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Mathematical Albumin Function for Neonates Undergoing Therapeutic Hypothermia in Comparison with Control Neonates

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Zoë Vander Elst, MD[1,2], Thibault Stultjens, MD[3], Pieter Annaert, PhD[4], Paul Clarke, MD[5,6], Isabek Iglesias-Platas, PhD[5], Elisabeth Agathos, MD[5], Gozdem Kaykı, MD[7], Annouschka Laenen, PhD[8], Nadir Yalçın, PhD[9], Anne Smits, PhD[1,2], and Karel Allegaert, PhD[1,10,11]

Abstract

Hypoxic-ischemic encephalopathy (HIE) resulting from perinatal asphyxia presents a substantial risk of mortality and long-term sequelae in neonates. Therapeutic hypothermia (TH) improves both short- and long-term outcomes in near-term/term neonates with moderate to severe HIE. While neonates with perinatal asphyxia and TH often require polypharmacy, the impact of both covariates on pharmacokinetics and pharmacodynamics is only partially described and quantified. In this pooled, multicenter retrospective study, longitudinal trends of human serum albumin (HSA, the major drug binding protein) and total protein (TP) concentrations in near-term/term neonates were described using linear mixed models and compared between cohorts (TH vs control neonates, and moderate vs severe HIE TH cases). A mathematical function for HSA concentrations in neonates with HIE undergoing TH was derived (AlbuCool function). The pooled dataset to estimate these functions contained 330 TH neonates and 425 controls with 1725 and 1415 HSA observations, respectively. The median (interquartile range) HSA concentration was 27.0 (23.0–31.0) g/L for the TH cohort, and 32.1 (28.4–35.7) g/L for the control cohort. Estimated mean HSA concentrations were significantly lower ($P < .001$) in TH compared to control cases, as well as in severe compared to moderate HIE cases ($P < .001$) over the first 7 postnatal days. The HSA function for neonates with HIE undergoing TH was: HSA (g/L) = $32.28 - 2.94 * \text{PNA} + 0.33 * \text{PNA}^2$ (PNA is postnatal age). The integration of this function in pharmacokinetic models holds the promise to improve the predictive performance of these models, and consequently, the pharmacotherapy of HSA-bound drugs in this vulnerable population.

Keywords

drug binding, human serum albumin, hypoxic-ischemic encephalopathy, neonate, physiologically based pharmacokinetics, therapeutic hypothermia

Introduction

According to the World Health Organization, perinatal asphyxia remains one of the leading causes of neonatal death worldwide, affecting approximately two to five newborns per 1000 births with a mortality of 15%–20% in developed countries.[1–3] Hypoxic-ischemic

[1]Department of Development and Regeneration, KU Leuven, Leuven, Belgium
[2]Neonatal Intensive Care Unit, University Hospitals Leuven, Leuven, Belgium
[3]Faculty of Medicine, KU Leuven, Leuven, Belgium
[4]Drug Delivery and Disposition, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium
[5]Neonatal Intensive Care Unit, Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, UK
[6]Norwich Medical School, University of East Anglia, Norwich, UK
[7]Division of Neonatology, Department of Pediatrics, Hacettepe University, Ankara, Turkey
[8]Leuven Biostatistics and Statistical Bioinformatics Centre (L-Biostat), KU Leuven, Leuven, Belgium
[9]Department of Clinical Pharmacy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey
[10]Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium
[11]Department of Hospital Pharmacy, Erasmus MC, Rotterdam, The Netherlands

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Corresponding Author:
Karel Allegaert, KU Leuven, Herestraat 49, 3000 Leuven, Belgium
Email: karel.allegaert@kuleuven.be

Zoë Vander Elst and Thibault Stultjens are shared first authors.

Anne Smits and Karel Allegaert are shared last authors.

encephalopathy (HIE) resulting from perinatal asphyxia presents a substantial risk of mortality and long-term sequelae in neonates, including neurodevelopmental delay, cerebral palsy, and epilepsy.[3–5]

Clinical examination tools such as the Thompson score or modified Sarnat classification allow for standardization of HIE severity assessment.[6,7] The Thompson score is a numeric scoring system correlated with early neonatal outcome in asphyxiated newborns.[6,8,9] Similarly, the severity of HIE can be categorized into three stages (mild, moderate, and severe) using the modified Sarnat classification.[7] Moderate HIE is hereby characterized by hypotonia and lethargy, while severe HIE is marked by stupor and flaccidity.[7,10]

Whole body therapeutic hypothermia (TH) initiated within 6 h after birth improves both short- and long-term outcomes in late preterm and term neonates (≥35 weeks gestational age, GA) with moderate to severe HIE.[3,11,12] This intervention involves lowering the core body temperature of neonates to 33.5°C for 72 h to mitigate brain damage and reduce mortality.[11,13] Neonates with perinatal asphyxia who receive TH often need polypharmacy, while the impact of both covariates on the inter- and intra-individual variability in pharmacokinetics (PK) and pharmacodynamics (PD) is only partially described and quantified.[14] In addition, add-on pharmacotherapy to further improve the outcome of neonates with HIE who receive TH are an active field of research.

