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Fetal and neonatal alloimmune thrombocytopenia: the proof of the pudding is in the eating

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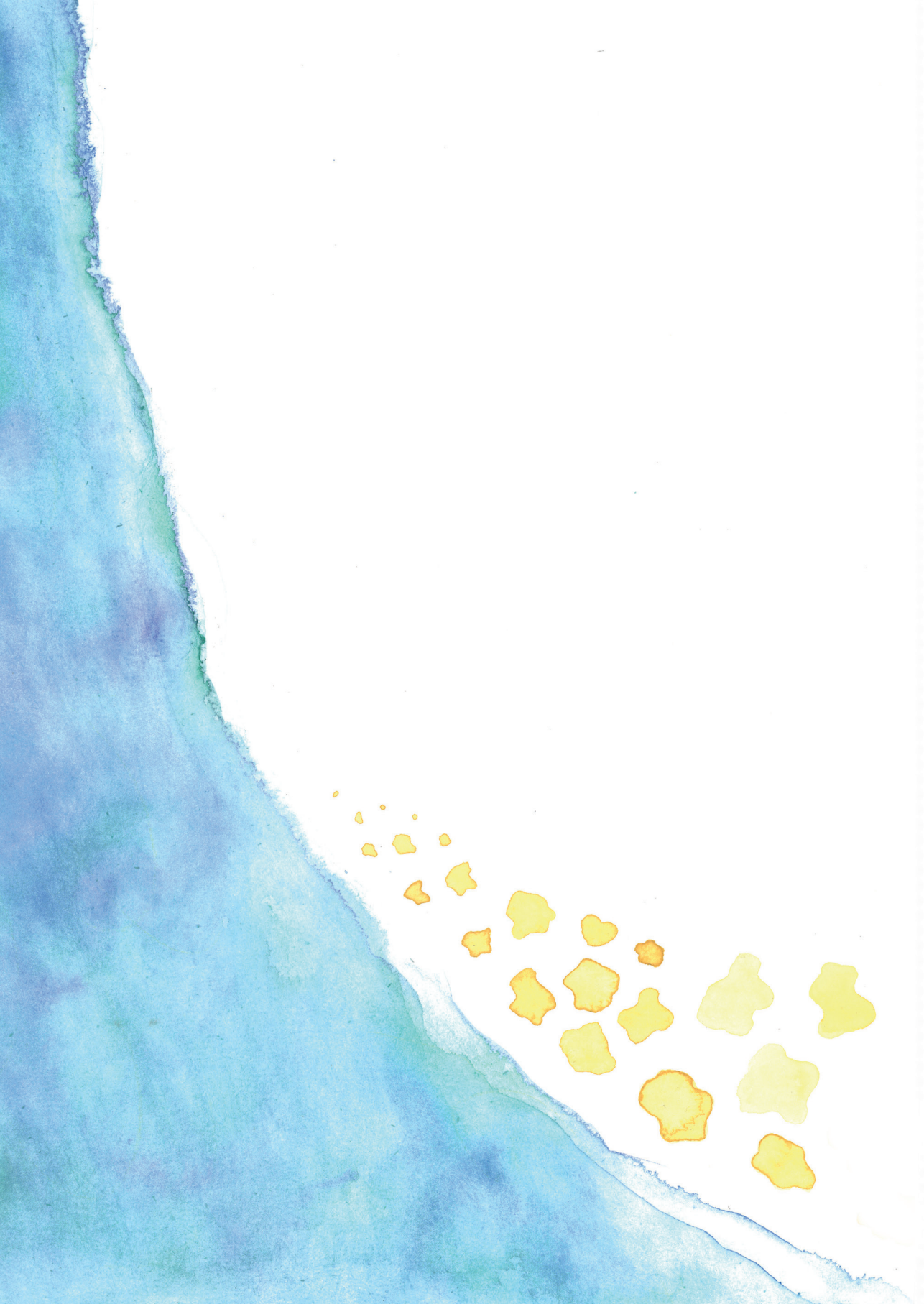
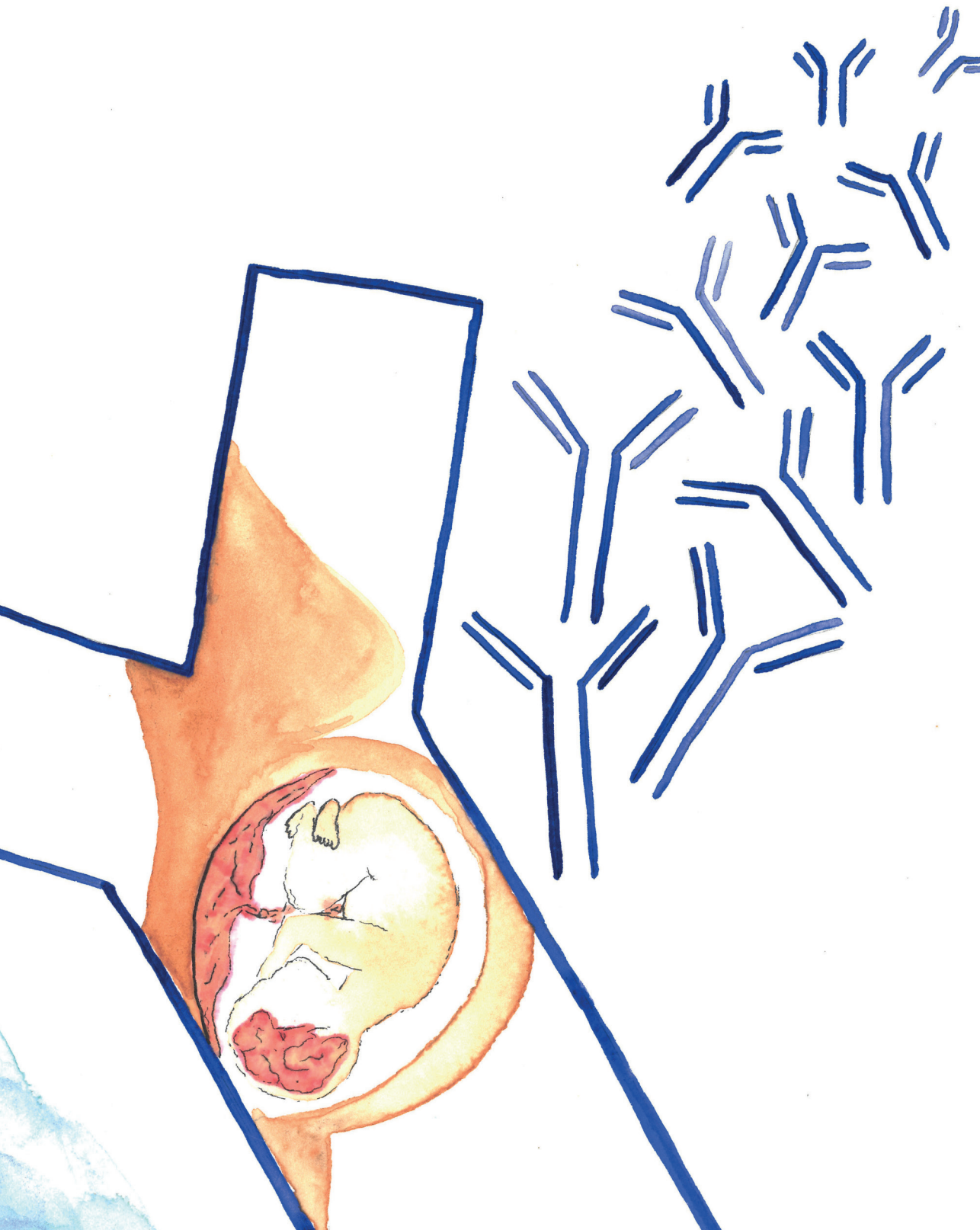


PART FOUR

Neonatal management



CHAPTER 6

Postnatal treatment for children with fetal and neonatal alloimmune thrombocytopenia: a multicentre, retrospective cohort study

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SUMMARY

Background

Children affected by fetal and neonatal alloimmune thrombocytopenia (FNAIT) are at risk for severe intracranial haemorrhage. Management in the postnatal period is based on sparse evidence. We aimed to describe the contemporary management and outcomes of patients with FNAIT in high-income countries.

Methods

In this multicentre, retrospective, cohort study, we set up a web-based registry for the collection of deidentified data on the management and course of neonates with FNAIT liveborn between January 1, 2010, and January 1, 2020. Eight centres from seven countries (Australia, Norway, Slovenia, Spain, Sweden, the Netherlands, and the USA) participated. Eligibility criteria comprised anti-human platelet antigen (HPA) alloantibodies in maternal serum, confirmed maternal and fetal HPA incompatibility, and bleeding detected at antenatal ultrasound, neonatal thrombocytopenia (<150 × 10^9 platelets per L), or both in the current or previous pregnancy. Clinical data were retrieved from local medical records of the first neonatal admission and entered in the registry. The key outcome was the type of postnatal treatment given to neonates with FNAIT. Other outcomes were daily median platelet counts in the first week of life, median platelet count increment after first unmatched versus first matched transfusions, and the proportion of neonates with mild or severe bleeding.

