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# Maternal antibodies against paternal class I human leukocyte antigens are not associated with foetal and neonatal alloimmune thrombocytopenia

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## Summary

The causative role of maternal, anti-human leukocyte antigen (anti-HLA) class I antibodies in foetal and neonatal alloimmune thrombocytopenia (FNAIT) remains controversial. Furthermore, in FNAIT cases caused by anti-human platelet antigen-1a (anti-HPA-1a) antibodies, the possible additive effect of maternal anti-HLA class I antibodies on outcomes is unclear. Among 817 mother–father–neonate trios of suspected FNAIT, we assessed the possible association of maternal anti-HLA class I antibodies with neonatal platelet count, and the incidence of FNAIT caused by anti-HPA-1a antibodies. In 144 FNAIT cases caused by anti-HPA-1a antibodies, we investigated the possible association of maternal anti-HLA class I antibodies with neonatal platelet count, birth weight, and the incidence of intracranial haemorrhage ($n$ = 16). Maternal anti-HLA class I antibodies were not associated with neonatal platelet count in suspected cases of FNAIT. There was no significant interaction between the presence of anti-HLA class I antibodies and anti-HPA-1a antibodies. In FNAIT cases caused by anti-HPA-1a antibodies, there was no association between the presence of anti-HLA class I antibodies and neonatal platelet count, birth weight, or occurrence of intracranial haemorrhage. This study's findings do not support the concept that maternal anti-HLA class I antibodies represent a risk factor of FNAIT or disease severity.

**Keywords:** pregnancy, HLA class I antibodies, foetal and neonatal alloimmune thrombocytopenia, intracranial haemorrhage, neonates.

Foetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by maternal antibodies against foetal platelet antigens inherited from the father. After transplacental transport to the foetal circulation, immunoglobulin G class antibodies may cause thrombocytopenia and bleeding complications in the foetus or newborn. The incidence of FNAIT is 1 in 1 000 live births. Its most severe consequence, intracranial haemorrhage (ICH), leading to death or neurologic disability, occurs in 10 per 100 000 neonates, commonly prior to birth (Kamphuis *et al.*, 2014). In Caucasians, most FNAIT cases are caused by maternal antibodies against the paternally inherited human platelet antigen (HPA)-1a. Although maternal anti-HPA-1a antibodies' (or other anti-HPA antibodies') causative role in foetal thrombocytopenia or bleeding complications is undisputed (Davoren *et al.*, 2004; Kroll *et al.*, 2005; Ghevaert *et al.*, 2007), antibodies' role against human leukocyte antigens (HLA) remains controversial (Taaning, 2000). Depending on assay sensitivity, anti-HLA antibodies are detectable through the lymphocytotoxicity test (LCT) and the more sensitive, solid-phase Luminex test in 18–30% (Regan *et al.*, 1991) and ≥50% (Masson *et al.*, 2013; Vilches & Nieto, 2015) of all pregnant women respectively. HLA class I antigens are expressed on platelets (Kao *et al.*, 1986; Santoso *et al.*, 1993), and antibodies against HLA class I antigens can cause refractoriness to platelet transfusion in platelet concentrate recipients (Stanworth *et al.*, 2015). Several cohort studies of pregnant women (Sharon & Amar, 1981; Skacel *et al.*, 1989; Marshall *et al.*, 1994; Panzer *et al.*, 1995; King *et al.*, 1996) have investigated whether maternal anti-HLA class I antibodies can cause neonatal thrombocytopenia. Four of the five studies (Sharon & Amar, 1981; Marshall *et al.*, 1994; Panzer *et al.*, 1995; King *et al.*, 1996) did not

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associate the presence of maternal anti-HLA class I antibodies and neonatal platelet count. A major limitation of these studies, involving unselected pregnant women, was the very low number or even absence of thrombocytopenic newborns. Despite the negative findings observed in unselected pregnant women, numerous case reports have been published in which neonatal thrombocytopenia, occasionally accompanied by severe bleeding complications, occurred in women with anti-HLA class I antibodies (Svejgaard *et al.*, 1967; Sternbach *et al.*, 1986; Evans, 1987; Chow *et al.*, 1992; Onishi *et al.*, 1992; del Rosario *et al.*, 1998; Sasaki *et al.*, 2001; Tanaka *et al.*, 2000; Saito *et al.*, 2003; Moncharmont *et al.*, 2004; Thude *et al.*, 2006; Gramatges *et al.*, 2009; Starcevic *et al.*, 2010; Hutchinson *et al.*, 2015; Nakamura *et al.*, 2015; Meler *et al.*, 2017; Wendel *et al.*, 2017). The authors suggested a causal relationship between maternal anti-HLA antibodies and foetal or neonatal disease. At term, pregnant women have a high prevalence of anti-HLA antibodies. Therefore, there is a strong chance of high association of these antibodies with thrombocytopenia in neonates, but whether this association is causal or coincidental remains to be determined.