To facilitate safe and effective drug dosing in special populations, simulation-based prediction tools such as physiology-based pharmacokinetic (PBPK) models are increasingly being developed and used to predict PK and PD changes.[14] To achieve adequate accuracy, such tools should be informed by real-world, longitudinal (patho)physiological data of the population of interest.[15]

As the major drug binding protein, human serum albumin (HSA) availability can influence both PK and PD of HSA-bound drugs. Consequently, it is one of the relevant physiological laboratory parameters that need to be taken into account in PK analyses.[16,17] Overall, neonates have higher unbound drug fractions compared to older populations due to lower HSA concentrations in early life, competing endogenous substances for HSA binding sites (e.g., bilirubin and free fatty acids), and non-maturational factors such as critical illness.[16,18,19] Concentrations of HSA are lower in neonates with HIE compared to control patients, however data on the magnitude and variability in differences are limited, while mathematical functions for HSA concentrations are still absent.[20,21]

To predict the impact of perinatal asphyxia and TH on HSA concentrations in neonates, and consequently on drug exposure, more accurate HSA trends, based on real-world data, are needed. Therefore, the aims of this study were (1) to retrospectively describe HSA trends in a large, multicenter cohort of HIE neonates undergoing TH compared to controls, and (2) to explore differences in HSA concentrations between moderate and severe HIE cases. Accordingly, trends in total plasma protein (TP) concentrations will also be explored. Control patients are available from a recently published dataset of Vander Elst et al (2024).[22]

Methods

Ethics

The study was performed with data provided by the University Hospitals Leuven (UZL, Leuven, Belgium), Hacettepe University Children's Hospital (HUC, Ankara, Turkey), and a previously published pooled dataset from four neonatal intensive care units (NICUs) in the United Kingdom (Norfolk and Norwich University Hospital, NNUH, Norwich; Addenbrooke's Hospital, AH, Cambridge; Liverpool Women's Hospital, LWH, Liverpool; St Mary's Hospital for Women and Children, SMH, Manchester). The individual data utilized in this study are not publicly accessible and were analyzed with the permission of all co-authors. All studies were approved by their local ethics committees: the Ethics Committee Research UZ/KU Leuven (study number S66227; real-world data in neonates undergoing therapeutic hypothermia, and S61706; control HSA dataset), the Ethics Committee Research Hacettepe University (study number SBA 23/282), and the National Research Ethics Service ethics committee UK (study number 11/EE/0349), respectively.[20] The study was conducted in compliance with the principles of the Declaration of Helsinki (current version, 2013), the principles of Good Clinical Practice (GCP), and all applicable regulatory requirements. Since the data in this study were collected retrospectively, informed consent was waived.

Study Design in Therapeutic Hypothermia and Control Patients

A retrospective study was performed with pooled data from six centers, that is, the NICUs of UZL (June 2010–November 2021), HUC (January 2013–October 2023), and four UK NICUs (July 2006–June 2011). The NICUs of UZL and HUC are level IV NICUs providing care for (extremely) preterm and term neonates, specialized surgical care, and acting as a referral center for complex congenital malformations. The four UK NICUs are all level III NICUs and neonatal surgical centers. Neonates admitted to one of these NICUs were eligible for inclusion if they were treated with TH for perinatal asphyxia (predominantly offered for infants ≥36 weeks gestational age), had a

postnatal age (PNA) of ≤10 days, and had at least one HSA determination during their NICU stay.

Control patients were selected from a recently published HSA and TP real-world dataset from the UZL NICU (N = 848 neonates). For the current analysis, neonates from this dataset were included if they had a GA of ≥35 weeks, and a PNA of ≤10 days.[22]

The following variables were extracted from the patients' medical health records: GA (weeks), PNA (days), birth weight (BW, grams), HSA (g/L), and TP (g/L). Gestational age was reported in full weeks. Day of birth was defined as PNA day 1. Specific for the TH patients, the Thompson score (numeric value between 0 and 22) or Sarnat classification (mild, moderate, and severe), and survival (yes/no) were recorded. In addition, Thompson scores were converted to Sarnat classification as suggested by Chansarn et al (2021) to standardize this covariate of HIE disease severity.[23]

Bio-Analytical Information

Determination of laboratory values in routine clinical care was performed on arterial or venous blood samples collected in lithium heparin tubes and analyzed at the laboratory departments of UZL (Leuven, Belgium), HUC (Ankara, Turkey), NNUH (Norwich, UK), AH (Cambridge, UK), LWH (Liverpool, UK), and SMH (Manchester, UK). HSA concentrations were determined with a colorimetric assay with bromocresol green in UZL, HUC and before 2008 in NNUH, and with a colorimetric assay with bromocresol purple in NNUH from 2008 onward. Additional bio-analytical information is presented in Table S1. Related to the retrospective design, specific bio-analytical information of AH, LWH, and SMH is missing.

Statistical Analysis

Statistical analyses were performed using SAS software (version 9.4, SAS System for Windows) and R software (version 4.4.0, R Core Team, 2024). Baseline continuous clinical characteristics were described by mean and standard deviation (SD), or median and interquartile range (IQR). Categorical variables were reported as incidence (count and percentage). To explore longitudinal trends of HSA and TP concentrations, linear mixed models for repeated measurements were applied. To deal with nonlinear trends of HSA over PNA, models were considered where PNA was modeled categorically, or continuous with a log-transformation or quadratic effect. Longitudinal data were visually presented as means and mean differences with 95% confidence intervals.

