*Xmn1*] polymorphism) of the patients. Normal serum ferritin concentration is 12–300 µg/L. Elevation over this represents iron overload, with various levels of risk. Normal haemoglobin concentration 13·5–17·5 g/dL for men, 12·0–15·5 g/dL for women. *Versus patients without this polymorphism.

***Table 2:*** **Survival analysis of patients with haemoglobin E thalassaemia**

| | No transfusion or on-demand transfusion (n=55) | Regular or irregular transfusion (n=54) | p value |
|---|---|---|---|
| Age at diagnosis (years) | 8 (4–18) | 3 (1–7) | <0·0001* |
| Age at present or death (years) | 31 (26–42) | 30 (22–36) | 0·21* |
| Sex | | | |
| Male | 34/55 (62%) | 20/57 (35%) | 0·017† |
| Female | 21/55 (38%) | 37/57 (65%) | .. |
| α-thalassaemia | 6/55 (11%) | 1/54 (2%) | 0·11‡ |
| rs7482144 (*Xmn1*) polymorphism (G→A) 5′ to *HBG2* | 11/55 (20%) | 3/54 (6%) | 0·042‡ |
| rs4671393 polymorphism (G→A) in intron 2 of the *BCL11A* gene | 20/52 (38%) | 10/53 (19%) | 0·045† |
| Splenectomy | 31/55 (56%) | 42/54 (78%) | 0·030† |
| Steady-state haemoglobin (g/dL) | 6·2 (5·8–7·0) | 5·4 (4·9–6·0) | <0·0001* |
| Most recent serum ferritin measurement (μg/L) | 787 (480–1157) | 1255 (811–2042) | 0·0012* |
| Liver iron concentration (mg/g dry weight) | 6·8 (3·4–12·4) | 10·5 (5·9–14·2) | 0·029* |
| Complications | | | |
| Gall stones | 25/47 (53%) | 28/53 (53%) | 1·0† |
| Pulmonary artery pressure >30 mm Hg | 13/40 (33%) | 22/48 (46%) | 0·29† |
| Leg ulcers | 18/51 (35%) | 17/52 (33%) | 0·94† |
| Fractures | 16/51 (31%) | 16/52 (31%) | 1·0† |
| Ejection fraction <60% | 16/51 (31%) | 15/50 (30%) | 1·0† |
| Hypothyroidism | 8/45 (18%) | 22/54 (41%) | 0·016† |
| Delayed menarche | 11/42 (26%) | 17/47 (36%) | 0·43† |
| Short stature (less than the third percentile) | 4/5 (80%) | 5/11 (45%) | 0·45† |
| Serious infections | 8/48 (17%) | 12/51 (24%) | 0·55† |
| Diabetes | 4/52 (8%) | 9/54 (17%) | 0·24‡ |
| Death | 19/55 (35%) | 13/54 (24%) | 0·32† |

Data are median (IQR) or n/N (%). *Mann-Whitney *U* test. †Pearson's $\chi^2$ test. ‡Fisher's exact test.

***Table 3:*** **Clinical characteristics and complications, stratified by transfusion group**

Survival in patients who, by the end of observation or death, were receiving no transfusions or on-demand transfusions (55 [50%] of 109 patients) did not differ significantly from that in those receiving scheduled transfusions (regular, 32 [29%] patients; irregular, 22 [20%] patients; table 3, appendix p 7). Compared with patients receiving scheduled transfusions, patients receiving no or on-demand transfusions were older at diagnosis and had a higher prevalence of homozygosity for the *Xmn1* polymorphism 5′ to *HBG2*, higher steady-state haemoglobin concentrations, lower estimated iron burdens, and a lower prevalence of hypothyroidism, suggesting a milder phenotype.

No venous thromboembolic events were recorded. The number of complications was associated with the number of genetic modifiers: one or more complications developed in 48 (92%) of 52 patients with no genetic modifiers, in 21 (81%) of 26 patients with one genetic modifier, and in all four (100%) patients with two genetic modifiers (p=0·41). Complications in the oldest surviving patients are detailed in the appendix (p 8).

