Clinica Chimica Acta 498 (2019) 68–75

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

A single-center investigational study of CD36 antigen deficiency and platelet alloantibody distribution in different populations in Northern China as well as platelet alloantibodies effect on pregnancy

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ARTICLE INFO

Keywords:
CD36 deficiency
Platelet alloantibody
Fetal/neonatal alloimmune thrombocytopenia
Platelet transfusion refractoriness

ABSTRACT

Background: Platelet antibodies can lead to clinical diseases such as platelet transfusion refractoriness (PTR), fetal/neonatal alloimmune thrombocytopenia (FNAIT), etc. This study is aimed at understanding CD36 expression, platelet alloantibody distribution in different populations in Northern China, and effects of platelet alloantibodies on pregnancy.

Study design and methods: Whole blood samples of 612 subjects including hematological patients, pregnant women, and blood donors were collected at a single center, then CD36 expressions were determined, followed by platelet antibody screening and characterization of platelet antibody specificity. A retrospective analysis was performed in 1552 pregnant women admitted to Department of Obstetrics, in order to investigate FNAIT occurrence.

Results: Rate of CD36 deficiency expression was 2.12% (13/612), all cases exhibited type II deficiency without type I deficiency being detected, and such rate is lower than that in Southern China (3.43%), Japanese (4.87%) and in the black people (4.18%), and higher than that in the White people (0.09%). Positive rates of platelet antibody screening in hematological patient group (6.86%, 14/204) and in pregnant women group (6.31%, 13/206) are higher than that in blood donor group (0.49%, 1/202), $P < .01$. Out of 1552 pregnant women, there were not children with FNAIT.

Conclusion: The frequency of CD36 deficiency in northern China was low, all of them were type II deficiency, and no CD36 antibody was detected. It is speculated that the risk of immune-related thrombocytopenia caused by CD36 deficiency in this population is very low. Platelet antibodies should be monitored early in patients with hematological and multiple miscarriages pregnant.

1. Introduction

Platelet alloantibodies primarily arise from blood transfusion, pregnancy or transplantation, and may lead to a number of clinical diseases of immune thrombocytopenia, such as fetal/neonatal alloimmune thrombocytopenia (FNAIT), platelet transfusion refractoriness (PTR), post-transfusion purpura (PTP), miscarriage or stillbirth in pregnant women. FNAIT is caused by maternal alloantibodies against human platelet antigens (HPAs) resulting from maternal alloimmunization after exposure to paternally derived antigens on fetal platelets. When HPA-alloantibodies enter the fetal circulation after passing the placenta through FcRn-mediated transport, they can destruct fetal platelets as well as damage endothelial cells, which may result in bleeding complications [1]. It's found in recent studies that level of anti-HPA-Ia antibody is associated with low birth weight in newborns, presumed to compromise fetal development [2,3].

PTR often occurs in hematological malignancies patients necessitating multiple transfusion, and is a circumstance that platelet count fails to increase as expected or even decreases instead in a patient after multiple transfusion of platelets [4]. HLA or HPA antibody result from immune factors such as transfusion, pregnancy or transplantation, and when transfused platelets contain corresponding antigen, these platelet

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https://doi.org/10.1016/j.cca.2019.08.009
Received 3 June 2019; Received in revised form 2 August 2019; Accepted 13 August 2019
Available online 14 August 2019

antibodies can bind to the platelets rapidly, resulting in exacerbated platelet disruption and decrease in platelet count, instead. Therefore, early identification of platelet alloantibody and selecting antigen negative donor's platelet before transfusion are main approaches to clinical prevention and response to PTR [5].

Currently, platelet alloantibodies detected mainly include anti-HLA antibody, anti-HPA antibody, and anti-CD36 antibody [6–9]. In addition, these antibodies have clinical significance and can lead to FNAIT, PTR, and PTP, etc. CD36 molecules were initially discovered in thrombocytopenia patients and identified as platelet glycoprotein [10], or called membrane glycoprotein IV (GPIV) or NaK^a antigen, which are a type of Class B (double transmembrane domain) scavenger receptor expressed on a variety of cells such as platelets, monocytes, adipocytes, myocytes, macrophages, hepatocytes, and vascular epithelial cells [11,12], playing an important role in platelet aggregation, anti-angiogenesis, and tumor immunology [6]. CD36 deficiency can be divided into two phenotypes: type I with absence of CD36 expression on both platelets and monocytes, and Type II with absence of CD36 on platelets but express on monocytes. Anti-CD36 antibody is mainly seen in subjects with type I CD36 antigen deficiency. Frequency of CD36 antigen deficiency varies with region, population, and race; there are more studies on CD36 expression in Southern China [9,13,14], but no related studies in Northern China have been reported. Therefore, in this study, for three different populations, blood donors, pregnant women, and hematological malignancies patients, in Northern China, CD36 expression, platelet antibody screening and characterization were performed to investigate into frequency of CD36 deficiency and platelet antibody distribution, so as to provide references for preventing and addressing clinical issues such as FNAIT, PTR, and PTP.