Findings

408 liveborn neonates with FNAIT were entered into the FNAIT registry, of whom 389 from Australia (n = 74), Norway (n = 56), Slovenia (n =19), Spain (n = 55), Sweden (n = 31), the Netherlands (n = 138), and the USA (n = 16) were included in our analyses. The median follow-up was 5 days (IQR 2–9). More neonates were male (241 [64%] of 379) than female (138 [36%]). Severe thrombocytopenia (platelet count <50 × 10^9 platelets per L) was reported in 283 (74%) of 380 neonates, and extreme thrombocytopenia (<10 × 10^9 platelets per L) was reported in 92 (24%) neonates. Postnatal platelet count nadir was higher in the no-treatment group than in all other groups. 163 (42%) of 389 neonates with FNAIT received no postnatal treatment. 207 (53%) neonates received platelet transfusions, which were either HPA-unmatched (88 [43%] of 207), HPA-matched (84 [41%]), or a combination of both (35 [17%]). The proportion of neonates who received HPA-matched platelet transfusions varied between countries, ranging from 0% (Slovenia) to 63% (35 of 56 neonates; Norway). Postnatal intravenous immunoglobulin treatment was given to 110 (28%) of 389 neonates (alone [n = 19] or in combination with platelet transfusions [n = 91]), with the proportion receiving it ranging from 12% (17 of 138 neonates; the Netherlands) to 63% (ten of 16 neonates; the USA) across countries. The median platelet increment was 59 × 10^9 platelets per L (IQR 35–94) after HPA-unmatched platelet transfusions and 98 × 10^9 platelets per L (67–134) after HPA-

matched platelet transfusions (*P*<0.0001). Severe bleeding was diagnosed in 23 (6%) of 389 liveborn neonates, with one having a severe pulmonary haemorrhage and 22 having severe intracranial haemorrhages. Mild bleeding was diagnosed in 186 (48%) neonates.

Interpretation

Postnatal management of FNAIT varies greatly between international centres, highlighting the absence of consensus on optimal treatments. Our data suggest that HPA-matched transfusions lead to a larger median platelet count increment than HPA-unmatched transfusions, but whether HPA matching is also associated with a reduced risk of bleeding remains unknown.

Funding

Sanquin

RESEARCH IN CONTEXT

- **EVIDENCE BEFORE THIS STUDY**

  We searched PubMed without language restrictions for studies published between database inception and April 27, 2022 reporting on postnatal treatment and outcomes in patients with fetal and neonatal alloimmune thrombocytopenia (FNAIT). Search terms related to postnatal management and outcomes of patients with FNAIT were used. We identified four prospective and ten retrospective cohort studies. Available studies had methodological limitations including small numbers of patients, no randomisation and no analyses of confounding factors. Optimal postnatal management is not known; current guidelines are based on sparse qualitative evidence owing to the rarity of the condition. None of the studies we found compared the differences in management between different countries. The standard of care and whether postnatal management varies between international referral centres is unknown.

- **ADDED VALUE OF THIS STUDY**

  To our knowledge, this multicenter study is the first to investigate postnatal treatment strategies and outcomes of neonates affected by FNAIT in different countries. We gathered data from seven different countries and 389 FNAIT cases and found great variation in postnatal management strategies, particularly in the use of human platelet antigen (HPA)-matched platelet transfusions and intravenous immunoglobulins.

- **IMPLICATIONS OF ALL THE AVAILABLE EVIDENCE**

  This study shows variation in postnatal treatment strategies for FNAIT. HPA-matched or HPA-unmatched platelet transfusions and intravenous immunoglobulins are frequently administered postnatally; however the efficacy of these treatment strategies is unknown. Our findings could motivate international collaboration trough multicentre, randomised trials aimed at improving the management and outcomes of patients with FNAIT. Data from this study can serve as a basis from which future clinical trials can be designed.

INTRODUCTION

Children affected by fetal and neonatal alloimmune thrombocytopenia (FNAIT) face an increased risk of bleeding during pregnancy and after birth. Incompatibility in human platelet antigens (HPAs) between mother and fetus can lead to a maternal alloimmune response, with the formation of HPA-alloantibodies. Platelet-directed antibodies (IgG) are actively transported across the placenta into the fetal circulation. These alloantibodies bind to platelets and possibly endothelial cells resulting in fetal thrombocytopenia and an increased risk of bleeding.[1-3] FNAIT is the leading cause of severe thrombocytopenia in otherwise healthy term neonates and occurs in approximately one in 1500 pregnancies.[4, 5]
The main goal of antenatal and postnatal management of FNAIT is to prevent severe fetal and neonatal intracranial bleeding and its long-term sequelae. Administration of intravenous immunoglobulin (IVIg) to the mother during pregnancy is often used as a first-line treatment in mothers during pregnancies subsequent to one where FNAIT was diagnosed.[6] However, quantitative evidence supporting postnatal treatment in FNAIT is sparse and there is no international consensus on neonatal management.[7] Given the rarity of this disease, large prospective randomised trials are not available. Guidelines are mostly based on small observational studies and expert opinion.[6] We therefore aimed to evaluate the international practices in postnatal treatment and the outcomes of patients with FNAIT.

MATERIAL AND METHODS

STUDY DESIGN AND PARTICIPANTS

For this retrospective cohort study, on September 1, 2020, we set up a multicentre, web-based registry for the collection of deidentified data on the postnatal management and course of liveborn neonates with FNAIT. Eight centres from seven countries with specific interest and expertise in FNAIT agreed to participate: the Australian Neonatal Alloimmune Thrombocytopenia registry Monash University, Melbourne, VIC, Australia), the Arctic University of Norway (Tromsø, Norway), University Medical Centre Ljubljana (Ljubljana, Slovenia), Blood and Tissue Bank (Barcelona, Spain), Karolinska University Hospital (Stockholm, Sweden), Leiden University Medical Center (Leiden, the Netherlands), Sanquin Diagnostics (Amsterdam, the Netherlands), Levine Children's Hospital (Charlotte, NC, USA), and Boston Children's Hospital (Boston, MA, USA; Supplemental Table 1). Investigators were supplied with personal credentials to enter clinical data into a secured online database (Castor Electronic Data Capture 2019). Participants were eligible if they were liveborn between January 1, 2010, and January 1, 2020, their mothers had anti-HPA alloantibodies in their serum, incompatibility between the maternal and fetal HPAs was confirmed,[8] and they (or a previous pregnancy) had bleeding detected at an antenatal ultrasound, neonatal thrombocytopenia ($<150 \times 10^9$ platelets per L), or both. The medical ethical committee of

Leiden-Delft-DenHaag provided a waiver of consent for the initiating country (G20.074). The requirement for informed consent was waived. International investigators obtained ethical consent according to national laws and regulations.

PROCEDURES

Between September 1, 2020 and September 1, 2021, the following information was retrieved from local medical records of the first neonatal admission and entered in the online registry: time of diagnosis (antenatal or postnatal), reason for suspecting FNAIT, anti-HPA alloantibody specificity, method of HPA-alloantibody detection, gravidity, parity, the presence of maternal thrombocytopenia, antenatal treatment, gestational age at birth, delivery mode, sex (as determined by caregiver directly after birth), birthweight (including percentile and small for gestational age), neonatal skin or organ bleeding, intracranial haemorrhage (including neuroimaging reports), neonatal mortality, lowest platelet count per day (up to nine platelet counts per participant), postnatal treatment per day during the first admission, and bleeding complications after start of treatment.

We defined five postnatal treatment groups: no treatment, platelet transfusion from HPA-unmatched donors, platelet transfusion from HPA-matched donors, HPA-unmatched and HPA-matched platelet transfusions, and postnatal intravenous immunoglobulin. Participants who received an HPA-matched platelet transfusion did so from donors who were HPA-typed and selected on the absence of the implicated HPA. Participants who received both a platelet transfusion and postnatal intravenous immunoglobulin treatment or steroids were not analysed separately but included in a treatment group on the basis of the platelet transfusion received. Platelet transfusion thresholds for participants with and without bleeding and the recommended transfusion doses from clinical guidelines for FNAIT postnatal treatment were reported for each centre.

Antenatal diagnosis of FNAIT was defined as the detection of anti-HPA alloantibodies in the mother's serum during the current or previous pregnancy, with confirmed HPA incompatibility between the mother and fetus in the current pregnancy. Postnatal diagnosis was defined as the detection of anti-HPA alloantibodies after birth. Small for gestational age was defined as a birthweight of less than the 10th percentile according to local or national growth charts. Neonates born from pregnancies during which mothers received antenatal treatment (intravenous immunoglobulins, corticosteroids, intrauterine platelet transfusions, or a combination thereof) are called antenatally treated cases.

