The impact of in utero transfusions on perinatal outcomes in patients with alpha thalassemia major: the UCSF registry

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Key Points

- Fetuses with ATM who receive ≥2 IUTs have resolution of hydrops with near-term birth and a short hospital stay.

- Children with ATM who received ≥2 IUTs have normal neurodevelopment; earlier transfusions correlate with higher scores.

Alpha thalassemia major (ATM) is a hemoglobinopathy that usually results in perinatal demise if in utero transfusions (IUTs) are not performed. We established an international registry (NCT04872179) to evaluate the impact of IUTs on survival to discharge (primary outcome) as well as perinatal and neurodevelopmental secondary outcomes. Forty-nine patients were diagnosed prenatally, 11 were diagnosed postnatally, and all 11 spontaneous survivor genotypes had preserved embryonic zeta-globin levels. We compared 3 groups of patients; group 1, prenatally diagnosed and alive at hospital discharge (n = 14), group 2, prenatally diagnosed and deceased perinatally (n = 5), and group 3, postnatally diagnosed and alive at hospital discharge (n = 11). Group 1 had better outcomes than groups 2 and 3 in terms of the resolution of hydrops, delivery closer to term, shorter hospitalizations, and more frequent average or greater neurodevelopmental outcomes. Earlier IUT initiation was correlated with higher neurodevelopmental (Vineland-3) scores (r = −.72, P = .02). Preterm delivery after IUT was seen in 3/16 (19%) patients who continued their pregnancy. When we combined our data with those from 2 published series, patients who received ≥2 IUTs had better outcomes than those with 0 to 1 IUT, including resolution of hydrops, delivery at ≥34 weeks gestation, and 5-minute appearance, pulse, grimace, activity, and respiration scores ≥7. Neurodevelopmental assessments were normal in 17/18 of the ≥2 IUT vs 5/13 of the 0 to 1 IUT group (OR 2.74; P = .01).

Thus, fetal transfusions enable the survival of patients with ATM and normal neurodevelopment, even in those patients presenting with hydrops. Nondirective prenatal counseling for expectant parents should include the option of IUTs.

Introduction

Alpha thalassemia is one of the most common hemoglobinopathies worldwide, with a carrier rate of up to 51% in Southeast Asia and 5% in the Americas.[1-3] Disease severity is proportional to the number of affected alpha-globin genes: 1-gene deletion results in a silent carrier, 2-gene deletions result in alpha thalassemia trait, 3-gene deletions result in hemoglobin H (Hb H) disease, and 4-gene deletions result in alpha thalassemia major (ATM).[4] Patients with ATM have the most severe genotype that often manifests on prenatal ultrasound as hydrops fetalis (characterized by fetal effusions, including ascites, pleural effusion, pericardial effusion, and generalized edema). In some patients, a combination of 2 alpha-globin gene deletions and a third nondeletional alpha-globin variant can also present with hydrops.[3,5] The term "Hb Bart's hydrops fetalis syndrome" has also been used to describe patients with 4-gene deletions or the more heterogeneous variants. Because fetal presentation is indiscernible, we used the term ATM to include both patient groups in this report.

Patients with ATM presenting with hydrops fetalis are at high risk of fetal demise. Thus, historically, parents of a fetus diagnosed with ATM have been counseled to terminate the pregnancy because of concerns about poor fetal prognosis and the risk of obstetric complications for the mother.[6,7] However, numerous case series have shown that in utero transfusions (IUTs) can enable survival to birth with improved neonatal outcomes.[8-11] After birth, the care of patients with ATM is similar to that for beta thalassemia major, that is, chronic transfusion therapy and iron chelation. Hematopoietic stem cell transplantation (HSCT) can be curative but requires myeloablative conditioning with cytotoxic agents that have attendant morbidities.[12] To improve the safety of HSCT, we are currently performing a phase 1 clinical trial (NCT02986698) for in utero HSCT in fetuses with ATM, using maternal donor cells.[13]

Despite the growing understanding that fetal therapy is an option for ATM, there is a relative reluctance to offer IUTs for this condition, partly because of the limited literature demonstrating the benefits of fetal therapy on postnatal outcomes. To address this knowledge gap, we established an international registry to evaluate genotype-phenotype correlations, pregnancy choices, and the impact of IUTs on outcomes. Here, we report the short- and long-term outcomes of patients with ATM who received IUTs, including the results of developmental assessment. To further understand the impact of fetal therapy on patients with ATM, we combined and analyzed our data with 2 large published series that evaluated the impact of IUTs.[9,10] Our results are important for evidence-based counseling of alpha thalassemia carriers and for supporting informed decision-making for patients who receive a prenatal diagnosis of ATM.

Methods

We established an international registry of patients with ATM (NCT04872179), capturing all cases referred to the University of California, San Francisco (UCSF) or our collaborating sites. This was accomplished through retrospective chart review and prospective enrollment. This study was approved by the UCSF Institutional Review Board (#17-22372, #19-29656).

Inclusion criteria

We included all singleton pregnancies affected by ATM referred to the UCSF Fetal Treatment Center between March 2000 and December 2020. We also included patients who were identified postnatally and referred to our team for inclusion in the registry. Although most patients had ATM, 4 patients with nondeletion genotypes were included because they also present with fetal anemia and hydrops fetalis.[3,5] For brevity, we refer to the entire cohort as affected by ATM but distinguish these cases in each graph using symbols.

Data collection

We collected data on maternal and paternal demographics, prenatal findings, number of IUTs, procedural details, neonatal outcomes, and postnatal treatment. The primary outcome of this analysis was survival to discharge. Secondary outcome measures were the resolution of hydrops at birth, gestational age (GA) at delivery, birth weight percentile, 5-minute appearance, pulse, grimace, activity, and respiration (APGAR) scores, need for mechanical ventilation, length of neonatal hospital stay, and average or greater neurodevelopmental outcomes. Patients were classified as follows: group 1 was prenatally diagnosed and alive at the time of discharge from initial hospitalization, group 2 was prenatally diagnosed and suffered perinatal demise, and group 3 was diagnosed postnatally and alive. To understand whether specific genetic factors affect prognosis and survival, we examined the patient genotypes. Postnatal outcomes and comorbidities were gathered through a combination of chart reviews and information obtained from the referring providers. The spectrum of hydrops was determined based on the presence of pericardial effusion, ascites, skin edema, and/or pleural effusion. Cardiomegaly was by report or evaluation of cardiothoracic ratio >0.33 by area method.[14] Birth weight percentile was determined using Fenton growth curves.[15,16] Small for gestation age was defined as less than the tenth percentile.[17] Short stature was considered growth less than the fifth percentile.

Neurobehavioral and neurodevelopmental testing

We evaluated neurodevelopment and neurobehavior with the Vineland Adaptive Behavior Scales Third Edition (Vineland-3) Comprehensive Interview (Q-global edition).[18] The Vineland-3 was completed by administration of the Parent/Caregiver Form by a single investigator (M.S.). All English-speaking parents of patients with ATM, regardless of their current age (n = 14), were invited to participate. One adult patient was offered participation directly.[19] Ten parents and 1 single adult patient responded; all agreed to participate. Consent was obtained and no compensation was provided. Testing was performed using a phone or a video platform

(Zoom). The Vineland-3 yields standard scores (scale, 20-160; mean, 100; and standard deviation, 15) in 3 domains: Communication, Daily Living Skills, and Socialization. A composite score is the sum of the raw scores of the 3 domains and correlates with an age-matched population-based percentile rank. A fourth domain, Motor Skills, was included for children aged <9 years old.[18] This tool assesses both fine and gross motor skills. The motor skills score was not incorporated into the composite score because this outcome is not used in the evaluation of intellectual disability risk. A favorable neurobehavioral outcome was defined as an age-corrected standard score of ≥70 on the Vineland-3. During the Vineland testing, parents of patients >2 years old were asked about their child's health-related quality of life using the validated PedsQL quality of life inventory.[20] In total, 8 parents were eligible based on the child's age and all completed the inventory.