2. Patients and methods

2.1. Population

Whole blood samples in this study came from patients visiting the First Medical Center of Chinese PLA General Hospital from February 2017 to October 2017 and blood donors participating blood donation. There were totally 612 subjects, 290 males and 322 females, including hematological malignancies patients ($n = 204$), pregnant women ($n = 206$), and blood donors ($n = 202$). From each subject, 5 mL of EDTA-anticoagulated venous blood was collected for analysis.

A retrospective analysis of medical records information of 1626 pregnant women was performed. Inclusion criteria: (1) pregnant women admitted to Department of Obstetrics, Chinese PLA General Hospital from February 2017 to October 2017; (2) for patients hospitalized many times, only medical records information of current hospitalization of those patients having pregnancy/delivery outcomes were included. Exclusion criteria: (1) patients with neither pregnancy nor delivery information; (2) patients undergoing actively induced abortion surgery due to any nonpathological cause (e.g., birth control); (3) Twin and multiple births were excluded to avoid weight bias. This study has been approved by the Medical Ethics Committee and informed consent from subjects has been obtained.

2.2. Flow cytometry

2.2.1. CD36 expression on platelets

5 mL of EDTA-anticoagulated fresh whole blood sample was centrifuged at a speed of 200 *g* for 10 min to result in platelet-rich plasma (PRP), centrifuged at a speed of 1200 *g* for 5 min to make a concentrated solution of platelets, and platelets were washed with PBS twice and the platelet concentration were adjusted to 1×10^7/mL for flow cytometry.

The 100 μL of platelet concentration were incubated with 10 μL of APC-anti-CD36 monoclonal antibody (MoAb; Clone, Mitenyi Biotec, Bergisch Gladbac, Germany) and 10 μL of fluorescein isothiocyanate (FITC) -anti-CD61 monoclonal antibody to detect CD36 expressed on platelets. APC-mouse immunoglobulin (Ig) G2α (Clone P3.6.2.8.1, Mitenyi Biotec) and FITC-mouse IgG1 isotype control (Clone P3.6.2.8.1, Mitenyi Biotec) were used as negative controls. The above materials were incubated away from light at 4 °C for 20 min, washed with PBS and resuspended, then loaded into a flow cytometer (FACSCalibur, BD, San Jose, CA) for analysis.

2.2.2. CD36 expression on monocytes

For samples with negative CD36 expression on platelets, CD36 expression on their monocytes was further detected. Into 50 μL of EDTA-anticoagulated fresh whole blood, 10 μL of APC-CD36 and 2 μL of PE-CD14 (MoAb; Clone REA5899, Mitenyi Biotec, Bergisch Gladbac, Germany) were added to detect CD36 expressed on monocyte; APC-mouse IgG2α and PE-human REA Control(S) (Clone P3.6.2.8.1, Mitenyi Biotec) isotype controls were negative controls; the above materials were incubated away from light at 4 °C for 20 min, into which 1 mL of RBC lysis buffer was added to enable lysis at room temperature for 10 min, washed with PBS buffer twice, resuspended and loaded into the flow cytometer for analysis. CD36 antigen expressions on platelets and monocytes were calculated from geometric mean fluorescence intensity using CELLQuest analysis software.

2.3. Assay of platelet alloantibodies in three populations

Solid-phase Coombs Test Kit for Platelet Antibody Assay (Lot 20170921 Changchun Bode Biotechnology Co., Ltd., Changchun, China) was used to detect platelet alloantibody in plasma of each sample, and the test was conducted in strict accordance with the procedure specified in package insert of the kit. For samples being positive for platelet antibody screening, PAKPLUS platelet typing kit (Lot 3005907 IMMUCOR, USA) was used for platelet antibody classification, this kit is able to further discriminate anti-HLA-I antibody and anti-GPIIb/IIIa, anti-GPIa/IIa, anti-GPIb/IX, and anti-GPIV, the assay was conducted in strict accordance with the procedure specified in package insert of the kit.

2.4. Comparison in CD36 expression deficiency

Literature on CD36 expression deficiency in populations from different regions in China and in different race populations in other countries is retrieved and compared with results of this study for differences in expression frequency among Chinese and other races in the world.

2.5. Retrospective analysis of pregnancy outcomes in pregnant women

206 pregnant women completing platelet antibody screening and CD36 expression assay were tracked and analyzed for presence of any adverse effect on pregnancy outcome, including occurrence of miscarriage/stillbirth/fetal diapause, neonatal weight, Apgar score, and occurrence of thrombocytopenia. Among which, 22 cases lost to follow-up due to not deliver babies in our hospital, and 184 cases had pregnancy outcomes.

