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Title page

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Abstract

Over 75% of severely thrombocytopenic neonates receive platelet transfusions, though little evidence supports this practice, and only 10% develop major bleeding. In a recent randomized trial, platelet transfusions given at a threshold of $50x10^9$/L compared to a threshold of $25x10^9$/L were associated with increased risk of major bleeding or mortality. These results emphasize the need for improved and individualized neonatal platelet transfusion guidelines, which require accurate prediction of bleeding risk. Therefore, the objective of this study was to develop a dynamic prediction model for major bleeding in thrombocytopenic preterm neonates. This model allows for calculation of bleeding risk at any time-point during the first week after onset of severe thrombocytopenia.

In this multicenter cohort study, we included neonates with a gestational age <34 weeks, admitted to a neonatal intensive care unit, who developed severe thrombocytopenia (platelet count $<50x10^9$/L). The study endpoint was major bleeding. We obtained predictions of bleeding risk using a proportional baselines landmark supermodel.

Of 640 included neonates, 71 (11%) developed major bleeding. We included the variables gestational age, postnatal age, intra-uterine growth restriction, necrotizing enterocolitis, sepsis, platelet count and mechanical ventilation in the model. The median cross-validated c-index was 0.74 (IQR 0.69-0.82).

This is a promising dynamic prediction model for bleeding in this population that should be explored further in clinical studies as a potential clinical decision support tool. The study was registered at www.clinicaltrials.gov (NCT03110887).

Introduction

Neonatal major bleeding occurs in approximately 5-15% of preterm neonates admitted to a neonatal intensive care unit (NICU) and can lead to lifelong disabilities and death. The most common type of bleeding is intraventricular hemorrhage (IVH).[1,2]

Since platelets are required for primary hemostasis, preterm neonates with severe thrombocytopenia are thought to be particularly at risk for major bleeding. However, the associations between thrombocytopenia, platelet transfusions and bleeding in preterm neonates are not clear. In a recently published systematic review, only six studies could be included. These provided insufficient evidence to assess whether platelet counts are causally related to major bleeding, or whether platelet transfusions reduce bleeding risk in thrombocytopenic preterm neonates.[3] Despite this lack of evidence, platelet transfusions are given to approximately 75% of thrombocytopenic preterm neonates.[4,5]

Recently, the first randomized trial assessing currently used platelet count thresholds in preterm infants was published. It showed that a prophylactic transfusion threshold of $50x10^9$/L was associated with increased risk of bleeding and mortality compared to a lower threshold of $25x10^9$/L, within 28 days after randomisation.[6] These results emphasize the need for improved and individualized neonatal platelet transfusion guidelines.

In addition to lack of evidence regarding transfusion thresholds and identification of platelet transfusion related harm, indications for platelet transfusions are based primarily on platelet count. However, two neonates with similar platelet counts but different clinical conditions may have a very different risk of bleeding, and benefit differently from platelet transfusions.[7] We need to be able to predict which neonates will develop major bleeding and quantify this bleeding risk, using a model that includes not only platelet count but also a set of relevant clinical variables. This prediction model could be used to define indications for transfusion in future studies, which is a first step towards individualized platelet transfusion therapy.

Some prediction models for bleeding in neonates have already been developed, but these models were not derived specifically for neonates with thrombocytopenia, and only allow for a risk assessment at baseline.[8–15] The disadvantage of baseline prediction models is that they do not take the clinical course of the neonate into account, which can change substantially over time, and may have a profound impact on bleeding risk. In dynamic prediction, the clinical course can be incorporated into the model. Therefore, the objective of this study was to develop a dynamic prediction model for major bleeding in thrombocytopenic preterm neonates.

Methods

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. 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.[16] An extended methods section is available in the Supplementary Materials, including the procedure for predictor selection, outcome definitions, a list of participating centers with an overview of clinical practice, description of the data acquisition process, sample size calculations, details on statistical methods and the role of the funding source.

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. 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.

Model development and statistics

The core research team drafted and approved a statistical analysis plan prior to data analysis. We developed a proportional baselines landmark supermodel, with bleeding within the next three days as outcome.[17] Variables included in the model were 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).

Model validation

We validated the model by internal calibration using the heuristic shrinkage factor by van Houwelingen et al.[18] 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

Our study 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 validation studies have been performed, we will develop an online calculator. 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.