Bleeding symptoms were divided into mild and severe bleeding. Mild bleeding was defined as any uncomplicated haemorrhage (eg, petechiae, haematomas, or a grade 1–2 intraventricular haemorrhage [grading system was adapted from Papile *et al*[9] and Inder *et al*[10]]. Severe bleeding was classified by a severe intracranial haemorrhage (a grade 3 intraventricular haemorrhage,

an intraventricular haemorrhage of any grade in combination with parenchymal involvement, a parenchymal haemorrhage or cerebellar haemorrhage, a subdural haemorrhage causing parenchymal compression, a subarachnoid haemorrhage, or an epidural haemorrhage), severe organ bleeding (life-threatening bleeding associated with shock or requiring volume boluses, red blood cell transfusions, or inotropes), or both. Asymptomatic cases were participants without any bleeding symptoms. In participants with a severe intracranial haemorrhage, we attempted to estimate the timeframe in which the intracranial haemorrhage could have occurred by recording the date of the latest (antenatal) ultrasound without intracranial haemorrhage and the date at which the intracranial haemorrhage was diagnosed.

OUTCOMES

Our primary aim was to describe current practice in the postnatal treatment of neonates with FNAIT per country. Our key endpoint was postnatal treatment during first admission, described in the five treatment groups. Other aims were to describe neonatal outcomes and platelet count increments. Endpoints were the daily median postnatal platelet counts in the first week of life in neonates with FNAIT per treatment group, the median change (increment) in platelet count after the first platelet transfusion in recipients of unmatched versus matched platelet transfusions, and the proportion of participants with mild or severe bleeding per treatment group.

STATISTICAL ANALYSES

Study sample size was not based on statistical hypothesis testing. To obtain an overview of contemporary treatment and differences in postnatal treatment between countries we aimed to recruit at least 200 neonates from at least six centres. Endpoints were analysed in a population of liveborn neonates with FNAIT who had available information on postnatal treatment. Median platelet counts per day are presented by treatment group and additionally stratified by antenatal treatment status.

To calculate the platelet count increment per transfusion type we subtracted the minimum platelet count on the day before transfusion and the day of transfusion (whichever was least) from the maximum platelet count on the day of transfusion or the day after transfusion (whichever was most). This choice was made because platelet counts exactly before and after transfusion and after transfusion were not available (only the lowest platelet count per day was documented). Participants who received transfusions the same day or had missing platelet counts for the days of interest were not included in these analyses. Only first transfusions were included in these analyses, subsequent transfusions were not included, because their effects could not be determined without taking into account the previous transfusion. We compared median platelet count increments after transfusion using Mann-Whitney *U* test (an unadjusted analysis). Because data on confounding variables such as the volume, concentration and duration of transfusions were not available, adjusted

analyses were not done. Platelet count increments after transfusion were calculated shown separately. We did a subgroup analysis of clinical outcomes and treatment by country to assess differences in FNAIT severity.

Statistical analyses were done in Stata (version 16) and SPSS (version 26.0). Data are presented as the numbers of participants and percentages or medians and IQRs. The distributions of postnatal treatment strategies are shown as pie charts and platelet counts are shown as dot plots with medians and IQRs. Figures were made with GraphPad Prism (version 9).

ROLE OF THE FUNDING SOURCE
The funder of the study had no role in the study design, data collection, data analysis, data interpretation or writing of the report.

RESULTS

408 liveborn neonates with FNAIT were entered in the FNAIT registry. We excluded 19 neonates (5%) who had missing information on postnatal treatment, leaving 389 (95%) in our analyses (Figure 1). 255 (66%) of 389 cases of FNAIT were diagnosed postnatally (Table 1). Postnatal suspicion of FNAIT was due to skin bleeding in 127 (33%) of 389 neonates, thrombocytopenia detected as a chance finding in 118 (30%), and severe bleeding in ten (3%). 134 (34%) neonates were diagnosed antenatally, of whom 117 were diagnosed after a diagnosis of FNAIT in the mother's previous pregnancy. FNAIT was diagnosed antenatally in a screening study or because of a family history of FNAIT in 12 (3%) of 389 neonates. For the remaining five (1%) neonates, FNAIT was suspected due to severe antenatal bleeding. The method of detecting anti-HPA antibodies differed between countries (Supplemental Table 2). Antenatal treatment was given in 105 (78%) of 134 pregnancies that were antenatally diagnosed; the maternal administration of intravenous immunoglobulin was started in all 105 pregnancies, intravenous immunoglobulin was combined with steroids in 12, and intravenous immunoglobulin and steroids were combined with intrauterine platelet transfusions in two. There was an over-representation of male neonates and small-for-gestational-age neonates (Table 1). Baseline characteristics, particularly the prevalence of antenatal treatment, varied between countries (Supplemental Table 3).

The median follow-up during admission was 5 days (IQR 2–9). 163 (42%) of 389 neonates with FNAIT received no postnatal treatment (Table 2). 207 (53%) neonates received platelet transfusions, which were either HPA-unmatched platelet transfusions (88 [43%] of 207), HPA-matched platelet transfusions (84 [41%]), or a combination of both (35 [17%]). In the no-treatment group, 77 (47%) of 163 mothers were treated antenatally. The median number of postnatal transfusions given in the total population was 1 (IQR 1–2). The proportion of neonates

who received HPA-matched platelet transfusions varied between countries, ranging from 0% (Slovenia) to 63% (Norway; Figure 1; Supplemental Figure 1).

TABLE 1. Clinical characteristics of liveborn neonates with FNAIT

Variable	Total (n = 389)
HPA specificity, n (%)	
HPA-1a	291 (75)
HPA-1b	3 (1)
HPA-2b	3 (1)
HPA-3a	3 (1)
HPA-5a	7 (2)
HPA-5b	46 (12)
HPA-15a	3 (1)
HPA-15b	5 (1)
HPA-1a and HPA-3a	1 (<1%)
HPA-1a and HPA-5b	8 (2)
HPA-1b and HPA-5b	2 (1)
Other	11 (3)
Unknown	6 (2)
Antenatal diagnosis, n (%)†	134 (34)
Postnatal diagnosis, n (%)	255 (66)
Antenatal treatment, n (%)	105/134 (78)
First pregnancy (primigravida), n (%)	82 (21)
Male sex, n (%)	241 (64)
Gestational age at birth (weeks) - median (IQR; min-max)	38 (37–40; 24–42)
Birthweight (g) - median (IQR; min-max)	3060 (2584–3441; 737–4520)
Small for gestational age (SGA), n (%)	77 (21)

All statistics and percentages are calculated based on the valid numbers. i.e. excluded the missing.
† 117 cases of FNAIT diagnosed antenatally after a diagnosis of FNAIT in a previous pregnancy diagnosed FNAIT pregnancy, 17 cases were newly diagnosed in this current pregnancy.
§ 82 (31%) of 272 women who had not been previously diagnosed with FNAIT were primigravida.
Abbreviations: HPA, human platelet antigen; IQR, interquartile range; g, grams.

Postnatal intravenous immunoglobulin treatment was given to 110 (28%) of 389 neonates (alone [n = 19] or in combination with platelet transfusions [n = 91]), with the proportion receiving it ranging from 12% to 63% across countries (Supplemental Figure 1). Four (4%) of 93 neonates with dosing information received a dose of 0.5 g/kg of bodyweight per day of postnatal intravenous immunoglobulin treatment, 85 (91%) received 1.0 g/kg per day, and four (4%) received 2.0 g/kg per day. The dose of postnatal intravenous immunoglobulin treatment was not known for 17 neonates. Steroid treatment was given to only four neonates from two countries (Spain and the Netherlands). Platelet transfusion guidelines varied between centres, with transfusion thresholds ranging from 20×10^9 platelets per L to 50×10^9 platelets per L in neonates without bleeding and from 50×10^9 platelets per L to 100×10^9 platelets per L in neonates with bleeding (Supplemental Table 4). Transfusion doses varied from 10 mL/kg to 20 mL/kg (Supplemental Table 4).