In retrospective analysis of medical records information of 1626 pregnant women admitted to Chinese PLA General Hospital, information collected from these pregnant women includes age, number of times of pregnancy, number of times of delivery, fetal age, occurrence of miscarriage/stillbirth/fetal diapause, neonatal weight, Apgar score, and occurrence of thrombocytopenia [15], and factors relating to abnormal pregnancy outcome were studied. Among 1626 cases, 31 cases had no delivery information, 40 cases had twin pregnancy, 1 case had triplet pregnancy, and 2 cases experienced drug-induced miscarriage due to birth control, such cases were excluded, and totally 1552 pregnant women were included in analysis.

2.6. Statistical analysis

For differences in CD36 expression deficiency, positive rate of platelet antibody screening among pregnant women group, hematological malignancies patient group, and blood donor group, and differences in CD36 expression deficiency among population in this study, populations in south regions in China, and other race populations in the world, Pearson's chi-squared test or Fisher's exact probability test was used; Spearman's nonparametric correlation test was used to analyze factors leading to positive platelet antibody screening; Spearman's nonparametric correlation test was also used to determine relevance of platelet antibody screening, CD36 expression deficiency, age, number of times of pregnancy, and number of times of delivery to abnormal pregnancy outcome; Mann-Whitney *U* test was used to study post-delivery differences in pregnancy outcome in pregnant women group, and SPSS 19.0 was used to process data in statistical analysis. $P < .05$ indicates presence of statistical difference.

3. Results

3.1. CD36 antigen expression

612 subjects, including hematological malignancies patients, pregnant women, and blood donors, were screened by flow cytometry; 13 cases were detected with CD36 antigen deficiency, including 3 hematological malignancies patients, 4 pregnant women, and 6 blood donors; all deficiencies belong to platelet expression deficiency (type II deficiency), and frequency of CD36 antigen deficiency was 2.12%. Frequencies of CD36 antigen deficiency are not statistically different among 3 test groups, as shown in Table 1, Fig. 2. Expressions of CD36 antigen on platelets and monocytes are shown in Fig. 1.

From literature retrieval, a total of 18 papers on research of CD36 expression deficiency in Chinese in Jiangsu, Zhejiang, Shanghai, Guangxi, Shenzhen, and Guangzhou in China (collectively referred to as population in Southern China), Chinese in Taiwan in China, Japanese, the White people, and the black people were found; the analysis shows that, rate of CD36 expression deficiency in this study was lower than that in the population in Southern China (2.12% vs 3.43%, $P = .083$), and the rate was lower than those in Japanese (4.87%, $P = .004$) and in the black people (4.18%, $P = .028$); but higher than that in the White people (0.09%, $P < .01$), as detailed in Table 2.

3.2. Platelet alloantibody assay

Out of 204 hematological malignancies patients, a total of 14 patients were positive for platelet antibody screening, positive rate was 6.86%, 3 patients with CD36 antigen deficiency were all negative for platelet antibody screening. For 14 positive patients, antibody classification was performed using PAKPLUS kit; as a result, anti-HLA-I was present in 6 cases, anti-GPIIb/IIIa was present in 2 cases, and 6 cases showed negative results, antibody specificity failed to be defined, and anti-CD36 antibody was not detected (Table 1). Spearman's nonparametric correlation test was used to analyze hematological malignancies patients for relevance of positive platelet antibody screening to age, sex, CD36 expression, number of times of RBC transfusion, and number of times of platelet transfusion; as a result, positive platelet antibody screening was relevant to number of times of RBC transfusion ($P = .023$) and number of times of platelet transfusion ($P = .008$), whereas its relevance to other factors was not statistically significant ($P > .05$), as shown in Table 3.

Among 206 pregnant women, 13 cases were positive for platelet antibody screening, positive rate was 6.31%, and 4 patients with CD36 antigen deficiency were all negative for platelet antibody screening. Positive platelet antibody screening was relevant to number of times of delivery ($P = .023$) (Table 3). For 13 patients being positive for platelet antibody screening, antibody classification was performed using PAKPLUS kit; as a result, HLA-I antibody was present in 11 cases, HLA-I antibody coupled with anti- GPIIb/IIIa antibody was found in 1 case, and 1 case showed negative result, its specificity failed to be defined, and anti-CD36 antibody was not detected (Table 1, Fig. 2).

Among 202 blood donors, 1 case was positive for platelet antibody screening, the antibody was tested to be HLA-I antibody, this blood donor is a woman having a history of 2 births, and no anti-CD36 antibody was detected. The rate of positive platelet antibody of hematological malignancies patient group and pregnant women group, were both higher than that in blood donor group ($P < .01$) (Table 1, Fig. 2).