In patients with evaluable growth records, final adult height exceeding the third percentile was achieved in 61 (69%) of 89 patients and mid-parental height was achieved in 40 (52%) of 77 patients. Median age at menarche, available in 49 (89%) of 55 women who had achieved puberty spontaneously, was 16 years (IQR 14–17), significantly older than the age at menarche of their mothers (14 years [13–15] years; p=0·002), available in 16 (29%) of 55 mother-daughter pairs. Tanner staging was not systematically recorded in most male patients.

In 20 patients aged 27–39 years, we observed a striking development of intolerance to anaemia (presenting as fatigue, exercise intolerance, or chest pain) following years of normal activities in the absence of scheduled transfusions (appendix p 9), unrelated to hypersplenism or accelerated red cell haemolysis. Scheduled transfusions were initiated or resumed in these patients.

## Discussion

To our knowledge, this is the first longitudinal analysis of survival, and factors influencing survival, in people with haemoglobin E thalassaemia, the most common form of severe β-thalassaemia worldwide. Survival in patients with haemoglobin E thalassaemia was shorter than that in people with other forms of thalassaemia[15,16] or in individuals without thalassaemia in Sri Lanka[17] (cumulative survival by age 50 years was estimated as 93·4% in a large cohort of patients with thalassaemia intermedia;[16] life expectancy in Sri Lanka at birth is estimated at 76·98 years).[17] Deaths and clinical complications were related to both chronic anaemia and iron overload. Some findings were unexpected and remain unexplained. Gallstones were associated with better survival. Venous thromboembolism was not observed in any patients in the study cohort, by contrast with findings in other studies.[18,19] Another unanticipated finding was the striking subacute development of intolerance to anaemia in older patients (aged 27–39 years) who had previously thrived without regular transfusions, consistent with our previous studies of age-related changes in adaptation to severe anaemia,[20] and similar to findings of a previous study of older patients with thalassaemia intermedia not involving haemoglobin E, all of whom had been started on scheduled transfusions after age 18 years, and were in a similar age range to our patients.[21] We hypothesise that this finding might be related to changes in responsiveness to erythropoietin with age, as previously observed;[20] measurements of serum erythropoietin are awaited to provide secure conclusions. Further findings will be published subsequently. Prospective studies could evaluate factors important in these changes in phenotype.

This is also the first study, to our knowledge, to report thresholds of risk of iron overload in haemoglobin E thalassaemia, potentially offering guidance for

management. The threshold of serum ferritin (1300 µg/L) associated with poorer survival was lower than that in thalassaemia major (2500 µg/L[22]), but higher than that in thalassaemia intermedia not involving haemoglobin E (800 µg/L).[23] The threshold of liver iron concentration associated with poorer survival (5 mg/g) was lower than that in thalassaemia major (15 mg/g)[14] and similar to that in thalassaemia intermedia not involving haemoglobin E.[24]

Two-thirds of patients underwent splenectomy. By contrast with previous findings,[25] in over half of these patients, splenectomy did not permanently mitigate the requirement for prescheduled transfusions. Although it might seem prudent to recommend avoidance of splenectomy in patients with haemoglobin E thalassaemia, shortages of blood throughout Asia complicate disease management,[2] such that steady-state haemoglobin concentrations sufficient to suppress splenic expansion are often not achieved.[26]

Regarding the fundamental question of which patients with haemoglobin E thalassaemia should have transfusions and on what regimens, our findings could guide future work. Judicious withdrawal of transfusions in this study permitted identification of approximately 50% of patients who were able to continue on-demand transfusions. Compared with patients requiring regularly scheduled transfusions, these patients appeared to have a milder phenotype, as suggested by several factors including a clinically modest but statistically significant higher mean steady-state haemoglobin concentration, consistent with our previous findings.[6] However, even in patients with this mild phenotype, the safety of on-demand transfusions might be questioned because of the high prevalence of complications that emerged in this study. Although maintenance of steady-state haemoglobin concentrations of 4·5 g/dL (the threshold for survival identified for haemoglobin E thalassaemia in this study) is not recommended, uncertainty persists as to the optimal target haemoglobin concentration, and by extension intensity of transfusions, in this disorder. In several countries, many affected children begin prescheduled transfusions at diagnosis; one study reported the primary, and arbitrary, indication for this approach was the country of birth.[27] Furthermore, traditional guidelines for transfusions in thalassaemia that propose maintenance of steady-state haemoglobin concentrations of 9·5–10·0 g/dL arise from one small retrospective study in European patients with thalassaemia major.[28] Whether such a regimen is appropriate in all patients with haemoglobin E thalassaemia, many of whom might adapt better to anaemia related to the decreased oxygen affinity of haemoglobin E[29] and who, even when irregularly transfused, have better survival than those with thalassaemia major,[8,9] remains unclear.