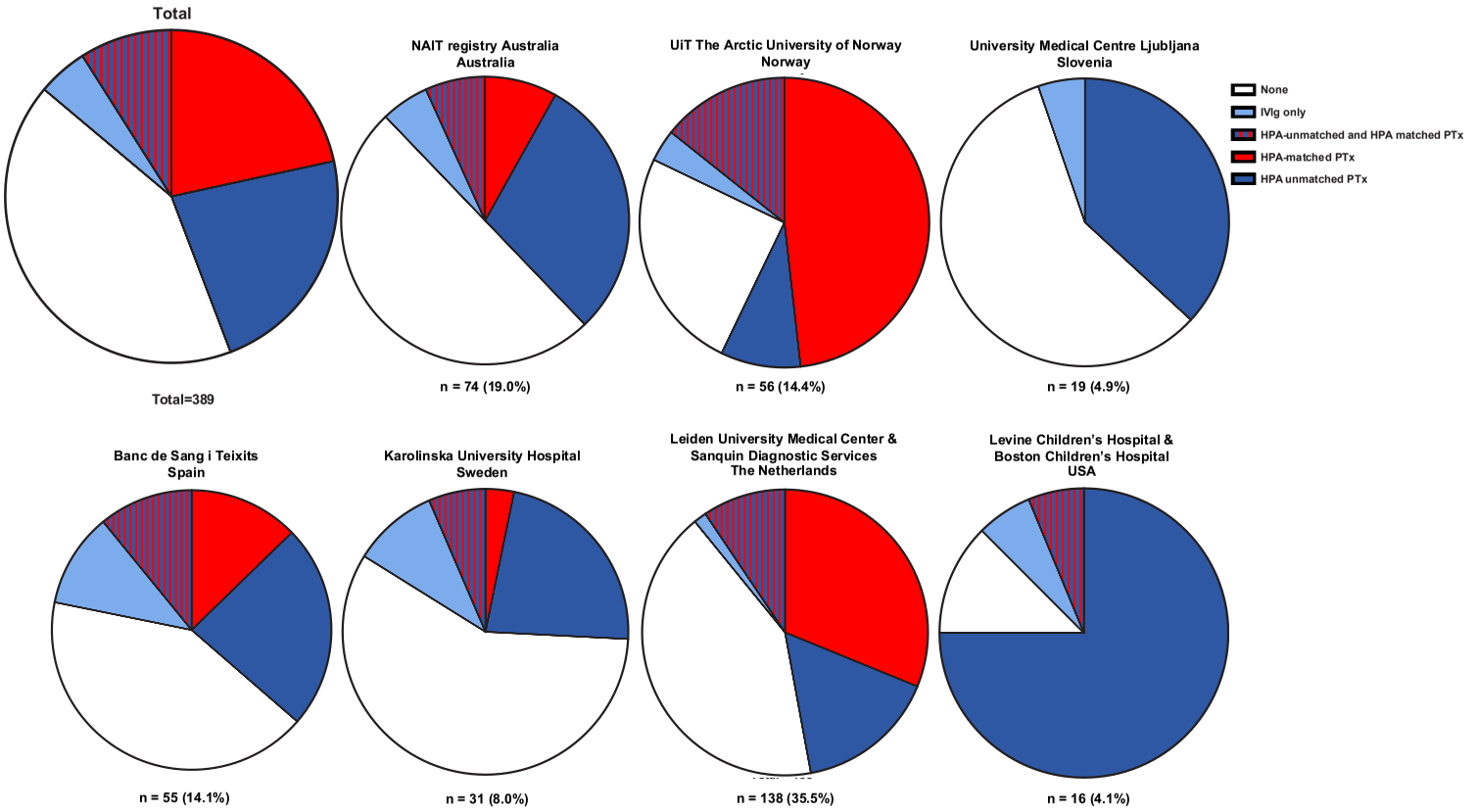

FIGURE 1. Postnatal treatment strategy per country

The distribution of treatment strategies applied in all cases and per centre. Cases that received no postnatal treatment were allocated in the no postnatal treatment group. Cases treated with HPA-unmatched donor platelets were allocated in unmatched platelet group (unmatched PTx). Cases treated with HPA typed and matched donor platelet transfusions were allocated in the matched platelet transfusion group (matched PTx). Cases that received both matched and HPA-unmatched donor platelets were allocated in both HPA-unmatched and HPA-matched platelet transfusion group (both HPA-unmatched and HPA-matched PTx). Cases that were treated with intravenous immunoglobulin (IVIg) only were allocated in the IVIg only group.

TABLE 2. Treatment and outcome of liveborn neonates with FNAIT per postnatal treatment strategy

Characteristic	No postnatal treatment (n = 163)	HPA-unmatched platelet transfusion (n = 88)	HPA-matched platelet transfusion (n = 84)	HPA-unmatched and HPA-matched platelet transfusions (n = 35)	Intravenous immunoglobulin only (n =19)	Total (n = 389)
Antenatal treatment - (n, %)	77 (47)	3 (3)	21 (25)	0	4 (21)	105 (27)
Postnatal treatment						
Additional IVIg treatment - (n, %)	-	42 (48)	24 (29)	25 (71)	19 (100)	110 (28)
Additional steroids - (n, %)	-	1 (1)	1 (1)	2 (6)	-	4 (1)
Platelet count						
Platelet count nadir (× 10^9 platelets per L) - median (IQR)	65 (34-162)	12 (8-19)	12 (7-18)	6 (3-14)	23 (12-38)	21 (10-51)
Thrombocytopenia (Platelet count <150 × 10^9 platelets per L) - (n, %)	111 (72)	88 (100)	84 (100)	35 (100)	19 (100)	337 (89)
Severe thrombocytopenia (Platelet count <50 × 10^9 platelets per L) - (n, %)	58 (38)	88 (100)	84 (100)	35 (100)	18 (95)	283 (74)
Very severe thrombocytopenia (Platelet count <25 × 10^9 platelets per L) - (n, %)	13 (8)‡	74 (84)	75 (89)	33 (94)	11 (58)	206 (54)
Extreme thrombocytopenia (Platelet count <10 × 10^9 platelets per L) - (n, %)	1 (1)‡	33 (38)	31 (37)	23 (66)	4 (21)	92 (24)
Severity of bleeding symptoms†						
Mild bleeding	39 (24)	57 (65)	58 (69)	24 (69)	8 (42)	186 (48)
Severe bleeding	2 (1)	10 (11)	5 (6)	6 (17)	0	23 (6)

All statistics and percentages are calculated based on the valid numbers. i.e. excluded the missing.
† Severe bleeding was defined as cases with severe organ bleeding and/or severe ICH, mild bleeding are cases with skin bleeding, mild organ bleeding and/or intraventricular haemorrhage (IVH) grade I of II.
‡ Reason why no treatment was given in these thrombocytopenic infants is unknown. Platelet count was below 25 × 10^9 per L for one day in all 14 cases.
Abbreviations: IVIg, intravenous immune globulin; IQR, interquartile range.

The median age at detection of platelet count nadir was 0 days (the day of birth; IQR 0–1). In 348 (92%) of 380 neonates with complete data, platelet count nadir was detected at 3 days or earlier. Severe thrombocytopenia was detected in 283 (74%) of 380 and extreme thrombocytopenia was detected in 92 (24%; Table 2). Postnatal platelet count nadir was higher in the no-treatment group than in all other groups (Table 2). Treatment groups differed in the proportion of neonates who were antenatally treated and in the proportion of neonates who were treated with (additional) postnatal intravenous immunoglobulin (Table 2). In all treatment groups, median platelet count increased in the first week after birth (Figure 2). The platelet counts of neonates receiving HPA-unmatched platelets were not different from those of neonates receiving HPA-matched platelets after day 3 (Figure 2). At day 4, the median platelet count of neonates receiving HPA-unmatched platelets (87 × 10^9 platelets per L [IQR 48–142]) did not differ from the median platelet count of neonates receiving HPA-matched platelets (125 × 10^9 platelets per L [70–172]; $P = 0.15$).

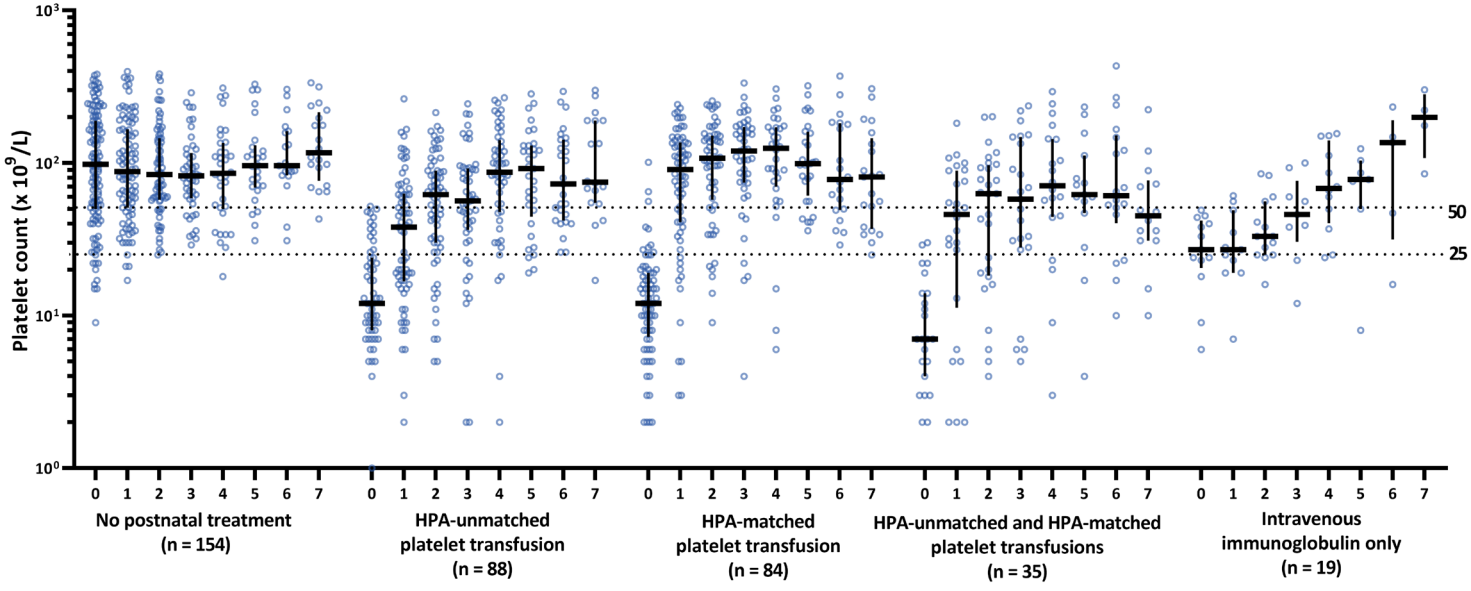

FIGURE 2. Platelet count per treatment strategy

The dot plot shows the lowest neonatal platelet count per day per treatment strategy. The dots represent individual platelet counts, the solid, black horizontal lines represent median values, ant the vertical solid black lines represent the IQRs. Dashed lines represent platelet counts of 50×10^9 per L and 25×10^9 per L.

