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Integrating HLA and HPA in Precision Transfusion: Insights from Platelet Transfusion Refractoriness Driven by Anti-CD36 Alloimmunization and Multifactorial Hemostatic Complications

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Abstract

Purpose: CD36, recognized as a distinct blood group system, encodes a class-B scavenger receptor essential for hemostasis and innate immunity. Alloantibodies against CD36, although often overlooked, are a significant cause of platelet transfusion refractoriness (PTR), fetal–neonatal alloimmune thrombocytopenia, and immune-mediated bleeding. The comparative effectiveness of platelet antibody assays remains unclear. We aim to propose a streamlined diagnostic algorithm for PTR.

Methods: This retrospective cohort study, conducted at a tertiary center, analyzed 2,453 patients undergoing parallel screening for platelet antibodies using both the Solid Phase Red Cell Adherence Assay (SPRCA) and qualitative ELISA over a period of nearly seven years. Confirmatory workflows included MAIPA, molecular genotyping, and flow cytometry for CD36 antigen expression. Six illustrative cases with genetically or phenotypically confirmed CD36 deficiency were comprehensively profiled.

Results: ELISA detected antiplatelet antibodies in 33.5% of samples, while SPRCA identified them in 18.1%, yielding a moderate overall concordance of 78.1% ($\kappa = 0.444$). Among SPRCA-positive samples, 81.9% were confirmed by ELISA; for SPRCA-negatives, 22.8% were ELISA-positive, underscoring substantial assay discordance. Notably, 18.7% of cases were exclusively ELISA-positive, compared to only 3.3% detected solely by SPRCA. Dual assay positivity was highly specific for clinically significant platelet alloimmunization and PTR.

Conclusion: Our findings highlight the critical role of combined ELISA and SPRCA screening, supplemented by confirmatory and molecular methods, in accurately identifying anti-CD36 alloimmunization. The study underscores the need for comprehensive diagnostic protocols, rare donor registry development and the broader implementation of CD36-negative platelet inventories to optimize personalized transfusion strategies and patient outcomes.

Keywords: CD36 deficiency, CD36 alloimmunization, platelet transfusion refractoriness, Monoclonal Antibody-specific Immobilization of Platelet Antigens (MAIPA), Solid Phase Red Cell Adherence Assay (SPRCA).

List of Abbreviations

Human Platelet Antigen (HLA/HPA), platelet transfusion refractoriness (PTR), fetal–neonatal alloimmune thrombocytopenia (FNAIT), Monoclonal Antibody-specific Immobilization of Platelet Antigens (MAIPA), Solid Phase Red Cell Adherence Assay (SPRCA), Enzyme-Linked Immunosorbent Assay (ELISA), lymphocytotoxicity test (LCT), disseminated intravascular coagulation (DIC), drug-induced thrombocytopenia (DITP)

Main Text

Introduction

CD36 (glycoprotein IV) is a heavily N-glycosylated class-B scavenger receptor expressed on platelets, monocytes, endothelium, and erythroid lineage cells [1, 2]. Early studies defined the Naka– (CD36-negative) phenotype [3] and established type I and type II deficiency (absent on platelets±monocytes) [4], providing the immunohematologic framework for today’s diagnostics [5]. Its polymorphic expression on red cells and null variants, now recognized by The International Society of Blood Transfusion supports classifying CD36 as a blood group system with clear clinical relevance [6, 7]. Anti-CD36 is an important, often under-recognized cause of FNAIT[8],[9],[10] and fetal anemia[6, 11], demonstrating effective prenatal therapy in preclinical models and editorially highlighting integration into perinatal workflows.¹² Mechanistically, platelet CD36 surface density correlates with prothrombotic signaling responses[12][13] (e.g., to oxidized low-density lipoprotein)[14, 15], helping to explain why deficiency or alloimmunization can materially alter hemostasis[16]. In transfusion practice, anti-CD36–mediated platelet transfusion refractoriness (PTR) [17][18], which should be considered when Human Leukocyte Antigen and Human Platelet Antigen (HLA/HPA) matching fails [18], especially in high-prevalence populations [5], and may contribute to post-transfusion purpura (PTP) [19, 20]. Finally, flow cytometry remains first-line for phenotyping [5, 21], while optimized Monoclonal Antibody-specific Immobilization of Platelet Antigens (MAIPA) capture clones (GZ-70/GZ-608) markedly improve anti-CD36 detection over legacy reagents, enabling more reliable case identification and targeted donor selection [22].

CD36 deficiency exhibits notable geographic differences, affecting 2–4% of Chinese[23, 24] populations, 3%-11% of Japanese[3] populations, 2.6% of Middle Eastern[25] populations, and up to 3% of African[26, 27] populations,

but remains rare(<0.4%) in Europeans, which explains the low rate of anti-CD36 alloimmunization in Western populations.

Recent advancements in high-throughput sandwich Enzyme-Linked Immunosorbent Assay (ELISA) have surpassed conventional methods for CD36 phenotyping, achieving intra- and inter-assay variations as low as 2.1–5.2% [23]. Diagnosis remains challenging due to the disorder's rarity and the need for multiplexed approaches beyond standard antibody screens, especially in patients with comorbidities that can mask or mimic clinical features. Accurate diagnosis depends on comprehensive tests, including flow cytometry [28], MAIPA [29], Solid Phase Red Cell Adherence Assay (SPRCA), ELISA, and molecular genotyping, to distinguish CD36-related platelet alloimmunization from other causes. Our clinical series underscores the importance of integrated diagnostics for accurate identification in complex hemostatic disorders and guides optimal transfusion management.

Accurate detection of antiplatelet antibodies is essential in managing Immune Thrombocytopenia, FNAIT, and PTR. At MacKay Memorial Hospital (MMH), since 2019, parallel testing using SPRCA and ELISA has revealed a persistent “gray zone” of detection discrepancies [30]. Integrating CD36 antigen typing and anti-CD36 antibody screening has been pivotal for elucidating PTR mechanisms, highlighting the need for standardized thresholds to improve diagnostic precision.

Methods

We would report that patients, including cases 1[31], 2-4[32], 5, and 6[32, 33], have CD36 deficiency and CD36-related refractoriness.

Patients

Case 1: A 78-year-old female patient with lung adenocarcinoma and a documented CD36 deficiency was admitted with respiratory distress caused by pneumonia. Initial treatment involved empiric antibiotics, notably teicoplanin (400 mg IV daily), administered from January 30 to February 7 and resumed on February 25. Piperacillin/tazobactam was given concurrently from January 30 to February 6. Meropenem was initiated on February 4 and continued until the patient's death. Ceftriaxone was added to the antimicrobial regimen from February 18 to February 25.