To explore differences in HSA and TP concentrations between cohorts (TH vs controls, and moderate vs severe HIE in TH cases), we first investigated the interaction between PNA and cohort. In case of a significant interaction, the difference between cohorts was estimated at each postnatal day. If the interaction was not significant, a main effects model was applied to estimate the mean difference between the cohorts. Additionally, to assess the impact of GA and BW on HSA and TP concentrations in TH patients, we investigated the interactions between PNA and GA, as well as PNA and BW. If the interaction was significant, the effect of the predictor variable on HSA or TP concentrations was analyzed and presented as a slope for each postnatal day. Conversely, if the interaction was not significant, a main effects model was applied to estimate the mean slope over PNA. A *P*-value of <.05 was considered statistically significant.

To facilitate implementation of these HSA-related findings in future pharmacometrics models, we derived a mathematical function for HSA concentrations in neonates with HIE undergoing TH (the "AlbuCool function"), and compared to the age-dependent mathematical HSA function for neonates in the PBPK modeling platform Simcyp (Simcyp simulator version 22, release 1, Sheffield, UK). Differences were reported as mean differences with 95% confidence intervals, and visually presented using boxplots. A paired t-test was used to investigate the significance of the differences between the predicted HSA concentrations from these mathematical functions and the observed HSA concentrations in our dataset.

Results

Clinical Characteristics

In total, 330 neonates who underwent TH were included in this study (UZL n = 45, HUC n = 64, and UK n = 221) with a median (IQR) GA of 40 (38–40.7) weeks and BW of 3337 (3000–3830) g. Additionally, 425 controls were included with a median GA of 38 (36–39) weeks and BW of 3070 (2610–3512) g. During the first 10 days of life, we had access to 1725 and 1415 HSA measurements, with a median number of measurements available per patient of 5 (4–7) and 3 (2–5) for the TH and control patients, respectively. The clinical characteristics of the included neonates are provided in Table 1.

HSA and TP Observations in TH Versus Control Patients

The median (IQR) HSA concentration was 27.0 (23.0–31.0) g/L for the TH cohort, and 32.1 (28.4–35.7) g/L for the control cohort. Median HSA concentrations over PNA for both cohorts are presented in Table 2, and median TP concentrations in Table S2. A decrease in HSA concentrations was observed over the first five postnatal days in the TH cohort, after which it increased again. Differences in mean HSA concentrations between the TH and control cohort were estimated at each PNA (Table 3). Estimated mean

Table 1. Clinical Characteristics of the Included Neonates with Perinatal Asphyxia Treated with Therapeutic Hypothermia (Cases) and Controls

Variable	Statistic	UZL	HUC	UK	Total cases	Controls
Gestational age (weeks)	**N**	**45**	**64**	**221**	**330**	**425**
	Mean	38.7	38.4	39.8	39.4	37.8
	SD	1.8	1.9	1.7	1.9	1.8
	Median	39.0	39.0	40.1	40.0	38.0
	IQR	(37.0–40.0)	(37.0–40.0)	(38.72–41.1)	(38.0–40.7)	(36.0–39.0)
	Range	(34.0–42.0)	(33.0–41.0)	(36.00–42.6)	(33.0–42.6)	(35.0–42.0)
Birth weight (g)	**N**	**45**	**64**	**221**	**330**	**425**
	Mean	3251.3	3172.3	3512.9	3411.2	3080.9
	SD	584.0	450.3	671.2	638.2	628.8
	Median	3160.0	3240.0	3500.0	3337.0	3070.0
	IQR	(3000–3460)	(2800–3450)	(3000–4020)	(3000–3830)	(2610–3512)
	Range	(2050–4900)	(1850–4130)	(1450–5200)	(1450–5200)	(1280–6000)
Thompson score	**N**	**32**	**8**	**0**	**40**	**0**
	Mean	8.9	6.1	NA	8.4	NA
	SD	2.4	3.8	NA	2.9	NA
	Median	9.0	4.5	NA	8.5	NA
	IQR	(7.0–11.0)	(3.5–9.0)	NA	(6.5–11.0)	NA
	Range	(4.0–14.0)	(2.0–13.0)	NA	(2.0–14.0)	NA
Sarnat						
1	n/N (%)	5/32 (15.6)	5/8 (62.5)	32/221 (14.5)	42/261 (16.1)	NA
2	n/N (%)	26/32 (81.3)	3/8 (37.5)	117/221 (52.9)	146/261 (55.9)	NA
3	n/N (%)	1/32 (3.1)	0/8 (0.0)	72/221 (32.6)	73/261 (28.0)	NA
Survival						
0	n/N (%)	11/45 (24.4)	9/64 (14.1)	27/221 (12.2)	47/330 (14.2)	NA
1	n/N (%)	34/45 (75.6)	55/64 (85.9)	194/221 (87.8)	283/330 (85.8)	NA

HIE, hypoxic-ischemic encephalopathy; HUC, Hacettepe University Children's Hospital; IQR, interquartile range; NA, missing data/not applicable; Sarnat 1 = mild HIE, 2 = moderate HIE, 3 = severe HIE; SD, standard deviation of the mean; Survival 0 = no, 1 = yes; UK, four NICUs in the United Kingdom; UZL, University Hospitals Leuven.

HSA concentrations were significantly lower ($P < .001$) in the TH cohort compared to controls during the first 7 postnatal days. Estimated mean HSA concentrations over PNA for both cohorts are plotted in Figure 1a. Estimated differences in mean TP concentrations between the TH and control cohort were statistically significant for each postnatal day 1–10, except for postnatal day 7 (Table S3 and Figure S1a).