50 neonates who did not receive antenatal or postnatal treatment had severe thrombocytopenia (platelet count <50 × 10^9 platelets per L) and 11 had very severe thrombocytopenia (platelet count <25 × 10^9 platelets per L), but none developed bleeding. Platelet counts during the first week of life stratified by antenatal treatment status are shown in Supplemental Figure 2. The median nadir platelet count was 110 × 10^9 platelets per L (IQR 33–191) for neonates who were antenatally treated and 17 × 10^9 platelets per L (8–20) for neonates who did not receive antenatal treatment. The median nadir platelet counts of neonates were 19 × 10^9 platelets per L (IQR 9–40) if their mothers had anti-HPA-1a antibodies, 55 × 10^9 platelets per L (28–146) if their mothers had anti-HPA-5b antibodies, and 39 × 10^9 platelets per L (16–46) if their mothers had anti-HPA-15b antibodies (the most commonly involved antigens).

207 neonates, of whom 24 (12%) were antenatally treated with intravenous immunoglobulin, received 367 postnatal platelet transfusions. We excluded 81 (39%) neonates from our analysis of post-transfusion platelet count increments, because they either received a second transfusion on the same day as the first transfusion (n = 15) or on the day after the first transfusion (n = 29) or had missing data (n = 37). The platelet increment after the first transfusion was calculated and compared between HPA-unmatched platelet transfusions (n = 60) and HPA-matched platelet transfusions (n=66). The median platelet increment was 59 × 10^9 platelets per L (IQR 35–94) after HPA-unmatched platelet transfusions and 98 × 10^9 platelets per L (67–134) after HPA-matched platelet transfusions (P<0.0001; Figure 3). Our results were similar when stratified by antenatal treatment status (Figure 3).

Severe bleeding was diagnosed in 23 (6%) of the 389 liveborn neonates. One neonate had a severe pulmonary haemorrhage; the other 22 neonates were diagnosed with severe intracranial haemorrhages (Supplemental Table 5). Three (14%) cases of intracranial haemorrhage were detected antenatally by ultrasound, 17 (77%) were detected postnatally, and the timepoint of detection was unknown for two (9%). In two of the three neonates who were diagnosed antenatally, antenatal intravenous immunoglobulin treatment was started after the detection of anti-HPA antibodies in the mother's serum at 20 weeks' gestational age and 32 weeks' gestational age, respectively. Estimating the timepoint of severe intracranial haemorrhage development was not possible in 16 (73%) of 22 neonates owing to missing data and the lack of serial ultrasound examinations during pregnancy and after delivery. In one neonate with a severe intracranial haemorrhage diagnosed postnatally, the bleeding worsened postnatally on MRI after the initial diagnosis. In this neonate, platelet count nadir was 19 × 10^9 platelets per L on day 5 after birth and the implicated antibody was directed against HPA-15b. No other worsening of severe bleeding was reported for the other neonates.

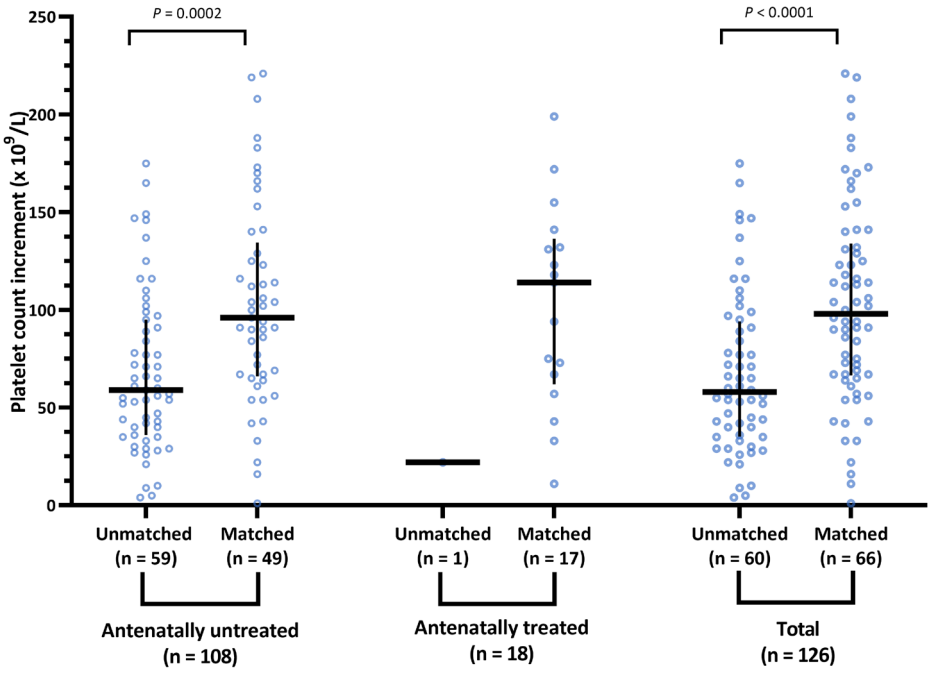

FIGURE 3. Platelet increment after first platelet transfusion

Platelet count increment was calculated of the first matched or the first HPA-unmatched platelet transfusion. The first set of plots shows cases that did not receive antenatal treatment (antenatally untreated). The second set of plots shows cases that received antenatal treatment during pregnancy (antenatally treated). The last set of plots shows platelet increment of all cases. Black lines represent medians with interquartile ranges. Median platelet count increments were compared by performing Mann Whitney U test (unadjusted analysis), statistical testing in the antenatally treated group was not possible; the HPA-unmatched group in this subgroup included 1 case only.

Mild bleeding was diagnosed in 186 (48%) of 389 neonates, of whom eight (4%) had a mild intracranial haemorrhage, eight (4%) had mild organ bleeding, and 170 (91%) had skin bleeding. Of the eight neonates who had a mild intracranial haemorrhage, five (63%) had a grade 1 intraventricular haemorrhage and three (38%) had an intracranial haemorrhage that was not specified. Five of the eight neonates who had mild organ bleeding had gastrointestinal bleeding, two had umbilical cord bleeding, and one had a retinal haemorrhage. None of the neonates who had a previous sibling diagnosed with FNAIT were diagnosed with intracranial haemorrhage. In our subgroup analysis assessing treatment and clinical outcome by country, differences were observed in the proportion of neonates who were antenatally treated, ranging from three (5%) of 56 in Norway to 47 (64%) of 74 in Australia (Supplemental Table 3). The proportion of neonates with severe bleeding was similar across all countries (Supplemental Table 3).

DISCUSSION

This multicentre, retrospective cohort study of 389 neonates with FNAIT born in a 10-year inclusion period in seven countries highlights the large variation in contemporary postnatal treatment strategies. Postnatal management strategies included frequent use of matched or unmatched platelets for transfusion, intravenous immunoglobulin, or a combination of treatments. Analysis of one of our outcomes showed differences in platelet count increments between treatment types, with the median platelet increment being significantly larger after matched platelet transfusions than after unmatched platelet transfusions. We could not analyse associations between treatment type and the occurrence of severe bleeding due to missing data on the timing of bleeding. This study presents new information on platelet responses to postnatal treatment for FNAIT, providing a starting point for a comparative clinical trial.

In a previous national cohort study in the Netherlands, we found a great diversity in postnatal treatment strategies in neonates with FNAIT, despite the availability of a national guideline.[11] To our knowledge, this is the first international study addressing postnatal treatment in neonates affected by FNAIT. More than half of the neonates were treated with platelet transfusions, which were either HPA-matched or HPA-unmatched, or a combination of both. Differences in the treatment strategy might be partly explained by variation in treatment guidelines between centres and in the availability of HPA-matched platelets per centre. For example, HPA-matched platelets are not routinely available in many countries (eg, Slovenia) and intravenous immunoglobulin is recommended as a first-line treatment in other countries (including some centres in the USA). Differences in treatment strategy might also be related to variation in clinical characteristics of the cases per country, although we did not investigate this possibility.

