Dynamic prediction of bleeding risk in thrombocytopenic preterm neonates

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SUPPLEMENTARY ONLINE ONLY MATERIALS

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Supplementary materials

Table S1: list of potential predictors identified in literature search (ranked by number of papers).

Description	Code	Number of papers	Description	Code	Number of papers
mode of delivery		100	sodiumbicarbonate	CO	11
gestational age		100	necrotizing enterocolitis		11
antenatal corticosteroids		99	coagulation	NM	11
birth weight		89	hematocrit		11
anyything related to ventilation		87	body temperature		11
Apgar scores		62	maternal smoking		10
chorioamnionitis		60	parity		10
surfactant		59	postnatal doppler	NM	10
gender		57	abruptio placentae	RA	9
anything related to hemodynamics / shock		54	phenobarbital	RA	9
patent ductus arteriosus		52	interleukin 6	NM	9
preeclampsia		44	red blood cells		9
includes ph, lactatae, BE, etc		44	nucleated red blood cells or erythroblasts		9
PROM		43	suspected fetal distress	NR	8
sepsis		34	beginning of labor (induced, spontaneous)		8
postnatal corticosteroids		31	nitric oxide	RA	8
respiratory distress syndrome		30	premature contractions	CO	7
platelets or platelet tx		29	timing of delivery		7
tocolysis		27	intubation in delivery room	CO	7
multifetal pregnancies		27	sodium		7
pneumothorax		23	white blood cell count		7
maternal age		22	clinical risk score for babies	NR	7
fetal heart rate reactivity	NR	21	prenatal care	NC	6
doppler		20	maternal fever	CO	6
inotropic agents		20	ethamsylate	RA	6
inborn versus outborn	NC	18	triplets	RA	6
twins	CO	16	resuscitation		6
interhospital transport	CO	16	seizures		6
maternal bleeding		15	SNAP score	NR	6
fetal position (breech, vertex)		15	abruptio placentae or placenta praevia	RA	5
indomethacin		15	chorionicity		5
SGA	CO	15	vitamin E	RA	5
Genes	NM	15	pulmonary hemorrhage		5
RBC transfusion		14	hypothermia	CO	5
antenatal magnesium		14	placenta	NM	4
resuscitation at birth		14	maternal diabetes		4
ethnicity		13	maternal phenobarbital	RA	4
Mode of conception		13	maternal alcohol use		4
maternal sepsis		12	antenatal indomethacin		4
IUGR	CO	11	meconium		4
maternal drugs		11	vitamin A	RA	4

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Description	Code	Number of papers	Description	Code	Number of papers
erythropoietin	RA	4	umbilical cord clamping	NR	2
opioids		4	MOD triplet	RA	2
hyperglycemia	CO	4	acidemia	CO	2
periventricular leukomalacia	TE	4	birthorder		2
thyroid	RA	4	antihypertensives	CO	2
ureaplasma infection	CO	4	head circumference	NC	3
gravida		4	bronchopulmonary dysplasia	TE	2
blood glucose disorders		4	apnea	NR	2
typecaregiver	NR	4	creatinemia	NM	2
NIRS en FTOE (fractional tissue oxygen extraction)	NM	4	insulin-like growth factor	NM	2
intraventricular hemorrhage	OT	4	neonatal leukemoid reaction	RA	2
vena cava superior flow	NM	4	creatine kinase	NM	2
ECMO	RA	4	AST, LDH, CK, HBDS, ASAT etc	NM	2
umbilical line placement	NC	4	interleukin 8	NM	2
maternal aspirin	RA	3	incubators	NR	2
maternal vitamin K	RA	3	type of NICU	NC	2
maternal race	NR	3	potential better practices	NR	2
fetal heart rate monitoring	NR	3	nurse practicioner vs pediatric resident	NR	2
birth asphyxia		3	TTS	RA	2
interval between fetuses in multifetal pregnancy	RA	3	clinical judgement (threatened, stable)	NR	2
active labor	NR	3	recurrent apnoe / bradycardia	NR	2
duration of labor	NR	3	maternal bethasone	CO	1
heparin	RA	3	maternal magnesium sulfate and aminophylline	RA	1
activin A	NM	3	maternal floor infarction	RA	1
bilirubin	NC	3	maternal transplantation	RA	1
neutropenia	CO	3	maternal hepatitis	RA	1
infectious agents		3	maternal beta sympathicomimetics	RA	1
potassium		3	maternal antiphospholipid syndrome	RA	1
C-reactive protein		3	perinatal care	NC	1
repeat suctioning	NR	3	maternal toxemia	CO	1
EEG	NM	3	maternal genital tract flora	NM	1
maternal SLE	RA	2	amount of amniotic fluid	NC	1
maternal asthma	RA	2	placenta weight		1
cerclage in triplet gestation	RA	2	placenta perfusion defect	NM	1
HELLP	CO	2	maternal medication	ND	1
maternal education	NR	2	antenatal corticosteroids in combination with antibiotics	CO	1
maternal infection as an indication for delivery	CO	2	maternal chronic disease (not specified)	RA	1
placenta infarction	RA	2	maternal pregnancy related disease	CO	1
idiopathic preterm labor	CO	2	cervical incompetence	NR	1
maternal anaesthetics		2	cervical cerclage	RA	1
maternal socio economic status	NR	2	amniocentesis	RA	1
maternal use of 17-hydroxyprogesterone	RA	2	PROM in combination with chorioamnionitis	CO	1
birth induction (iatrogenic preterm birth)	CO	2	maternal drugs and smoking	CP	1