Results

Baseline characteristics

Of 9333 neonates with a gestational age <34 weeks, 927 had at least one platelet count <50x10^9/L. Of these, 67 were excluded due to spurious platelet count and 29 because thrombocytopenia occurred only during a readmission. Of the remaining 831 neonates, 191 were excluded based on major bleeding prior to thrombocytopenia (55), previous admission to other NICU (51), congenital malformations (47), missing medical files (35) and because thrombocytopenia occurred exclusively during exchange transfusion (3). The remaining ~~a~~ 640 neonates (7%) were included in the study. (Figure 1) The median gestational age at birth was 28.1 weeks and median birth weight was 900 grams (Table I and Figure S1 and S2). 73% of neonates received at least one platelet transfusion. No cases of fetal and neonatal alloimmune thrombocytopenia (FNAITP) were identified. Lowest platelet counts during study for neonates with and without major bleed are reported in Figure S3.

Major bleeds

A total of 71 (11%) major bleeds occurred, of which 55 were intraventricular hemorrhages and other intracerebral hemorrhages, twelve were pulmonary hemorrhages and four were gastro-intestinal hemorrhages (Table II). The major bleeds occurred at a median of 1 day (interquartile range 1-4) after onset of severe thrombocytopenia. At the end of the ten day follow up period, 73 patients (11%) had died, 63 (10%) had developed major bleeding and 93 (15%) had been discharged or transferred (Figure 2). Of the 93 discharged neonates, 76 (82%) were discharged to a stepdown unit. 91% of neonates underwent at least one ultrasound scan, with a mean of two scans during the ten days follow up period. In four neonates, major intracranial hemorrhage was already diagnosed on the first ultrasound scan after birth, on the first day of life.

Model development

The model contained 12 variables: all seven selected variables, plus the interaction term between platelet count and transfusion, plus interactions between time and IUGR and time and platelet count (both linear and quadratic). Platelet count was converted to a log-scale. The number of major bleeds included in the model was 63, because eight bleeds occurred more than ten days after T_0 (Table II).

Final model

The median c-index of the final model was 0.74 (interquartile range 0.69 - 0.82) (figure 3). This indicates good predictive performance. An example of a risk-estimation by the model is shown in Figure 4, a plot of bleeding risk of two neonates with a distinct risk profile. During study day 1-3, the predicted risk of major bleeding within

the next 3 days in Child A is substantially higher than in Child B, indicating that use of this prediction model during that time-period would have correctly identified Child A as being at high risk of bleeding. This image also illustrates that bleeding risk can increase or decrease rapidly. Table III shows the details of the model. A hazard ratio > 1 indicates that increase of the risk factor is associated with higher risk of bleeding, and a hazard ratio < 1 indicates that increase of the risk factor is associated with lower risk of bleeding. The effects of platelet count and IUGR varied over time, while the effects of all other variables were constant over time. Table IV shows predicted risks of bleeding for different clinical scenarios.

Sensitivity analyses

None of the sensitivity analyses resulted in substantial changes in hazard ratios for the individual covariates, indicating that our model is robust (Table SIII).

Discussion

In this study, we developed a dynamic prediction model for major bleeding in thrombocytopenic preterm neonates. The model has good predictive performance with a median c-index of 0.74.

To our knowledge, this is the first dynamic prediction model for bleeding in preterm neonates. The importance of using a dynamic model is illustrated by a recent survey assessing at which thresholds clinicians would administer a platelet transfusion to a preterm neonate with a gestational age of 27 weeks at birth.[19] The study showed that if this neonate was two days old and in stable condition, most (European) clinicians would transfuse at a threshold of $30x10^9$/L. However, if the same neonate was septic, mechanically ventilated and receiving vasopressors, most clinicians would transfuse at a threshold of $50x10^9$/L. This illustrates that although neonates may have a comparable clinical status at baseline (gestational age 27 weeks), their clinical course in the following days is perceived as an important determinant of bleeding risk. We have developed a model that allows clinicians to quantify bleeding risk and adjust it as the clinical situation of the neonate changes.

Future validation studies should externally validate and preferably expand the model, to improve its predictive accuracy. Once a larger, externally validated model has been developed, it can be used to study the effect of platelet transfusion indications based on predicted risk of bleeding in an impact study. Ultimately, this is a first step towards individualized platelet transfusion guidelines. Individualized guidelines are important, because several studies have shown that there is a large discrepancy between the number of thrombocytopenic neonates receiving platelet transfusions (75%) and the number of neonates who develop major bleeding (9%).[5,20] These numbers are comparable to our results, where 70% of neonates received transfusions and 11% developed major bleeding. In addition, results of a recent randomized trial indicate platelet transfusion related harm when using a platelet count threshold of $50x10^9$/L compared to $25x10^9$/L. Although the overall results of this study show benefit associated with the low threshold, not all neonates in the high threshold group developed major bleeding or died. Moreover, 19% of neonates in the low threshold group died or developed major bleeding. This indicates that a platelet count based transfusion threshold does not accurately separate neonates whose bleeding or death will be prevented by a platelet transfusion. A threshold that includes clinical variables, such as one based on our dynamic prediction model, might perform better and thereby improve outcome.