To our knowledge, this is the first systematic study of the possible role of maternal antibodies against paternal HLA class I antigens in a large cohort of unselected cases of neonatal thrombocytopenia.

## Materials and methods

### *Subjects*

We screened 817 families (mother–father–neonate trios) of suspected FNAIT cases, referred to our Center for Fetomaternal Incompatibility between January 2000 and April 2016, for inclusion. In Germany, routine post-natal platelet count in the neonate is not the standard of care. Therefore, all patients represent clinically overt cases of foetal or neonatal thrombocytopenia, or cases in which neonatal thrombocytopenia was incidentally detected. We defined two study groups (Fig 1): (i) FNAIT excluded, and (ii) FNAIT proven. The former group included mothers in whom HPA-1a antibodies were not detected. Mothers with genotype HPA-1bb without detection of HPA-1a antibodies at the time of postpartum blood sampling ($n = 20$) were excluded. *HLA-DRB3**01:01 genotype was not determined prospectively, and follow-up samples were not regularly screened in HPA-1bb mothers. The risk of HPA-1a immunisation after delivery of an HPA-1a-positive child is 12·7% in women who are *HLA-DRB3**01:01 positive but only 0·5% in women who lack this allele (Kjeldsen-Kragh *et al.*, 2019). Thus, the group of HPA-1bb mothers may include by chance a proportion of cases without any immunisation to HPA-1a as well as cases with low-affinity antibodies to HPA-1a that escaped detection. Therefore, this group was designated 'FNAIT possible'

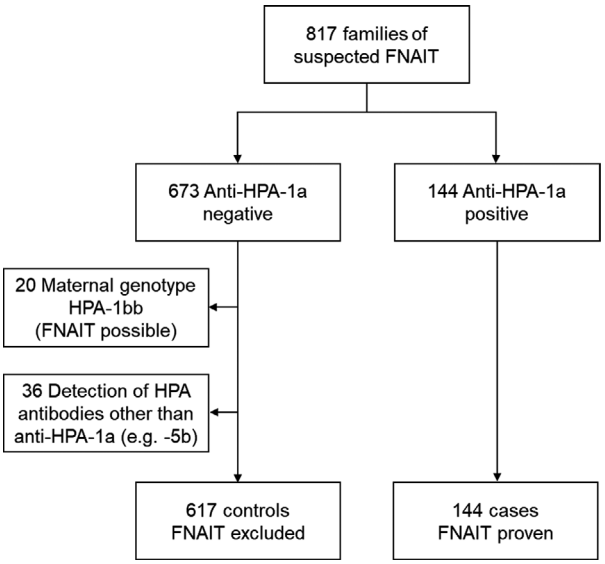



**Fig 1.** Screening and definition of controls and cases. Six women in the groups of cases had additional anti-HPA antibodies, mainly anti-HPA-5b.

(Fig 1) and excluded from further analysis. Furthermore, women with other anti-HPA antibodies (e.g. anti-HPA-5b, anti-HPA-3a) were excluded. The majority of cases with other anti-HPA antibodies had anti-HPA-5b antibodies [$n = 25$]. There is a striking difference in clinical presentation between FNAIT cases due to anti-HPA-1a and due to anti-HPA-5b antibodies (Ghevaert *et al.*, 2007); for example neonatal platelet counts in FNAIT cases with anti-HPA-5b are higher than in FNAIT cases mediated by anti-HPA-1a. Differences in cell type distribution of antigen may explain the disparity of biological consequences. To avoid mixing of different entities, we included exclusively the large group of mothers with anti-HPA-1a antibodies in the group 'FNAIT proven'. This group included all women who were HPA-1a-negative (HPA-1bb), had an HPA-1a-positive offspring and had anti-HPA-1a antibodies. Six women in this group had additional anti-HPA antibodies, mainly anti-HPA-5b. Finally, we included 617 cases in the FNAIT excluded group (control) and 144 cases in the FNAIT proven group (FNAIT due to anti-HPA-1a antibodies). All data were retrieved from the in-house laboratory information system and our laboratory's medical records, including the referring physician's letter.