3.3. Follow-up and investigation of pregnancy outcome in pregnant women group

Out of 184 pregnant women, there were 4 cases of miscarriage, 25 cases of preterm birth, 155 cases of normal delivery. Compare prenatal information and pregnancy outcomes of 171 pregnant women in the group of negative platelet antibody screening and 13 pregnant women in the group of positive platelet antibody screening, and it's found that number of times of pregnancy in the positive group was higher than that in the negative group, $P = .046$; Apgar scores of newborns in the positive group were significantly lower than those in the negative group, $P < .014$. (Table 4). In this study, among newborns delivered by 13 pregnant women screened to be positive for platelet antibody screening. There were 10 cases were full-term birth, while the remaining 3 cases were preterm birth but all the platelet counts of 13 cases newborns were normal without FNAIT, Fig. 2. Retrieval of medical history of 13 pregnant women being positive for platelet antibody

Table 1
Distribution of CD36 expression deficiency and platelet antibody in different test groups.

	Hematological malignancies		Pregnant women		Blood donors		
	Type I/II CD36 deficiency ($n = 3$)	CD36 nodeficiency ($n = 201$)	Type I/II CD36 deficiency ($n = 4$)	CD36 nodeficiency ($n = 202$)	Type I/II CD36 deficiency ($n = 6$)	CD36 nodeficiency ($n = 196$)	Total
Negative (n)	0/3	187	0/4	189	0/6	195	584 (95.42%)
Positive (n)	0	14 (6.86%)	0	13 (6.31%)	0	1 (0.49%)	28 (4.58%)
Anti-HLA-I (n)	/	6	/	11	/	1	18
Anti-GPIIb/IIIa (n)	/	2	/	0	/	0	2
Anti HLA-I with GPIIb/IIIa (n)	/	0	/	1	/	0	1
Nonspecific (n)	/	6	/	1	/	0	7

Note: Based on chi-squared test, percentages of CD36 deficiency are not statistically different among three groups, $\chi^2 = 1.148$, $P > .05$. Pearson's chi-squared test shows that, positive rates of platelet antibody screening in populations of three groups were significantly different, $\chi^2 = 11.569$, $P < .01$; positive rate in hematological malignancies patient group was significantly higher than that in blood donor group, $\chi^2 = 11.567$, $P = .001$; positive rate in pregnant women group was significantly higher than that in blood donor group, $\chi^2 = 10.410$, $P = .001$; positive rate in hematological malignancies patient group was not significantly different from that in pregnant women group, $\chi^2 = 0.51$, $P = .822$.