Case 2 was a 32-year-old pregnant woman. Case 3 was a 60-year-old man with metastatic small-cell carcinoma, Sjögren's syndrome, and an unexpected PTR. Case 4 was a 21-year-old woman with acute leukemia, cellulitis, disseminated intravascular coagulation (DIC), and PTR. Case 5 was a 46-year-old man with severe cardiovascular disease, ischemic gangrene requiring multiple amputations, acute respiratory failure, and repeated surgical interventions. Case 6 was an 86-year-old woman with chronic lymphoma, recurrent infections, dementia, chronic heart failure, and atrial fibrillation.

Additionally, our retrospective analysis of 2,453 samples tested using SPRCA and ELISA under standardized protocols at MMH from the beginning of January 2019 to the middle of November 2025 found that positive results from either method prompted clinical interpretation. Discrepancies were categorized as either concordant (positive/negative) or discordant (ELISA-only/SPRCA-only).

Methods

Clinical Assessment

Patients were evaluated for CD36 alloimmune risk using transfusion history, pregnancy records, platelet increment data, and pertinent clinical information.

Platelet Antibody Screening and Confirmation of Specificity

Primary detection of platelet alloantibodies, including anti-CD36, was performed using SPRCA. Wells were coated with anti-thrombocyte antibody, then incubated with PRP from 12 random donors and patient serum; indicator red blood cells were used to identify antibody binding. SPRCA was supported by MAIPA, lymphocytotoxicity test (LCT), or chloroquine-modified SPRCA when results were ambiguous.

Supplemental ELISA: PAKPLUS

The PAKPLUS ELISA (Immucor) evaluated serum reactivity against key platelet glycoproteins (GPIIb/IIIa, GPIa/IIa, GPIb/IX, GPIV/CD36, HLA) using kit controls; results were determined by measuring optical density.

Flow cytometric assessment of CD36 expression

Using fluorescence-activated cell sorting (FACS), platelet CD36 was measured after dual labeling of PRP with FITC–anti-CD36 and PerCP–anti-CD41a; 5,000 CD41a+ events were analyzed. Monocyte CD36 levels were assessed in lysed whole blood using FITC–anti-CD36 and PerCP–anti-CD14, with 2,000 CD14+ events analyzed.

Molecular typing: CD36 exon 5 sequencing

Genomic DNA was extracted from EDTA-anticoagulated blood (MagCorePlus automated platform) from two CD36-deficient samples. Exon 5 and the surrounding intronic regions of CD36 were amplified using exon-specific primers (Forward 5'-AGATCTAATGTTTCACATATG-3'; Reverse 5'-GATTAATTACATGAGTTCTAG-3'). PCR cycling included 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 60 seconds, and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 10 minutes. Amplicons were sequenced using Sanger sequencing on an Applied Biosystems 3730xl DNA Analyzer at an external core facility, and sequence analysis followed standard bidirectional trace review with quality control of base calling.

Quality control and interpretation

All serologic assays included both kit-provided and in-house positive and negative controls; any runs that did not meet control acceptance criteria were repeated. Ambiguous serology results were clarified through orthogonal testing (chloroquine-modified SPRCA, MAIPA, and LCT) and by correlating with cellular CD36 expression and the CD36 exon 5 sequence, when available.

Ethics approval and consent to participate

The Institutional Review Board (IRB) of MacKay Memorial Hospital approved this study.

Results

Case 1: Clinical Presentation

During hospitalization, the patient quickly developed worsening, confluent, and prominent bilateral ecchymoses with significant soft tissue swelling on the axillae, chest, and limbs. The cutaneous surface demonstrated widespread disruptions, persistent serous exudation, and widespread macular lesions on the thigh. On February 7, she experienced an episode of acute, massive hematochezia exceeding 800 mL, along with sharp drops in both hemoglobin and platelet counts. Despite prompt transfusions of packed red blood cells, fresh frozen plasma, and pooled platelets, the cytopenia remained resistant to treatment. An urgent colonoscopy revealed a large colonic perforation, with widespread bruising observed across multiple areas.

Targeted transfusion of CD36-negative platelets caused a temporary but significant rise in platelet count (Figure 2B). Supportive care was aggressive, including broad-spectrum antimicrobials (Figure 2A), hemodialysis, and

plasma exchange (Figure 1). However, hemorrhagic activity remained uncontrolled, and disseminated intravascular coagulation (DIC) worsened, leading to progressive multi-organ failure.

Laboratory Findings

Laboratory evaluation revealed significant thrombocytopenia and coagulopathy [34], including prolonged prothrombin time (PT/INR), elevated total and direct bilirubin, D-dimer, and fibrin degradation products, with low fibrinogen (Figure 2C) [35]. Haptoglobin (<30 mg/dL) indicated acute hemolysis or massive consumptive bleeding (Figure 2B). Renal and hepatic functions were impaired (Figure 1), consistent with multi-organ involvement.

Diagnostic Assessment

Considering her known CD36 deficiency and history of multiple transfusions, immunologic studies were conducted. ELISA and flow cytometry detected an anti-CD36 (anti-Naka) alloantibody. Platelets showed no expression of the CD36 antigen, and MAIPA screening confirmed the deficiency of CD36. She received a transfusion of CD36-negative platelets, which led to sustained platelet recovery by February 19. Flow cytometric fluorescence profiles verified the absence of detectable anti-CD36 antibodies in the patient's serum (Figure 3A-C).

This presentation illustrates the clinical features of severe, resistant DIC in the context of PTR. It is characterized by catastrophic gastrointestinal bleeding, extensive skin involvement, and significant vascular damage. These dermatologic signs, such as increasing ecchymoses and blister rupture, along with widespread mucocutaneous systemic bleeding, emphasize the severity of vascular injury and widespread capillary fragility, highlighting the overlapping hemorrhagic and thrombotic disorders that characterize the final stage of PTR complicated by advanced DIC.

Since Case 2 was pregnant and diagnosed with NAITP, maternal flow cytometry clearly showed complete CD36 deficiency on platelets and monocytes, confirming maternal Type I CD36 deficiency. The paternal CD36 phenotype tested positive, establishing an antigenic incompatibility that could trigger maternal alloimmunization. (Figure 4 A-F) Additional serological tests using SPRCA assays identified maternal anti-CD36 antibodies, with IgG specifically binding to CD36-positive platelets. Crossmatch serology confirmed these findings, showing that maternal serum bound only to CD36-positive platelets without reactivity to CD36-negative platelets. Neonatal serum showed no independent antibody activity, indicating passive transfer of maternal antibodies.

The neonate was born with severe thrombocytopenia, with an initial platelet count of $40 \times 10^3/\mu\text{L}$, presenting as petechiae. The platelet counts naturally rose to $332 \times 10^3/\mu\text{L}$ within eight days, following a recovery pattern typical of NAIT. Meanwhile, progressive anemia was observed, with hemoglobin dropping from 12.6 g/dL at birth to 9.1 g/dL by day 8; however, this anemia was not directly attributable to anti-CD36 antibodies. Additionally, intermittent episodes of neonatal desaturation occurred, likely due to perinatal stress or infection rather than antibody-mediated mechanisms.