### *Work-up of suspected FNAIT families*

The platelet count was determined in ethylenediaminetetraacetic acid-anticoagulated whole blood, using a haematology analyser (KX-21N, Sysmex Corporation, Kobe, Japan). A platelet count $<10 \times 10^9$/l was controlled microscopically in a counting chamber. ABO blood groups were determined for the mother, father and newborn. DNA was

isolated from maternal, paternal and neonatal whole blood and genotyped for HPA-1, -2, -3, -5, -9 and -15 using a real-time polymerase chain reaction (TaqMan; Applied Biosystems/Thermo Fisher Scientific, Darmstadt, Germany). Maternal serum was used to detect platelet-reactive alloantibodies. Postpartum samples were collected soon after delivery when FNAIT was suspected by the referring physician. We did not include follow-up serum samples of the same mothers to avoid bias by evanescence or affinity maturation of antibodies not reflecting the maternal humoral immune response during the actual pregnancy. Paternal platelets and platelets from donors with known HPA genotypes were isolated to perform the indirect monoclonal antibody immobilisation of platelet antigens (MAIPA) assay. Maternal platelets were isolated from mothers with thrombocytopenia (platelet count $<100 \times 10^9$/l) for detection of maternal autoantibodies in a direct MAIPA. The MAIPA assay was essentially performed as previously described by Kiefel *et al.* (1987). A MAIPA crossmatch (maternal serum with paternal platelets) was performed using monoclonal antibodies (MoAbs) Gi5, FMC25, Gi9, TAE/D2 and Gi18 specific for platelet membrane glycoproteins (gp) gp IIb/IIIa, gp Ib/IX, gp Ia/IIa, CD 109 and CD31, respectively. Maternal serum was also tested with at least three panel cells with known genotypes (two homozygous and one heterozygous test cell for frequent HPAs) in an indirect MAIPA. Test sensitivity was controlled using a World Health Organization anti-HPA-1a reference reagent (National Institute for Biological Standards and Control 05/106). Direct MAIPA with maternal platelets was performed using MoAbs Gi5, FMC25 and Gi9 against glycoproteins, gp IIb/IIIa, gp Ib/IX and gp Ia/IIa, respectively, to exclude maternal autoantibodies as a FNAIT cause. The presence of maternal anti-HLA class I antibodies was investigated in indirect MAIPA with two pooled panel cells containing 20 different blood donors, as well as in the crossmatch (maternal serum with paternal platelets) employing MoAb B1G6 directed to β2-microglobulin. MAIPA positive results were grouped through semi-quantitative scores (1 to 4) based on delta optical density (DOD = OD test − OD blank). A DOD >0·2 was scored as 1, a DOD >0·4 as 2, a DOD >0·8 as 3, and DODs >1·2 as 4.

### *Statistical analysis*

Data were managed and analysed using Excel (Microsoft Office 365; Microsoft Corporation, Redmond, WA, USA) and Prism 8 (GraphPad Software, Inc., San Diego, CA, USA) software packages. For descriptive purposes, both groups' characteristics were presented as medians and interquartile ranges (IQRs). The putative risk factors' effect on the outcomes, neonatal platelet count, birth weight and ICH was assessed descriptively using the Mann–Whitney *U* test, Kruskal–Wallis test, or Fisher´s exact test, as appropriate. Missing values are indicated throughout.

### *Meta-analysis*

We searched for eligible publications in the National Center for Biotechnology Information PubMed database. The search was restricted to publications written in English. Additional publications were identified through cross-referencing. We selected all reports on screening studies with data regarding maternal anti-HLA antibodies and neonatal platelet count in unselected cohorts of pregnant women. The outcome of the meta-analysis was the risk ratio of maternal anti-HLA antibodies on neonatal thrombocytopenia (platelet count $<150 \times 10^9$/l). This analysis was performed using Review Manager 5·3 (Review Manager, 2014).

### *Study approval*

The study was approved by the Ethics Committee of the Medical Faculty, Justus-Liebig-University (Giessen, Germany) (file number 82/09).

## Results

### *Alloimmunisation against paternal HLA class I antigens is not associated with maternal immune response to HPA-1a*

We compared the prevalence of maternal anti-HLA class I antibodies in controls (FNAIT excluded) and cases (anti-HPA-1a antibody positive) to test the hypothesis that alloimmunisation against paternal HLA class I antigens reflects a general propensity to mount an immune response to foetal alloantigens (Table I). Cases and controls were matched for maternal age, whereas male sex was overrepresented in controls (61%) and cases (72%; $P = 0{\cdot}02$). Maternal antibodies' prevalence against paternal HLA class I antigens did not differ between controls (43%) and cases (46%).

### *The absence or presence of maternal anti-HLA class I antibodies is not associated with platelet count in thrombocytopenic neonates*

The median platelet count in thrombocytopenic neonates did not differ according to the absence or presence of maternal antibodies against paternal HLA class I antigens (Fig 2). However, the platelet count of neonates born to mothers immunised against the foetal/neonatal HPA-1a antigen was significantly lower compared with that recorded in thrombocytopenic neonates, in whom FNAIT was excluded (median: $67 \times 10^9$/l; IQR: 37–112 versus median: 45·5; IQR 18–89·5; $P < 0{\cdot}0001$). We plotted the neonatal platelet count in controls, according to a negative or positive result of the HLA class I-specific cross match, between maternal serum and paternal platelets. Positive results were grouped through semi-quantitative scoring from 1+ to 4+ (Fig 2). There was no association detected between the semi-quantitative scores