Some children also underwent neurodevelopmental testing using the Bayley Scales of Infant and Toddler Development, third edition (Bayley-3). These results, as well as clinician and/or school assessments, were collected when available. All available data were reviewed by a single pediatric neurologist (D.G.) who evaluated the entire history to define "average or greater neurodevelopment" using parameters such as a Vineland-3 age-corrected composite score of ≥70, clinician assessments, children in grade for age (if no other measurement tool was available), and lack of cerebral palsy or functional motor deficit.

Combined analysis with previous case series

We analyzed perinatal outcome data from our series combined with 2 contemporary series of patients with ATM that detailed prenatal management, including data on the number of IUTs (Chan et al 2018,[9] Zhang et al 2021[10]). The 4 UCSF patients with nondeletion genotypes were not included in the combined analysis because these genotypes were absent from the other 2 series. All 3 series evaluated the neurological outcomes, although they used different types of testing. The current study used the Vineland-3. Chan et al used intelligence quotient (IQ, assessment tool not reported), clinician assessment, and academic achievement.[9] Zhang et al used academic achievement and Wechsler Intelligence Scale for Children fourth edition or Wechsler Preschool and Primary Scale of Intelligence fourth edition.[10]

Optimal transfusion (OT) vs suboptimal transfusion analysis (SOT). For the combined analysis with previous series, after establishing that there was 100% survival in pregnancies that received ≥2 IUTs, we separated the cohort into patients who received ≥2 IUTs (optimal transfusions, OT) and those who received 0 to 1 IUT, including those diagnosed postnatally (suboptimal transfusions, SOT). Patients who had undergone medical abortion were excluded from the outcome analysis. The OT group corresponded to group 1 in the UCSF series, whereas the SOT group corresponded to groups 2 and 3 in the UCSF series.

Statistics

Pearson's Chi-squared test was used to compare categorical variables across groups. The Kruskal-Wallis one-way analysis of variance test was used to compare the continuous variables across the 3 groups, and the Wilcoxon Rank sum test and Fisher's exact test were used to compare the 2 groups. P-values <.05 were considered statistically significant. No multiple testing adjustments were performed. R (version 4.0.5) was used for the analyses and graphing was performed using GraphPad Prism (version 9.3.1). For the combined analysis with the 2 additional case series, Mantel-Haenszel random effects models were fitted using the restricted maximum likelihood method. Syntheses included results when at least 2 studies included the outcome of interest and followed the guidelines of the Cochran Handbook Version 6.3 2022. Forest plots with 95% confidence intervals were created for each variable using mean differences for continuous variables, and log odds ratios for categorical variables were back-transformed to present the odds ratios in the figures. All analyses were performed using Stata version 16.1 (StataCorp, College Station, TX).

Results

Maternal and fetal characteristics

We enrolled 60 singleton patients in the UCSF registry through December 2020: 56 with ATM and 4 with other nondeletional variants (1 patient with homozygous Zurich-Albisrieden, 3 patients with SEA/Hb H Adana (Figure 1A and B). Two patients underwent prenatal stem cell transplantation at UCSF as part of an ongoing phase 1 clinical trial (NCT02986698). Filipino or Chinese ancestry were the most represented. Patients were referred from the following countries: United States, Canada, Thailand, Qatar, Philippines, New Zealand, Taiwan, and Brazil; the distribution of patients living in lower middle, upper middle, or high income countries of origin are detailed in Table 1.[21] In total, 49 patients (82%) were diagnosed prenatally at a mean GA of 20.3 weeks (range, 12.7-27.4 weeks). Among these patients, 30 elected medical abortions at a mean GA of 21 weeks (range, 17-26.7 weeks). Eleven patients were diagnosed postnatally and were referred to us specifically for inclusion in the registry. Forty-one percent (20/49) had at least 1 prior pregnancy affected by ATM. Three patients were previously described by our group.[8] Seven patients were previously described by other groups.[11,22,23]

Next, we compared the main 3 groups of patients defined by the primary outcome (survival to hospital discharge after delivery) and the time of diagnosis (prenatal or postnatal). Among prenatally diagnosed patients who did not undergo medical abortion, 14 survived to hospital discharge (group 1), and all fetuses received ≥2 IUTs. In contrast, 5 fetuses received 0 to 1 IUTs and all suffered perinatal demise (group 2); 1 patient received a single IUT at 27 weeks, delivered prematurely 1 week later, and died after a 6-month hospital stay; 3 patients did not received IUT and suffered fetal demise, and one fetus received a single IUT and died at 2 days of age after parents opted for palliative care. In addition, 11 patients were diagnosed postnatally and therefore did not receive any prenatal therapy (group 3).