53% of the neonates received platelet transfusions, which is in line with a systematic review reporting that 51% of neonates with FNAIT receive postnatal platelet transfusions.[7] Unmatched platelet transfusion products are often readily available, whereas platelets matched for HPA-1, HPA-5, or both are not always available from hospital stocks or from blood centres due to donor availability and logistical challenges. With 98% HPA-1a positivity and 20% HPA-5b positivity in the population,[12] there is a high chance that HPA-unmatched platelet products will be incompatible. Whether matched platelet products yield better clinical outcomes than unmatched platelet products is still unclear. No randomised clinical trials have investigated the differences in clinical outcome and bleeding risk between the two treatments. We observed significantly larger median platelet count increment in patients receiving matched versus unmatched platelet transfusions. These data confirm results from previous smaller studies showing that the median platelet increment ranged from 116-170×10^9 per L after matched transfusions and from 27-68×10^9 per L after unmatched donor

transfusions.[13-16] As in these previous studies, we did not adjust for possible confounding factors between the two groups. However, our cohort had a large sample size and we used only data from first transfusions in patients who received no further transfusions on the following day. First matched platelet transfusions led to larger platelet increments than first unmatched platelet transfusions, but platelet counts after day 3 were similar. However whether matched platelets prevent severe bleeding better than unmatched platelets remain undocumented. Circulating maternal HLA-antibodies in the neonate may also cause the destruction of transfused platelets.[17] These HLA-antibodies were not considered in this study. Approximately a third of neonates received intravenous immunoglobulin postnatally, either alone or in combination with platelet transfusions. Administration of intravenous immunoglobulin to the mother during pregnancy decreases pathogenic IgG transport from the mother to the fetus due to competition at the receptor level in the placenta and has been shown to reduce the risk of fetal intracranial haemorrhage.[6] The mechanism of action of postnatal intravenous immunoglobulin treatment in FNAIT is not clear. Although our study was not designed to compare the outcomes of treatment regimens, platelet counts in the groups receiving platelet transfusions increased more rapidly than that in the intravenous immunoglobulin only group, in line with previous smaller studies.[18, 19]

We found a lower prevalence of severe bleeding (6%) compared with other cohort studies, which have reported rates from 10% to 25%.[5, 20] Several explanations are possible. First, we included only liveborn neonates with FNAIT and therefore, unlike other studies, did not include fetuses that had died or pregnancies that had been terminated due to intracranial haemorrhage. As a result, calculating mortality rates was also not possible. Second, about a quarter of neonates in our study received antenatal intravenous immunoglobulin treatment, which could possibly have prevented the occurrence of intracranial haemorrhage. Finally, the definitions of bleeding differed between studies; we classified eight cases of intracranial haemorrhage as mild bleeding, whereas the other studies reported on all neonates with intracranial haemorrhages without stratifying by severity.

In our study, worsening of the intracranial haemorrhage after postnatal diagnosis was reported in only one neonate, suggesting that this event is rare. This finding is consistent with earlier studies suggesting that intracranial haemorrhage develops predominantly during pregnancy rather than postnatally.[21] Given the small proportion of neonates with FNAIT and thrombocytopenia who develop intracranial haemorrhage, it is unlikely that thrombocytopenia is the sole cause of intracranial haemorrhage—another factor might potentially increase the risk of bleeding. In a 2022 cohort study,[22] genetic screening was done in 194 fetuses antenatally diagnosed with intracranial haemorrhage. Pathogenic variants of *COL4A* and *COL4A2* (encoding basement membrane proteins) were found in 36 (19%) of 194 fetuses, emphasising the heterogeneity in the causes of fetal intracranial haemorrhage. Additionally, most HPAs are expressed by endothelial cells.[23, 24] Several studies using *in vitro*

and murine models have shown that HPA-antibodies can bind to the endothelium which might increase the risk of ICH.[1-3]

This study yielded two additional interesting findings. First, we found an over-representation of male neonates with FNAIT, confirming a similar finding in a previous study.[25] In maternal RhD alloimmunisation, male neonates are also reported to be more severely affected than female neonates.[26] The reason for the difference in sex distribution in neonates affected by FNAIT is not clear. Possible explanations can be found in transplantation medicine. Studies in this field have shown that sex-mismatch is a risk factor for transplant rejection, possibly due to recognition of Y chromosome-encoded peptides by the maternal immune system.[27] Second, we found that a large proportion of neonates were born small for gestational age, consistent with a previous report.[28] This may partly be due to selection bias because additional routine diagnostics tests, including a full blood count, are often done in neonates born with low birthweight, which can lead to the detection of thrombocytopenia. Alternatively anti-HPA-1a could have bound to placental cells that express HPA-1a leading to placental damage and dysfunction, and leading hence to fetal growth restriction.[29, 30]

There is no international consensus on the optimal postnatal treatment strategy for FNAIT. Different platelet transfusion thresholds and volumes were recommended in the guidelines of the participating centres. The safety and benefits of platelet transfusions in neonates have been questioned after a large, randomised trial in preterm neonates showed that a restrictive transfusion policy (transfusion at $<25\times10^9$ platelets per L) was associated with an improved outcome compared to a more liberal strategy (transfusion at $<50\times10^9$ platelets per L).[31, 32] Whether platelet transfusions in neonates with FNAIT could also have deleterious effects, is not known and requires further investigation.

As shown in this study, platelet counts in most neonates with FNAIT increased to more than 50×10^9 platelets per L in the first week of life. Neonates with FNAIT in the no-treatment group were less likely to have severe disease than those in the treatment groups, because guidelines recommend the administration of platelet transfusions to neonates with very low platelet counts, bleeding, or both. Platelet counts in neonates in the no-treatment group increased spontaneously within the first week of life. None of the 50 neonates with severe thrombocytopenia who did not receive antenatal or postnatal treatment developed bleeding and their platelet count increased.

Our study had several limitations. First, the results of our study should be interpreted in light of its retrospective design and probable selection bias, which is highlighted by the small numbers of neonates at several centres. Second, different antibody screening methods were used to detect HPA-antibodies. Differences in the sensitivity of these tests may have influenced the composition of our cohort.[8] Third, we were not able to analyse the association

between bleeding, the time of treatment initiation, and platelet transfusion thresholds, because we could not identify the time of bleeding onset in most neonates. Finally, it is difficult to compare treatment groups owing to confounding by indication, hampering the study of eventual causal effects of the treatments on clinical outcomes. To identify optimal postnatal treatment strategies, information on the timing of the intracranial haemorrhage or other types of severe bleeding in FNAIT is essential. In neonates who develop intracranial haemorrhage postnatally, the time at which bleeding develops can only be assessed if serial neuroimaging examinations are frequently done throughout pregnancy and throughout the neonatal period. However, because FNAIT is predominantly diagnosed after birth, these data might only become available if a screening program is in place to detect HPA-alloimmunised pregnancies.

The true effect of different postnatal treatments can only be reliably established with a randomised study design. In the absence of antenatal screening programmes to detect pregnancies at risk for FNAIT in a timely manner, FNAIT is hugely underdiagnosed, hampering inclusion rates for such a study.[33] However, this large, multicentre cohort study of neonates with FNAIT evaluated postnatal treatment strategies in seven countries, which varied greatly, provides valuable information for clinicians and researchers on FNAIT treatments and outcomes, and shows the potential of international collaboration in a future clinical trial. Although our data suggest that HPA-matched transfusions lead to higher platelet count increment than HPA-unmatched transfusions, whether HPA matching reduces the risk of bleeding is unclear. This study highlights the urgent need for further trials to establish evidence-based guidelines for the management of neonates with FNAIT.

DATA SHARING STATEMENT

Requests for data can be send to the corresponding author and will be reviewed by the scientific committee of the initiating centre and primary investigators of participating centres. If approval is given, data will be shared via a secure portal. Data sharing requests can be sent to the corresponding author beginning 3 months and ending 36 months after publication. The study protocol will be made available upon reasonable request to the corresponding author.

AUTHORSHIP CONTRIBUTIONS

TWdV contributed to data curation, investigation, project administration, writing – original draft DW contributed to investigation and writing – review & editing, CCD contributed to formal analysis, methodology and writing – review & editing, VA, MZ, VY, HBH contributed to investigation and project administration, JGvdB, DO, CEvdS, MSV, ET, EMW contributed to writing – review & editing, CCS, ED, HEH, JLK, ZKM, EMD, NN, LP, MS, HT, contributed to investigation and writing – review & editing, MH contributed to conceptualisation and writing – review & editing, EL contributed to methodology, supervision and writing – review

& editing. TWdV, DW, VA, CCS, ED, HEH, HBH, JLK, NN, MS, MSV, LP, ET, HT, VY, MZ had access to, verified and interpreted the data of their centre and entered the data in the secured online database. TWdV and CCD had access to the complete database and verified and analysed the data. All authors read and approved the manuscript. TWdV and EL had final responsibility to submit the manuscript for publication.

DECLARATION OF INTERESTS

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SUPPLEMENTARY INFORMATION

SUPPLEMENTAL TABLE 1. Participating sites.