In **Case 3**, anti-CD36 was detected using support from SPRCA, and a biallelic CD36 mutation confirmed the genetic basis of homozygosity (Figure 5E), explaining the type I CD36 deficiency. This genetic evidence validated the immunophenotypic absence of CD36 on platelets and monocytes, as shown by flow cytometry in Figures 5A-D. The patient experienced severe anemia from melena, severe thrombocytopenia ($\sim 13\text{--}15\text{K}/\mu\text{L}$), and developed anti-CD36 alloantibodies after transfusions, which explained the persistent PTR. His cardiac and metabolic assessments revealed elevated NT-proBNP, indicating worsening cardiometabolic stress, along with hyponatremia. He also reported fatigue, chest pain, and exercise intolerance.

Case 4: Anti-CD36 was conclusively detected by SPRCA and showed absence of CD36 on platelets and monocytes assessed by flow cytometry (type I deficiency) (Figure 6A-D). Biallelic CD36 mutations confirmed the CD36 deficiency (Figure 6E). She also developed anti-CD36, resulting in severe thrombocytopenia ($\sim 15\text{K}/\mu\text{L}$), transfusion dependence, and refractoriness to platelet support. Her cardiac issues included cardiomegaly, elevated BNP, and acute heart failure.

Case 5: The patient's clinical course was characterized by severe platelet refractoriness, starting with an initial platelet count of $27 \times 10^3/\mu\text{L}$. After surgery, his count rebounded significantly, reaching up to $758 \times 10^3/\mu\text{L}$, a response that intensified with repeated surgical stress, ischemic conditions, and systemic inflammatory reactions caused by extensive tissue necrosis. Although such fluctuations are typically indicative of consumptive coagulopathy, DIC, or reactive thrombocytosis following major surgery, recent specialized testing provides crucial clarification. Flow cytometry performed on the patient's platelets and monocytes revealed markedly reduced or absent CD36 expression, confirming the diagnosis of CD36 deficiency. (Figure 7 A-H) Additionally, serological testing detected the presence of specific anti-CD36 antibodies through MAIPA and SPRCA, confirming alloimmunization.

He exhibited a significantly elevated NT-proBNP level (1710 pg/mL), consistent with acute heart failure. The patient's history of hypertension,[11] mellitus with complications, stroke, and ARDS-induced pneumonia indicated severe cardiovascular and metabolic stress.

Case 6: The 86-year-old patient presented with thrombocytopenia primarily related to her underlying lymphoma, chronic infections, and age-related conditions, which made it difficult to definitively interpret CD36 involvement. Despite significant cardiomegaly and metabolic symptoms, typical signs directly caused by CD36 deficiency were not clear, and no anti-CD36 antibodies were detected.

Clinical Interpretation of ELISA versus SPRCA: In this cohort of 2,453 evaluations, ELISA demonstrated a higher detection rate of antiplatelet antibodies (33.5%) compared to SPRCA (18.1%). The agreement between the two methods was moderate, with an overall concordance of 78.1% and a Cohen's kappa of 0.444, adjusting for chance. Notably, only 14.8% of all cases were concordant double-positives, while discordant results mainly involved ELISA-only positives (18.7%) versus SPRCA-only positives (3.3%). Method-specific analysis further emphasized this imbalance: 81.9% of SPRCA-positive samples were confirmed by ELISA, and nearly a quarter (22.8%) of SPRCA-negative cases were classified as positive by ELISA.

Discussion

1. Clinical relevance

The clinical vignette (Case 1) illustrates the disastrous convergence of three distinct but synergistic pathophysiological processes: anti-CD36 alloimmunization, drug-induced thrombocytopenia (DITP), and DIC [34]. DITP [36], notably linked to medications such as antibiotics, further complicates clinical management. Teicoplanin-associated DITP typically appears 5-7 days after exposure, aligning with the patient's thrombocytopenic episode and the timeline of clinical deterioration. The sequential administration of multiple broad-spectrum antibiotics, including meropenem and ceftriaxone reflects increasing concern for multidrug-resistant infections and the management of sepsis. The pathogenesis involves teicoplanin-dependent antibodies that target epitopes on GPIIb/IIIa or GPIb/V/IX complexes, leading to antibody-mediated platelet destruction.[37] Studies demonstrate a 4.6% incidence of teicoplanin-induced thrombocytopenia in clinical populations, with a median onset of 5 days and maximum platelet decrease within 8 days.

Immunological assessment, including CD36 antigen typing and anti-CD36 antibody testing, clarified that immune-mediated PTR was caused by massive transfusions of standard random donor platelets due to the high immunogenicity of CD36 and its widespread presence on donor platelets. This likely existed prior to sensitization and contributed to the development of anti-CD36 alloantibodies. Consequently, CD36-negative platelet transfusions are required for transient clinical improvement. However, persistent and consumptive coagulopathy DIC significantly increased the risk of PTP, a massive hemorrhage and severe thrombocytopenia involving alloimmune and consumptive mechanisms. This complicated the effectiveness of supportive treatments, including infection management, coagulation correction, and targeted immunotherapy (e.g., IVIG for CD36 isoimmune PTR; platelet washing [38], leukoreduction [39], plasma exchange attempted to remove antibodies [40-42]).

This scenario highlights the need for a systematic approach to severe thrombocytopenia, including the timing of drug exposures by increasing clinician awareness of DITP and promptly discontinuing the offending drug [37, 43], and avoiding preexposure [44]. Additionally, it involves screening for alloantibodies, establishing registries for CD36-deficient donors, and assessing for DIC to guide appropriate therapy. Further research into treatments for PTR complicated by anti-CD36 alloimmunization and DITP is essential to improve clinical guidelines and patient outcomes.

Case 2: This clinical scenario underscores the urgent need to quickly identify maternal CD36 deficiency and its link to alloimmunization in neonates presenting with unexplained severe thrombocytopenia. Accurate serologic detection of anti-CD36 antibodies allows for early intervention and appropriate clinical management, potentially preventing serious neonatal complications.[11] Furthermore, differentiating NAIT from non-immune etiologies is crucial for making optimal treatment decisions and accurately predicting outcomes.[10] Therefore, increased clinical awareness and involving an expert panel throughout the prenatal and postnatal stages[45, 46] and conducting comprehensive serologic assessments are strongly recommended.

Early recognition of this anti-CD36-mediated refractoriness thrombocytopenia enables precise and restrictive transfusion strategies[47], such as selecting CD36-negative donors to source CD36-negative platelet units[48] and enhancing clinical monitoring, prophylactic measures during pregnancy, the antenatal administration of IVIG[49, 50] and/or steroids[50, 51], and/or deg-mAb 32-106[11], to help optimize patient outcomes and enhance transfusion

safety and effectiveness. Postnatal maternal platelets for neonatal transfusion should be washed to remove HPA antibodies and irradiated to prevent GVHD [52].