Covariate Effects on HSA and TP Concentrations in TH Patients

HSA concentrations in neonates with moderate HIE were significantly higher than those with severe HIE (Table 4). There was no significant difference between moderate and severe HIE for TP concentrations. Mean HSA concentrations over PNA for moderate and severe HIE are plotted in Figure 1b, and for TP concentrations in Figure S1b.

The interaction between PNA and GA was significant. Therefore, the effect of GA on HSA and TP concentrations was presented per postnatal day (Table S4). The slope indicates an increase in concentration (g/L) for an increase of GA with one week. The interaction between PNA and BW was not significant. Therefore, a main effects model was applied to estimate the mean slope over PNA (Table S5). The slope indicates an increase in concentration (g/L) per kilogram increase in BW. There was no significant association between GA or BW and HSA concentrations. However, a statistically significant association ($P < .05$) was demonstrated between GA and TP, except for postnatal days 8 and 10, and between BW and TP ($P = .0009$).

Mathematical HSA Function for Neonates with HIE Undergoing TH

A mathematical HSA function, relevant to integrate in PK modeling efforts (e.g., PBPK models), for neonates with HIE undergoing TH was developed (Equation 1):

$$\text{HSA (g/L)} = 32.28 - 2.94*\text{PNA} + 0.33*\text{PNA}^2 \quad (1)$$

(AlbuCool function, Function 1). Similarly, a mathematical function for TP was developed (Table S6).

The developed HSA function of this study was compared to the existing HSA function in the available PBPK modeling software Simcyp (Simcyp simulator version 22, release 1, Sheffield, UK): HSA = 33.746 + 1.1287*log(PNA) (Simcyp function). This Simcyp function represents the general neonatal population, not specifically HIE cases. HSA concentrations were predicted using the two functions for both TH and

Table 2. Observed Human Serum Albumin (g/L) Concentrations Over Postnatal Age (Days) in Neonates Undergoing Therapeutic Hypothermia Versus Controls

Postnatal age (days)		1	2	3	4	5	6	7	8*	9*	10*
Therapeutic hypothermia (HSA, g/L)	N	296	304	292	271	226	163	124	23	18	8
	Mean	29.7	27.7	26.8	25.3	25.3	25.5	26.5	32.0	32.4	34.1
	SD	6.3	5.8	5.1	4.8	4.8	5.2	5.6	4.9	4.5	3.6
	Median	**30.0**	**27.3**	**27.0**	**25.0**	**25.3**	**25.0**	**27.0**	**32.2**	**31.1**	**34.2**
	IQR	(25.0–34.4)	(24.0–32.0)	(23.0–30.6)	(22.0–28.9)	(22.0–28.9)	(21.0–29.5)	(23.0–31.0)	(29.1–35.2)	(29.2–37.0)	(30.9–37.3)
	Range	(10.0–45.7)	(10.0–42.0)	(5.3–42.0)	(12.0–38.0)	(7.0–39.0)	(13.0–38.8)	(12.0–38.0)	(22.9–41.6)	(23.9–39.8)	(29.4–38.5)
Controls (HSA, g/L)	N	241	191	197	183	147	129	101	87	74	65
	Mean	34.8	33.5	32.1	31.5	31.0	30.5	30.4	30.3	31.1	30.9
	SD	4.5	4.8	4.7	5.0	5.1	5.2	5.0	5.7	5.7	6.0
	Median	**35.1**	**34.0**	**32.7**	**31.5**	**31.2**	**30.1**	**30.2**	**30.1**	**30.5**	**29.7**
	IQR	(32.1–37.6)	(30.2–37.1)	(29.0–35.6)	(28.1–34.9)	(27.4–34.8)	(27.2–33.4)	(26.5–33.8)	(26.4–33.4)	(26.7–33.8)	(26.8–34.1)
	Range	(14.8–56.7)	(18.4–44.9)	(16.2–43.1)	(19.1–44.6)	(19.0–43.7)	(19.3–45.7)	(18.5–48.1)	(19.0–55.9)	(22.0–55.1)	(18.9–51.4)

HSA, human serum albumin; IQR, interquartile range; SD, standard deviation of the mean.
*HSA concentrations of postnatal days 8–10 were only available for patients with a Sarnat score of 1 or 2.

Table 3. Estimated Mean Human Serum Albumin Concentrations (g/L) by Postnatal Age (days): Difference Between Therapeutic Hypothermia and Control Cohort

PNA (days)	Estimated mean HSA [95% CI]		Difference [95% CI]	*P*-value
	TH cohort	Controls		
1	29.5 [28.9–30.2]	35.1 [34.5–35.8]	5.6 [4.7–6.5]	<.001
2	27.9 [27.3–28.4]	34.0 [33.4–34.7]	6.2 [5.3–7.0]	<.001
3	26.8 [26.2–27.3]	33.1 [32.5–33.7]	6.4 [5.6–7.1]	<.001
4	25.5 [24.9–26.0]	32.5 [31.9–33.0]	7.0 [6.2–7.8]	<.001
5	25.5 [25.0–26.1]	32.1 [31.5–32.7]	6.6 [5.8–7.4]	<.001
6	26.5 [25.8–27.1]	31.9 [31.2–32.6]	5.4 [4.5–6.4]	<.001
7	27.8 [27.1–28.5]	32.0 [31.3–32.8]	4.2 [3.2–5.2]	<.001
8	30.7 [29.1–32.3]	32.1 [31.2–33.0]	1.4 [−0.4 to 3.3]	.1276
9	31.1 [29.2–32.9]	32.7 [31.7–33.7]	1.6 [−0.5 to 3.8]	.1326
10	33.9 [30.8–36.9]	33.1 [31.9–34.3]	−0.8 [−4.1 to 2.5]	.6353