Genotype analysis (Figure 1B) indicated that the majority of prenatally diagnosed patients were homozygous for the Southeast Asian gene deletion (-SEA; 59%) or heterozygous for the Filipino/Southeast Asian (-FIL/-SEA; 33%) or Thai/Southeast Asian (-THAI/-SEA; 2%) gene deletions. However, none of the 11 infants who survived to birth without IUT (group 3) had -FIL or -THAI deletions (which extended into the embryonic zeta-globin locus), compared with 34.7% (17/49) of prenatally diagnosed patients (Fisher's exact test, $P = .02$).
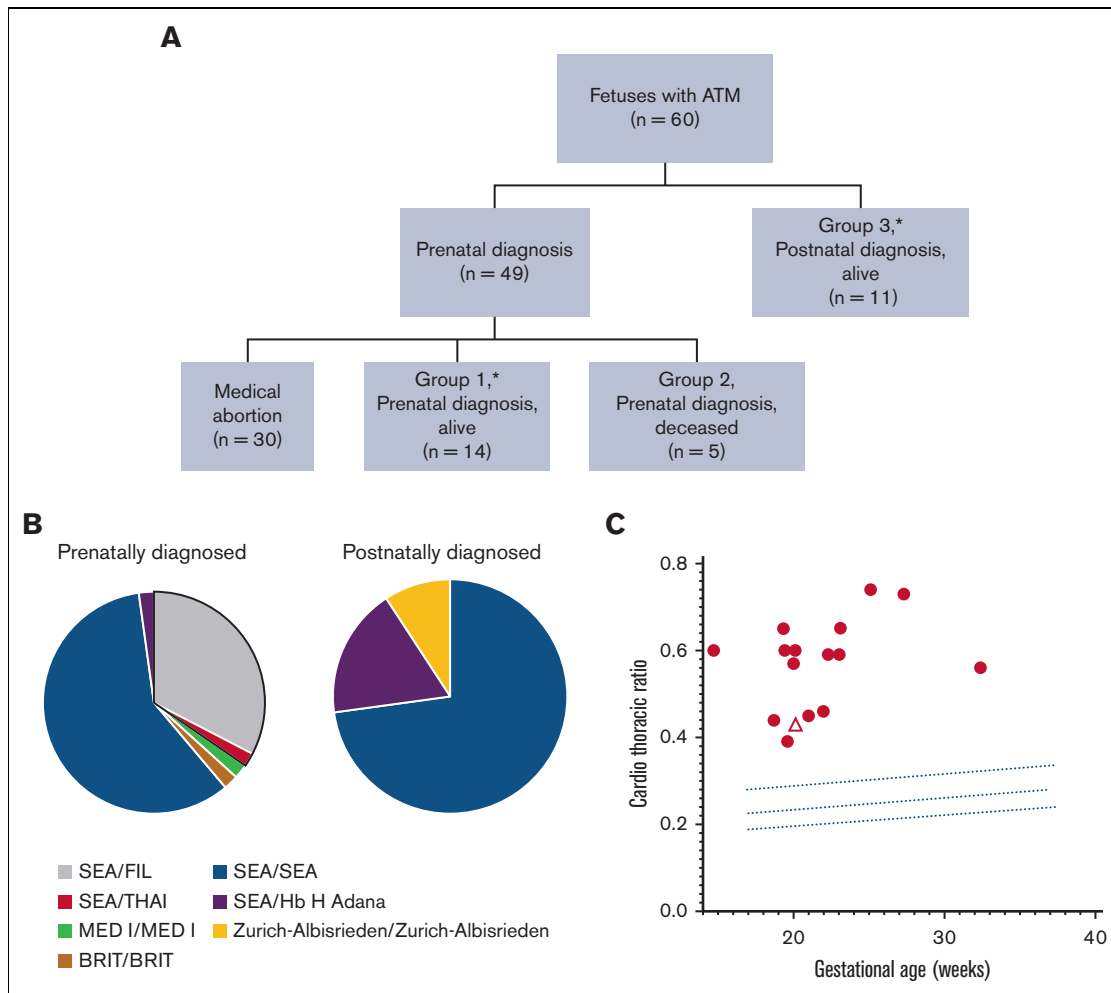
Figure 1. Patient characteristics. (A) Flowchart depicting all patients with ATM in the UCSF registry and the 3 groups included in the statistical analyses (group 1: prenatal diagnosis, alive (n = 14); group 2: prenatal diagnosis, deceased (n = 5), and group 3, postnatal diagnosis, alive (n = 11). Group 1 included one patient with a nondeletion variant (SEA/Hb H Adana), and group 3 included 3 patients with alpha thalassemia nondeletion variants (homozygous Zurich-Albisrieden [n = 1], SEA/Hb H Adana [n = 2]). *Previously published patients: 4 patients in group 1[8,22] and 6 patients in group 3.[11,23] (B) Genotypes of patients prenatally or postnatally diagnosed. The prenatally diagnosed group included 49 patients with the following genotypes: homozygous SEA (n = 29), SEA/FIL (n = 16), SEA/THAI (n = 1), homozygous MED I (n = 1), homozygous BRIT (n = 1), and SEA/Hb Adana (n = 1). The postnatally diagnosed group included 11 patients with the following genotypes: SEA/SEA (n = 8), SEA/Hb Adana (n = 2), and homozygous Zurich-Albisrieden (n = 1). The black outline in the prenatal diagnosis group indicates genotypes that have deletions spanning the zeta-globin gene (THAI, FIL), which were not observed in the postnatal diagnosis group (*P* = .02, Fisher's exact test). (C) Cardiothoracic ratio observed by ultrasonography at the indicated GAs. Each data point is a unique fetus, the lines indicate the fifth, fiftieth, and ninety-fifth percentiles.[14] The triangle represents a patient with a nondeletion variant.

IUT details

For all patients who continued their pregnancy and underwent IUT (n = 16), the mean GA at first IUT was 23.8 ± 3.07 (mean ± SD) weeks (Table 2). IUTs resulted in resolution of hydrops; the average time from first IUT to hydrops resolution was 3.9 ± 2.55 weeks. Transfusion volumes for the first IUT varied widely (29.08 ± 16.40 mL, n = 12 with data available). However, simple linear regression demonstrated a significant correlation between transfusion volume and GA for this initial IUT (*P* = .016) and most patients received a volume within the 95% confidence interval of the best fit line, demonstrating interinstitutional concordance in performing IUTs (supplemental Figure 1A).

IUTs can be associated with technical complications such as fetal demise and preterm delivery. Nineteen patients underwent IUTs,

with 69 transfusion procedures performed in total. Among these, 16 patients continued their pregnancy and could be evaluated for the risk of preterm labor; 3 patients (19%) delivered preterm (<36 weeks). Two patients in group 1 delivered at 29.4 and 33.7 weeks after their last IUT secondary to chorioamnionitis. One patient in group 2 delivered at 28.7 weeks after the onset of preterm labor 9 days after IUT only.

Ultrasound characteristics

Ultrasound data were available for 52/60 patients (Table 3), of which 46/52 (88%) presented with hydrops. Information regarding specific effusions was available for 35 patients. Pericardial effusions (32/35; 91%) and ascites (26/35; 74%) were the most common, followed by pleural effusion (11/35; 31%) and skin

Table 1. Demographics of pregnancies affected by ATM. 'n' represents the number of pregnancies for which the indicated data point is available. Income level of country was determined using the World Bank Atlas[21]

Characteristics	Pregnancies n (%)
Maternal ancestry (n = 60)	
Filipino	23 (38%)
Chinese	11 (18%)
Vietnamese	6 (10%)
Thai	5 (8%)
Laotian	1 (2%)
Mediterranean	2 (3%)
Taiwanese	1 (2%)
Pakistani	1 (2%)
Unknown	10 (17%)
Country of origin* (n = 60)	
Lower middle income	1 (2%)
Upper middle income	7 (12%)
High income	52 (87%)
GA at prenatal diagnosis, mean in wks (range) (n = 40)	20.3 (12.7-27.4)
Method of diagnosis (n = 60)	
Amniocentesis	32 (53%)
Chorionic villus sampling	7 (12%)
Percutaneous umbilical vein sampling	4 (7%)
Hydrops in known carrier couple	4 (7%)
Products of conception	2 (3%)
Postnatal diagnosis	11 (18%)
Prior pregnancy history (n = 49)*	
No prior affected	29 (59%)
One prior affected	16 (33%)
Two prior affected	4 (8%)

*Two mothers are represented twice as they had 2 pregnancies that are in the registry.

edema (11/35; 31%). Cardiomegaly was observed in 31/52 (60%) patients. Specific cardiothoracic ratio values were available for 16 patients, all of which were above the 95 percentile for healthy fetuses (mean cardiothoracic ratio = 0.57 ± 0.03, Figure 1C). Amniotic fluid abnormalities were also observed (19/52; 37%), including polyhydramnios (n = 6) and oligohydramnios/anhydramnios (n = 12). One patient initially had polyhydramnios and later had oligohydramnios. Echogenic bowel was observed in 19/52 (37%) patients at the time of diagnosis; 9 of these patients were in the surviving group, and none had consequences at birth.

Other anatomic findings

Postnatal evaluation to corroborate prenatal ultrasound findings was available for 26 patients. Nine of 14 males (64%) had hypospadias, as previously reported for patients with ATM.[24] Digital deformities were not observed in our cohort. Minor structural cardiac anomalies (atrial and ventricular septal defects) were observed in 11/25 (44%) patients. In addition, 1 patient had cardiac rhabdomyoma (molecular diagnosis of tuberous sclerosis),

and one patient had a suspected Ebstein anomaly; both patients pursued medical abortion with no pathology confirmation. Growth data were available for 19 patients, of whom 9 (47%) were considered to have short stature (defined as a height less than the fifth percentile).