Cases 3 and 4 emphasize the essential role of specialized diagnostics in managing rare platelet alloimmunization and how CD36 deficiency intersects with complex diseases, transfusion, and cardiometabolic care. Flow cytometry and sequencing confirmed CD36 deficiency which complicates underlying diseases and can worsen heart failure through impaired myocardial fatty acid metabolism [53, 54], potentially increasing vulnerability to cardiac dysfunction under metabolic stress [55]. This critically impacts patient management by complicating transfusion therapy, raising the risk of PTR, and potentially aggravating cardiometabolic instability in severe underlying diseases.

Minimize Platelet Transfusion; Immunosuppression/Desensitization

In the Case 4 scenario, an AML patient has anti-CD36. The immune PTR in patients with hematologic malignancies varies widely, ranging from 7% to 34% [56]. Minimizing prophylactic platelet transfusions and transfusing only during chemotherapy-induced aplasia unless bleeding occurs is an optimized approach[47, 57] and can help reduce ongoing destruction of incompatible platelets [58], limit further immune stimulation [4], and temporary suppress anti-CD36 production. Regimens have included rituximab, bortezomib [59], and plasmapheresis to remove antibodies. In one severe case, these measures only modestly lowered antibody levels (anti-CD36), with the mean fluorescence intensity falling but rebounding within days [4], underscoring that current desensitization is often inadequate. Furthermore, high CD36 expression is independently associated with worse overall survival and event-free survival [60], likely through altered lipid uptake, metabolic reprogramming, and enhanced leukemic persistence stem cells [60, 61]. CD36-expressing metastasis-initiating cells directly contribute to aggressive disease biology [62] and correlate with poor prognosis in multiple carcinomas. Targeted inhibition of CD36 disrupts metabolic and signaling pathways essential for metastatic colonization, demonstrating a tangible reduction in tumor dissemination and disease progression [63]. Overall, these findings position CD36 not only as a predictor of therapeutic response but also as a potential therapeutic target [64] to overcome drug resistance and improve long-term outcomes in AML patients [65]. An "effector-silencing" anti-CD36 monoclonal antibody has been proposed to block the patient's antibodies experimentally [58], thereby allowing safe transfusion.

In case 5, with severe PTR, was caused by undetected CD36 alloimmunization.[17] Specifically, despite multiple platelet transfusions, his post-transfusion counts remained near zero until an anti-CD36 isoantibody was identified, which caused rapid clearance of transfused platelets. In known CD36-deficient patients undergoing surgery or therapy, it is crucial to minimize allogeneic transfusions to prevent triggering anti-CD36 alloimmunization.

CD36 deficiency was indicated complex metabolic interactions and provides a protective metabolic effect [66] regarding diabetic cardiomyopathy and atherosclerosis [67, 68]. However, the patient's severe phenotype underscores the multifactorial nature of cardiovascular diseases, especially in chronic diabetic [55] and hypertensive contexts.[46]

Beyond transfusion reactions, CD36 plays a vital role in platelet activation and lipid metabolism and has been proposed as a therapeutic target to reduce thrombotic risk in individuals [47, 67, 69] with dyslipidemia. Type I CD36 deficiency is linked to atherosclerosis [70], altered glycolipid metabolism and ischemia-reperfusion myocardial damage [71], and cardiomyopathy [72]. Clinically, patients with type I CD36 deficiency, especially those without prior anti-CD36 alloimmunization, require careful perioperative and transfusion management [73] due to potential metabolic and hemostatic alterations [74].

In studies of platelet function, the findings suggest that CD36 deficiency might protect against acute ischemic events like myocardial infarction or stroke in certain situations and improved outcomes in stroke models [75, 76], and improved functional recovery after stroke[77].

These cases underscore advanced platelet antibody detection techniques in distinguishing CD36-related conditions from thrombocytopenia caused by other chronic diseases are vital. The presence of severe underlying conditions, particularly lymphoma and extensive surgical trauma, significantly influences the clinical signs of CD36 deficiency. Accurate detection of anti-CD36 antibodies using MAIPA [22] and SPRCA assays allows for the development of tailored management strategies, emphasizing the importance of high diagnostic suspicion and specialized testing in complex cases.

In contrast, as shown in Case 6 with lymphoma-related thrombocytopenia, a CD36-negative patient without alloantibodies can initially receive standard platelets without immediate destruction, because no anti-CD36

antibodies are present. However, her transfusion refractoriness likely results from non-immune factors, such as splenomegaly or HLA antibodies, rather than CD36 alloantibodies.

In summary, CD36 deficiency complicates transfusion outcomes by forming an often-overlooked immune barrier. If left unrecognized, it results in refractory thrombocytopenia, despite adequate platelet doses [78]. Once identified, clinicians should tailor transfusion support by sourcing rare donors or using immunosuppressants to prevent alloimmune destruction and employing leukoreduction, which can reduce alloimmune platelet refractoriness from 14% to 4% in patients receiving chemotherapy for acute leukemia or stem cell transplantation [39, 60]. The profound refractoriness observed with anti-CD36 (Case 5) compared to standard management of thrombocytopenia without such antibodies (Case 6) underscores the clinical importance of CD36 alloimmunization in hematology. Ultimately, the immune dysregulation and metabolic effects of CD36 deficiency have major implications for cancer treatment. CD36 is a pattern-recognition scavenger receptor on monocytes, macrophages, and dendritic cells that binds danger signals and pathogen component [79]. It aids in phagocytosing bacteria and cell debris and modulates cytokine production during infections. CD36 is expressed in various cancer cells and immune cell subsets, where it exerts both pro- and anti-tumorigenic roles [80].

In patients with hematologic malignancies, maintaining an intact innate immune response is crucial as they are often neutropenic or lymphopenic due to chemotherapy [24]. Therefore, the optimal function of macrophages and other remaining immune cells is essential for fighting infection. CD36 deficiency can impair innate immunity, for example, by reducing macrophage uptake of Gram-negative bacteria and diminishing Toll-like receptor signaling, resulting in reduced cytokine release. This could make an immunocompromised cancer patient even more vulnerable to sepsis or fungal infections [81, 82].

2. Recommended Diagnostic Strategy

Overview of Agreement Metrics and Clinical Implications

A comparative analysis of 2,453 paired samples revealed ELISA detected antiplatelet antibodies in 33.5% of samples, while SPRCA identified them in 18.1%, yielding a concordance (overall agreement) of 78.1% and moderate agreement (The Cohen's Kappa value; $\kappa = 0.444$) indicates that relying on a single platform may either overestimate or underestimate the alloimmunization risk, emphasizing significant assay-specific differences.