It is important to note that individual covariates in the model should not be interpreted as causal associations, because the associations may be confounded in multiple ways. For example, IUGR was associated with lower predicted bleeding risk in our model, but we cannot conclude that IUGR protects against bleeding. Firstly, because IUGR is also a risk factor for thrombocytopenia, and we restricted our population to neonates with thrombocytopenia. It is possible that other causes of thrombocytopenia, for example viral infections, are associated with higher risk of bleeding than IUGR. A neonate with thrombocytopenia as a result of IUGR is therefore not protected by IUGR, but has lower bleeding risk because the thrombocytopenia was not caused by a viral infection. This is an epidemiological concept called collider stratification bias.[21] Secondly, perhaps neonates with IUGR received more treatments intended to decrease risk of bleeding as compared to neonates without IUGR, as neonatologists perceived them to be at higher risk of bleeding (confounding by indication). And lastly, because the number of events in our study was limited, we have not been able to correct for all possible confounders. In short, the association between IUGR and bleeding is complex, our model only indicates that it is a good predictor for bleeding, but we cannot draw any causal conclusion based on this information. This applies to all individual covariates in the model.

Various possible limitations of our study need to be discussed. Firstly, we could not externally validate our model because a similar database is currently not available. Secondly, identification of prognostic variables could possibly have been improved with a prior systematic review assessing all potential predictors. However, despite this limitation, our model contains variables generally considered best candidates for predicting major bleeding, as many of them were included in various existing baseline models. Some variables, such as mean platelet volume and immature platelet count, could not be included in our model because they were not routinely measured. Thirdly, the time a major bleed occurs is not similar to the time it is diagnosed on an ultrasound scan, because major intracranial bleeds in neonates are often asymptomatic, and detected during routine screening. To address this issue, we performed two additional sensitivity analyses, one in which we corrected time of bleeding based on whether or not minor bleeding was visible on prior ultrasound scans, and one in which we removed events of which we could not determine whether they occurred prior to or after the bleeding. Results of these analyses showed minor changes in hazard ratios of individual coefficients, suggesting that this problem does not substantially affect the predictive power of our model (Table SIII). Fourthly, after day six, the c-index drops below 0.60, possibly due to a lower event rate, therefore the model should be applied with caution after day six. We hypothesize that the variation in predictive accuracy over time as depicted in figure 2 may be caused by a

balance between having enough clinical information to predict (difficult on day 1 and 2), and enough events to fit a good model (difficult after day 4). Fifthly, the risk of bleeding in neonates in our population may have been affected by treatment with platelet transfusions. Therefore, the risks calculated using our model may be an underestimation of the 'true' risk (without transfusion). However, there are no cohorts available in which platelet transfusions were not administered and various studies including the previsouly described randomized controlled trial suggest that the effect of platelet transfusions on bleeding risk may be limited.[6,22–24] We therefore estimate that our model's predictions are accurate. Finally, four neonates had a gestational age at birth of less than 24 weeks. In addition, local policies differed with respect to active support for neonates born at a gestational age between 24+0 and 25+6 weeks. Therefore, the neonates with a gestational age less than 26 weeks in our population might be a selection of neonates for whom good outcomes were expected. The model should thus be applied with caution in neonates less than 26 weeks gestational age.

Strengths of our study are the size of the cohort and the fact that we have selected the predictors prior to data analysis and have not performed stepwise selection. In addition, we have performed meticulous data collection and multiple additional sensitivity analyses to confirm the robustness of our model. Our model is easy to apply, because we have used clear and simple definitions of the covariates. Once the model has been externally validated, we will develop an online calculator, with which it should only take a few minutes to enter the variables and calculate absolute risk of bleeding.

In short, this dynamic prediction model allows clinicians to quantify bleeding risk and adjust it as the clinical situation of the neonate changes. Risk can be predicted at any timepoint during the first week after onset of severe thrombocytopenia. This is a promising model that should be explored in future studies, as it is a first step towards individualized platelet transfusion guidelines.

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Authorship

Contribution**:** SFFG, KF, EL and JGvdB designed the study. SFFG and IR collected the data, in collaboration with PA, EJH, CVH, WO, AAK, DCV and EL. CCD prepared the data for analysis. HP analysed the data. SFFG, KF, EL, HP and JGvdB interpreted the data and wrote the report. All authors revised and approved the final report.