Our study has limitations. During the 20-year period, approaches to management shifted, complicating some evaluations. There is little information on deaths, and no post-mortem data. Quantitative measurements of body iron burden were were not regularly assessed, because of resource constraints. Baseline haemoglobin F concentrations were not assessed in most patients. Resting ejection fractions provide little information about cardiac reserve or iron loading. Haemoglobin and serum ferritin concentrations changed over the life course of patients; although we considered haemoglobin and recent ferritin concentrations, time-dependent analysis could be considered in future. Another limitation was that, before the study, transfusions had been started in many patients without adequate iron-chelation therapy; it, therefore, became the priority to reduce, where possible, transfusions in patients with iron burdens associated with increased risk of death. Access to chelation therapy continued to be problematic, limiting transfusion approaches in many patients.

Throughout Asia where the burden of thalassaemia is increasing,[30] most resources for its management continue to be scarce.[2,4] Programmes to reduce the incidence of thalassaemia in neonates, which are ongoing in several countries,[2] have not yet had a substantial impact in Sri Lanka, possibly related to challenges in coordination.[9] In parallel, although cures are offered to patients in higher-income countries,[31] resources for most of the world's patients are fragmented, and survival remains poor.[2,32] Indeed, survival of patients with haemoglobin E thalassaemia in other Asian countries, which lack the publicly financed, freely delivered health-care system of Sri Lanka,[33] is likely to be poorer than that reported in this study. Particularly in countries with ongoing blood shortages, controlled clinical trials to evaluate the benefits and risks of different transfusion regimens, in parallel with evaluation of drugs to increase fetal haemoglobin[34] or total haemoglobin,[35] are crucially needed to address these fundamental questions in this under-studied disease.

#### Contributors

DJW, APP, SdS, and NFO conceived and planned the study. DSE, APP, AA, VM, GM, CF, RCY, AK, SK, SA, AS, and NFO curated and analysed the data. DJW, APP, TSP, and NFO acquired funding for the study and provided resources. DJW, DR, APP, AA, TSP, RCY, DJW, and NFO were responsible for follow-up of patients and clinical assessments. DSE, AS, APP, DJW, and NFO were responsible for methodology and software. APP, AA, SdS, WP, NK, MA, SM, NJ, RC, DB, SPL, DJW, and NFO helped administer the project. DJW, APP, SPL, SM, and NFO provided resources. APP, SdS, WP, NK, MA, SM, DB, SA, RCY, SPL, TSP, DJW, and NFO supervised the work. APP, AS, TSP, DR, AA, VM, SA, DJW, and NFO validated the data. APP, DSE, SM, AA, SA, AS, and NFO drafted the manuscript. All authors had access to all the data in the study. APP, DSE, NFO, SA, and AA had final responsibility for the decision to submit for publication. NFO, APP, and DSE verified the underlying data. All authors reviewed and approved the final manuscript.

#### Declaration of interests

TSP reports a grant from Novartis, a discount on services and consultancy funds to his institution from Resonance Health, and patents issued to Resonance Health for experimental methods (US6605943 and US2004222792), outside the submitted work. All other authors declare no competing interests.

**Data sharing**

Data for this study, including individual participant data and the data fields in the set, may be shared, depending on the request. Deidentified participant data, access to relevant patient demographics, the statistical analysis plan, and the informed consent forms will be shared for specific analyses within an agreed-upon collaboration under the terms of a signed access agreement with the report's three senior authors (APP, DSE, and NFO); agreement with all senior authors will be required for access to any data. Data will be available to publicly funded investigators not receiving support from private companies. Priority access will be provided to investigators in low-income and middle-income countries. Data will be made available after further work on these data has been completed after January, 2023, up to December, 2023.