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Description	Code	Number of papers	Description	Code	Number of papers
history of abortion	RA	1	nuchalcord	RA	1
maternal epidural paincontrol	CO	1	deliveryrisk	ND	1
maternal urinary tract infection	CO	1	homebirth	RA	1
previous adverse pregnancy outcome	NR	1	DOB		1
uncomplicated pregnancy	NR	1	TOB		1
maternal body mass index	NR	1	wrap after birth for temperature control		1
maternal weight gain	NR	1	umbilical cord milking	NR	1
maternal Hb	NM	1	trial of labor after CS	RA	1
maternal Ht	NM	1	probiotics	RA	1
maternal platelet	NM	1	amphotericin	RA	1
mproteinuria	CO	1	EACA during ECMO	RA	1
idiopathic preterm labor or PROM	CO	1	emollient	RA	1
cervical width on admission	NR	1	ascorbicacid	RA	1
length of prepartum hospital stay	NR	1	alpha proteinase inhibitor	RA	1
maternal anti epileptics	RA	1	immuneglobulins	RA	1
maternal trombocytopenia	RA	1	tranexamic acid	RA	1
maternal serum thromboxane B2 concentrations	NM	1	ibuprofen		1
antenatal corticosteroids in combination with vit K	NC	1	docosahexaenoic acid	RA	1
PROM and oligohydramnios	NR	1	dopamin vs hydrocortison	CO	1
twinantcorts	CO	1	epinephrine	CO	1
antcortstoco	CO	1	diuretics	RA	1
Other causes for preterm birth, (eg prenat diagn malformation)	ND	1	antibiotics		1
unknown cause of preterm birth	CO	1	opioids plus muscle relaxant	RA	1
fetal inflammatory response (placenta histology)	CO	1	musclerelaxants	RA	1
biophysical profile	CO	1	tolazoline	RA	1
antenatal thyroid releasing hormone	NM	1	alkali	RA	1
maternal hyperuricemia	RA	1	vitaminK	NC	1
month of birth	NA	1	ambroxol	RA	1
PPROM guideline	NR	1	buffer	RA	1
bruising postpartum	NR	1	analgesia	RA	1
MOD in hemophilia	RA	1	fluconazol		1
umbilical cord abnormal	ND	1	insulin	RA	1
prolonged second stage of labor	NR	1	macrosomy	CO	1
shoulder dystocia	RA	1	twin with 1 anomalous fetus	RA	1
mode of labor	CO	1	congenital anomaly	RA	1
prolonged labor	NR	1	reduced multifetal pregnancy	RA	1
precipitous delivery (quick delivery, <3 hours)	NR	1	discordant twins (vs non-discordant)	RA	1
unattended delivery	RA	1	postconceptional age	CO	1
placenta accreta plus meconium	RA	1	discordant triplets (vs non-discordant)	RA	1
placenta infarction plus amnionitis	RA	1	meningitis	RA	1
prolapsed cord	RA	1	pathological icterus (nieuwe variabele)	NC	1
no spontaneous respiration at 5 min		1	diffuse intravascular coagulation		1
			retinopathy of prematurity	TE	1
			pulmonary interstitial emphysema	CO	1