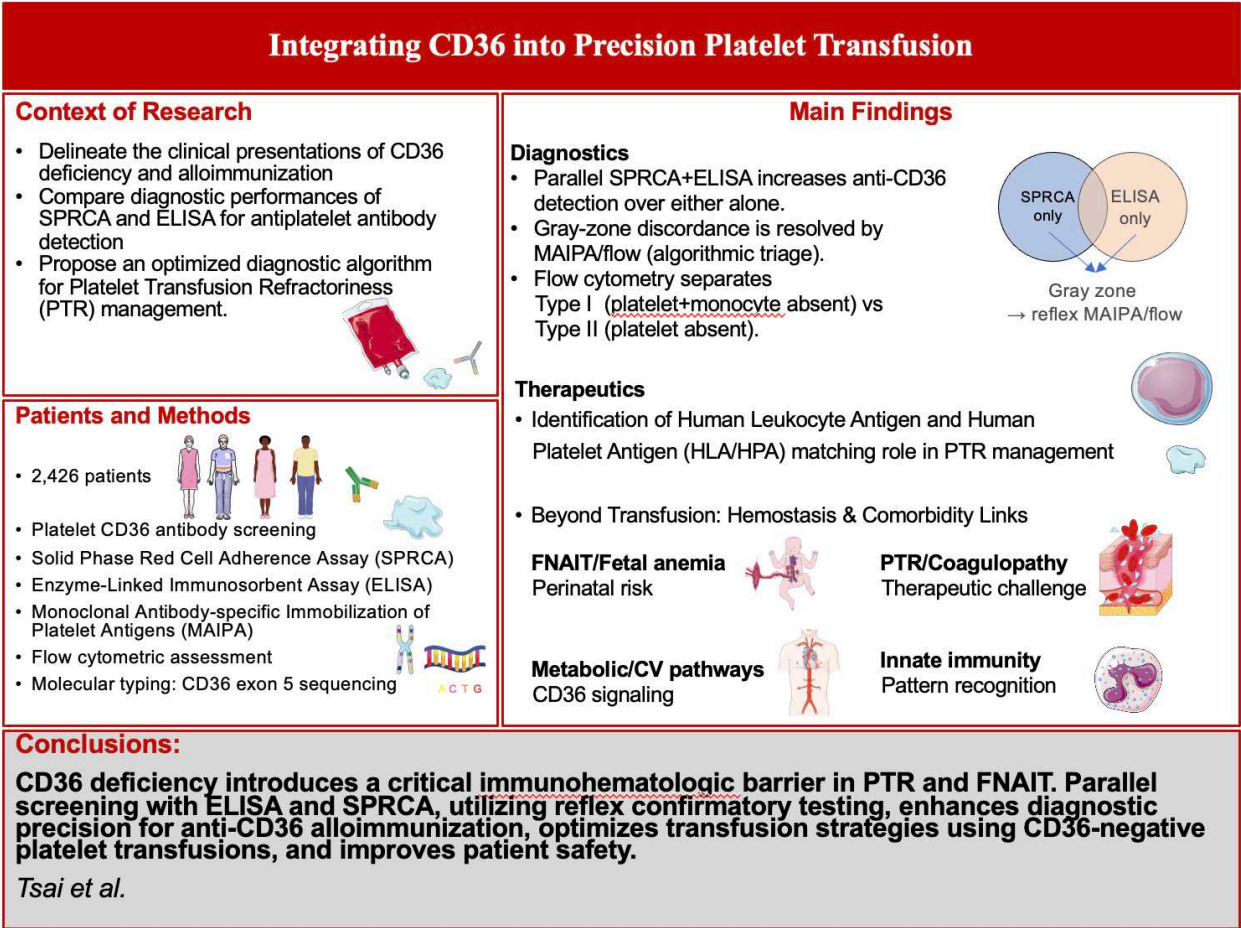
Notably, only 14.8% of all samples were concordantly positive by both assays. Since there is no definitive gold standard for antiplatelet antibody detection, neither method was considered the reference. Instead, we examined method-specific concordance: Among SPRCA-positive samples, 81.9% were confirmed by ELISA; for SPRCA-negatives, 22.8% were ELISA-positive, underscoring substantial assay discordance. Notably, 18.7% of cases were exclusively ELISA-positive, likely reflecting increased sensitivity for low-affinity or non-complement-fixing antibodies of uncertain clinical relevance. Meanwhile, the only 3.3% detected solely by SPRCA probably represent alloantibodies with highly specific for clinically significant platelet alloimmunization and PTR, consistent with SPRCA's dependence on antigen density and its lower susceptibility to nonspecific binding. These findings underscore the superior sensitivity of ELISA in this setting and highlight the need for method-specific interpretation of discordant results and the complementary nature of ELISA and SPRCA rather than their interchangeability. Specifically, ELISA provides broad antibody detection, while SPRCA enhances detection of functionally relevant signals. In the context of platelet transfusion refractoriness, relying on only one assay risks patient misclassification and the selection of inappropriate donors. Therefore, a sequential, tiered diagnostic approach combining high-throughput screening with ELISA, targeted confirmation with SPRCA, and molecular tools such as flow-cytometric CD36 phenotyping and next-generation HPA/HLA genotyping [22] offers opportunities to refine antibody characterization [83], link serologic patterns with donor genetics, and ultimately improve matching strategies for highly alloimmunized patients.

GP-specific antibody assays most effectively detect anti-Nak (a). However, false negatives can occur due to monoclonal antibody interference that blocks human antibody binding. Conventional platforms, such as Luminex bead assays and the MAIPA assay, often miss anti-CD36 because of epitope competition with capture antibodies (clone FA6-152). Employing ELISA for initial broad screening is recommended due to its efficiency and scalability. Subsequently, all ELISA-positive samples should be confirmed with SPRCA or other high-specificity assays, such as MAIPA [22] or flow cytometry [24], which are crucial for clinical decisions. Dual positive results on ELISA and SPRCA strongly indicate pathogenicity, while discordant cases require a detailed review of clinical and laboratory data, including transfusion history and symptoms. This integrated approach enhances the accuracy of anti-CD36 detection, supports thrombocytopenia management, and aligns with emerging evidence. Interpretation guidelines for clinical decisions should be considered that samples positive by both ELISA and SPRCA are highly valid, indicating true positivity. Discordant results (ELISA-positive/SPRCA-negative or ELISA-negative/SPRCA-

positive) should undergo further reflex testing through clinical correlation, including a review of the transfusion history, symptoms, and other relevant laboratory results.

Conclusions

In practice, prevention, rapid triage, and targeted support should serve as the foundation of managing immune PTR [84]. Establish a universal pre-storage leukoreduction standard to limit HLA alloimmunization [39, 85]. In cases of refractoriness, especially when HLA/HPA-matched platelets fail, use parallel SPRCA and ELISA with reflex MAIPA/flow to determine specificity (including anti-CD36) and the phenotype/genotype of CD36 (Type I/II). Transfuse to prevent antibody formation: prioritize crossmatch-compatible or HLA-matched platelets. For confirmed anti-CD36, source CD36-negative units ($\pm$ HLA/HPA matching) [10, 84]. When rapid intervention is clinically necessary, consider plasma exchange or immunoadsorption combined with IVIG for short-term reduction of pathogenic Fc-mediated clearance [86]. Reserve rituximab [68], and, in carefully managed rescue cases, plasma-cell-targeted therapies (e.g., bortezomib, daratumumab [87]) for high-titer, persistent cases. Finally, ensure institutional readiness by accessing CD36-negative donor registries, utilizing standardized MAIPA capture clones [22], implementing predefined reflex algorithms, and routinely monitoring CCI/bleeding outcomes [88] to support continuous quality improvement.