**Acknowledgments**

This study was supported by the Wellcome Trust (053267/Z98F), Medical Research Council (4050189188), US March of Dimes, the Anthony Cerami and Ann Dunne Foundation for World Health, and Hemoglobal. We wish to acknowledge the support rendered by the many medical officers, nursing officers, laboratory personnel, clinic volunteers, and support staff of the National Center, Kurunegala, Sri Lanka. We are grateful for the interest, support, and contributions of the patients and the families, both past and present, attending the National Center. We thank Martin Pippard for analysis of liver iron concentrations in liver biopsies.

**References**

1 Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. *Blood* 2010; **115:** 4331–36.

2 Eleftheriou A, Angastiniotis M. Global thalassaemia review. Thalassemia International Federation, 2021. https://thalassaemia.org.cy/what-we-do/global-thalassaemia-review/ (accessed Oct 21, 2021).

3 Michlitsch J, Azimi M, Hoppe C, et al. Newborn screening for hemoglobinopathies in California. *Pediatr Blood Cancer* 2009; **52:** 486–90.

4 Olivieri NF, Pakbaz Z, Vichinsky E. HbE/β-thalassemia: basis of marked clinical diversity. *Hematol Oncol Clin North Am* 2010; **24:** 1055–70.

5 Pootrakul P, Sirankapracha P, Hemsorach S, et al. A correlation of erythrokinetics, ineffective erythropoiesis, and erythroid precursor apoptosis in Thai patients with thalassemia. *Blood* 2000; **96:** 2606–12.

6 Premawardhena A, Fisher CA, Olivieri NF, et al. Haemoglobin E beta thalassaemia in Sri Lanka. *Lancet* 2005; **366:** 1467–70.

7 Traivaree C, Monsereenusorn C, Nujkijyanont P, Prasertsin W, Boonyawat B. Genotype–phenotype correlation among beta-thalassemia and beta-thalassemia/HbE disease in Thai children: predictable clinical spectrum using genotypic analysis. *J Blood Med* 2018; **9:** 35–41.

8 Viprakasit V, Jansutjawan S. Survival and causes of death in patients with alpha- and beta-thalassaemia in a developing country: the first report from Thailand. Abstract P383 EHA Library 2015. 20th Congress of the European Hematology Association; June 11–14, 2015. https://library.ehaweb.org/eha/2015/20th/100623/vip.viprakasit.survival.and.causes.of.death.in.patients.with.lpha-.and.html (accessed Oct 21, 2021).

9 Premawardhana AP, Mudiyanse R, De Silva ST, et al. A nationwide survey of hospital-based thalassemia patients and standards of care and a preliminary assessment of the national prevention program in Sri Lanka. *PLoS One* 2019; **14:** e0220852.

10 de Silva S, Fisher CA, Premawardhena A, et al. Thalassaemia in Sri Lanka: implications for the future health burden of Asian populations. Sri Lanka Thalassaemia Study Group. *Lancet* 2000; **355:** 786–91.

11 Lettre G, Sankaran VG, Bezerra MA et al. DNA polymorphisms at the *BCL11A*, *HBS1L-MYB*, and β-*globin* loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. *Proc Natl Acad Sci U S A* 2008; **105:** 11869–74.

12 Olivieri N, Muraca GM, O'Donnell A, Premawardhena A, Fisher C, Weatherall DJ. Studies in haemoglobin E beta-thalassaemia. *Br J Haematol* 2008; **141:** 388–97.

13 St Pierre T, Clark PR, Chua-anusorn W, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood* 2005; **105:** 855–61.

14 Brittenham GM, Griffith PM, Nienhuis AW, et al. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. *N Engl J Med* 1994; **331:** 567–73.

15 Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica* 2004; **89:** 1187–93.

16 Musallam KM, Vitrano A, Meloni A, et al. Survival and causes of death in 2,033 patients with non-transfusion-dependent beta-thalassemia. *Haematologica* 2021; **106:** 2489–92.