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Description	Code	Number of papers	Description	Code	Number of papers
hypoglycemia	CO	1	extubation	CO	1
pneumonia		1	biochemical pulmonary assessment	NM	1
neonpulmcompl	CO	1	paralysis during ventilation	RA	1
metalloprotease	NM	1	biochemical long maturity and gestational age	NM	1
lymphocytes	NM	1	irregular respiration	NR	1
mannose-binding lectin	NM	1	fresh frozen plasma		1
hemopoietic stem cells	NM	1	based on genetic mutations and homocysteine levels	RA	1
Erythropoietine and interleukin 6	NM	1	conjunctival hemorrhage	RA	1
immune proteins and cytokines	NM	1	retinal hemorrhage	RA	1
Free radicals	NM	1	exchange transfusion	RA	1
lactate and base excess	CO	1	plasmanate	CO	1
genetic polymorphisms of antioxidant enzymes	NM	1	periventricular bleeding	TE	1
homocysteine	NM	1	gastro-intestinal surgery	OT	1
ADAMTS13	NM	1	rectal bleeds guideline	NR	1
paCO2	CO	1	vaccinations	TE	1
antioxidants	NM	1	HELPP and Preterm	CO	1
antithrombin III	NM	1	MOD in triplets	RA	1
enolase	NM	1	weight improvement program	NR	1
IL1a	NM	1	digital cervical examination	NR	1
IL1b	NM	1	corticosteroids both antenatal and postnatal	CO	1
tumor necrosis factor	NM	1	intrauterine myelomeningokele repair	RA	1
osmolality	NM	1	candida infection	RA	1
calcium	NM	1	nasal CPAP + minimal handling	NR	1
hypoxanthin	NM	1	influence of birth weight on bleeding risk during ECMO	RA	1
xanthin	NM	1	multiple risk factors for bleeding during ECMO	RA	1
VEGF	NM	1	cathether position	NR	1
adrenomedullin	NM	1	renal injury in asphyxiated newborn infants	RA	1
S100protein	NM	1	enteral feeding	NC	1
brain derived neurotrophic factor	NM	1	antenatal and postnatal phenobarbital	CO	1
interleukin 12	NM	1	cardiac arrest before ECMO	RA	1
nursing excellence	NR	1	mode of ECMO	RA	1
after-hours in house senior physician cover	NR	1	breast milk	NR	1
environmental temperature	NR	1	bpm	NR	1
organizational quality of NICU	NR	1	cardiac markers e.g. troponin, pro-BNP	NM	1
fetal vs neonatal growth charts	OT	1	enrollment bias	OT	1
height of NICU	NC	1	weight during ECMO	RA	1
study participation	OT	1	consanguin parents	NR	1
individualized care	NR	1	age at intubation	CO	1
outpatientcare	CO	1	age at admission to NICU	NC	1
outborn	CO	1	age at surfactant administration	CO	1
active IVH surveillance methods	NR	1	surgery	OT	1
minimal handling	NR	1	congenital heart disease	RA	1
IVH prevention protocol	NR	1			

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Description	Code	Number of papers
chesttubes	RA	1
healthy versus entire population (BW curves study)	OT	1
full fontanel	NR	1
abnormal eye signs (e.g. nystagmus)	NR	1
decreased tone	NR	1
change in activity (spontaneous movement)	NR	1
abnormal movement or posture	NR	1
targeted neonatal echocardiography	NM	1
	NM	1
fentanyl versus dexmedetomidine	RA	1
laboratory samples drawn from placenta vs baby	NM	1
neonatal resuscitation program team training	NR	1
NAITP	RA	1
enemas	RA	1
maternal BMI impact on triplets	CO	1
discordant doppler velocimetric findings in twins	RA	1
neonatal status score	NR	1
outpatient and chorioamnionitis	RA	1
guideline for preeclampsia	NR	1