Impact of IUTs on perinatal (secondary) outcomes

We examined secondary perinatal outcome measures in the 3 patient groups (Figure 2 and supplemental Table 1). Comparing the outcomes of patients in group 1, who were prenatally diagnosed and received >2 IUTs, with those of group 2 (prenatally diagnosed with perinatal demise, all of whom had 0-1 IUT) and 3 (postnatally diagnosed and alive) allowed us to discern the impact of IUTs on each of these outcome measures.

Hydrop resolution was evaluated in patients for whom prospective ultrasound data were available; 12/12 patients in group 1 had resolution of hydrops, whereas resolution of hydrops was less common in groups 2 (2/3) or 3 (1/4) (Figure 2A). The median GA at delivery was higher in group 1 patients than in groups 2 and 3 patients (Figure 2B). The median APGAR scores at 5 minutes were higher in group 1 than in group 2 and 3 patients (Figure 2C). Fewer (50%) patients in group 1 required invasive mechanical ventilation (Figure 2D), whereas all patients in groups 2 and 3 required invasive ventilation. The median length of hospitalization after birth was shorter in group 1 patients than in group 2 and 3 patients (Figure 2E). Average or greater neurodevelopment was observed in all patients in group 1 vs 54.5% in group 3 (Figure 2F). There were no differences in the median birthweight percentiles or in the incidence of small stature among the groups (supplemental Figure 1B,C).

Neurodevelopmental outcomes in ATM

We offered Vineland-3[18] to all English-speaking patients and performed testing on those who consented (10 in group 1 and 1 in group 3 (supplemental Table 2). Among the patients in group 1, the mean composite percentile was 59 ± 7%. The subdomain percentile scores were 49.2 ± 8.5% for communication, 62.5 ± 6.1% for daily living skills, and 63.2 ± 7.1% for socialization. Notably, all composite scores were within the normal ranges. However, there was a negative correlation between GA at the first IUT and the Vineland composite percentile score, with patients transfused earlier with higher composite scores (Figure 3A, r = −0.72, P = .02), and with the communication subdomain percentile (Figure 3B, r = −0.82, P = .04), but not with the subdomains of daily living or socialization (supplemental Figure 2A,B). Although 4 patients in this group were born preterm (before 37 weeks), GA at birth was not correlated with the Vineland composite score (supplemental Figure 2C, r = 0.2, P = .55). School data available for 4 patients indicated that 2 children were electively held back 1 academic year by their parents. Both children had normal Vineland-3 scores at follow-up and neither required ongoing modifications at school. Only 1 patient required speech therapy and none required physical or occupational therapy.

For patients who did not have Vineland data, we analyzed other measures, including scores on the Bayley Scales of Infant Development-3[25] (n = 2), schooling assessment (n = 6), and clinician assessment (n = 3). Only 5 had multiple measures available. Based on all available assessments, neurodevelopmental outcomes at the

Table 2. Characteristics of IUTs

Patient ID	Genetic diagnosis	Total # of IUTs	GA at 1st IUT	1st IUT Volume transfused	1st IUT Opening Hematocrit	Wks from 1st IUT to hydrops resolution
8	−SEA/−SEA	1	28.4	62.00	23.1	4.5
44	−SEA/−SEA	1	27.5	35.00	21.3	1.0
32	−SEA/−SEA	4	25.6	50.00	26	
66	−FIL/−SEA	5	25.6	25	20.4	
7	−FIL/−SEA	5	25.0			2.00
67	−SEA/−SEA	5	25.3	17	23.7	4.80
4	−FIL/−SEA	3	24.0	21.00	19.8	5.10
15	−SEA/−SEA	5	20.1	20.00	14.7	
21	−FIL/−SEA	3	23.9	47.00		9.80
57	−SEA/−SEA	3	20.0			6.00
58	−FIL/−SEA	3	28.7			4.00
61	−FIL/−SEA	5	22.1	8.00	24	5.00
60	−SEA/−SEA	5	19.4		17.1	
12	−SEA/−SEA	6	20.6	13.00	18.9	2.40
20	−SEA/Hb H Adana	5	20.4	21	8.7	0.70
62	−SEA/−SEA	6	24.0	30.00	21.3	1.9
Mean ± SD		4 ± 1.63	23.8 ± 3.07	29.08 ± 16.40	19.92 ± 4.71	3.9 ± 2.55

Volume in mL, Hematocrit in percentage.
SD, standard deviation; GA, gestational age; IUT, in utero transfusion.

Table 3. Prenatal ultrasound abnormalities and postnatal anatomic findings. Data reflect numbers from patients for whom detailed data were available

	Group 1: prenatal diagnosis, alive	Group 2: prenatal diagnosis, perinatal demise	Group 3: postnatal diagnosis, alive	Group 4: medical abortion	Total
Prenatal					
Cases with available data/total in group	14/14 (100%)	5/5 (100%)	8/11 (73%)	25/30 (83%)	52/60 (87%)
Cardiomegaly	11/14 (71%)	4/5 (80%)	5/8 (63%	11/25 (44%)	31/52 (60%)
Amniotic fluid abnormalities	6/14 (43%)	1/5 (20%)	4/8 (50%)	5/25 (20%)	19/52 (37%)
Echogenic bowel	8/14 (57%)	1/5 (20%)	2/8 (25%)	8/25 (32%)	19/52 (37%)
Ambiguous genitalia (males)	1/4 (25%)	1/3 (33%)	1/4 (25%)	2/6 (33%)	5/17 (29%)
Cardiac abnormalities	0/14 (0%)	0/5 (0%)	0/8 (0%)	2/25 (8%)	2/52 (4%)
Total with hydrops	12/14 (86%)	5/5 (100%)	8/8 (100%)	21/25 (84%)	46/52 (88%)
Hydrops* (nonspecific)	1/12 (8%)	1/5 (20%)	3/8 (36%)	6/21 (29%)	11/46 (24%)
Single effusion	3/11 (27%)	0/4 (0%)	2/5 (40%)	6/15 (40%)	11/35 (31%)
≥2 effusions	8/11 (73%)	4/4 (100%)	3/5 (60%)	9/15 (60%)	24/35 (69%)
Pericardial effusion	10/11 (91%)	4/4 (100%)	4/5 (80%)	14/15 (93%)	32/35 (91%)
Ascites	7/11 (64%)	3/4 (75%)	4/5 (80%)	12/15 (80%)	26/35 (74%)
Pleural effusion	2/11 (18%)	3/4 (75%)	0/5 (0%)	6/15 (40%)	11/35 (31%)
Skin edema	3/11 (27%)	3/4 (75%)	0/5 (0%)	5/15 (33%)	11/35 (31%)
Postnatal					
Hypospadias (males)	4/4 (100%)	1/3 (33%)	4/7 (57%)		9/14 (64%)
Cardiac abnormalities	5/14 (36%)	0	6/11 (55%)		11/25 (44%)
short stature	5/9 (55%	n/a	4/10 (40%)		9/19 (47%)