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Declarations

Ethics approval and consent to participate

The Institutional Review Board (IRB) of MacKay Memorial Hospital approved this study.

When consent was obtained: Written informed consent was obtained from the patient (or the patient's legal guardian) for participation in the study and for publication of the clinical details (including any relevant images), in accordance with institutional and international ethical guidelines. When consent could not be obtained, the IRB approved a waiver of informed consent in accordance with ethical guidelines, as the study involved a retrospective analysis of de-identified clinical data and posed no more than minimal risk to participants.

Consent for publication

The authors agree with the publication of this paper.

Availability of data and materials

Data are available from the authors upon reasonable request and with permission of MacKay Memorial Hospital, and the Taiwan Blood Services Foundation.

Declaration of originality

The authors confirm that this manuscript is original and independent from other submitted or published works.

Ethics and Transparency

All clinical investigations were conducted under the oversight of the institutional IRB. Selected elements of this work were previously presented in abstract form at the 2024 AABB Annual Meeting and are accepted for presentation at the 2025 ISBT Regional Congress. The complete manuscript has not been published or submitted elsewhere. Prior abstract dissemination does not constitute prior publication and complies with the prevailing policies of conferences and journals.

References

1. Hoosdally, S.J., et al., *The Human Scavenger Receptor CD36: glycosylation status and its role in trafficking and function*. J Biol Chem, 2009. **284**(24): p. 16277–16288.
2. Martin, C.A., et al., *Cd36, a class B scavenger receptor, functions as a monomer to bind acetylated and oxidized low-density lipoproteins*. Protein Sci, 2007. **16**(11): p. 2531–41.
3. Tomiyama, Y., et al., *Identification of the platelet-specific alloantigen, Naka, on platelet membrane glycoprotein IV*. Blood, 1990. **75**(3): p. 684–7.
4. Pedini, P., et al., *A case of platelet transfusion refractoriness in a CD36-negative patient with acute myeloid leukemia: Diagnostic and therapeutic management*. Transfusion, 2022. **62**(10): p. 2148–2150.
5. Yamamoto, N., et al., *A platelet membrane glycoprotein (GP) deficiency in healthy blood donors: Naka-platelets lack detectable GPIV (CD36)*. Blood, 1990. **76**(9): p. 1698–703.
6. Alattar, A.G., J.R. Storry, and M.L. Olsson, *Evidence that CD36 is expressed on red blood cells and constitutes a novel blood group system of clinical importance*. Vox Sang, 2024. **119**(5): p. 496–504.
7. Storry, J.R. and S. Azouzi, *An uncommon MALady: is the AnWj puzzle complete?* Blood, 2024. **144**(26): p. 2688–2689.
8. Curtis, B.R., et al., *Isoimmunization against CD36 (glycoprotein IV): description of four cases of neonatal isoimmune thrombocytopenia and brief review of the literature*. Transfusion, 2002. **42**(9): p. 1173–9.
9. Curtis, B.R., et al., *Isoimmunization against CD36 (glycoprotein IV): description of four cases of neonatal isoimmune thrombocytopenia and brief review of the literature*. Transfusion, 2002. **42**(9): p. 1173–1179.
10. Stanworth, S.J. and A.D. Mumford, *How I diagnose and treat neonatal thrombocytopenia*. Blood, 2023. **141**(22): p. 2685–2697.
11. Xu, X., et al., *Successful prenatal therapy for anti-CD36-mediated severe FNAIT by deglycosylated antibodies in a novel murine model*. Blood, 2021. **138**(18): p. 1757–1767.
12. Zhu, W., W. Li, and R.L. Silverstein, *Advanced glycation end products induce a prothrombotic phenotype in mice via interaction with platelet CD36*. Blood, 2012. **119**(25): p. 6136–6144.
13. Ruggeri, Z.M., *Platelets in atherothrombosis*. Nature medicine, 2002. **8**(11): p. 1227–1234.
14. Yang, M. and R.L. Silverstein, *CD36 signaling in vascular redox stress*. Free Radical Biology and Medicine, 2019. **136**: p. 159–171.

15. Ghosh, A., et al., *Platelet CD36 surface expression levels affect functional responses to oxidized LDL and are associated with inheritance of specific genetic polymorphisms*. Blood, 2011. **117**(23): p. 6355–6366.
16. Ahlen, M.T., *Fighting anti-CD36-mediated FNAIT*. Blood, 2021. **138**(18): p. 1650–1652.
17. Sekayan, T., et al., *Management challenge of a rare concomitant platelet glycoprotein IV/CD36 and IIb/IIIa deficiencies: Case illustration*. Transfusion, 2025. **65**(4): p. 767–772.
18. Nahirniak, S., V. Nadarajan, and S.J. Stanworth, *How I treat patients who are refractory to platelet transfusions*. Blood, 2025. **145**(20): p. 2293–2302.
19. Bierling, P., et al., *Posttransfusion purpura-like syndrome associated with CD36 (Naka) isoimmunization*. Transfusion, 1995. **35**(9): p. 777–782.
20. Xu, X., et al., *Studies on CD36 deficiency in South China: Two cases demonstrating the clinical impact of anti-CD36 antibodies*. Thromb Haemost, 2013. **110**(6): p. 1199–206.
21. Cserti, C.M., et al., *Quantitative Flow Cytometric Assessment of CD36 (Platelet Glycoprotein IV) Expression on Monocytes and Platelets: Assay Development, Role in Plasmodium falciparum Risk Assessment, and Relationship to Sick Hemoglobin*. Blood, 2007. **110**(11): p. 3857–3857.
22. Xu, X., et al., *Improvement of Anti-CD36 Antibody Detection via Monoclonal Antibody Immobilization of Platelet Antigens Assay by Using Selected Monoclonal Antibodies*. Ann Lab Med, 2023. **43**(1): p. 86–91.
23. Xia, W., et al., *CD36 deficiency among South-East Asian populations*. ISBT Science Series, 2016. **11**(S2): p. 33–36.
24. He, H., et al., *High-Throughput CD36 Phenotyping on Human Platelets Based on Sandwich ELISA and Mutant Gene Analysis*. Transfus Med Hemother, 2024. **51**(1): p. 32–40.
25. Flesch, B.K., et al., *Platelet CD36 deficiency is present in 2.6% of Arabian individuals and can cause NAIT and platelet refractoriness*. Transfusion, 2021. **61**(6): p. 1932–1942.
26. Lee, K., et al., *CD36 deficiency is frequent and can cause platelet immunization in Africans*. Transfusion, 1999. **39**(8): p. 873–9.
27. Curtis, B.R. and R.H. Aster, *Incidence of the Nak(a)-negative platelet phenotype in African Americans is similar to that of Asians*. Transfusion, 1996. **36**(4): p. 331–4.
28. Flesch, B., et al., *Successful autologous hematopoietic progenitor cell transplantation in a patient with an isoantibody against CD36 (glycoprotein IV, Naka)*. Bone Marrow Transplant, 2008. **42**(7): p. 489–91.