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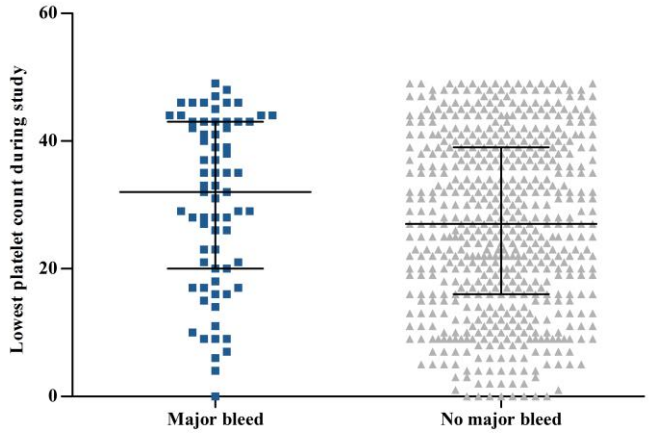
Table S2: additional information about the model variables.

Variable	Definition	What was entered into the model at each landmark point
Postnatal age	Age in hours since time of birth	Age in hours (baseline variable)
Gestational age	Gestational age as reported in medical files	Gestational age in days (baseline variable)
IUGR	Birthweight below the 10^{th} centile according to Dutch national birth weight curves	IUGR yes/no (baseline variable)
Mechanical ventilation	A neonate was deemed as being mechanically ventilated when he or she was intubated, irrespective of ventilation type, ventilator settings and duration of ventilation.	Mechanically ventilated yes/no
Platelet count	Every platelet count was recorded in the database as count $x10^9$/L.	Most recent platelet count
Platelet transfusion	Every platelet transfusion was recorded in the database, including dose.	Transfusion given within 2 hours after landmark point yes/no[1]
NEC/sepsis (combined)	NEC was defined as ≥ grade IIA as per Bell's criteria.[1] Sepsis was defined as culture positive sepsis or culture negative sepsis where antibiotics are given for a minimum of 5 complete days	NEC/sepsis yes/no. If either NEC or sepsis, are present, answer yes.

[1] We included transfusion after, not before, the landmark point into the model, because we wanted clinicians to be able to calculate bleeding risk with and without giving a platelet transfusion. This could potentially induce immortal time bias, but since the time interval is relatively short compared to our prediction window (2 versus 72 hours), we deemed this risk negligible. We did not present this feature of the model in the main paper, because the combined hazard ratios of transfusion and the interaction term of transfusion and platelet count suggest that transfusions are associated with increased bleeding risk in all neonates. We hypothesise that this is partially caused by the fact that we did not adjust for all possible confounders, due to the limited number of events in our study, though a true adverse effect of transfusion cannot be ruled out.

Figure S1: lowest platelet count during study for neonates with and without major bleed.



Legend figure S1. This scatterplot represents the lowest platelet count during study for neonates with and without major bleeding. For neonates with major bleeding, end of study was defined as the major bleed, therefore this platelet count represents the lowest platelet count prior to major bleeding. Lines represent median and interquartile ranges.

Figure S2: gestational age at birth in neonates with and without major bleeding.

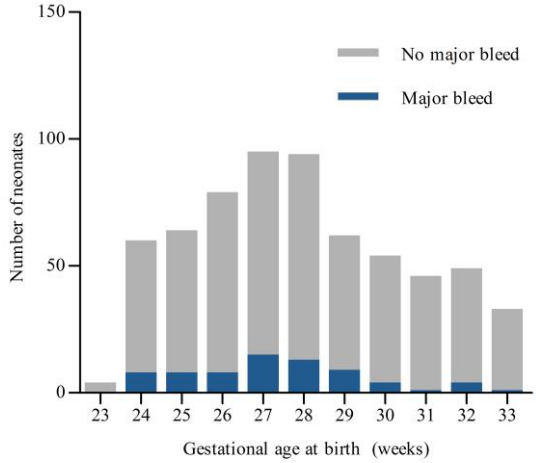


Figure S3: postnatal age at onset of severe thrombocytopenia in neonates with and without major bleeding.