17 Trading Economics. Life expectancy at birth (total) years, Sri Lanka: https://tradingeconomics.com/sri-lanka/life-expectancy-at-birth-total-years-wb-data.html (accessed Oct 21, 2021).

18 Natesirinilkul R, Charoenkwan P, Nawarawong W, et al. Hypercoagulable state as demonstrated by thromboelastometry in hemoglobin E/beta-thalassemia patients: association with clinical severity and splenectomy status. *Thromb Res* 2016; **140:** 125–31.

19 Ekwattanakit S, Siritanaratkul N, Viprakasit V. A prospective analysis for prevalence of complications in Thai nontransfusion-dependent Hb E/β-thalassemia and α-thalassemia (Hb H disease). *Am J Hematol* 2018; **93:** 623–29.

20 O'Donnell A, Premawardhena A, Arambepola M, et al. Age-related changes in adaptation to severe anemia in childhood in developing countries. *Proc Natl Acad Sci USA* 2007; **104:** 9440–44.

21 Ricchi P, Meloni A, Pistoia L, et al. Longitudinal follow-up of patients with thalassaemia intermedia who started transfusion therapy in adulthood: a cohort study. *Br J Haematol* 2020; **191:** 107–14.

22 Olivieri NF, Nathan DG, Macmillan JH, et al. Survival in medically treated patients with homozygous beta-thalassemia. *N Engl J Med* 1994; **331:** 574–78.

23 Musallam K, Cappellini M, Daar S, et al. Serum ferritin level and morbidity risk in transfusion-independent patients with β-thalassemia intermedia: the ORIENT study. *Haematologica* 2014; **99:** e218–21.

24 Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. *Lancet* 2018; **391:** 155–67.

25 Mandal P, Ghosh M, Bhattacharyya M. Does profile of hemoglobin Eβ-thalassemia patients change after splenectomy? Experience of a tertiary thalassemia care centre in eastern India. *Indian J Hematol Blood Transfus* 2015 **31:** 446–52.

26 Bansal D. Splenectomy for β-thalassemia major in resource challenged settings: often a Hobson's choice! *Indian J Pediatr* 2015; **82:** 1082–83.

27 Rees DC, Styles J, Vichinsky EP, Clegg JB, Weatherall DJ. The hemoglobin E syndromes. *Ann N Y Acad Sci* 1998; **850:** 334–43.

28 Cazzola M, Borgna-Pignatti C, Locatelli F, Ponchio L, Beguin Y, De Stefano P. A moderate transfusion regimen may reduce iron loading in beta-thalassemia major without producing excessive expansion of erythropoiesis. *Transfusion* 1997; **37:** 135–40.

29 Allen A, Fisher C, Premawardhena A, et al. Adaptation of anemia in hemoglobin E-B thalassemia. *Blood* 2010; **116:** 5368–70.

30 The Lancet Haematology. The global burden of haematological diseases. *Lancet Haematol* 2020; **7:** e851.

31 Thompson A, Walters M, Kwiatkowski J, et al. Gene therapy in patients with transfusion-dependent β-thalassemia. *N Engl J Med* 2018; **378:** 1479–93.

32 Dhanya R, Sedai A, Ankita K, et al. Life expectancy and risk factors for early death in patients with severe thalassemia syndromes in south India. *Blood Adv* 2020; **4:** 1448–57.

33 Smith O. Sri Lanka: achieving pro-poor universal health coverage without health financing reforms. Universal Health Coverage Studies Series no 38. Washington, DC: World Bank, 2018. https://openknowledge.worldbank.org/handle/10986/29175 (accessed Oct 21, 2021).

34 Ansari SH, Lassi ZS, Khowaja SM, Adil SO, Shamsi TS. Hydroxyurea (hydroxycarbamide) for transfusion-dependent β-thalassaemia. *Cochrane Database Syst Rev* 2019; **3:** CD012064.

35 Cappellini M, Viprakasit V, Taher A, et al. A phase 3 trial of luspatercept in patients with transfusion-dependent β-thalassemia. *N Engl J Med* 2020; **382:** 1219–31.