*Patients with reported hydrops and no further specific data available were classified as hydrops (nonspecific) and not included in the analysis of number and frequency of specific effusions.
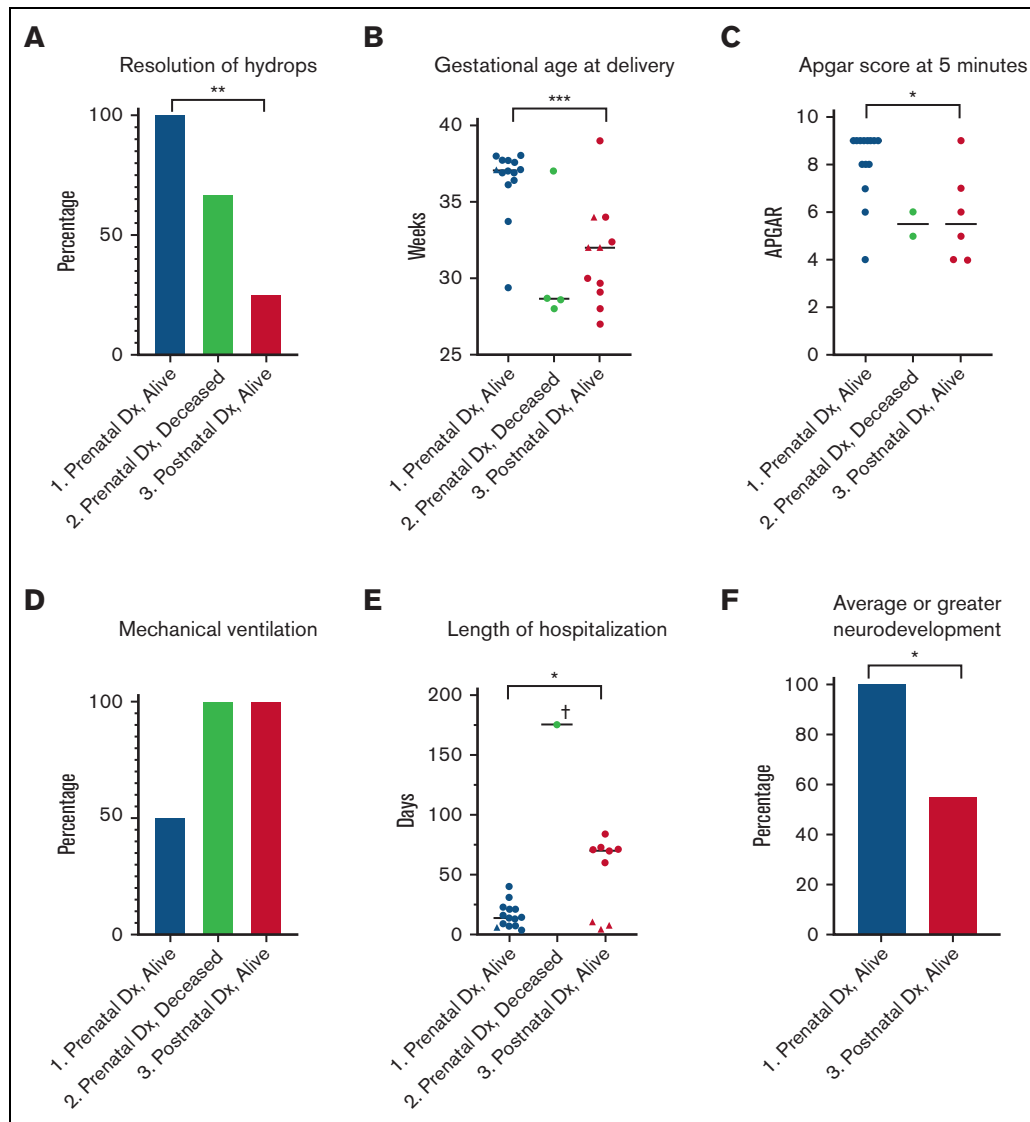
Figure 2. The effect of IUTs on perinatal outcomes. We compared outcomes in fetuses who were diagnosed prenatally and survived (group 1, blue fill) with those diagnosed prenatally who had a perinatal demise (group 2, green fill) and those who were postnatally diagnosed and alive (group 3, red fill). (A) Percentage of fetuses in whom hydrops had resolved at the time of birth (group 1: n = 12, group 2: n = 3, group 3: n = 4 with data available). $P = .005$ across groups, $P = .01$ between groups 1 and 3. (B) GA at delivery in weeks (group 1: n = 14, group 2: n = 4, and group 3: n = 11 with data available). $P = .004$ across groups, $P = .003$ between groups 1 and 3, $P = .022$ between groups 1 and 2. (C) APGAR scores at 5 minutes (group 1: n = 14, group 2: n = 2, group 3: n = 6 with data available). $P = .03$ across groups, $P = .03$ between groups 1 and 3. (D) Percentage of neonates who required invasive mechanical ventilation after birth (group 1: n = 14, group 2: n = 2, group 3, n = 8 with available data) $P = .03$ across groups, $P = .052$ between groups 1 and 3). (E) Length of neonatal hospitalization (group 1: n = 14, group 2: n = 1, group 3, n = 9 with data available) $P = .04$ across groups, $P = .049$ between groups 1 and 3. (F) Percentage with average or greater neurodevelopment (group 1: n = 11, group 3: n = 11 with data available). $P = .04$ between groups 1 and 3. In panels B, C, and E, each symbol represents 1 patient; triangles represent patients with nondeletion variants (Zurich-Albisrieden, Hb H Adana); and the horizontal line indicates the median. For comparisons between groups 1 and 3, $*P \leq .05$, $**P \leq .01$; $***P \leq .005$. † Represents a patient who died after a 175-day hospital stay. (Kruskal-Wallis one-way analysis of variance test with pairwise comparisons was used to compare the continuous variables across the 3 groups, and Fisher's exact test was used for ordinal variables).

last follow-up (median age 33 months, IQR 17-67 months) were classified as average or greater neurodevelopment in 11/11 from group 1 and 6/11 from group 3 patients ($P = .042$).

We also assessed the health-related quality of life in 8 English-speaking patients (all in group 1) by administering the Pediatric Quality of Life Inventory (PedsQL).[20] The mean score was 89.2 ± 3.6 in this cohort (supplemental Figure 2D), which is comparable with previously reported scores[26] for children without chronic diseases (83.84 ± 12.65) and children with diabetes (80.35 ± 12).