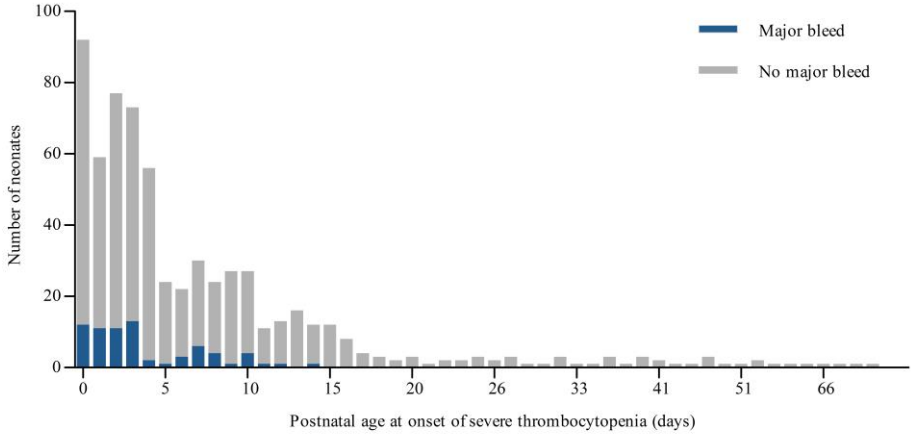


Table S2: sensitivity analyses

Name	Description	Results and interpretation
Timing accuracy	In our primary analysis, all variables were included irrespective of whether time of event was known exactly (+/- five minutes), or was estimated (range: +/- 30 minutes to +/- 12 hours). In this sensitivity analysis, we only included patients if 100% of their event times had a maximum uncertainty of +/- 30 minutes.	This left 308 neonates in the model, with 41 major bleeds. Minor changes in covariate hazard ratios indicate that timing inaccuracies did not substantially influence our primary model.
Major bleed plus mortality	In our primary analysis, our outcome was major bleeding. In this sensitivity analysis, our outcome was a composite of major bleeding and mortality.	136 neonates reached this composite endpoint within ten days after T_0. Minor changes in covariate hazard ratios indicate that our model predicts a composite outcome of major bleeding and mortality as well as it predicts major bleeding alone.
Model without grey areas	In our primary model, events that occurred after an ultrasound that showed no major bleed, but prior to an ultrasound that showed a major bleed, the so-called *grey area*, were included. In this sensitivity analysis, we excluded those, because we could not know whether these happened prior to or after the bleed.	Grey areas ranged from zero to ten days. Minor changes in covariate hazard ratios indicate that the uncertainty of the timing of events within these 'grey area's' did not substantially influence our primary model.
Revised start-time of major bleeding	In our primary analysis, the time of major bleed was defined as the time on which a bleeding was classified as major for the first time. In this sensitivity analysis we looked at the ultrasounds prior to the major bleeding to see if the bleeding had already started (minor bleed on previous ultrasound scan). If so, we changed the time of major bleed accordingly.	This left 635 neonates in the model, with 65 major bleeds. Minor changes in covariate hazard ratios indicate that improving our estimation of the time of bleed did not substantially improve our primary model.
Thrombocytopenic episode only	In our primary analysis, neonates reached end of study at time of discharge, death or major bleeding. In this sensitivity analysis, end of study is defined as the end of severe thrombocytopenia plus an additional three days, a window of time during which the effect of thrombocytopenia might still be present.	This left 58 major bleeds in the model. Minor changes in covariate hazard ratios indicate that our model has good predictive power even after platelet counts return to normal.
Landmarks every hour	In our primary analysis, landmarks were set at every two hours. In this sensitivity analysis, landmarks were set at every hour, to assure accurateness of order of events (events prior to or after landmark points).	Minor changes in covariate hazard ratios indicate that changing the number of landmarks did not substantially impact our model.

Table S3: sensitivity analysis (continued)