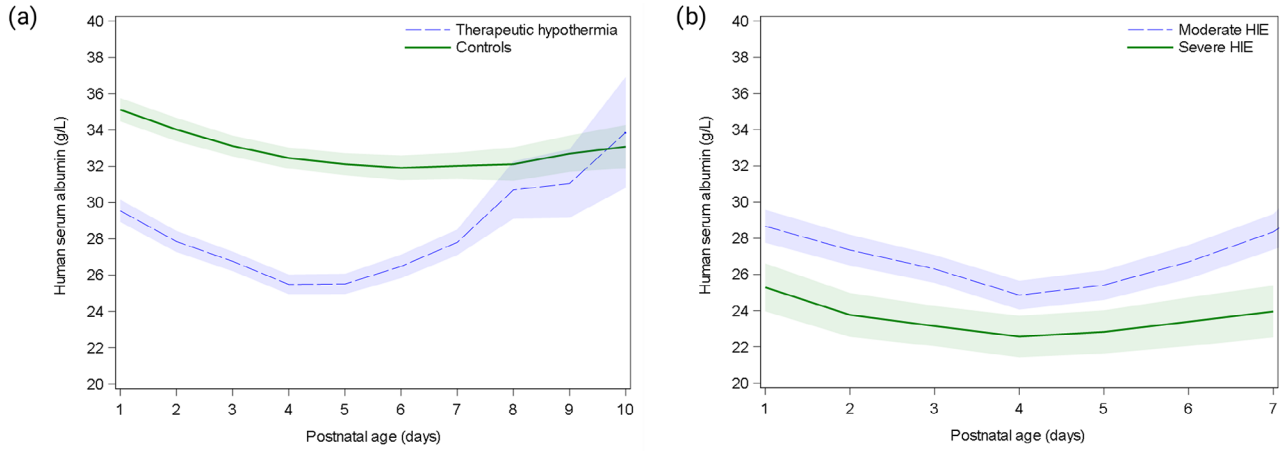
CI, confidence interval; HSA, human serum albumin (g/L); PNA, postnatal age (days); TH, therapeutic hypothermia (g/L).

control neonates (Figure 2). Predicted HSA concentrations of the AlbuCool function decreased in the first 5 postnatal days, after which they increased again. In contrast, Simcyp predicted HSA concentrations steadily increased from postnatal day 1 through 10. The difference between Simcyp and AlbuCool predictions is presented in Table 5. Additionally, the predicted HSA concentrations were compared to the observed HSA concentrations from our dataset for both TH and control neonates (Figure 3). For the TH cases, the mean difference [95% CI] between the observed HSA concentrations and AlbuCool predictions (Equation 1) was −0.3 [−0.5 to +0.0] g/L (P-value = .06). In contrast, the mean difference [95% CI] between the Simcyp predictions and observed HSA concentrations was −7.9 [−8.2 to −7.7] g/L (P-value < .001). For the control neonates, the mean difference [95% CI] between the AlbuCool or Simcyp predictions and observed HSA concentrations was 3.7 [3.4–4.0] g/L and −3.4 [−3.7 to −3.1] g/L, respectively (P-value < .001 for both).

Discussion

In this large multicenter study of pooled datasets, we have described the HSA trends of 330 neonates with HIE undergoing TH and compared them to those of 425 NICU-admitted controls. Furthermore, we have explored and quantified the effect of moderate versus severe HIE on HSA concentrations in TH patients. This is the first time a mathematical HSA and TP function has been developed for neonates with HIE undergoing TH.

We observed a decrease in HSA concentrations over the first 5 postnatal days in the TH cohort, after which these concentrations increased again. In a smaller retrospective study of Toptan et al (2024) a similar decrease in HSA concentrations was demonstrated in neonates undergoing TH between time points 0–6 and 60–72 h



HIE = hypoxic-ischemic encephalopathy
Full/dashed line = estimated mean human serum albumin concentration (g/L); Shaded area = 95% confidence interval

Figure 1. Estimated mean (with 95% confidence interval) human serum albumin concentrations (g/L) over postnatal age of (a) the therapeutic hypothermia versus control cohort and (b) neonates with moderate versus severe hypoxic-ischemic encephalopathy.