**Table I.** Baseline characteristics of thrombocytopenic neonates.

| Characteristic | Controls* ($n$ = 617) | FNAIT† cases ($n$ = 144) | $P$ value§ |
|---|---|---|---|
| Maternal age — years | | | |
| Median | 31 | 31 | n.s. |
| Range | 26–34 | 26–35 | |
| Missing values — $n$ (%) | 98 (16) | 14 (10) | |
| Sex of the neonate | | | |
| Male sex — $n$ (%) | 379 (61) | 104 (72) | 0·02 |
| Missing values — $n$ (%) | - | - | |
| Platelet count of the neonate | | | |
| Median ($10^9$/l) | 67 | 45·5 | <0·0001 |
| Interquartile range | 37–112 | 18–89·5 | |
| Missing values — $n$ (%) | 12 (2) | 12 (8) | |
| Maternal HLA class I antibodies | | | |
| Positive — $n$ (%) | 231 (43) | 54 (46) | n.s. |
| Missing values — $n$ (%) | 77 (12) | 28 (19) | |

*Thrombocytopenic neonates; foetal and neonatal alloimmune thrombocytopenia excluded.
†FNAIT, foetal and neonatal alloimmune thrombocytopenia; diagnosis of FNAIT due to maternal anti-HPA-1a antibodies confirmed.
§All $P$ values are two-sided and are based on Fisher´s exact test (for categorial variables) or the Mann–Whitney $U$ test (for continuous variables).

of the HLA class I-specific MAIPA assay and neonatal platelet count.

### *The absence or presence of maternal anti-HLA class I antibodies is not associated with platelet count, birth weight, or ICH in FNAIT cases*

We tested the hypothesis that maternal anti-HLA class I antibodies may worsen pre-existing FNAIT's clinical course. For this purpose, neonatal platelet counts (Fig 3), birth weight (Fig 4) and absence or presence of ICH (Fig 5) were grouped according to the absence or presence of maternal antibodies against paternal HLA class I antigens. ICH occurred in 10 of the 81 cases (12·35%) without, and in six of the 56 cases (10·71%) with, detection of maternal anti-HLA class I antibodies (Fig 5). In conclusion, an association between the presence or absence of maternal anti-HLA class I antibodies and the tested clinical endpoints was not detected.

### *Meta-analysis of studies regarding the possible association of maternal anti-HLA antibodies and neonatal thrombocytopenia in normal pregnancies*

Table II presents the results of a meta-analysis of studies regarding the possible association of maternal anti-HLA antibodies with neonatal thrombocytopenia in normal pregnancies. Sharon and Amar (1981) screened 1 507 women immediately after delivery for panel reactive lymphocytotoxic antibodies. Of those, 32·8% were positive. Neonatal platelet counts were performed only when bleeding was suspected.

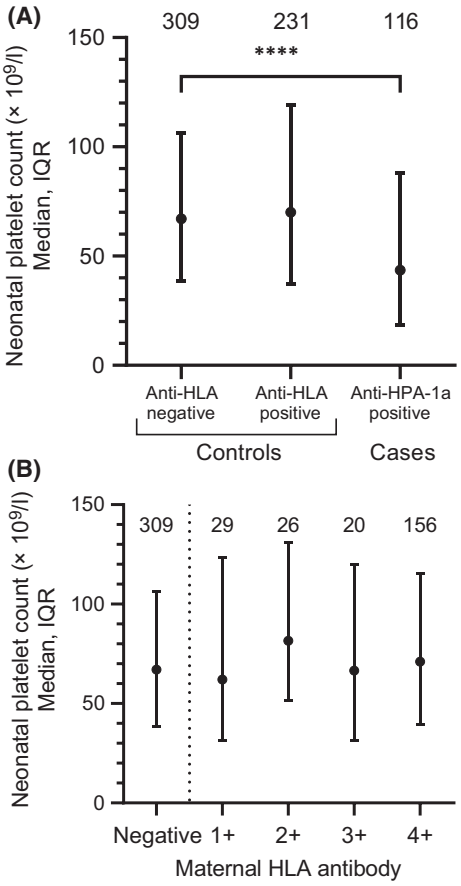



**Fig 2.** (A) Platelet counts of 540 controls (FNAIT excluded) and 116 cases (FNAIT due to maternal anti-HPA-1a antibodies proven). The median platelet count (+/− interquartile range, IQR) in controls is plotted according to a negative or positive cross match between the maternal serum and paternal platelets, determined through the β2-microglobulin-specific MAIPA assay (anti-HLA negative or positive). $P < 0{\cdot}0001$; Kruskal–Wallis test. (B) Platelet count of 540 thrombocytopenic neonates in whom foetal and neonatal alloimmune thrombocytopenia were excluded. The median platelet count (+/− IQR) is plotted according to a negative or positive crossmatch between the maternal serum and paternal platelets, determined through the β2-microglobulin-specific MAIPA assay (maternal HLA antibodies negative or positive). Positive results are grouped according to semi-quantitative scoring from 1+ to 4+. Not significant; Kruskal–Wallis test.