Sensitivity analysis model	Timing accuracy	Major bleed plus mortality	Model without grey areas	Revised start time of major bleeding	Thrombocytopenic episode only	Landmark every hour
Covariates with time-constant effects						
Gestational age (days)	1·01 (0·99 – 1·04)	0·99 (0·98 – 1·01)	1·00 (0·99 – 1·02)	1·00 (0·99 – 1·02)	1·01 (0·99 – 1·02)	1·00 (0·98 – 1·02)
Postnatal age (days)	0·95 (0·89 – 1·01)	0·96 (0·93 – 1·00)	0·88 (0·82 – 0·94)	0·89 (0·84 – 0·95)	0·89 (0·84 – 0·94)	0·88 (0·83 – 0·94)
Mechanical ventilation	7·47 (2·82 – 19·78)	3·87 (2·34 – 6·40)	4·43 (1·81 – 10·80)	4·82 (2·04 – 11·35)	5·29 (2·18 – 12·82)	4·18 (1·83 – 9·52)
NEC/sepsis	0·86 (0·38 – 1·94)	0·72 (0·47 – 1·08)	0·89 (0·43 – 1·84)	0·72 (0·37 – 1·42)	0·81 (0·41 – 1·59)	0·80 (0·42 – 1·53)
Platelet transfusion	0·58 (0·15 – 2·20)	0·55 (0·26 – 1·13)	0·39 (0·05 – 3·03)	0·88 (0·30 – 2·57)	1·10 (0·38 – 3·21)	1·05 (0·35 – 3·14)
Interaction platelet count and transfusion	1·89 (0·78 – 4·56)	1·73 (1·06 – 2·82)	1·67 (0·46 – 6·00)	1·35 (0·67 – 2·72)	1·18 (0·59 – 2·37)	1·17 (0·57 – 2·42)
Covariates with time-varying effects						
IUGR Constant	0·53 (0·14 – 1·97)	0·48 (0·23 – 0·99)	0·23 (0·05 – 1·04)	0·61 (0·21 – 1·77)	0·49 (0·16 – 1·52)	0·57 (0·20 – 1·68)
IUGR Time-varying: LM	0·59 (0·19 – 1·86)	1·05 (0·59 – 1·87)	0·41 (0·09 – 1·82)	0·28 (0·09 – 0·93)	0·35 (0·11 – 1·08)	0·25 (0·08 – 0·85)
IUGR Time-varying: LM2	1·10 (0·92 – 1·31)	1·01 (0·96 – 1·15)	1·21 (0·98 – 1·51)	1·26 (1·05 – 1·50)	1·21 (1·02 – 1·42)	1·28 (1·07 – 1·53)
Log10 platelet count Constant	2·89 (0·66 – 12·56)	0·96 (0·43 – 2·11)	2·08 (0·65 – 6·64)	2·42 (0·91 – 6·44)	2·17 (0·76 – 6·15)	2·07 (0·84 – 5·14)
Log10 platelet count Time-varying LM	0·24 (0·08 – 0·71)	0·44 (0·27 – 0·72)	0·37 (0·16 – 0·87)	0·25 (0·13 – 0·48)	0·28 (0·14 – 0·58)	0·28 (0·14 – 0·56)
Log10 platelet count Time-varying: LM2	1·19 (1·00 – 1·41)	1·09 (1·03 – 1·17)	1·10 (0·98 – 1·23)	1·17 (1·06 – 1·30)	1·16 (1·03 – 1·30)	1·16 (1·05 – 1·29)

Coefficients are expressed as hazard ratio (95% confidence interval).

Extended methods section

The study protocol was published online on www.clinicaltrials.gov (NCT03110887). The institutional review board of the Academic Medical Center Amsterdam approved the study and waived the need for informed consent, since the study involves retrospective datacollection. The study was conducted in accordance with the Declaration of Helsinki and reported according to The Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) guidelines.[2]

Population

We performed a cohort study among consecutive preterm neonates with thrombocytopenia admitted to any one of seven participating NICU's in the Netherlands between January 2010 and January 2015. The cohort comprised all neonates with gestational age at birth < 34 weeks and at least one platelet count < $50x10^9$/L. The NICU's were located in the Leiden University Medical Center, Academic Medical Center Amsterdam, Máxima Medical Center Veldhoven, Isala Zwolle, Erasmus Medical Center Rotterdam, University Medical Center Utrecht and University Medical Center Groningen. We excluded patients with 1) severe congenital malformations; 2) a high suspicion of spurious platelet count (e.g. clots in the sample, or spontaneous platelet count recovery within six hours, or a platelet count labelled as spurious in the medical file); 3) thrombocytopenia occurring exclusively in the context of exchange transfusion; 4) prior admission to another NICU or readmission, and 5) major bleeding prior to severe thrombocytopenia. Neonates with major bleeding after end of follow up were not excluded, but registered as not having experienced major bleeding during the study.