Table 4. Mean Human Serum Albumin Concentrations (g/L) of Neonates with Moderate Versus Severe Hypoxic-Ischemic Encephalopathy

PNA (days)	Mean HSA [95% CI]		Difference [95% CI]	*P*-value
	Moderate HIE	Severe HIE		
1	28.7 [27.8–29.6]	25.3 [24.0–26.6]	3.4 [1.8–5.0]	<.001
2	27.4 [26.5–28.2]	23.8 [22.6–25.0]	3.6 [2.1–5.1]	<.001
3	26.3 [25.5–27.1]	23.2 [22.0–24.3]	3.2 [1.8–4.5]	<.001
4	24.9 [24.1–25.7]	22.6 [21.4–23.7]	2.3 [0.9–3.7]	.0014
5	25.4 [24.6–26.2]	22.8 [21.6–24.0]	2.6 [1.1–4.0]	.0005
6	26.7 [25.8–27.6]	23.4 [22.0–24.7]	3.3 [1.7–4.9]	<.001
7	28.4 [27.4–29.3]	24.0 [22.5–25.4]	4.4 [2.7–6.1]	<.001

CI, confidence interval; HIE, hypoxic-ischemic encephalopathy; HSA, human serum albumin (g/L); PNA, postnatal age (days)

after start of TH.[21] Overall, HSA concentrations were higher in the TH cohort of Toptan et al (2024) (median [IQR] HSA concentration of 35.0 [30.5–39.0] g/L) compared to the TH cohort in this study (median HSA concentration of 30.0 [25.0–34.4] g/L) on postnatal day 1.[21] Possible explanations for this difference might be differences in fluid management, laboratory analytical methods, or albumin administration between both studies.

In the present study, neonates undergoing TH had significantly lower ($P < .001$) HSA concentrations compared to controls during the first 7 postnatal days. Control patients were selected from a previously published albumin dataset and included neonates with a GA ≥35 weeks since there were neonates with a GA of 35 weeks in the TH dataset.[22] However, most neonates receiving TH are older. Therefore, mean GA of TH cases was higher than control neonates. Additionally, HSA concentrations were significantly lower in neonates with severe HIE compared to moderate HIE ($P < .001$). These multicenter results confirm the results of Muniraman et al (2017) where infants with more severe HIE had lower HSA concentrations.[20] This difference is probably attributable to critical illness, which is known to reduce HSA concentrations, as HSA functions as a negative acute-phase reactant.[24,25]

The difference in real-world data for HSA between neonates undergoing TH and NICU controls, and between moderate and severe HIE illustrates that neonatal disease and therapeutic interventions reflect acute changes in their physiology. This will subsequently alter the PK and PD of drugs, with implications that dosage adjustments may be indicated. In a recent open access repository on PBPK modeling publications, 72 papers explored the impact of changes in HSA in diverse scenarios (e.g., critical illness, cancer, cirrhosis, pediatrics, or pregnancy) or compartments (e.g., subcutaneous, central nervous system, and tumor).[26] However, this repository does not yet include data on neonates undergoing TH.

Obviously, HSA is only one covariate, as drug dosing in neonates becomes even more complex due to maturation-dependent physiological changes, such as the increase in total body weight and maturation of organs with increasing age.[27] Since both perinatal asphyxia and TH influence neonatal physiology, the I-PREDICT project is developing a PBPK framework to support drug development and dose precision.[14,28] I-PREDICT investigates a PBPK model to predict drug exposure in asphyxiated neonates undergoing TH.[14,28] Similarly, the CreaCool study described serum creatinine (SCr) patterns in neonates with HIE treated with TH.[29] Patterns over PNA were significantly higher in TH neonates compared to controls, illustrating the importance of population-specific data to better

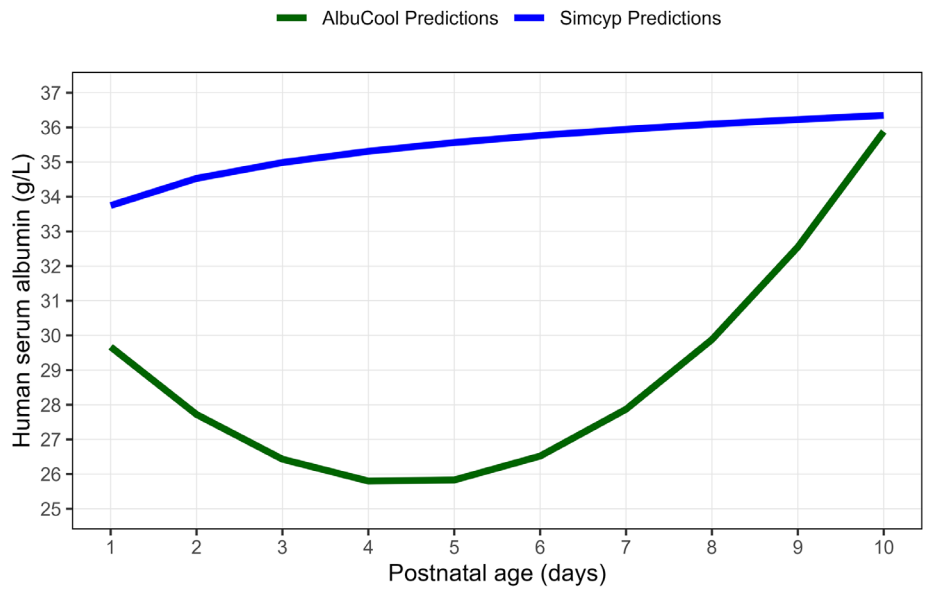


Figure 2. AlbuCool and Simcyp predicted (Simcyp simulator version 22, release 1) human serum albumin concentrations (g/L) over postnatal age.