In this series, most of the patients (19/25) underwent serial transfusions. Seven patients underwent bone marrow transplantation, 6 of whom were successful, and transfusions were no longer required.
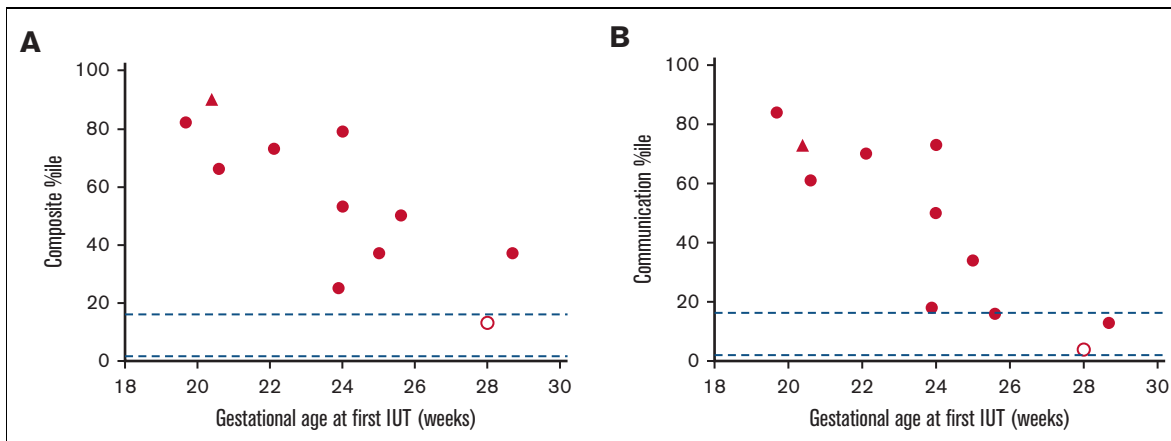
Figure 3. Vineland-3 percentile score and correlation with GA at the first IUT. (A) Composite Vineland percentile score vs GA at first IUT (Pearson r = −0.72, P = .02). (B) Communication subdomain Vineland percentile (Pearson r = −0.82, P = .004). In each graph, the filled circles represent patients in group 1, and the triangle represents a group 1 patient with a nondeletion variant (Hb H Adana). The empty circle represents a patient who was born spontaneously at 28 weeks and diagnosed after birth (this patient was not included in the statistical analysis to evaluate the impact of IUTs on neurodevelopment). The superior dotted line corresponds to 1 standard deviation below the mean (sixteenth percentile); which signifies the risks of developmental delay. The inferior dotted line corresponds to 2 standard deviations below the mean (2.5th percentile); which signifies the risks for intellectual disability and is not seen in any patient in the transfused cohort.

Combined analysis of UCSF registry with contemporary case series

During the registry enrollment period, 2 case series that detailed prenatal management of patients with ATM were published (Chan et al 2018,[9] Zhang et al 2021[10]). We performed a combined analysis of the published data from these series. A third registry of 69 patients[11] was not included because IUT details were unavailable. The combined analysis consisted of a total of 48 patients with ATM (supplemental Table 3). Based on the finding of improved outcomes in patients in group 1 in our cohort, we compared patients in all 3 series who received ≥2 IUTs ("OT," n = 27) with those who had 0 to 1 IUTs ("SOT," n = 21).

Perinatal outcomes

Data on hydrops resolution were available for 27 patients with ATM. By the time of birth, hydrops had resolved in 17/18 OT and 3/9 SOT fetuses (Figure 4A). The number of births at >34 weeks was significantly higher in the OT patients than that in the SOT patients (Figure 4B). OT patients were significantly more likely than SOT patients to have reassuring APGAR scores (≥7) at 5 minutes (Figure 4C). There was a trend for a decreased need for mechanical ventilation at birth in OT patients compared with SOT patients (supplemental Figure 3A). Similarly, the length of hospitalization was available for 29 patients and tended to be shorter in the OT than in the SOT patients (supplemental Figure 3B). There was no difference in the percentage of patients with small stature (defined as lesser than the fifth percentile) (supplemental Figure 3C) or percentage of patients small for GA (defined as birthweight less than the tenth percentile) (supplemental Figure 3D.)

Neurodevelopmental outcomes

Outcome data were available for 18 OT patients and 13 SOT patients. Nearly all (17/18 (94.4%) patients with OT had an average or greater level of neurodevelopment compared to 5/13 (38.5%) SOT patients (Figure 4D). A single patient in the OT group

with a history of developmental delay had normalized and attended mainstream school.

Other anatomic findings

In this combined cohort, hypospadias were observed in 14/22 males. Digital deformities were seen in of 2/48 patients. Cardiac manifestations were reported in 18/48 patients, 16 with minor structural anomalies (atrial and ventricular septal defects). One patient had type 3 jejunal atresia and one had anodontia/malocclusion.

Discussion

Although alpha thalassemia variants are highly prevalent worldwide, there are limited data regarding the impact of fetal therapy in patients with ATM. The objective of this international registry was to understand the impact of fetal transfusions on perinatal and long-term neurodevelopmental outcomes in patients who received fetal therapy. We found that fetuses with ATM who received at least 2 IUTs had excellent survival, resolution of hydrops, delivery near term, and normal neurodevelopmental outcomes. These findings are important for counseling expectant families.

Concern regarding neurodevelopmental compromise is a critical consideration in decision-making for pregnancies diagnosed with ATM. This series demonstrates that IUTs not only result in hydrops resolution in patients with ATM, but also in age-appropriate developmental function, even if children require early intervention therapies. This finding was demonstrated in the UCSF cohort, where we performed detailed testing and substantiated in the combined analysis. Although detailed neuropsychological testing would be required to determine subtle differences in language, higher level cognition, and executive function, it is encouraging to note that survivors who receive adequate transfusions do not exhibit clinically significant impairment in learning and are functional at the age and/or grade level. These findings are consistent with case reports of patients with ATM who were transfused in utero and similarly revealed minimal to no delay.[8,27-29] It is of note that
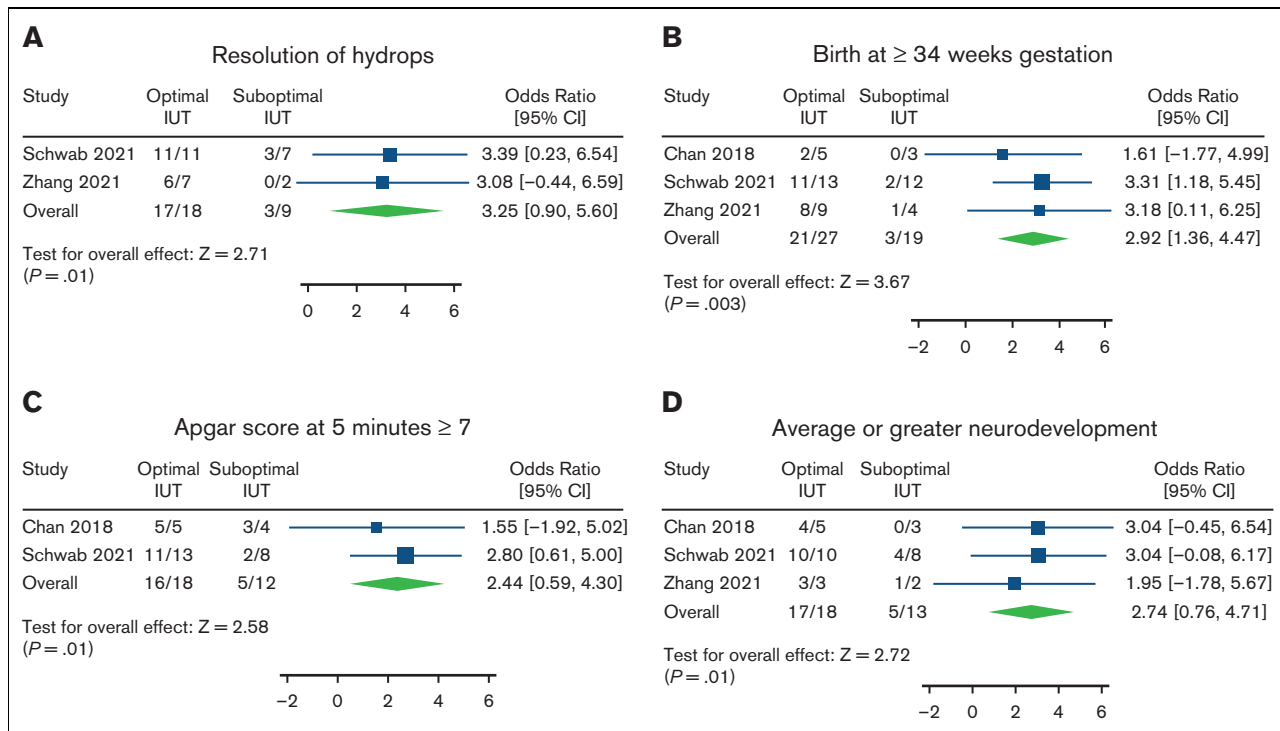
Figure 4. Combined analysis of perinatal outcomes in our case series and those reported in 2 previously published case series (Chan et al 2018, Zhang et al 2021). Forest plots with 95% confidence intervals comparing patients who received optimal IUTs (OT, ≥2 IUTs) and those with suboptimal IUTs (SOT, 0-1 IUT). Denominators for each analysis reflect the number of patients for whom the data were available. (A) At the time of birth, hydrops resolved in 17/18 (94.4%) OT and 3/9 (33.3%) SOT fetuses (OR 3.25 [0.90, 5.60]; P = .01). (B) The number of births at ≥34 weeks was 21/27 (77.8%) in OT vs 3/19 (15.8%) in SOT patients (OR 2.92 [1.36, 4.47]; P = .003). (C) OT patients were significantly more likely than SOT patients to have reassuring APGAR scores (≥7) at 5 minutes (OT: 16/18 (88.2%) vs SOT: 5/12 (41.6%) (OR 2.44 [0.59, 4.30]; P = .01). (D) OT patients were significantly more likely than SOT patients to have average or greater neurodevelopment (OT: 17/18 (94.4%) vs SOT 5/13 (38.5%) (OR 2.74 [0.76, 4.71]; P = .01).