Among the neonates, 57 (3·8%) were thrombocytopenic, and the investigators attributed this to ABO incompatibility or sepsis. The anti-HLA status of mothers in these 57 cases was not provided. An association between maternal anti-HLA antibodies and neonatal thrombocytopenia was not reported in this study. Skacel *et al.* (1989) screened for panel reactive lymphocytotoxic anti-HLA antibodies in sera, obtained at 34 weeks of gestation from 142 women, and performed platelet counts using the corresponding cord blood samples. HLA antibodies' presence was not associated with the cord blood platelet count. However, six counts in the group with HLA antibodies were <150 x $10^9$/l compared with one count

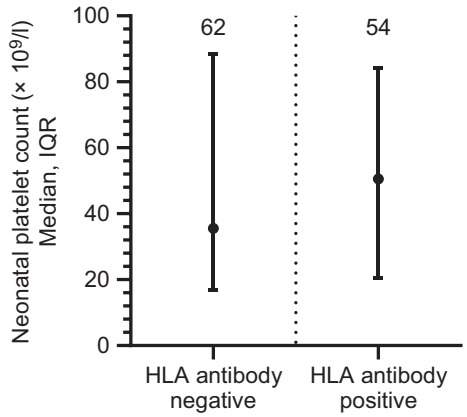



**Fig 3.** Platelet count of 116 thrombocytopenic neonates with confirmed diagnosis of FNAIT (maternal anti-HPA-1a antibodies detected, mother HPA-1bb, offspring HPA-1ab). The median platelet count (+/− interquartile range, IQR) is plotted according to a negative or positive crossmatch between the maternal serum and paternal platelets, determined through the β2-microglobulin-specific MAIPA assay (HLA antibody negative or positive). Not significant; Mann–Whitney *U* test.

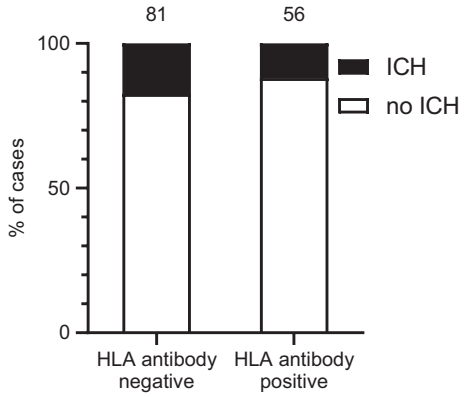



**Fig 5.** Intracranial haemorrhage among 137 thrombocytopenic neonates with confirmed diagnosis of FNAIT (maternal anti-HPA-1a antibodies detected, mother HPA-1bb, offspring HPA-1ab). The bars display the proportion of cases with (black) and without (white) intracranial haemorrhage (ICH). Cases are grouped according to a negative or positive crossmatch between the maternal serum and paternal platelets, determined through the β2-microglobulin-specific MAIPA assay (HLA antibody negative or positive). Not significant; Fisher´s exact test.

in the group without HLA antibodies. Marshall *et al.* (1994) screened 600 women at their first antenatal visit (i.e. 8–20 weeks of gestation) for panel reactive anti-HLA antibodies using platelet flow cytometry. Of those tested, 95 women (15·8%) had anti-HLA antibodies alone. A follow-up of 62 infants born to these women did not reveal any cases of thrombocytopenia. Panzer *et al.* (1995) evaluated the incidence of anti-platelet antibodies in 933 mother–child pairs. Panel reactive lymphocytotoxic anti-HLA antibodies were determined in 35 thrombocytopenic neonates (3·8%), five of whom tested positive for anti-HLA antibodies. King *et al.* (1996) studied 493 births and the possible association of maternal panel reactive anti-HLA antibodies with neonatal platelet counts. The HLA antibodies were detected through the enzyme-linked immunosorbent assay employing purified HLA class I antigens prepared from platelet concentrates. Of the mothers, 139 (31%) were immunised to HLA class I antigens. There was no association between HLA antibodies and neonatal platelet count. In addition, cord blood sera from neonates whose mothers had anti-HLA antibodies were tested to determine the presence of anti-HLA antibodies in the circulation of the neonate. HLA antibodies were detected in eight of 60 neonates (13%). Six of the 47 neonates (12·8%) in the group without thrombocytopenia, versus two of the five neonates (40%) in the group with thrombocytopenia, tested positive; however, the difference between the two groups was not statistically significant. Owing to the lack of events (e.g. neonatal thrombocytopenia) or missing data in controls, we were able to calculate the possible effect of the presence of maternal anti-HLA antibodies on neonatal thrombocytopenia in only two of the five prospective studies. The overall effect was not statistically significant ($P = 0{\cdot}51$).