Selection of potential predictors

We chose the predictors for our model prior to data analysis~~,~~ under supervision of a professor of clinical epidemiology and head of clinical transfusion research center. Five experts (a paediatric hematologist and senior investigator with extensive experience in neonatal hematology studies, a pediatric hematologist and transfusion specialist in training, two neonatologist (of which one senior investigator with extensive experience in neonatal hematology studies) and a PhD student with an MD degree selected variables from a literature-based list of potential prognostic factors. The list was based on an large literature search yielding over 8000 abstracts. 360 risk factors were identified from the abstracts and ranked according to number of publications per risk factor (Table SI). A variable was excluded from this list when it was not consistently documented in medical records, when few studies concerning this variable had been published, when a strong interaction with another variable was expected, when it was rare or too prevalent (occurring in <5% or >95% of our study population) or when the

variable was not measured routinely in clinical practice. All remaining risk factors (n=74) were further reviewed by the experts, who then voted for risk factors deemed to be good predictors for major bleeding. Based on the number of votes per risk factor we included the following variables in the model: gestational age, intra uterine growth retardation (IUGR), mechanical ventilation, platelet count, platelet transfusion, postnatal age at inclusion, and necrotizing enterocolitis (NEC) and/or sepsis (combined) (Table SII). Despite the lack of evidence for a direct causal assocation between platelet count and bleeding, platelet count was included, because ultimately, our aim is to investigate which (if any) subgroups of neonates with thrombocytopenia benefit from platelet transfusions. Therefore it is essential for platelet count to be part of the prediction model. Platelet transfusion within the next two hours following the moment of bleeding risk prediction was included in the model to allow for calculation of two bleeding risks: one with and one without administration of a transfusion. NEC was defined as ≥ grade IIA as per Bell's criteria.[1] Sepsis was defined as culture positive sepsis or culture negative sepsis where antibiotics are given for a minimum of 5 complete days, to allow for use of the prediction model early in the course of sepsis, when culture results are not yet available. NEC and sepsis were combined because at onset, it is often difficult to distinguish between NEC and sepsis. Combining them allows for use of the prediction model despite this uncertainty.

Main outcome definition

The main outcome of this study was major bleeding, defined as either one of the following:

1. Intraventricular hemorrhage (IVH) grade 3 (according to the Papile grading system);[3]
2. IVH of any grade in combination with parenchymal involvement;
3. Parenchymal hemorrhage (without IVH) visible on ultrasound scan;
4. Cerebellar hemorrhage visible on ultrasound scan;
5. Pulmonary hemorrhage, defined as fresh blood from the endotracheal tube in combination with increased ventilatory requirements;
6. Any other type of hemorrhage, if major. A bleeding was considered major if it required or if it was associated with either one of the following: a) red blood cell transfusion, b) volume boluses, c) need for inotropes (either start of inotrope therapy, or increased dose of current therapy), d) significant drop in blood pressure (mean blood pressure less than gestational age).

Clinical practice in the seven participating centers

In general, national protocols recommended that cranial ultrasound scans in preterm neonates were made on day of life 1, 3, 7 and then biweekly until discharge, and additional scans when clinically indicated. National platelet

transfusion protocols recommended transfusion at a platelet count threshold of $20x10^9$/L. A higher threshold of $50x10^9$/L was recommended in case of active bleeding, surgery, after exchange transfusion, or for a clinically unstable neonate of <1500 grams and <32 weeks gestational age at birth. A threshold of $100x10^9$/L was recommended prior to exchange transfusion. No national guidelines existed with regards to frequency of platelet count measurements, except for counts immediately prior to and within 24 hours after platelet transfusion. There was some variation in discharge policies between centers, depending on the presence of high care neonatal units in the vicinity of the NICU.