- 495 29. Porcelijn, L., E. Huiskes, and M. de Haas, *Progress and development of platelet antibody detection.*
496 Transfusion and Apheresis Science, 2020. **59**(1): p. 102705.
- 497 30. Shin-Yi Tsai, et al. Unmasking the Gray Zone: Five-Year Discrepancy Patterns Between SPRCA and
498 ELISA in Antiplatelet Antibody Detection. Presented at : Blood 2025 and the 36th Regional Congress of the ISBT;
499 2025 Oct 26 - 29; Perth, Australia. Oral presentation. Abstract 762.
- 500 31. Shin-Yi Tsai, Hsiao-Lin Chang, Yung-Shiu Chan, Ching-Hsiang Yu, Chen-Yu Jiang, Lin. M-L. A Case of
501 Platelet Transfusion Refractoriness in a CD36-Deficiency Patient With Drug-Induced Thrombocytopenia.
502 *Transfusion.* 2024;64(S3):157A.
- 503 32. Shin-Yi Tsai, et al. How CD36 Deficiency Intersects with Complex Diseases and Confounds Transfusion
504 and Cardiometabolic Care: Three Cases Illustrating Diagnostic Challenges. Presented at : Blood 2025 and the 36th
505 Regional Congress of the ISBT; 2025 Oct 26 - 29; Perth, Australia. Poster presentation. Abstract 758.
- 506 33. Shin-Yi Tsai, et al. Diagnostic challenges and clinical impacts of CD36 deficiency in patients with multiple
507 comorbidities: insights from two illustrative cases. Presented at : Blood 2025 and the 36th Regional Congress of the
508 ISBT; 2025 Oct 26 - 29; Perth, Australia. Poster presentation. Abstract 517.
- 509 34. Lee, B.-C., et al., *Thromboelastography characterized CD36 null subjects as slow clot formation and*
510 *indicative of hypocoagulability.* Thrombosis Research, 2019. **178**: p. 79–84.
- 511 35. Iba, T., et al., *Updated definition and scoring of disseminated intravascular coagulation in 2025:*
512 *communication from the ISTH SSC Subcommittee on Disseminated Intravascular Coagulation.* Journal of
513 Thrombosis and Haemostasis, 2025. **23**(7): p. 2356–2362.
- 514 36. Marini, I., et al., *Treatment of drug-induced immune thrombocytopenias.* Haematologica, 2022. **107**(6): p.
515 1264–1277.
- 516 37. George, J.N. and R.H. Aster, *Drug-induced thrombocytopenia: pathogenesis, evaluation, and management.*
517 Hematology Am Soc Hematol Educ Program, 2009: p. 153–8.
- 518 38. Sahai, T., et al., *Platelet Refractoriness Due to Anti-CD-36(NAKa) Ameliorated By Platelet Washing.*
519 Blood, 2016. **128**(22): p. 3857–3857.
- 520 39. Seftel, M.D., et al., *Universal prestorage leukoreduction in Canada decreases platelet alloimmunization*
521 *and refractoriness.* Blood, 2004. **103**(1): p. 333–339.

40. Hawkins, J., R.H. Aster, and B.R. Curtis, *Post-Transfusion Purpura: Current Perspectives*. J Blood Med, 2019. **10**: p. 405–415.
41. Hirunwidchayarat, P., et al., *Post-transfusion purpura in a 36-year-old woman*. Cmaj, 2025. **197**(23): p. E640–e644.
42. Cimo, P.L. and R.H. Aster, *Post-transfusion purpura: successful treatment by exchange transfusion*. The New England journal of medicine., 1972. **287**(6): p. 290–292.
43. Bakchoul, T. and I. Marini, *Drug-associated thrombocytopenia*. Hematology, 2018. **2018**(1): p. 576–583.
44. Nguyen, V.D., et al., *Rapid-Onset Thrombocytopenia Following Piperacillin-Tazobactam Reexposure*. Pharmacotherapy, 2015. **35**(12): p. e326–30.
45. Lieberman, L., et al., *Fetal-Neonatal Alloimmune Thrombocytopenia (FNAIT): Guidance to Reduce the Risk of Intracranial Bleeding*. Blood, 2018. **132**(Supplement 1): p. 4717–4717.
46. Lieberman, L., et al., *Fetal and neonatal alloimmune thrombocytopenia: recommendations for evidence-based practice, an international approach*. British Journal of Haematology, 2019. **185**(3): p. 549–562.
47. Billion, E., et al., *Lowering platelet-count threshold for transfusion in preterm neonates decreases the number of transfusions without increasing severe hemorrhage events*. Eur J Pediatr, 2024. **183**(10): p. 4417–4424.
48. Winkelhorst, D., D. Oepkes, and E. Lopriore, *Fetal and neonatal alloimmune thrombocytopenia: evidence based antenatal and postnatal management strategies*. Expert Rev Hematol, 2017. **10**(8): p. 729–737.
49. Ni, H., et al., *A novel murine model of fetal and neonatal alloimmune thrombocytopenia: response to intravenous IgG therapy*. Blood, 2006. **107**(7): p. 2976–2983.
50. Winkelhorst, D., et al., *Antenatal management in fetal and neonatal alloimmune thrombocytopenia: a systematic review*. Blood, 2017. **129**(11): p. 1538–1547.
51. Murphy, M.F. and J.B. Bussel, *Advances in the management of alloimmune thrombocytopenia*. Br J Haematol, 2007. **136**(3): p. 366–78.
52. Lieberman, L., et al., *Fetal and neonatal alloimmune thrombocytopenia: recommendations for evidence-based practice, an international approach*. Br J Haematol, 2019. **185**(3): p. 549–562.
53. Glatz, J.F.C., L.C. Heather, and J.J.F.P. Luiken, *CD36 as a gatekeeper of myocardial lipid metabolism and therapeutic target for metabolic disease*. Physiol Rev, 2024. **104**(2): p. 727–764.