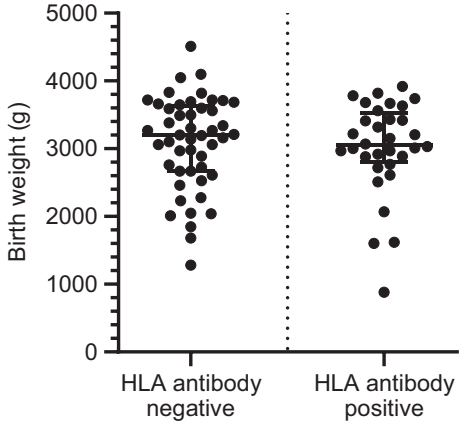



**Fig 4.** Birth weight of 80 thrombocytopenic neonates with confirmed diagnosis of FNAIT (maternal anti-HPA-1a antibodies detected, mother HPA-1bb, offspring HPA-1ab). The median birth weight (+/− interquartile range, IQR) is plotted according to a negative cross match (median birth weight: 3 198 g; IQR: 2 670–3 638 g) or positive crossmatch (median birth weight: 3 050 g; IQR: 2 798–3 528) between the maternal serum and paternal platelets, determined through the β2-microglobulin-specific MAIPA assay (HLA antibody negative or positive). Not significant; Mann–Whitney *U* test.

## Discussion

To the best of our knowledge, this is the first systematic study of the possible role of maternal antibodies against paternal HLA class I antigens in an unselected cohort of suspected FNAIT cases. In this study, the β2-microglobulin MAIPA was used to detect anti-HLA class I antibodies. This method was validated in a prospective study investigating the association between anti-HLA class I antibodies and

refractoriness to platelet transfusion, comparing β2-microglobulin MAIPA and LCT in 55 patients and 141 platelet transfusion episodes (Kurz *et al.*, 2001). The β2-microglobulin MAIPA and LCT's sensitivity to predict refractoriness to platelet transfusion (corrected count increment <5 at 16–20 h after transfusion) was 0·61 (36/59) and 0·17 (10/59), respectively. The specificity was 0·89 (73/82) and 0·99 (81/82), respectively. All HLA antibodies detected using the LCT were also detected through the β2-microglobulin MAIPA assay. Perhaps a more sensitive test than the β2-microglobulin MAIPA assay (e.g. a Luminex binding assay) is warranted to detect the possible association between maternal HLA antibodies and neonatal thrombocytopenia. Analysis of serum samples from patients that were originally included in the Trial to Reduce Alloimmunisation to Platelets study (Trial to Reduce Alloimmunisation to Platelets Study Group, 1997) showed that low levels of HLA antibodies, detectable through the Luminex binding assay alone, were not associated with refractoriness to platelet transfusion (Jackman *et al.*, 2013). The results of the β2-microglobulin MAIPA assay crossmatch between maternal serum and paternal platelets may not reflect the presence of maternal HLA class I antibodies that are directed against the neonate's class I antigens. The antibodies may be directed against non-inherited paternal antigens induced in a previous pregnancy. However, Dahl *et al.* (2017) demonstrated, through epitope mapping, that in most pregnant women maternal anti-HLA class I antibodies are directed towards paternally inherited foetal epitopes of the actual pregnancy. Furthermore, a strong correlation between the optical density value, obtained from the β2-microglobulin MAIPA assay and the mean fluorescence intensity, obtained from a Luminex assay for the detection of anti-HLA class I antibodies (FlowPRA), has been reported (Dahl *et al.*, 2016). In conclusion, the crossmatch between maternal serum and paternal platelets, using the β2-microglobulin MAIPA assay, reflects cognate maternal immunisation against paternal HLA class I antigens. Most of these antibodies may be directed against paternally inherited HLA class I antigens of the present offspring (Dahl *et al.*, 2017).

We did not detect an association between the presence of maternal anti-HLA class I antibodies and the immune response to the foetal HPA-1a antigen. Recently, HLA sensitisation was reported as a predictor for the formation of anti-HPA antibodies in a small series of nine pregnant women immunised against HPA (Reiher *et al.*, 2017). We could not confirm this observation in a large case series of FNAIT cases caused by maternal anti-HPA-1a antibodies. Instead, recent genetic association studies confirmed that the propensity to mount a maternal immune response to the foetal HPA-1a antigen is strongly associated with the maternal HLA allele *HLA-DBR3**01:01 (Sainio *et al.*, 2017; Wienzek-Lischka *et al.*, 2017; Kjeldsen-Kragh *et al.*, 2019). In the presence of maternal anti-HPA-1a antibodies, the neonatal platelet count was significantly lower than that observed in thrombocytopenic neonates without FNAIT. The association of platelet alloimmunisation with severe neonatal thrombocytopenia, compared with other causes of neonatal thrombocytopenia in which severe thrombocytopenia is a rare finding, has been reported in a large cross-sectional study (Burrows & Kelton, 1993).