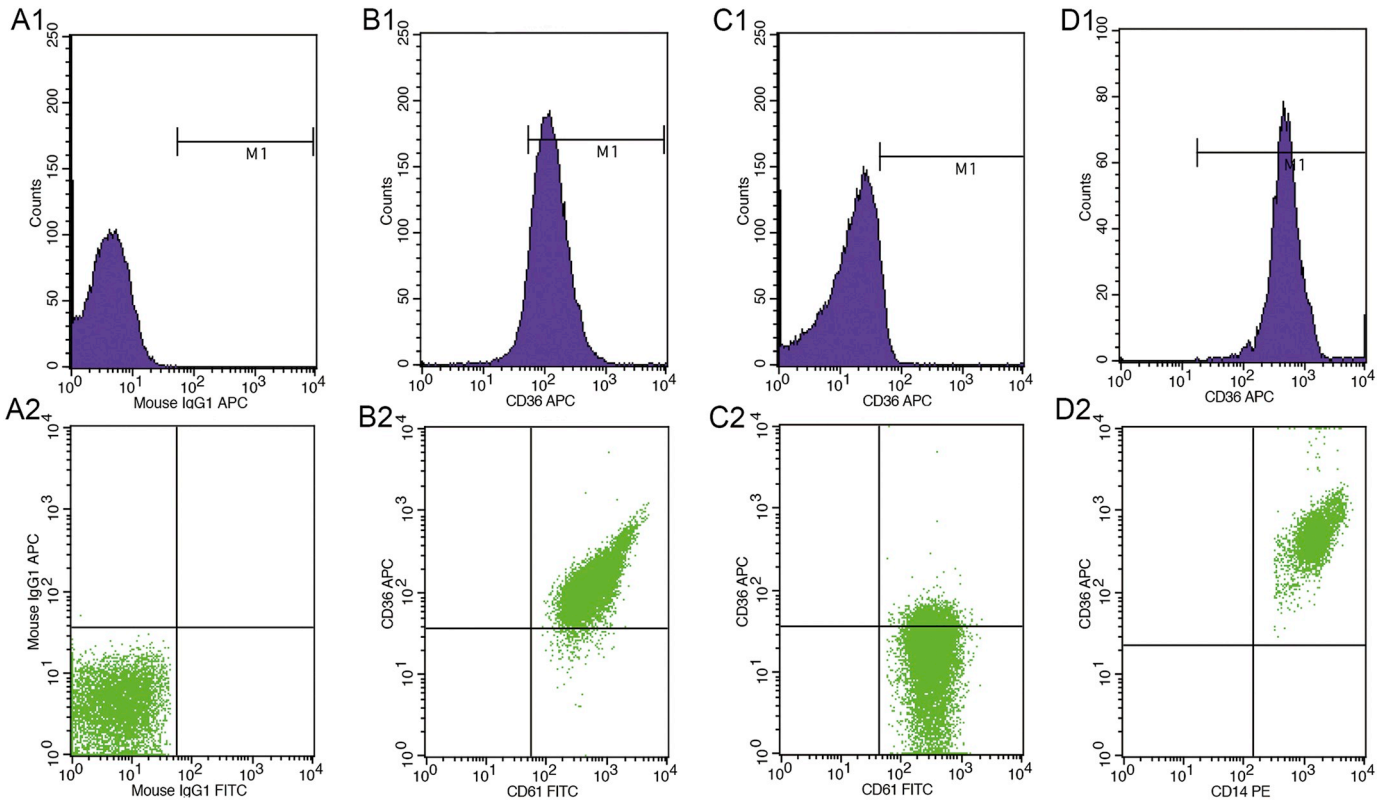

Fig. 1. Typical flow cytometric plots of CD36 expressed on platelets and monocytes.
A1-A2: isotype control of normal subjects;
B1-B2: CD36 expression on platelets is positive;
C1-C2 and D1-D2: subjects with type-II CD36 deficiency, C1-C2 indicates that CD36 expression on platelets is negative while D1-D2 indicates that CD36 expression on monocytes is positive.

screening shows that, 5 pregnant women having HLA-I and/or anti-GPIIb/IIIa antibody had a history of pregnancy abnormalities such as fetal diapause and miscarriage. In addition, the 3 cases of premature infants mentioned above occurred in these 5 pregnant women. Fortunately, however, these 5 pregnant women with a history of adverse pregnancy, although there were 3 premature births in this pregnancy, but no neonatal thrombocytopenia occurred in 5 newborns. However, we cannot rule out whether the adverse pregnancy history of these 5 pregnant women is caused by platelet antibodies, in other words, whether FNAIT were occurred. Information of 13 pregnant women with positive platelet alloantibody is detailed in Supplementary materials S-Table 1.

3.4. Retrospective analysis of medical records information in department of obstetrics

Since this study only detected and tracked the information of 206 pregnant women, in order to avoid the deviation caused by small sample size, we retrospectively analyzed the information of pregnant women in our hospital for one year to further investigate the occurrence of FNAIT in our hospital. Pregnancy outcomes of 1552 pregnant women include: 124 (7.99%) cases experienced miscarriage/stillbirth/diapause; 1428 (92.01%) cases delivered babies, including 1216 full-term infants (1216/1552, 78.35%) and 212 preterm birth infants (212/1552, 13.66%); 5 (0.32%) newborns had a laboratory indicator PLT $< 150 \times 10^9$/L, and were all preterm birth infants with a fetal age of 28–33 weeks, platelet alloantibodies in such newborns and their mothers failed to be detected as this part of study is a retrospective analysis, but further analysis of medical records information shows that mothers of such 5 newborns delivered preterm birth newborns due to underlying diseases diabetes mellitus, severe eclampsia, IgA nephropathy, premature rupture of membrane, and placenta previa, respectively, so it can be ruled out from clinical diagnosis that these 5 thrombocytopenia infants experienced FNAIT. With miscarriage and preterm birth as abnormal pregnancy outcomes, the age, number of times of pregnancy, number of times of delivery, newborn weight, Apgar score, and occurrence of neonatal thrombocytopenia in pregnant women were all correlated to abnormal pregnancy outcome, as shown in Table 5.

Table 2
CD36 expression deficiency in this study versus CD36 expression deficiency in different races in various regions.

	This study	Chinese in Southern China	Chinese in Taiwan, China	Japanese	White people	Blacks
Number of cases studied	612	7549	740	1621	4372	958
Number of cases with CD36 deficiency	13	259.00	14	79	4	40
Frequency of CD36 deficiency (%)	2.12%	3.43%	1.89%	4.87%	0.09%	4.18%
P value		0.083	0.761	0.004	< 0.01	0.028
Reference		[12,13,15–19]	[20,21]	[9,22–24]	[7,25–27]	[7,25]

Table 3
Correlation analysis of positive platelet antibody screening in hematological malignancies patient group and pregnant women group.