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Tables

Table I. Baseline characteristics (N=640)

	Total cohort (n=640)		Major bleed (n=71)		No major bleed (n=569)	
At birth						
Gestational age in weeks: median (IQR)[1]	28.1	(26.4-30.4)	27.7	(26.1-29.1)	28.1	(26.4-30.6)
Birth weight in grams: median (IQR)	900	(710-1180)	945	(760-1200)	900	(705-1178)
Intra uterine growth retardation, n (%)	206	(32)	14	(20)	192	(34)
At onset of severe thrombocytopenia						
Postnatal age in days: median (IQR)	3.9	(1.6-9.25)	2.6	(1.0-6.8)	4.1	(1.8-9.8)
Platelet count $x10^9$/L, median (IQR)	38	(29-45)	39	(31-44)	38	(28-45)
Mechanical ventilation, n (%)	329	(51)	49	(69)	280	(49)
Necrotizing enterocolitis/sepsis, n (%)	330	(52)	39	(55)	291	(51)
Sepsis, n (%)	293	(46)	37	(52)	256	(45)
Necrotizing enterocolitis, n (%)	73	(11)	5	(7)	68	(12)

IQR = interquartile range.

[1] In 5 cases the exact gestational age could not be determined due to uncontrolled pregnancies. It was estimated in full weeks.

Table II. Types of bleeding

Major bleeds, n (%)	71	(11)
Type of major bleeding, n (%)		
Uni-/bilateral IVH grade 3 with or without parenchymal involvement	32	(45)
IVH grade 1 or 2 (uni- or bilateral) with parenchymal involvement	4	(6)
Solitary (non-cerebellar) parenchymal hemorrhage	4	(6)
Cerebellar parenchymal hemorrhage	11	(15)
Subdural hemorrhage	4	(6)
Pulmonary hemorrhage	12	(17)
Gastrointestinal hemorrhage	4	(6)

Eight bleeds (of 71) were excluded from the model because they occurred more than ten days after T_0.: 1 cerebellar, 1 IVH grade 1 plus infarction basal ganglia, 1 IVH grade 1 and grade 2 plus infarction basal ganglia, 1 gastro-intestinal bleed, 1 pulmonary bleed, 1 bilateral IVH grade III, 1 frontal-parietal bleed and 1 subdural hemorrhage.

IVH = intraventricular hemorrhage.

Table III. The dynamic prediction model

	Hazard ratio	95% CI
Covariates with time-constant effects		
Gestational age (days)	1.00	0.98 – 1.02
Postnatal age (days)	0.88	0.83 – 0.94*
Mechanical ventilation	5.08	2.03 – 10.65*
NEC/sepsis	0.85	0.43 – 1.58
Platelet transfusion	1.06	0.38 – 2.95
Interaction term log10 platelet count and platelet transfusion	1.23	0.63 – 2.38
Covariates with time-varying effects		
LM (2 hour intervals)	2.30	0.89 – 5.94
LM^2 (2 hour intervals)	0.85	0.74 – 0.98*
IUGR Constant	0.51	0.17 – 1.59
IUGR Time-varying: LM	0.31	0.09 – 1.14
IUGR Time-varying: LM^2	1.22	1.04 – 1.44*
Log10 platelet count Constant	1.74	0.72 – 4.24
Log10 platelet count Time-varying LM	0.35	0.19 – 0.63*
Log10 platelet count Time-varying: LM^2	1.12	1.03 – 1.21*

A hazard ratio > 1 indicates that increase of the risk factor is associated with higher risk of bleeding. E.g. a mechanically ventilated neonate has a 5.08 times higher bleeding risk than a neonate who is not mechanically ventilated.

CI = confidence interval. If both ends of the confidence interval are either higher than 1 or lower than 1, the variable is a statistically significant predictor, indicated by *. LM = landmark time, linear interaction. LM^2 = landmark time, quadratic interaction. LM or landmark time refers to time since onset of severe thrombocytopenia (time-dependent variable), in 2 hour time intervals. Postnatal age refers to the postnatal age at the onset of severe thrombocytopenia (baseline variables).

Time-varying covariates should not be confused with time-dependent covariates, such as platelet count or platelet transfusion, where the value of the variable is not fixed (it is not a baseline variable) but can change over time. In time-varying covariates, the effect of the covariate can change over time, for example, the strength and direction of a potential association of IUGR with bleeding could be different immediately after onset of thrombocytopenia compared to a few days after onset of thrombocytopenia, due to interactions with other risk factors and changes in the clinical situation of the neonate.