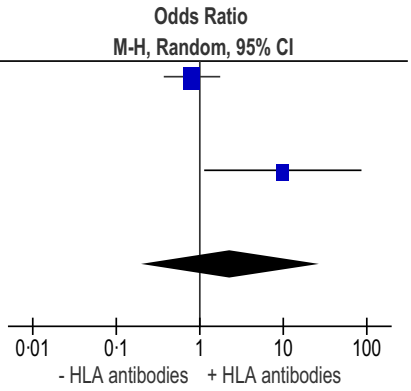
Prospective cohort studies in unselected pregnancies (Sharon & Amar, 1981; Skacel *et al.*, 1989; Marshall *et al.*, 1994; Panzer *et al.*, 1995; King *et al.*, 1996) did not associate maternal anti-HLA antibodies with neonatal thrombocytopenia, with one exception. Our systematic meta-analysis demonstrated that an important limitation of currently available studies was the small number, or even absence, of thrombocytopenic neonates in the study sample (Table II). Thus, we investigated maternal antibodies' possible role against paternal HLA class I antigens in a large cohort of suspected FNAIT cases. We did not detect an association of maternal anti-HLA class I antibodies with the neonatal platelet count in cases where FNAIT was excluded. Moreover, in FNAIT cases, we did not detect an association with the neonatal platelet count, birth weight, or ICH occurrence.

The presence of HLA antibodies (leukoagglutinins) in sera obtained from pregnant women was first demonstrated by van Rood (van Rood *et al.*, 1958) and Payne (Payne & Rolfs, 1958) in 1958. Anti-HLA antibodies are detected through LCT in 18–30% of pregnant women (Regan *et al.*, 1991). Regan *et al.* (1991) tested the serial blood samples of 306 pregnant women, demonstrating that anti-paternal cytotoxic antibodies appeared at 28 weeks of gestation or later in most cases. In this study, 32% had anti-paternal antibodies after successful completion of pregnancy; cytotoxic antibodies disappeared between pregnancies in most cases. Through the use of more sensitive solid-phase Luminex assays, the incidence of anti-HLA immunisation during pregnancy was ≥50% (Masson *et al.*, 2013; Vilches & Nieto, 2015). Consequently, there is a high possibility of finding maternal HLA antibodies in thrombocytopenic neonates attributed to chance. In 1962 and 1964, Shulman *et al.* (1962) and Pearson *et al.* (1964) respectively investigated neonatal thrombocytopenia cases. Through complement fixation, they detected maternal antibodies directed against paternally inherited antigens on neonatal platelets, granulocytes and lymphocytes. Subsequently, it was shown that these antibodies (anti-PlGrLy$^{B1}$ and anti-PlGrLy$^{C1}$) were directed against HLA class I antigens. After these initial reports, an ongoing series of case reports was published, in which maternal antibodies directed against HLA class I antigens of the neonate were associated with neonatal thrombocytopenia (Svejgaard *et al.*, 1967; Sternbach *et al.*, 1986; Evans, 1987; Chow *et al.*, 1992; Onishi *et al.*, 1992; del Rosario *et al.*, 1998; Tanaka *et al.*, 2000; Sasaki *et al.*, 2001; Saito *et al.*, 2003; Moncharmont *et al.*, 2004; Thude *et al.*, 2006; Gramatges *et al.*, 2009; Starcevic *et al.*, 2010; Hutchinson *et al.*, 2015; Nakamura *et al.*, 2015; Meler *et al.*, 2017; Wendel *et al.*, 2017). Owing to the high incidence of maternal anti-HLA class I antibodies at term, these observations may be coincidental.

**Table II.** Meta-analysis of studies investigating the association of maternal HLA class I antibodies with the risk of neonatal thrombocytopenia (events: platelet count <150 x $10^9$/l).†

| | + HLA antibodies | | - HLA antibodies | | | Odds Ratio | |
|---|---|---|---|---|---|---|---|
| Study or Subgroup | Events | Total | Events | Total | Weight | M-H, Random, 95% CI | Year |
| King 1996 | 10 | 139 | 27 | 308 | 58·2% | 0·81 [0·38, 1·72] | 1996 |
| Panzer 1995 | 5 | 0 | 30 | 0 | | Not estimable | 1995 |
| Marshall 1994 | 0 | 62 | 0 | 505 | | Not estimable | 1994 |
| Skacel 1989 | 6 | 57 | 1 | 85 | 41·8% | 9·88 [1·16, 84·45] | 1989 |
| Sharon 1981 | 0 | 495 | 0 | 1012 | | Not estimable | 1981 |
| **Total (95% CI)** | | **753** | | **1910** | **100·0%** | **2·30 [0·20, 26·78]** | |
| Total events | 21 | | 58 | | | | |
| Heterogeneity: Tau² = 2·55; Chi² = 4·78, df = 1 (P = 0·03); I² = 79% | | | | | | | |
| Test for overall effect: Z = 0·67 (P = 0·51) | | | | | | | |