	Hematological malignancies patient group	*P* value	Pregnant women group	*P* value
Age (years)		0.907		0.593
Median (min–mix)	46 (3–88)		31 (24–44)	
Sex		0.350		/
Male	126		0	
Female	78		206	
CD36 expression		0.068		0.461
Deficiency	3		4	
Expression	201		202	
Number of times of RBC transfusion	3 (0 − 20)	0.023	/	/
Number of times of PLT transfusion	1 (0–18)	0.008	/	/
Number of gestational weeks	/	/	35 (7–41)	0.085
Number of times of pregnancy	/	/	2 (1–7)	0.068
Number of times of delivery	/	/	0 (0–2)	0.023

Note: Spearman's nonparametric correlation test shows that, number of times of platelet transfusion and number of times of RBC transfusion in hematological malignancies patient group were relevant to positive rate of platelet antibody screening, with $P < .05$; and number of times of delivery in pregnant women group was relevant to positive platelet antibody screening, with $P < .05$.

4. Discussion

The earliest report of anti-CD36 was from one PTR patient in Japan in 1989. This patient presented with PTR even after being transfused with HLA-matched platelets, presence of anti-CD36 antibody in his serum was identified, and platelet count increased following transfusion of platelets with negative CD36 antigen [10]. Later, relevance of CD36 to transfusion began to attract public attention, and Ohto et al. reported fetal/neonatal hemolytic disease or stillbirth in pregnant women with CD36 antigen deficiency following formation of CD36

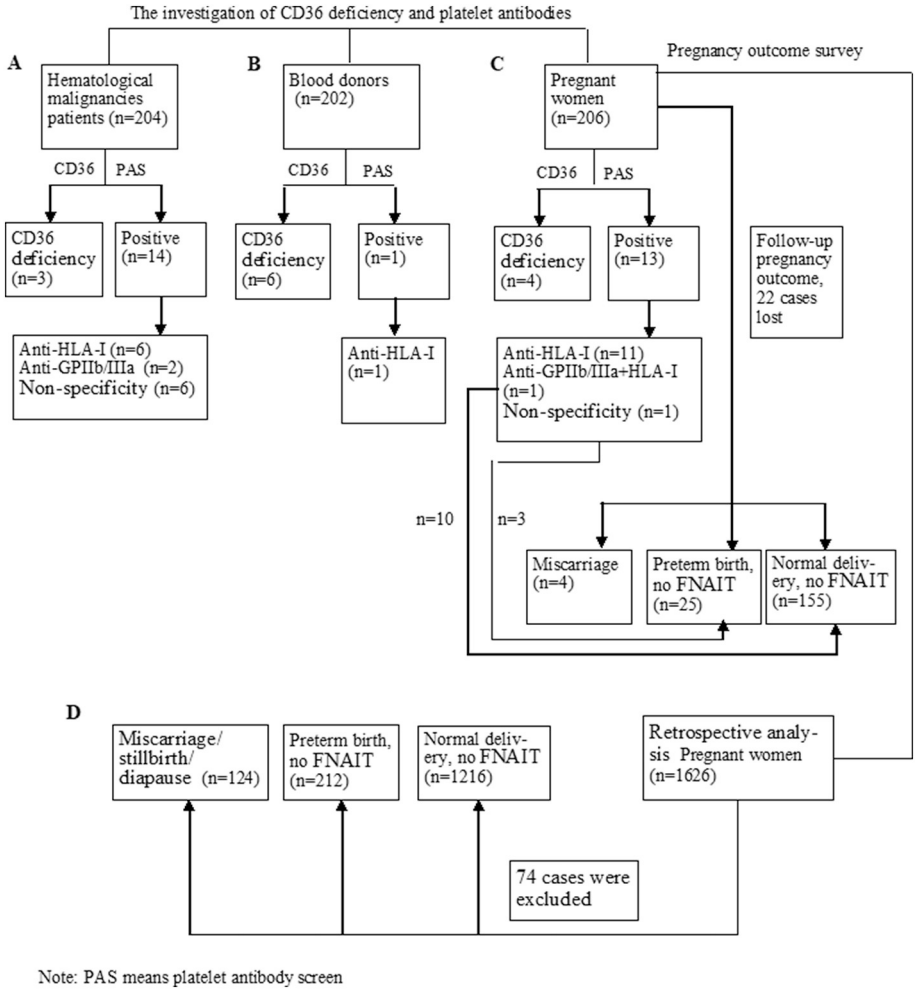

Fig. 2. The investigation process of platelet antibody screen and CD36 expression in difference group and survey of maternal pregnancy outcomes.
A: Study of platelet antibody screen and CD36 expression in patients with hematological disease; B: Study of platelet antibody screen and CD36 expression in blood donors;
C: Study of platelet antibody screen and CD36 expression in pregnant women, and pregnancy outcomes for this group of subjects Tracking;
D: Retrospective survey of maternal pregnancy outcomes in obstetrics.

Table 4
Follow-up statistics of pregnancy outcome in 184 pregnant women.