Country	Centre	Principle Investigator	Patients enrolled
The Netherlands	Leiden University Medical Centre, Leiden & Sanquin Diagnostic Services, Amsterdam	Enrico Lopriore & Masja de Haas	138
Australia	NAIT registry, Monash University, Melbourne	Erica Wood	93
Norway	UiT The Arctic University of Norway, Tromsø	Heidi Tiller	56
Spain	Blood and Tissue Bank, Barcelona	Núria Nogués	55
Sweden	Karolinska University Hospital, Stockholm	Emöke Deschmann	31
Slovenia	University Medical Centre Ljubljana, Ljubljana	Jana Lozar Krivec	19
United States	Levine Children's Hospital, Charlotte	Matthew Saxonhouse	13
United States	Boston Children's Hospital, Boston	Martha Sola-Visner	3

SUPPLEMENTAL TABLE 2. HPA-antibody detection method

	Australia (n = 74)	Norway (n = 56)	Slovenia (n = 19)	Spain (n = 55)	Sweden (n = 31)	The Netherlands (n = 138)	USA (n = 16)	Total (n = 389)
Antibody detection method - (n, %)								
MAIPA-assay	40 (54)	56 (100)	0	54 (98)	31 (100)	133 (98)	0	314 (91)
PIFT	25 (34)	0	11 (58)	2 (4)	0	127 (93)	0	165 (43)
Pak Lx assay (Immucor)	26 (35)	5 (9)	5 (26)	18 (33)	3 (10)	5 (4)	0	62 (16)
MACE	0	0	12 (63)	38 (69)	0	0	5 (31)	55 (14)
Flowcytometry	0	0	0	0	0	0	1 (6)	1 (1)
Unknown	12 (16)	0	5 (26)	0	0	0	10 (63)	27 (7)

Percentages do not add up to 100 because in 55% (214/389) of cases two or more antibody detection methods were used.
Abbreviations: HPA, human platelet antigen; USA, United states of America; MAIPA, monoclonal antibody specific immobilization of platelet antigen; PIFT, platelet immunofluorescence test; MACE, modified antigen capture ELISA.

SUPPLEMENTAL TABLE 3. Treatment and outcome of liveborn neonates with FNAIT per country

Characteristic	Australia (n = 74)	Norway (n = 56)	Slovenia (n = 19)	Spain (n = 55)	Sweden (n = 31)	The Netherlands (n = 138)	USA (n = 16)	Total (n = 389)
HPA antibody specificity - (n, %)								
HPA-1a	56 (76)	54 (96)	16 (84)	37 (67)	24 (77)	98 (71)	6 (38)	291 (75)
HPA-5b	6 (8)	1 (2)	3 (16)	5 (9)	3 (10)	28 (20)	0	46 (12)
Other	12 (16)	1 (2)	-	13 (24)	4 (13)	12 (9)	10 (62)	52 (13)
Pregnancy characteristics								
First pregnancy (primigravida) - (n, %)	9 (12)	14 (25)	6 (32)	15 (27)	4 (13)	34 (25)	0	82 (21)
FNAIT diagnosed in previous pregnancy - (n, %)	30 (41)	18 (32)	3 (16)	12 (21)	12 (39)	39 (28)	3 (19)	117 (30)
Antenatally treated - (n, %)	47 (63)	3 (5)	5 (26)	11 (20)	12 (39)	44 (32)	3 (19)	105 (27)
Neonatal characteristics								
Male sex - (n, %)	39 (59)	34 (61)	14 (74)	38 (69)	24 (77)	83 (61)	9 (56)	241 (64)
SGA - (n, %)	5 (8)	16 (29)	2 (11)	13 (24)	15 (48)	22 (17)	4 (25)	77 (21)
Platelet count								
Platelet count nadir (× 10^9 per L) - median (min. – max.)	25 (1–305)	15 (2–106)	26 (6–375)	27 (2–322)	39 (5–353)	20 (3–382)	14 (4–297)	21 (1–382)
Very severe thrombocytopenia (PC<25×10^9 per L) - (n, %)	33 (50)	39 (70)	8 (50)	27 (49)	11 (35)	78 (57)	10 (63)	206 (54)
Severity of bleeding symptoms†								
Mild bleeding - (n, %)	33 (45)	36 (64)	11 (58)	22 (40)	14 (45)	64 (46)	6 (38)	186 (48)
Severe bleeding - (n, %)	3 (4)	3 (5)	1 (5)	5 (9)	2 (7)	8 (6)	1 (6)	23 (6)

All statistics and percentages are calculated based on the valid numbers. i.e. excluded the missing.

† Severe bleeding was defined as cases with severe organ bleeding and/or severe ICH, mild bleeding are cases with skin bleeding, mild organ bleeding and/or intraventricular haemorrhage (IVH) grade I of II.

Abbreviations: USA, United States of America; HPA, human platelet antigen; FNAIT, fetal neonatal alloimmune thrombocytopenia; SGA, small for gestational age; L, litre; PC, platelet count.

SUPPLEMENTAL TABLE 4. Transfusion guidelines per centre in neonates affected by FNAIT during the study period (2010-2020)

	Transfusion threshold in cases without bleeding	Transfusion threshold in cases with bleeding	Recommended platelet transfusion dosage
Monash University, Australia	30×10^9/L in a term infant 50×10^9/L in a preterm infant	100×10^9/L for intracranial bleeding 50×10^9/L for other sites of bleeding	10 – 20 mL/kg
UiT The Arctic University of Norway, Norway	25×10^9/L	50×10^9/L	15 mL/kg
University Medical Centre Ljubljana, Slovenia	30×10^9/L until 72 hours after birth 20×10^9/L after 72 hours after birth	100×10^9/L for intracranial bleeding 50×10^9/L for other sites of bleeding	15 – 20 mL/kg
Banc de Sang i Teixits, Spain	50×10^9/L	100×10^9/L	20 mL/kg
Karolinska University Hospital, Sweden	30×10^9/L 50×10^9/L in neonates with birthweight <1500 grams during the first week of life	100×10^9/L for intracranial bleeding 50×10^9/L for other sites of bleeding	10 – 15 mL/kg
Leiden University Medical Center & Sanquin Diagnostics, The Netherlands†	25×10^9/L	50×10^9/L	10 mL/kg
Levine Children's Hospital, United States of America	30×10^9/L in a term infant 50×10^9/L in a preterm infant	100×10^9/L for intracranial bleeding	15 mL/kg
Boston Children's Hospital, United States of America	30×10^9/L in a term infant 50×10^9/L in a preterm infant	100×10^9/L for intracranial bleeding	15 mL/kg

† Random donor platelet transfusions are composed of material from five donors. Matched HPA transfusion products are composed of a single donor. The concentration of platelets in HPA matched transfusions are not different from HPA unmatched donor transfusions.

SUPPLEMENTAL TABLE 5. Characteristics of neonates with severe intracranial haemorrhage

Variable	Total (n = 22)
First pregnancy - n (%)	13 (59)
HPA antibody specificity - n (%)	
HPA-1a	14 (63)
HPA-1b	1 (5)
HPA-3a	1 (5)
HPA-5a	1 (5)
HPA-5b	2 (9)
HPA-15b	2 (9)
Unknown	1 (4)
Type of ICH - n (%)	
Intraparenchymal	10 (45)
Intraventricular grade III or IV	5 (23)
Subarachnoid	5 (23)
Subdural	1 (4)
Subpial	1 (4)
Detection of ICH - n (%)	
Antenatal, 23 weeks' GA	1 (4)
Antenatal, 32-34 weeks' GA	2 (9)
Postnatal, day of birth	2 (9)
Postnatal, 1 day after delivery	9 (41)
Postnatal, 2 days after delivery	3 (14)
Postnatal, 3 or more days after delivery	3 (14)
Unknown	2 (9)
Skin bleeding - n (%)	12 (55)
Platelet count nadir, median (IQR; min-max)	11 (7 – 26; 2 – 158)

All statistics and percentages are calculated based on the valid numbers. i.e. excluded the missing

† Case with platelet count 158 × 10^9/L received antenatal treatment.

Abbreviations: HPA, human platelet antigen; ICH, intracranial haemorrhage; GA, gestational age; IQR, interquartile range.