Table 5. Comparison Between AlbuCool and Simcyp Predicted (Simcyp Simulator Version 22, Release 1) HSA Concentrations

PNA (days)	AlbuCool HSA predictions (g/L)	Simcyp HSA predictions (g/L)	Difference [95% CI]
1	29.7	33.8	4.1 [2.2–6.0]
2	27.7	34.5	6.8 [4.9–8.7]
3	26.4	35.0	8.6 [6.7–10.5]
4	25.8	35.3	9.5 [7.6–11.4]
5	25.8	35.6	9.7 [7.8–11.6]
6	26.5	35.8	9.3 [7.4–11.1]
7	27.9	35.9	8.1 [6.2–10.0]
8	29.9	36.1	6.2 [4.3–8.1]
9	32.6	36.2	3.7 [1.8–5.6]
10	35.9	36.4	0.5 [−1.4 to 2.4]

CI, confidence interval; HSA, human serum albumin (g/L); PNA, postnatal age (days).

understand the pathophysiological changes and develop representative and clinically relevant PBPK models.[29] This is of utmost importance, as neonatal PBPK models currently have limited predictive performance due to suboptimal representation of (patho)physiological data in PBPK platforms.

Innovative in this study was therefore the development of a mathematical HSA function for neonates with HIE undergoing TH. The comparison of our function to the Simcyp function for TH cases illustrates the suboptimal representation of HSA data for neonates with HIE undergoing TH in this commercial platform. The AlbuCool function captured the data properly, with a mean difference of 0.25 g/L. However, results should be interpreted with caution since the dataset used to develop the function also served as the basis for the evaluation. Validation with an external dataset is therefore needed to confirm these results. For control neonates, both the AlbuCool and Simcyp function did not perform appropriately. The AlbuCool function underpredicted HSA concentrations in the first postnatal week, explainable by the fact that it was developed for HIE neonates undergoing TH, who exhibit lower HSA concentrations. Conversely, the Simcyp function generally overpredicted HSA concentrations in control neonates. This discrepancy may be attributed to the composition of our control dataset, which consists of neonates admitted to the NICU and may therefore not be representative for the broader, healthy neonatal population. Our AlbuCool function could significantly enhance the predictive performance of future neonatal PBPK models for neonates with HIE undergoing TH, thereby optimizing the pharmacotherapy of HSA-bound drugs in this vulnerable population. Drug–protein binding is an important factor in the pharmacotherapy of, especially, highly protein-bound drugs, as it influences a drug's PK–PD relationship.[16]

We recognize that the clinical relevance of drug–protein binding in pharmacotherapy has been disputed in the literature.[30–32] However, the contention seems mostly to surround a drug's degree of plasma protein binding and distribution volume.[33] Consequently, there is no general guideline indicating the clinical relevance of drug–protein binding and its effect on a drug's pharmacological activity. Rather, a drug-specific approach considering the individual patient's characteristics and pathology should be considered.[30]

Hence, this debate reiterates the utility of PBPK models in the pharmacotherapy of protein-bound drugs. Anti-epileptics such as phenytoin, midazolam, and diazepam have a high protein-bound percentages