	Platelet antibody screening		*P* value
	Positive (n = 13)	Negative (n = 171)	
Age (years)	31 (26–40)	32 (24–43)	0.303
Number of times of pregnancy	4 (1–7)	2 (1–6)	0.046*
Number of times of delivery	1 (1–3)	1 (0–3)	0.822
Abortion (n)	0	4	0.302
Gestational age			0.578
Premature (n)	3	22	
Term infants (n)	10	149	
Fetal weight (g)	3350 (1820–4000)	3400 (1200–4500)	0.493
Apgar score	10 (6–10)	10 (8–10)	0.014*

Note: Age, Number of times of pregnancy, Number of times of delivery, Fetal weight and Apgar score are expressed in the form of median (min–mix). * means $P < .05$.

Table 5
Correlation analysis of pregnancy outcome in pregnant women.

	Pregnant women (n = 1552)	*P* value
Age (years)	32 (17–47)	0.008
Number of times of pregnancy	2 (0–9)	< 0.001
Number of times of delivery	1 (0–4)	< 0.001
Gestational age	39 (25–41)	< 0.001
Abortion (n)	124	< 0.001
Fetal weight (g)	3350 (800–5000)	< 0.001
Apgar score	10(6–10)	< 0.001
NTP (n)	5	< 0.001

Note: Age, Number of times of pregnancy, Number of times of delivery, Gestational age, Fetal weight and Apgar score are expressed in the form of Median (min–mix).

antibody [16]. CD36-deficient people might produce anti-CD36 alloimmune antibodies by immune stimulation mechanisms such as blood transfusion, pregnancy, and transplantation, and present with clinical symptoms of hemorrhage due to reduction in platelet count, such as PTR and FNAIT [17,18]. Moreover, it's reported that transfusion reactions would occur when any blood product containing anti-CD36 antibody is transfused into people with normal CD36 phenotype. As reported by Katsuya Morishita et al. [19] and Okajima et al. [20], after transfusion of anti-CD36 antibody-containing plasma, CD36-positive patients presented with transfusion reactions including anaphylaxis and significant reduction in platelet count. Nakajima et al. [21] reported cases of transfusion-related acute lung injury (TRALI) due to anti-CD36 antibody, and proposed that anti-CD36 antibody might be closely associated with pulmonary microvascular endothelial dysfunction. Therefore, anti-CD36 antigen and antibody assay should not be ignored for both blood recipients and blood donors.

By now, two phenotypes of CD36 deficiency have been found: type I deficiency where neither platelets nor monocytes express CD36, and type-II deficiency where CD36 deficiency occurs in platelets alone. As reported, frequency of CD36 antigen deficiency varies somewhat with region, population and race. CD36 deficiency mainly occurs in colored races, such as Japanese, Koreans, and Chinese in Taiwan, China, CD36 deficiency frequencies are 3%–11%, type-II deficiency is dominant, while type-I CD36 deficiency merely accounts for 0.1%–0.56% [8,22–24]; CD36 deficiency frequency in African American population is about 8%, and that in the Caucasian population is < 0.3% [25,26]. 13 of 612 subjects in this study experienced type-II CD36 deficiency at a frequency of 2.12%, and it's found from literature analysis that CD36 deficiency frequency in Northern China is lower than those in Southern China, Japanese and the blacks, indicating that CD36 expression varies with region and race. In this study, 13 CD36-deficient subjects did not produce anti-CD36 antibody. It's considered in current studies that, subjects producing anti-CD36 antibody come generally from populations with type-I CD36 deficiency, while subjects with type-II CD36 deficiency do not produce anti-CD36 antibody to result in clinical symptoms [27,28], which concurs with findings in previously reported studies. Knowing CD36 deficiency frequency in local population would be a guidance on health care of pregnant women and platelet transfusion in patients to some extent. As shown in this study, CD36 deficiency rate in Northern China was lower, all cases were of type II deficiency, and neither pregnant women nor hematological malignancies patients produced anti-CD36 antibody, thus it's not necessary for medical staff in this region to detect excessively and show much concern for CD36 in pregnant women. Hematological malignancies patients are presumed to have a very low probability of generating anti-CD36 platelet antibody, however, it's recommended to screen blood donors from native population in Southern China once presence of anti-CD36 antibody is confirmed. In addition, as only 612 subjects in this study were detected to have CD36 expression, any deviation in results cannot be ruled out due to small sample size.