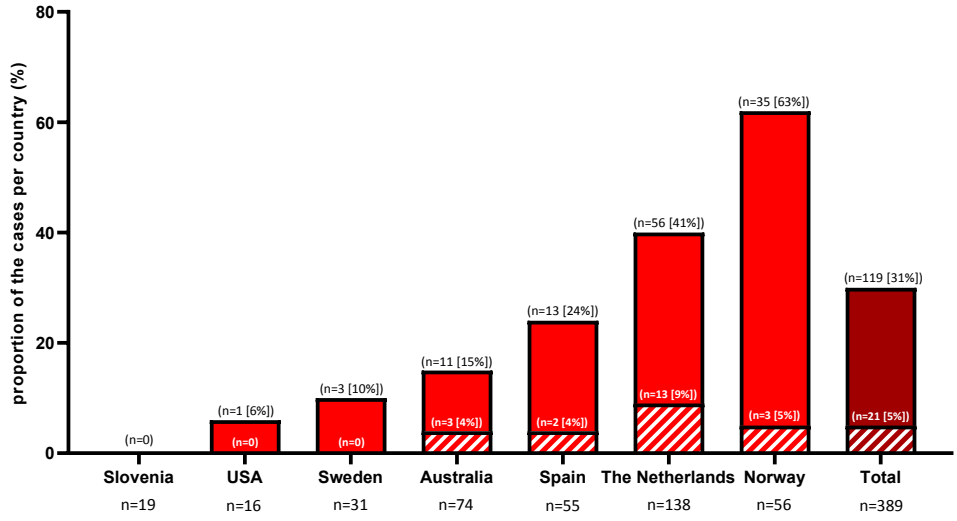

FIGURE 1A. Matched platelet transfusions

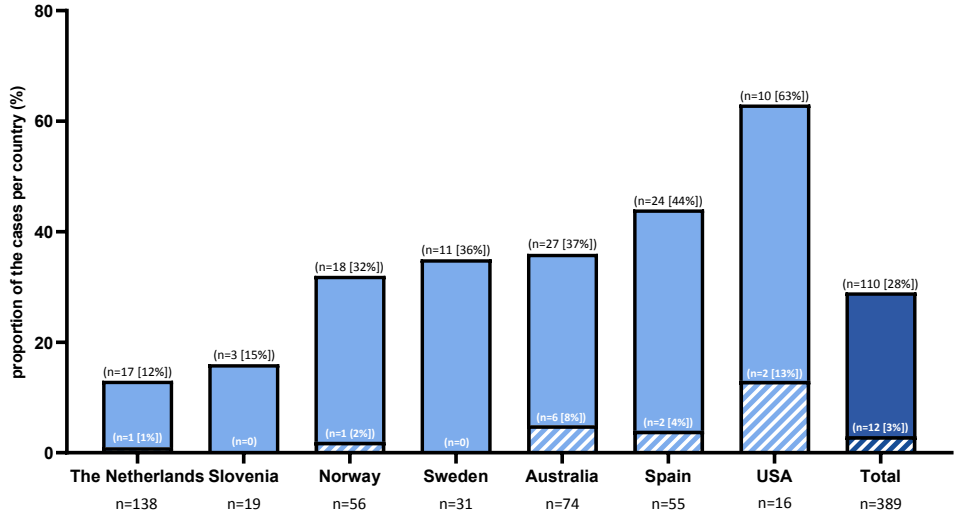

FIGURE 1B. Postnatal treatment with intravenous immune globulin (IVIg)

SUPPLEMENTAL FIGURE 1. Treatment with matched platelet transfusions and IVIg per country

Figure 1A shows the proportion of cases treated with HPA typed and matched donor platelet transfusions. **Figure 1B** shows the proportion of cases that were treated postnatally with intravenous immune globulin (IVIg) either in combination with platelet transfusions or IVIg alone. Striped parts in both graphs represent cases that were treated antenatally

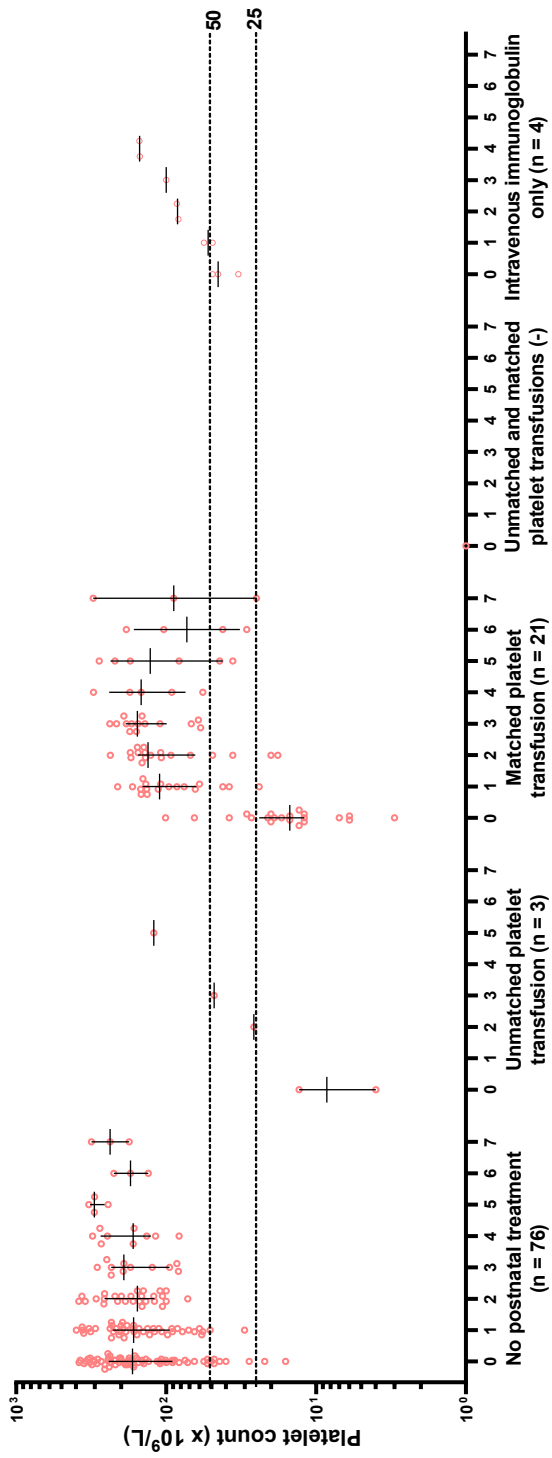

FIGURE 2A. Antenatally treated cases only

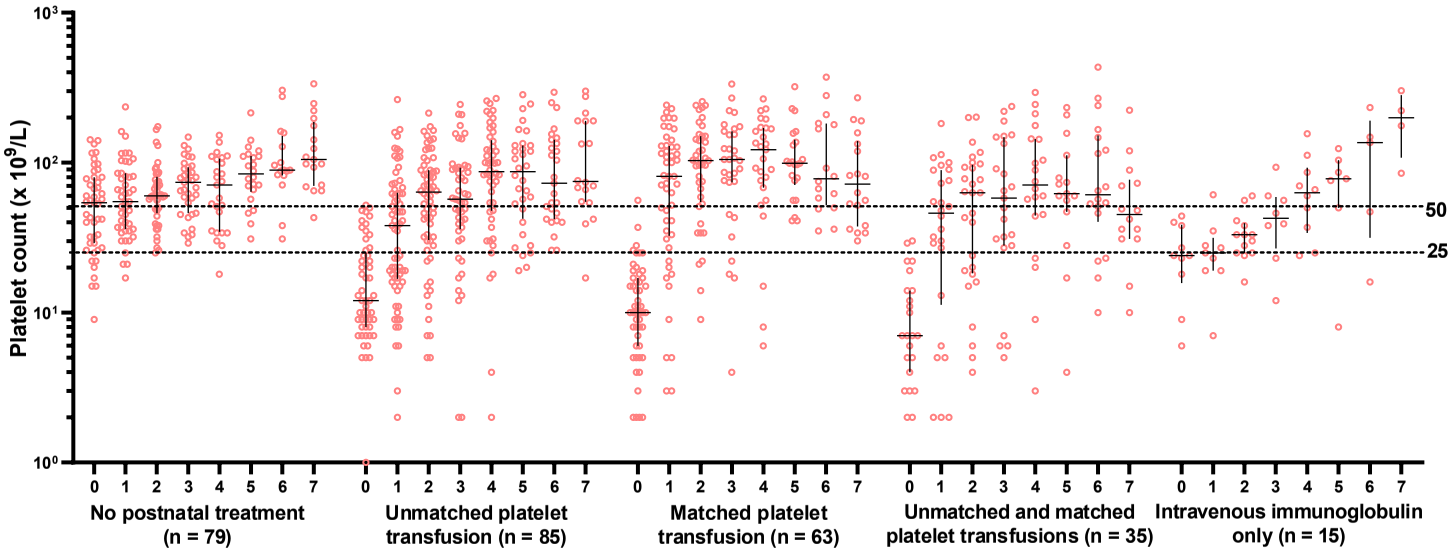

FIGURE 2B. Antenatally untreated cases only

SUPPLEMENTAL FIGURE 2. Platelet count per treatment strategy (stratified by antenatal treatment)

Supplemental Figure 2A shows the lowest neonatal platelet count per day per treatment strategy group of cases that were antenatally treated with IVIg. Missing data for one case in the no-treatment group. **Supplemental Figure 2B** shows the lowest neonatal platelet count per day per treatment strategy group of cases that were not antenatally treated with IVIg. Missing data for eight cases in the no treatment group. The horizontal lines in the graph represent platelet count of 50×10^9 per L and 25×10^9 per L for the upper and lower line respectively. Dots represent platelet counts, for each day the median and interquartile range are depicted with black lines.

Abbreviations: PTx, platelet transfusion; IVIg, intravenous immunoglobulin;