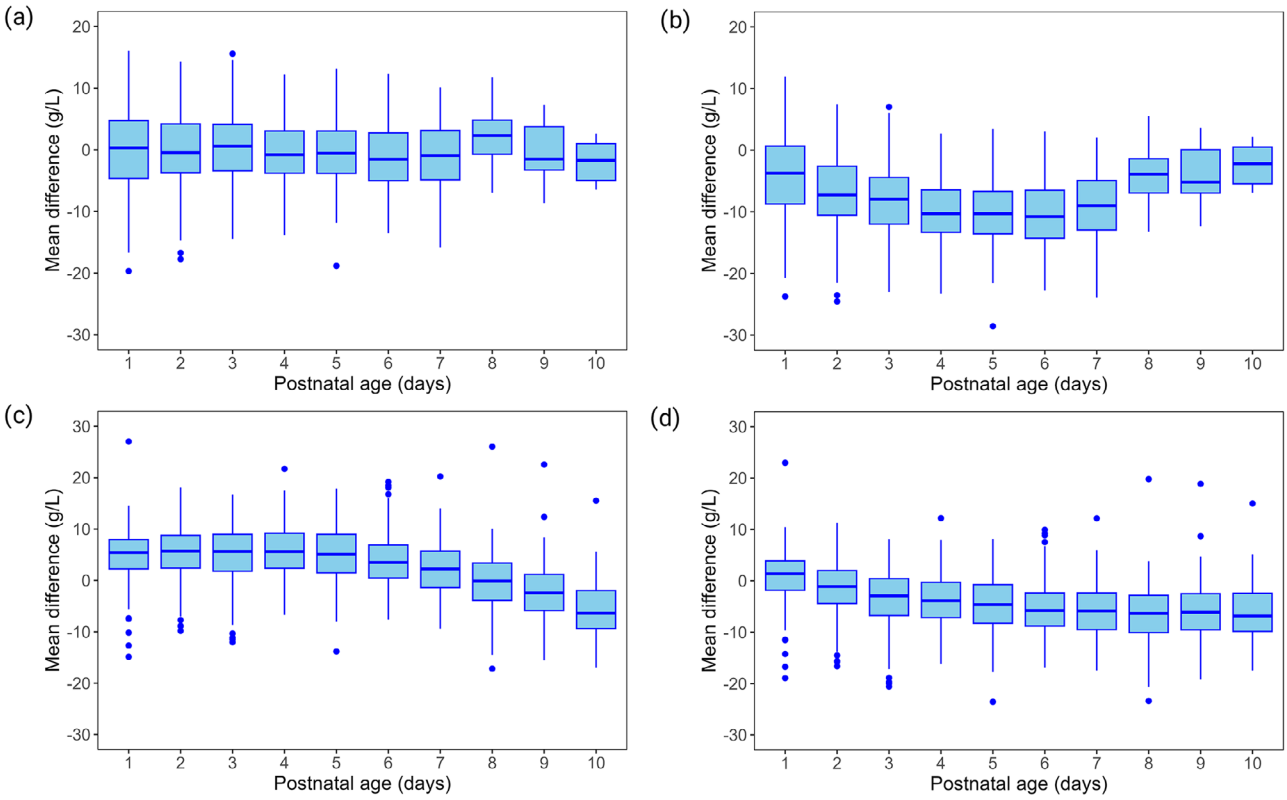


Figure 3. Mean difference in human serum albumin concentrations (g/L) between observed concentrations of the therapeutic hypothermia cohort and (a) AlbuCool predictions and (b) Simcyp predictions (Simcyp simulator version 22, release 1); and between observed concentrations of the control cohort and (c) AlbuCool predictions and (d) Simcyp predictions over postnatal age. Data are presented as boxplots.

(92%, 97%, and 97.2%, respectively) and are frequently used in the management of neonatal and pediatric seizures.[33,34] Antibiotics such as vancomycin or cefazolin have both been shown to have higher unbound fractions in neonates and to be partly influenced by albuminemia.[18,35,36] A PBPK model may help optimize dosing adjustments of these drugs for asphyxiated neonates undergoing TH.

Albuminemia is not the only factor influencing drug–protein binding in neonates. Bilirubin displacement decreases albumin binding because the association constant of bilirubin for albumin was reported to be 100 to 1000 times higher than for most drugs.[31] Thus, neonatal hyperbilirubinemia may result in a higher unbound drug fraction, thus warranting a lower dose to provide a similar therapeutic drug effect as in adults.[31] Additionally, modifications of the N-terminus of HSA may occur in ischemic conditions, resulting in ischemia-modified albumin which is considered a diagnostic marker for different types of ischemia.[37]

Obviously, this study has its limitations. Foremost, clinical data were collected retrospectively and may be subject to research bias. Second, no additional data were collected concerning co-existing diagnoses or therapeutic interventions (such as concomitant medications, albumin supplementation, total parenteral nutrition, blood product transfusions, and fluid administration practices), all potentially influencing HSA concentrations. Third, a different HSA assay was used in NNUH from 2008 onward. The assay with bromocresol purple typically gives lower values compared to bromocresol green.[38,39] Fourth, bio-analytical information from three UK hospitals (AH, LWH, and SMH) were unavailable, necessitating caution when extrapolating study results. Finally, the AlbuCool function proposed in this paper has not yet been subjected to external validation, raising hesitation regarding the generalizability of results. We recently published recommendations for standardized reporting of laboratory data of neonates.[40] These recommendations outlined a set of core information that we consider should always be included in scientific publications reporting laboratory data of neonates. However, the data presented in our present study were collected prior to the publication of these recommendations, and we concede that much of this core information was therefore missing in our dataset. As a result, certain key elements such as blood product transfusions and bio-analytical information for certain hospitals could not be reported in this study.

While we have failed to fully respect these recommendations for the current study, we strongly advise that researchers consult these recommendations before starting data collection to ensure that all essential information be captured, resulting in interpretable and actionable results. The strengths of this study include the collection and analysis of a large, multicenter dataset focusing on a special patient population of near-term/term neonates with HIE undergoing TH.

The future perspective of this study is to integrate this function in PK models, and to contribute to the prospective development of a representative and clinically relevant PBPK framework, based on real-world data, to improve the pharmacotherapy in neonates with HIE undergoing TH. A next step would be to validate the AlbuCool function with external datasets, and to apply the function in PK drug exposure predictions. To accomplish this goal, additional real-world data in this specific population should be collected and analyzed.

Conclusions

By compiling a large, real-world, multicenter dataset of neonates with HIE undergoing TH, we have analyzed HSA and TP trends compared to controls. We developed and present a mathematical HSA and TP function for neonates with HIE undergoing TH for future integration in PK models, to optimize the predictive performance of these models, and consequently, the pharmacotherapy of HSA-bound drugs in this special and vulnerable patient population.

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Author Contributions

Protocol development: Zoë Vander Elst, Thibault Stultjens, Anne Smits, and Karel Allegaert. Data acquisition: Zoë Vander Elst, Thibault Stultjens, Paul Clarke, Gozdem Kaykı, Nadir Yalçın, Anne Smits, and Karel Allegaert. *Data analysis*: Annouschka Laenen, Zoë Vander Elst, Thibault Stultjens, Anne Smits, and Karel Allegaert. *Draft of manuscript*: Zoë Vander Elst, Thibault Stultjens, Anne Smits, and Karel Allegaert. *Review of manuscript*: Zoë Vander Elst, Thibault Stultjens, Pieter Annaert, Paul Clarke, Isabek Iglesias-Platas, Elisabeth Agathos, Gozdem Kaykı, Nadir Yalçın, Annouschka Laenen, Anne Smits, and Karel Allegaert. All authors critically reviewed the manuscript and agreed to publish it.

Conflicts of Interest

The authors declare no conflicts of interest.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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