Table IV: Risk predictions for different clinical scenarios

Patient characteristics: GA 28 weeks, **platelet count 10x10^9/L** at day 3 of life (first time <50x10^9/L), no transfusion

		Ventilation	No ventilation
NEC/sepsis	IUGR	8%	2%
No NEC/sepsis	No IUGR	17%	3%
NEC/sepsis	No IUGR	14%	3%
No NEC/sepsis	IUGR	9%	2%

Patient characteristics: GA 28 weeks, **platelet count 50x10^9/L** at day 3 of life (first time <50x10^9/L) , no transfusion

		Ventilation	No ventilation
NEC/sepsis	IUGR	11%	2%
No NEC/sepsis	No IUGR	24%	5%
NEC/sepsis	No IUGR	20%	4%
No NEC/sepsis	IUGR	13%	3%

NEC = necrotizing enterocolitis. IUGR = intra uterine growth retardation. GA = gestational age

Figure legends

Legend figure 1: **CONSORT flow chart.** CONSORT = consolidated standards of reporting trials. NICU = neonatal intensive care unit.

Legend figure 2: **Number of neonates reaching the different study endpoints (major bleeding, death or discharge/transfer) in the first 10 days after the onset of severe thrombocytopenia**. T_0 is the day on which platelet counts dropped below $50x10^9$/L for the first time. Neonates who developed a major bleeding and then died were only registered as major bleeding (no overlap between major bleeding and mortality).

Legend figure 3: **Dynamic, cross-validated c-index.** This graph represents the dynamic, cross-validated c-index of the main model. A c-index of 1 resembles a model that discriminates perfectly between patients with and without a major bleeding, while a c-index of 0.5 indicates that the prediction is as good as chance. For each timepoint, the number at risk at the beginning of that day have been reported, as well as the total number of major bleeds that occurred during these 24 hours. E.g. at the start of day one, 604 patients were still at risk, and during this day 22 neonates developed a major bleed.

Legend figure 4: **Change in probability of developing a major bleeding within three days for two example patients.** Day 0 is the day of onset of severe thrombocytopenia (T_0). Characteristics of child A: gestational age (weeks+days) 27+2, birthweight 1100 grams, 2 days old at T_0, sepsis, mechanical ventilation, 2 platelet transfusions, platelet counts 41-104-47-88 $x10^9$/L. Bilateral grade III IVH on day 2. Characteristics of child B: gestational age (weeks+days) 32+3, birth weight 1175 grams, 5 days old at T_0, sepsis, no mechanical ventilation, no platelet transfusions, platelet counts 4-53-49-63-195-376 $x10^9$/L. No major bleed. Day 3-7 not shown because no substantial change in bleeding risk occurred. During study day 1-3, the predicted risk of major bleeding within the next 3 days in Child A is substantially higher than in Child B, indicating that use of this prediction model during that time-period would have correctly identified Child A as being at high risk of bleeding.