Data acquisition

Neonatologists, PhD students and medical students collected the data in an online GCP approved database. All received training to ensure data quality. We collected data from electronic and paper patient records on site. Start of the study (T_0) was defined as the first moment at which platelet counts dropped $<50x10^9$/L . End of study was defined as the time of a major bleed, death or discharge/transfer, whichever occurred first. All events were recorded with date and time in hours and minutes. If the exact time of an event was unknown, an estimate was reported. We included neonates once their platelet count dropped below $50x10^9$/L, and followed them for 10 days, irrespective of their platelet counts. If they developed another episode of thrombocytopenia after these 10 days, they were not re-included. Every ultrasound scan report was entered into the database. MRI results were not used to identify major bleeding, as only a small selected subset of neonates receives MRI scans, and ultrasound scans are generally considered to detect major bleeding accurately. Antepartum scan results were not recorded. We extracted platelet counts from the electronic hospital systems and checked for spurious platelet counts. Several hospitals provided electronic baseline data (e.g. GA, birth weight, date of birth, etc) from a national neonatal database, which we extracted and uploaded into the study database. We manually entered all additional clinical data. Discharge letters and ultrasound scan reports were screened for major hemorrhages.Site principal investigators reviewed the data concerning major bleeds to confirm accuracy of grading and timing.

Sample size calculation

Various studies showed bleeding incidences in premature neonates of 7-11%.[4–7] Assuming an event rate of ten percent, and using an event per variable ratio of ten, we would need to include 100 neonates for each variables included in the model. Data were available from 7 NICU's over a period of 5 years. Each year, 2800 neonates are admitted to the participating NICUs, of which approximately five percent have severe thrombocytopenia. Therefore, we expected 140 eligible neonates each year, and a sample size of 700.

Statistics

The core research team drafted and approved a statistical analysis plan prior to data analysis. We developed a proportional baselines landmark supermodel, as described elsewhere, with bleeding within the next three days as outcome.[8] At each two hour timepoint, all available data were entered into the model (Table SII). We used a full model approach and did not remove non-significant predictors.[9] We included 7 main variables and an interaction term between platelet transfusion and platelet count, because we hypothesized that the association between platelet transfusions and bleeding may become stronger when platelet counts are lower. In order to test for time-varying covariate effects, significant interactions between covariates and landmark times (both linear and quadratic) were also included in the model. Missing data were replaced by missing indicators. With this predictionmodel, risk of bleeding at any time point within seven days could be calculated. Because the last model (at day seven) also predicts bleeding within three days, the total duration of follow up was ten days. Follow up was stopped after 10 days for two reasons: 1) we expected the number of neonates to develop major bleeding after more than 10 days of onset of thrombocytopenia to be low, and 2) after 10 days, many neonates would be discharged, and follow up would be very incomplete, hampering accurate analysis.

We validated the model by internal calibration using the heuristic shrinkage factor by van Houwelingen et al.[10] When calculating bleeding risk probabilities from the model, we accounted for competing risk due to death using the Aalen-Johansen estimator.[11] We did not correct for discharge or transfer, as we assumed that neonates who were discharged or transferred did not develop a major bleed. We performed various sensitivity analyses in order to test the robustness of the model. (Table SIII)

We evaluated the model's accuracy in correctly discriminating between patients with and without major bleeding using the dynamic cross-validated c-index. A c-index of 1.0 indicates perfect discrimination, while a c-index of 0.5 is obtained when the model performs as well as chance. We calculated a c-index at each two hour timepoint, and reported this series of c-indices as a graph.

Analyses were carried out using SPSS (version 24.0), Stata (version 14.1) and R (version 3.4.2).

Clinical applicability of the model

The process from initial prediction model development to implementation into clinical practice can be divided into multiple steps, as explained in the TRIPOD statement paper. The TRIPOD statement is a prediction model development checklist, which was endorsed by a large number of prominent medical journals.[2] The first step (model development studies) is the development of a basic first model in a cohort. The next step is validation of this model in another cohort (model validation studies). Finally, the model needs to be tested in a randomized controlled trial (impact studies), because we cannot assume that prediction based treatment will invariably

improve outcome.[12] Our study is a model development study. It is a first, basic prediction model for major bleeding in preterm neonates with severe thrombocytopenia. Due to the dynamic nature of the model, it cannot be fully summarized in one table, but once model validation studies have been performed, we will develop an online calculator. This calculator will perform the complex mathematical procedures required to convert the input of the seven variables into an absolute bleeding risk for a specific neonate at a specific time. We have chosen not to publish the calculator along with this paper, in order to prevent inappropriate premature use of the model in clinical practice. The model is available upon request for researchers looking to perform model validation and impact studies.

Role of the funding source

The funding source was not involved in the design, data collection, analyses and publication of this study. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

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