none of the children in this series had evidence of brain injury in utero or after delivery on available neuroimaging; however, such complications may occur and result in an increased risk of neurodevelopmental delay and disability. Importantly, our quality of life evaluation also indicated that survivors enjoy a quality of life that is comparable to those without chronic diseases.[26]

In our registry, the mean gestation for the first IUT was 24 weeks, and earlier initiation of IUT correlated with higher Vineland composite scores. These findings demonstrate the importance of identifying at-risk couples to ensure early prenatal diagnosis and the opportunity for them to consider all options for pregnancy, including the timely initiation of IUTs. The American College of Obstetricians and Gynecologists recommends that all pregnant patients undergo screening with a complete blood count and assessment of mean cell volume, mean corpuscular hemoglobin and Hemoglobin electrophoresis/high performance liquid chromatography, and that molecular genetic testing for alpha thalassemia be performed in patients with a microcytic anemia not explained by iron deficiency.[30] Recognizing the high incidence of both iron deficiency anemia and thalassemia syndromes in many parts of the world, simultaneous evaluation for iron deficiency anemia (serum ferritin levels and zinc protoporphyrin) should also be pursued for patients of at-risk ancestry with a microcytic anemia.[31]

With respect to the IUT technique, most centers transfuse a volume similar to that used for alloimmunised fetuses; however, there are currently no specific guidelines for IUTs for patients with ATM. Additional data from this registry will allow us to develop a disease-specific protocol, including the time interval between transfusions and whether a correction factor (to account for the percentage of nonfunctional Bart's Hemoglobin) should be applied for the calculation of transfusion volumes.

We identified 11 patients diagnosed in infancy who were liveborn without prenatal intervention. None of these patients had a deletion overlapping the zeta-globin gene (ie, none were heterozygous for the larger -FIL or -THAI deletions). These findings support the hypothesis that the homozygous presence of zeta-globin, essential for the formation of embryonic Hb, may be protective and improve prenatal survival.[32]

In this series, we noted that fetuses with ATM often have ultrasound findings of hydrops at the time of diagnosis as well as echogenic bowel, cardiomegaly, or amniotic fluid abnormalities. Some patients had minor structural anomalies, such as atrial septal defects and hypospadias, as previously reported.[24,33-35] Many of these infants were small for GA at birth and had short stature; our combined analysis did not demonstrate an effect of IUTs on this outcome.

The strengths of this study are the large number of patients with ATM from multiple fetal treatment centers in North America and Southeast Asia and the focus on the impact of fetal therapy. We also performed a validated neurodevelopmental test to evaluate the

long-term outcomes. Limitations include a lack of complete information for all patients, in part because of the contributions from many different centers, which may have had different prenatal and neonatal management protocols. The international enrollment also contributes to varying assessments of school progress and other neurodevelopmental details (for example, we could only administer the Vineland to English-speaking patients, who are largely in group 1). Finally, for the combined analysis, we were limited to using only 2 additional small retrospective case series (only those with detailed prenatal management of IUTs). The combined neurodevelopmental evaluation included different testing methods (formal IQ and neuropsychiatric testing, self/parent Vineland-3 reporting, clinical assessment, and academic achievement). However, we accounted for this variability by determining the composite neurodevelopmental outcomes.

Providing adequate fetal therapy for patients with ATM has important cost implications, given the high cost of neonatal intensive care stays. A GA of 36 weeks at delivery compared with 32 weeks and a 2-week neonatal hospital stay compared with a 2-month stay may have a significant impact on health care costs. Ultimately, the objective of fetal therapy for patients with ATM is to increase the potential for meaningful survival, while reducing the medical and financial burden of the disease.

Decreasing the lifelong burden of disease for survivors with ATM remains essential. Only 6 patients in our series were cured by stem cell transplantation; the remaining patients remained transfusion-dependent. This may be due to multiple variables including the challenges of identifying a suitable donor for postnatal stem cell transplantation.[36] Recognizing the challenges of identifying a donor and the associated morbidity of this treatment, we are currently testing the safety and efficacy of in utero transplantation for ATM.[13] Gene therapy or editing strategies to enable alpha-globin production in red blood cells could also be developed. Since we noted the salutary effect of zeta-globin production in spontaneous survivors in our series, upregulation of zeta-globin may also be considered, analogous to the upregulation of gamma globin used to treat beta hemoglobinopathies.

We anticipate a continued increase in the prevalence of alpha thalassemia throughout North America due to the increasing international migration to this region.[37] It remains imperative to address the unmet needs of this patient population through an increased understanding of the impact of fetal therapies, as well as the development of curative therapies such as gene therapy or editing. Maintaining global alpha thalassemia registries will continue to improve our knowledge of the nuances of this disease and its impact on future therapies. As indicated in a recent consensus statement by a multidisciplinary team, it is critical to diagnose this condition early in pregnancy and provide nondirective counseling for all pregnancy options, including fetal transfusions.[38] The dramatically improved perinatal and neurodevelopmental outcomes of fetuses with ATM who received 2 or more IUTs demonstrates a clear rationale for fetal therapy for this historically fatal disease.

Authorship

Contribution: M.S., B.L., D.G., E.V., and T.M. contributed in conception and designing of the study; M.S. and B.L. collected the data; M.S., B.L., D.G., J.G.V., E.V., and T.M. analyzed the data; M.S., B.L., D.G., and T.M. contributed in manuscript writing; and M.S., B.L., D.G., J.G.V., E.V., and T.M. contributed in manuscript revision; all the other authors contributed patient data and approved the final manuscript.

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References

1. Piel FB, Weatherall DJ. The alpha-thalassemias. *N Engl J Med*. 2014;371(20):1908-1916.

2. Goh LPW, Chong ETJ, Lee PC. Prevalence of Alpha(alpha)-Thalassemia in Southeast Asia (2010-2020): a meta-analysis involving 83,674 subjects. *Int J Environ Res Public Health*. 2020;17(20):7354.