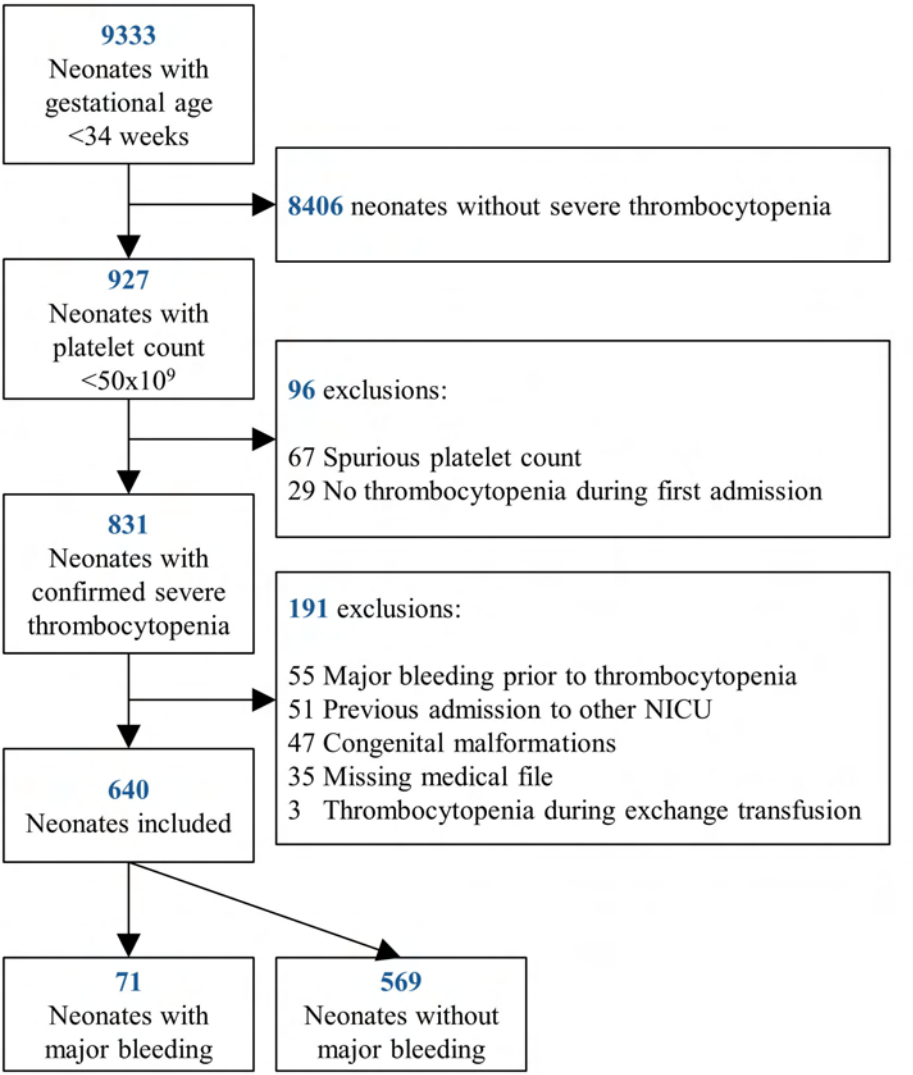

9333
Neonates with gestational age <34 weeks
8406 neonates without severe thrombocytopenia
927
Neonates with platelet count <50x10^9
96 exclusions:
67 Spurious platelet count
29 No thrombocytopenia during first admission
831
Neonates with confirmed severe thrombocytopenia
191 exclusions:
55 Major bleeding prior to thrombocytopenia
51 Previous admission to other NICU
47 Congenital malformations
35 Missing medical file
3 Thrombocytopenia during exchange transfusion
640
Neonates included
71
Neonates with major bleeding
569
Neonates without major bleeding

Figure 1

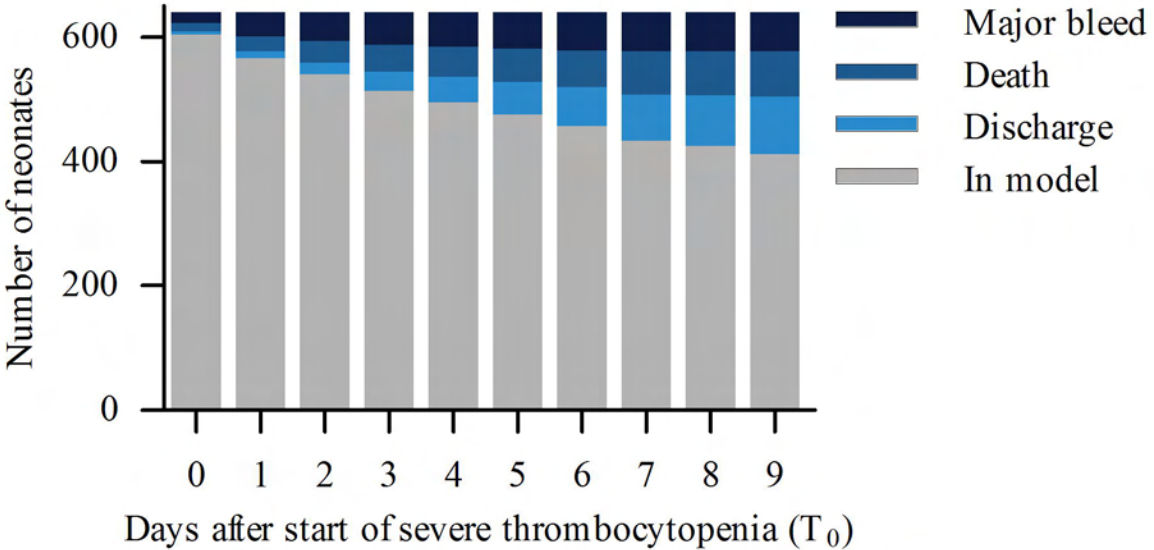
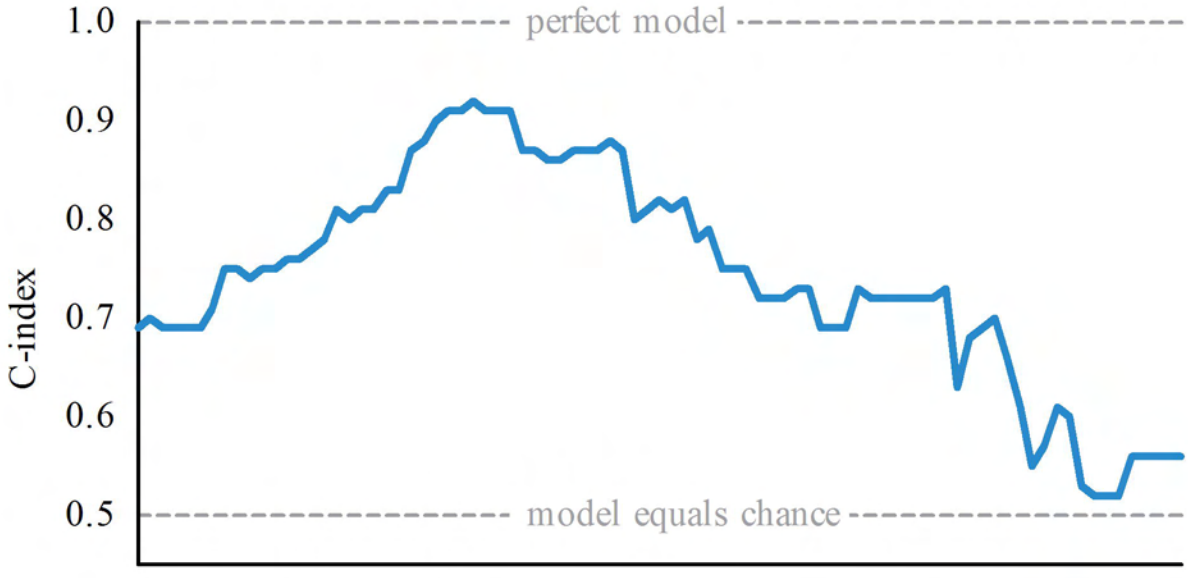