Platelet alloantibodies are primarily generated due to immune stimulation including transfusion, pregnancy and transplantation, resulting in PTR, PTP, FNAIT, miscarriage or stillbirth [9,29,30]. In this study, frequencies of platelet antibody production in hematological malignancies patient group and in pregnant women group were significantly higher than that in blood donor group. The resulting platelet alloantibodies were mainly HLA-I antibody, followed by anti-GPIIb/IIIa antibody, while no anti-CD36 antibody was detected. Hematological malignancies patients have to undergo multiple platelet transfusions for a long time, and thus readily produce platelet alloimmune antibody, leading to PTR [5]. J.Wang et al. [31] reported 204 hematological malignancies patients with PTR, 55.88% of which presented with PTR due to HLA and HPA platelet alloantibodies. It's found in this study that platelet antibody generation in hematological malignancies patient group was significantly relevant to number of times of platelet transfusion and number of times of RBC transfusion. As both leukocytes and platelets express HLA antigen, both residual leukocytes and platelets in RBC blood would lead to formation of HLA and/or HPA antibodies in patients. To cope with PTR patients, many clinicians normally increase transfusion volume, shorten transfusion interval, and increase number of times of transfusion; as a result, blood resource will be wasted on one hand, and there will be higher risk of generating other alloantibodies in patients on the other hand, hence transfusion effect will further deteriorate, type matching will be more difficult, resulting in a vicious circle. For blood transfusion in hematological malignancies patients, in addition to selection of platelets matching the blood type of a patient, leukocytes in blood should be filtered to minimize immune stimulation of patient body by residual leukocytes in blood and reduce probability of platelet antibody formation.

In this study, 6 hematological malignancies patients were positive for platelet antibody screening, but antibody assay results were all negative, and platelet antibody specificity was not identified. Investigation of medical records shows that they were 4 lymphoma patients and 2 patients with multiple myeloma, presumably plasmacyte hyperplasia led to in vivo globulin abnormalities that interfered with results of platelet antibody screening, meanwhile the patients were treated with chemotherapy drugs in clinical practice, so effects of the chemotherapy medication on results of platelet antibody screening cannot be ruled out.

During gestation or prenatal period, FNAIT is the most common cause of severe thrombocytopenia and intracranial hemorrhage occurring in fetuses and newborns [17,32,33]. Over 75% of FNAIT cases in the White people are induced by alloantibody for human platelet antigen HPA-1a, and the most common antibody in FNAIT cases in Japanese is HPA-4b alloantibody. In China, more attention is paid to reports of FNAIT due to formation of CD36 alloantibodies in mothers with type-I CD36 deficiency [34]. FNAIT cases due to HLA antibody are also

reported, though they are rare and role of HLA antibody in FNAIT remains controversial [32,35]. Therefore, we screened pregnant women population for platelet antibody and CD36 expression, finding that no CD36 antibody was detected in type-II CD36 deficiency mothers and no FNAIT case occurred, either. Positive rate of platelet antibody screening in pregnant women group was higher than that in blood donor group, positive platelet antibody screening was primarily associated with number of times of delivery, presumably some women of childbearing age would opt to induced abortion in 1–2 months in the first trimester of any unplanned pregnancy against birth control policy in China, and as maternal hemorrhage usually occurs in the late term or prenatal period, thus such a short history of pregnancy is not enough to stimulate a maternal body to produce platelet alloantibody. Follow-up investigation of pregnancy outcome in the population shows that, the platelet antibody positive group had significantly lower Apgar score than the negative group, and women with a history of adverse pregnancy were all positive for platelet antibody screening, presumably platelet antibodies might have influenced birth quality of newborns.

In light of the influence of sample deviation, we further analyzed retrospectively medical records information of 1552 pregnant women and did not find FNAIT cases in normal delivery women, but the possibility of FNAIT in cases of abortion, stillbirth or fetal cessation cannot be ruled out, because of a retrospective analysis, the pregnant women were not screened for platelet antibodies. So, based on statistical analysis, patients having a history of multiple times of pregnancy or delivery, particularly a history of abnormal pregnancy, platelet antibody assay should be performed to find or exclude at an earlier time that they were caused by platelet alloantibodies, while for pregnant women not having a history of abnormal pregnancy, routine screening should be not necessary.

As this study is limited in sample size, no case with type-I CD36 deficiency was detected. Besides, in both hematological malignancy patient group and pregnant women group, cases being positive for platelet antibody screening were found but identified as negative in PAKPLUS assay, for which no further validation was conducted as this study is not intended to evaluate methodological differences. Solid-phase RBC capture method was used for platelet antibody screening, platelet antibody specificity was determined using PAKPLUS kit by ELISA, and as kits differ to some extent in anti-interfering capability and sensitivity and specificity to platelet antibody detection [31], patient disease, medication, and limited antigen spectrum of PAKPLUS kit may lead to inconsistent results.

In this study, it was found that the frequency of CD36 deficiency in northern China was lower than that in southern China, and all the CD36 deficiency were type II. Anti-CD36 antibodies were not detected in these populations with type II CD36 deficiency. In addition, we found that frequencies of platelet alloantibody formation in hematological malignancies patient groups and in pregnant women group were evidently higher than that in healthy blood donors, so it's recommended to investigate and study platelet antibody screen in hematological malignancies patients and pregnant women having a history of abnormal pregnancy at an earlier time. More attention should be paid to HLA-I and HPA antibodies rather than CD36 antibodies in people in northern China.

Declaration of Competing Interest

All authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.08.009.

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