*In Sharon's study, thrombocytopenia was not observed in 28 cases, in which monospecific anti-HLA antibodies reacted with paternal lymphocytes. In this series, 57 infants were thrombocytopenic, and this was related to ABO incompatibility or sepsis, as judged by the investigators. The anti-HLA status of mothers in these 57 cases was not provided.

In this study, we did not detect an association of birth weight with the presence or absence of maternal anti-HLA class I antibodies in newborns with FNAIT. An association between the levels of maternal anti-HLA class I antibody and reduced birth weight was reported in 50 thrombocytopenic neonates, in whom maternal anti-HPA antibodies were excluded and maternal anti-HLA antibodies were detected (Dahl *et al.*, 2016). Interpreting these results is difficult considering the lack of a matched control group of thrombocytopenic neonates born to mothers without anti-HLA class I antibodies. A recent meta-analysis did not report a significant effect of maternal anti-HLA class I or class II antibodies on any of the investigated pregnancy outcomes (Lashley *et al.*, 2013).

Refsum *et al.* (2017) investigated 23 mother–child pairs with thrombocytopenic newborns, in whom FNAIT due to anti-HPA antibodies was excluded and maternal anti-HLA class I antibodies were detected. There was no significant correlation between the newborn platelet count nadir or clinical outcomes and antibody strength (i.e. median mean fluorescence intensity). Interpreting these results is difficult considering the lack of a matched control group of mother–child pairs without maternal anti-HLA class I antibodies.

The transfer of maternal anti-platelet antibodies to the foetus may resemble a transfusion of plasma-containing blood products from donors with platelet antibodies. Thrombocytopenia secondary to passive transfer of anti-platelet antibodies is a rare blood transfusion complication (Swain *et al.*, 2018). All published cases involved passive transfer of anti-HPA antibodies, with anti-HPA-1a antibodies reported in almost all cases (Pavenski *et al.*, 2008; Collins *et al.*, 2013; Swain *et al.*, 2018).

In transfusion-associated acute lung injury (TRALI), induced after infusion of anti-HLA class I antibodies, mild thrombocytopenia has been observed in patients (Ausley, 1987). This finding was also reported in an anti-major histocompatibility complex class I TRALI mouse model (Looney *et al.*, 2009). A similar observation was noted after transfusion of granulocyte-specific antibodies (anti-HLA class I antibodies excluded) (Ausley, 1987), and sequestration of platelets in the lung has been attributed to mild thrombocytopenia in severe TRALI (Looney *et al.*, 2009). Several retrospective studies investigating the prevalence of transfusion reactions in recipients of donor components, with and without anti-HLA antibodies, did not report thrombocytopenia in recipients (Maślanka *et al.*, 2007; Fadeyi *et al.*, 2008; Kleinman *et al.*, 2011). Collectively, the results indicate that the infusion of plasma-containing blood products with anti-HLA class I antibodies seemingly does not lead to thrombocytopenia in the recipient, except in cases of severe TRALI in whom sequestration of platelets in the lung may cause mild thrombocytopenia.

Based on the present study's data and the experimental and clinical data available in the literature, we conclude that anti-HLA class I antibodies can destroy platelets in the *host-versus-graft* direction (i.e. in the setting of platelet transfusion). In this setting, cognate HLA antibodies exclusively interact with HLA class I antigens on foreign platelets and may lead to platelet destruction through different mechanisms. In the *graft-versus-host* direction (i.e. transfer of maternal HLA antibodies to the foetus or transfer of HLA antibodies via plasma-containing blood products), destruction of platelets is not observed. The abandoned expression of soluble and membrane-bound HLA class I molecules in the host may neutralise any potential pathogenic effect on platelets.

This study's results suggest that, in pregnant women with a history of suspected FNAIT due to maternal anti-HLA class I antibodies, invasive diagnostic measures (i.e. foetal blood sampling) and administration of intravenous immunoglobulin are not evidence-based practice.

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## Author Contributions

UJS, SWL and GB designed the study. YD, DQ and WH analysed the data. YD, WH and GB completed the statistical analysis of the data. GB, UJS, SWL, NC, SS and BB contributed to the first draught of the manuscript. GB assumed the final responsibility to submit the manuscript for publication. All authors had full access to all of the data, carefully reviewed the manuscript and approved the final version.

## Conflict of Interest

The authors declare that they have no conflicts of interest relevant to the manuscript.

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