Number of neonates
600
400
200
0
0 1 2 3 4 5 6 7 8 9
Days after start of severe thrombocytopenia (T_0)
Major bleed
Death
Discharge
In model

Figure 2



Days since start of thrombocytopenia	0	1	2	3	4	5	6	7
N at risk	640	604	566	540	514	495	476	456
N bleed	17	22	7	6	3	3	3	1

Figure 3

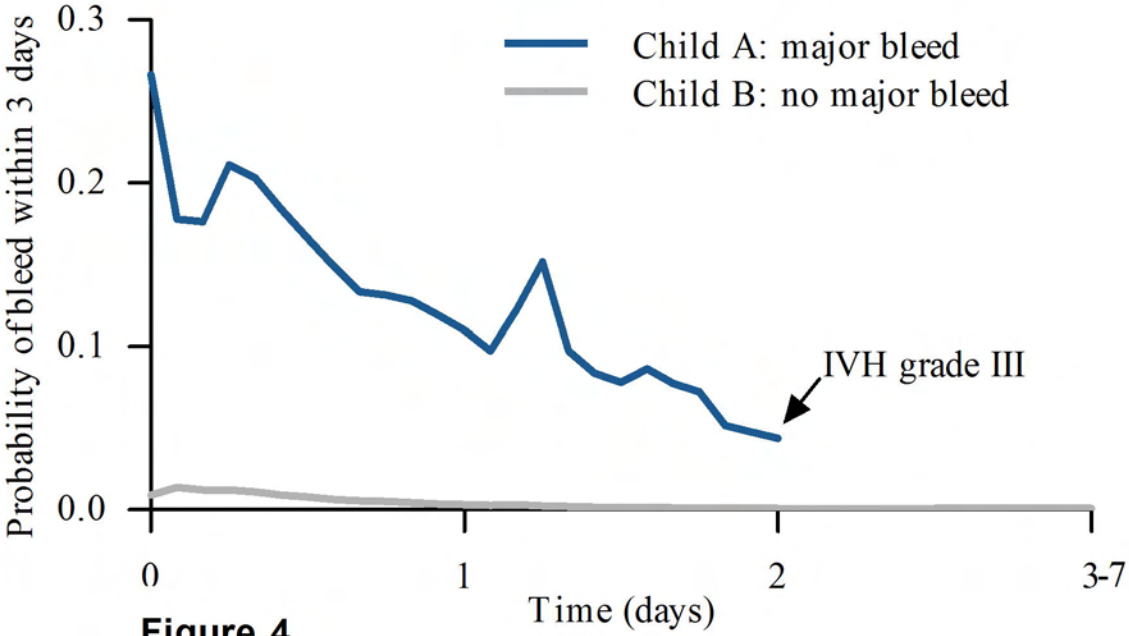

Child A: major bleed
Child B: no major bleed
IVH grade III
Probability of bleed within 3 days
0.3
0.2
0.1
0.0
0
1
2
3-7
Time (days)

Figure 4

Dynamic prediction of bleeding risk in thrombocytopenic preterm neonates.