3. Vichinsky EP. Alpha thalassemia major–new mutations, intrauterine management, and outcomes. *Hematology Am Soc Hematol Educ Program*. 2009: 35-41. https://doi.org/10.1182/asheducation-2009.1.35

4. Vichinsky E. Complexity of alpha thalassemia: growing health problem with new approaches to screening, diagnosis, and therapy. *Ann N Y Acad Sci*. 2010;1202:180-187.

5. Farashi S, Harteveld CL. Molecular basis of alpha-thalassemia. *Blood Cells Mol Dis*. 2018;70:43-53.

6. Tongsong T, Charoenkwan P, Sirivatanapa P, et al. Effectiveness of the model for prenatal control of severe thalassemia. *Prenat Diagn*. 2013;33(5): 477-483.

7. Yang Y, Li DZ. A survey of pregnancies with Hb Bart's disease in Mainland China. *Hemoglobin*. 2009;33(2):132-136.

8. Kreger EM, Singer ST, Witt RG, et al. Favorable outcomes after in utero transfusion in fetuses with alpha thalassemia major: a case series and review of the literature. *Prenat Diagn.* 2016;36(13):1242-1249.

9. Chan WY, Leung AW, Luk CW, Li RC, Ling AS, Ha SY. Outcomes and morbidities of patients who survive haemoglobin Bart's hydrops fetalis syndrome: 20-year retrospective review. *Hong Kong Med J.* 2018;24(2):107-118.

10. Zhang HJ, Amid A, Janzen LA, et al. Outcomes of haemoglobin Bart's hydrops fetalis following intrauterine transfusion in Ontario, Canada. *Arch Dis Child Fetal Neonatal Ed.* 2021;106(1):51-56.

11. Songdej D, Babbs C, Higgs DR, Consortium BI. An international registry of survivors with Hb Bart's hydrops fetalis syndrome. *Blood.* 2017;129(10): 1251-1259.

12. Horvei P, MacKenzie T, Kharbanda S. Advances in the management of alpha-thalassemia major: reasons to be optimistic. *Hematology Am Soc Hematol Educ Program.* 2021;2021(1):592-599.

13. MacKenzie T. In utero hematopoietic stem cell transplantation for Alpha-thalassemia major (ATM). https://clinicaltrials.gov/ct2/show/NCT02986698

14. Sompagdee N, Anuwutnavin S, Burapasikarin C, Ruangvutilert P, Thongkloung P. Nomograms of fetal cardiothoracic ratio from 17 to 37 weeks' gestation as assessed by three different measurement techniques and their correlation with gestational age. *Prenat Diagn.* 2021;41(13):1658-1667.

15. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr.* 2013;13:1-13, 59.

16. Chou JH, Roumiantsev S, Singh R. PediTools electronic growth chart calculators: applications in clinical care, research, and quality improvement. *J Med Internet Res.* 2020;22(1):e16204.

17. Battaglia FC, Lubchenco LO. A practical classification of newborn infants by weight and gestational age. *J Pediatr.* 1967;71(2):159-163.

18. Sparrow SBD, Cicchetti D. *The Vineland Adaptive Behavior Scales: Interview edition, Survey form.* American Guidance Service; 1984.

19. Voelker SL, Shore DL, Brown-More C, Hill LC, Miller LT, Perry J. Validity of self-report of adaptive behavior skills by adults with mental retardation. *Ment Retard.* 1990;28(5):305-309.

20. Varni JW, Burwinkle TM, Seid M, Skarr D. The PedsQL 4.0 as a pediatric population health measure: feasibility, reliability, and validity. *Ambul Pediatr.* 2003;3(6):329-341.

21. Bank TW. World Bank country and lending groups. https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups

22. Curran M, Mikhael M, Sun WD, et al. Perinatal management of Bart's hemoglobinopathy: paradoxical effects of intrauterine, transplacental, and partial exchange transfusions. *AJP Rep.* 2020;10(1):e11-e14.

23. Bianchi DW, Beyer EC, Stark AR, Saffan D, Sachs BP, Wolfe L. Normal long-term survival with alpha-thalassemia. *J Pediatr.* 1986;108(5 Pt 1):716-718.

24. Dame C, Albers N, Hasan C, et al. Homozygous alpha-thalassaemia and hypospadias—common aetiology or incidental association? Long-term survival of Hb Bart's hydrops syndrome leads to new aspects for counselling of alpha-thalassaemic traits. *Eur J Pediatr.* 1999;158(3):217-220.

25. N B. *Bayley scales of infant development.* Second Edition 1993.

26. Varni JW, Limbers CA, Burwinkle TM. Impaired health-related quality of life in children and adolescents with chronic conditions: a comparative analysis of 10 disease clusters and 33 disease categories/severities utilizing the PedsQL 4.0 Generic Core Scales. *Health Qual Life Outcomes.* 2007;5:43.

27. Chmait RH, Baskin JL, Carson S, Randolph LM, Hamilton A. Treatment of alpha(0)-thalassemia (−(SEA)/−(SEA)) via serial fetal and post-natal transfusions: can early fetal intervention improve outcomes? *Hematology.* 2015;20(4):217-222.

28. Sohan K, Billington M, Pamphilon D, Goulden N, Kyle P. Normal growth and development following in utero diagnosis and treatment of homozygous alpha-thalassaemia. *BJOG.* 2002;109(11):1308-1310.

29. Joshi DD, Nickerson HJ, McManus MJ. Hydrops fetalis caused by homozygous alpha-thalassemia and Rh antigen alloimmunization: report of a survivor and literature review. *Clin Med Res.* 2004;2(4):228-232.

30. Committee opinion no. 691: carrier screening for genetic conditions. *Obstet Gynecol.* 2017;129(3):e41-e55.

31. Porter J, Taher A, Cappellini MD, Farmakis D. *Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT). Nicosia (Cyprus)*: Thalassemia International Federation; 2021.

32. King AJ, Higgs DR. Potential new approaches to the management of the Hb Bart's hydrops fetalis syndrome: the most severe form of alpha-thalassemia. *Hematology Am Soc Hematol Educ Program.* 2018;2018(1):353-360.

33. Fung TY, Kin LT, Kong LC, Keung LC. Homozygous alpha-thalassemia associated with hypospadias in three survivors. *Am J Med Genet.* 1999;82(3):225-227.

34. Zhao QM, Niu C, Liu F, Wu L, Ma XJ, Huang GY. Spontaneous closure rates of ventricular septal defects (6,750 consecutive neonates). *Am J Cardiol.* 2019;124(4):613-617.

35. McMahon CJ, Feltes TF, Fraley JK, et al. Natural history of growth of secundum atrial septal defects and implications for transcatheter closure. *Heart.* 2002;87(3):256-259.

36. Pecker LH, Guerrera MF, Loechelt B, et al. Homozygous alpha-thalassemia: challenges surrounding early identification, treatment, and cure. *Pediatr Blood Cancer.* 2017;64(1):151-155.

37. Vichinsky EP, MacKlin EA, Waye JS, Lorey F, Olivieri NF. Changes in the epidemiology of thalassemia in North America: a new minority disease. *Pediatrics.* 2005;116(6):e818-e825.

38. MacKenzie TC, Amid A, Angastiniotis M, et al. Consensus statement for the perinatal management of patients with alpha thalassemia major. *Blood Adv.* 2021;5(24):5636-5639.