SUPPLEMENTARY ONLINE ONLY MATERIALS

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Contents

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			

NR = not registered in medical files. NC = no contrast (risk factor present in <5% or >95% of population. NM= not measured. RA = rare event. TE = timing of event problematic. When event occurs after risk period for bleeding (e.g. BPD). CO = risk factor combined with other risk factor (e.g. hyperglycemia and glucose disorders). ND = risk factor not well defined. NA = no association with bleeding (checked in a selection of papers). OT = other. Grey highlights: variables selected for further review (n=74).

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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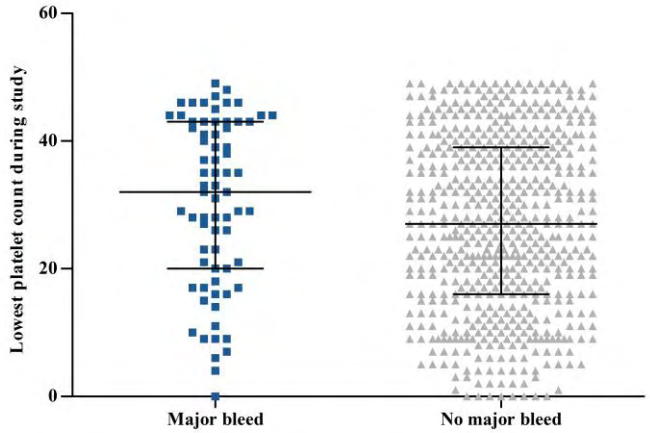
Table S2: additional information about the model variables.

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.

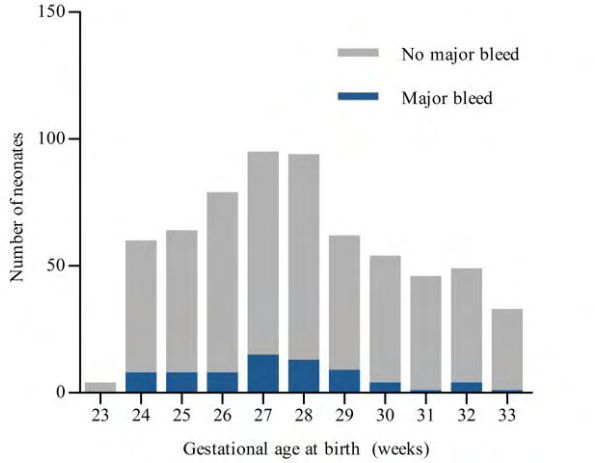


Figure S3: postnatal age at onset of severe thrombocytopenia in neonates with and without major bleeding.

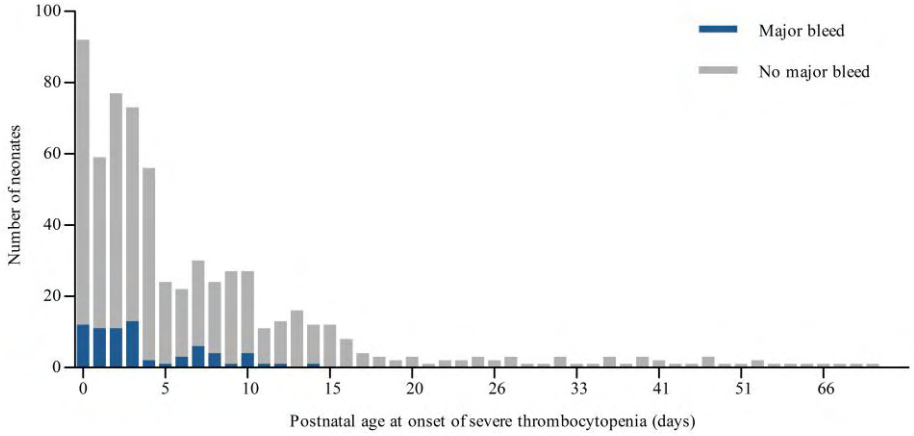


Table S2: sensitivity analyses

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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