54. Ashaq, M.S., et al., *The regulatory role of CD36 in hematopoiesis beyond fatty acid uptake*. Life Sciences, 2024. **339**: p. 122442.
55. Moon, J.S., et al., *The Role of CD36 in Type 2 Diabetes Mellitus: β -Cell Dysfunction and Beyond*. Diabetes Metab J, 2020. **44**(2): p. 222–233.
56. Bonstein, L., R. Lauterbach, and N. Haddad, *High Incidence of Alloimmunization to Platelets and Platelet Transfusion Refractoriness during Induction Therapy in Patients Diagnosed with Acute Myeloid Leukemia*. Blood, 2015. **126**(23): p. 3484–3484.
57. Coletti, K., et al., *Standardized Prophylactic Platelet Transfusion Dose for Infants Decreases Platelet Exposure without Increasing Bleeding or Transfusion Frequency*. Blood, 2024. **144**: p. 1268.
58. Metcalf, R.A., et al., *Platelet Transfusion: 2025 AABB and ICTMG International Clinical Practice Guidelines*. JAMA, 2025. **334**(7): p. 606–617.
59. Ratnasingam, S., et al., *Bortezomib-based antibody depletion for refractory autoimmune hematological diseases*. Blood Advances, 2016. **1**(1): p. 31–35.
60. Cohn, C.S., *Platelet transfusion refractoriness: how do I diagnose and manage?* Hematology, 2020. **2020**(1): p. 527–532.
61. Rattigan, K.M., M.M. Zarou, and G.V. Helgason, *Metabolism in stem cell–driven leukemia: parallels between hematopoiesis and immunity*. Blood, 2023. **141**(21): p. 2553–2565.
62. Yang, P., et al., *CD36-mediated metabolic crosstalk between tumor cells and macrophages affects liver metastasis*. Nature Communications, 2022. **13**(1): p. 5782.
63. Pascual, G., et al., *Targeting metastasis-initiating cells through the fatty acid receptor CD36*. Nature, 2017. **541**(7635): p. 41–45.
64. Yue, N., et al., *CD36: a promising therapeutic target in hematologic tumors*. Leuk Lymphoma, 2024. **65**(12): p. 1749–1765.
65. Amantéa, M.C., et al., *CD36 as a marker of acute myeloid leukemia prognosis: A systematic review*. Hematol Transfus Cell Ther, 2025. **47**(3): p. 103861.
66. Love-Gregory, L., et al., *Common CD36 SNPs reduce protein expression and may contribute to a protective atherogenic profile*. Hum Mol Genet, 2011. **20**(1): p. 193–201.

577 67. Bendas, G. and M. Schlesinger, *The Role of CD36/GPIV in Platelet Biology*. Semin Thromb Hemost, 2024.
578 50(2): p. 224–235.

579 68. Zhao, L., et al., *CD36 and lipid metabolism in the evolution of atherosclerosis*. British Medical Bulletin,
580 2018. 126(1): p. 101–112.

581 69. Favalaro, E.J., L. Pasalic, and G. Lippi, *Editorial Compilation XIV*. Semin Thromb Hemost, 2023. 50(02):
582 p. 151–156.

583 70. Tian, K., et al., *CD36 in Atherosclerosis: Pathophysiological Mechanisms and Therapeutic Implications*.
584 Curr Atheroscler Rep, 2020. 22(10): p. 59.

585 71. Shu, H., et al., *The Role of CD36 in Cardiovascular Disease: A Critical Review*. 2024. p. 49–84.

586 72. Fujita, S., et al., *Cardiomyopathy Associated with CD36 Deficiency: Role of (18)F-Fluorodeoxyglucose*
587 *Positron Emission Tomography in the Diagnosis*. Intern Med, 2024. 63(22): p. 3059–3064.

588 73. Tsubokawa, T., et al., *Perioperative Management of a Patient With CD36 Deficiency Undergoing Urgent*
589 *Cardiac Surgery*. Journal of Cardiothoracic and Vascular Anesthesia, 2022. 36(8): p. 3149–3151.

590 74. Glatz, J.F.C., L.C. Heather, and J.J.F.P. Luiken, *CD36 as a gatekeeper of myocardial lipid metabolism and*
591 *therapeutic target for metabolic disease*. Physiological Reviews, 2023. 104(2): p. 727–764.

592 75. Li, W., et al., *CD36 participates in a signaling pathway that regulates ROS formation in murine VSMCs*.
593 The Journal of Clinical Investigation, 2010. 120(11): p. 3996–4006.

594 76. Kim, E.-H., et al., *Targeting CD36-Mediated Inflammation Reduces Acute Brain Injury in Transient, but*
595 *not Permanent, Ischemic Stroke*. CNS Neuroscience & Therapeutics, 2015. 21(4): p. 385–391.

596 77. Balkaya, M., et al., *CD36 deficiency reduces chronic BBB dysfunction and scar formation and improves*
597 *activity, hedonic and memory deficits in ischemic stroke*. J Cereb Blood Flow Metab, 2021. 41(3): p. 486–
598 501.

599 78. Pedini, P., et al., *A case of platelet transfusion refractoriness in a CD36-negative patient with acute*
600 *myeloid leukemia: Diagnostic and therapeutic management*. Transfusion, 2022. 62: p. 2148–2150.

601 79. Chen, Y., et al., *CD36, a signaling receptor and fatty acid transporter that regulates immune cell*
602 *metabolism and fate*. Journal of Experimental Medicine, 2022. 219(6): p. e20211314.

603 80. Jiang, M., et al., *CD36 as a double-edged sword in cancer*. Immunology Letters, 2024. 265: p. 7–15.

81. Olonisakin, T.F., et al., *CD36 Provides Host Protection Against Klebsiella pneumoniae Intrapulmonary Infection by Enhancing Lipopolysaccharide Responsiveness and Macrophage Phagocytosis*. The Journal of Infectious Diseases, 2016. **214**(12): p. 1865–1875.
82. Baranova, I.N., et al., *Role of human CD36 in bacterial recognition, phagocytosis, and pathogen-induced JNK-mediated signaling*. J Immunol, 2008. **181**(10): p. 7147–56.
83. Salama, O.S., et al., *Evaluation of platelet cross-matching in the management of patients refractory to platelet transfusions*. Blood Transfus, 2014. **12**(2): p. 187–94.
84. Liu, Y., et al., *Current Status of and Global Trends in Platelet Transfusion Refractoriness From 2004 to 2021: A Bibliometric Analysis*. Front Med (Lausanne), 2022. **9**: p. 873500.
85. Slichter, S.J., et al., *Evaluation of different methods of leukoreduction of donor platelets to prevent alloimmune platelet refractoriness and induce tolerance in a canine transfusion model*. Blood, 2005. **105**(2): p. 847–854.
86. van Osch, T.L.J., et al., *Fc galactosylation of anti-platelet human IgG1 alloantibodies enhances complement activation on platelets*. Haematologica, 2022. **107**(10): p. 2432–2444.
87. Usmani, S.Z., et al., *Clinical efficacy of daratumumab monotherapy in patients with heavily pretreated relapsed or refractory multiple myeloma*. Blood, 2016. **128**(1): p. 37–44.
88. Khatri, S.S., B.R. Curtis, and C. Yamada, *A case of platelet transfusion refractoriness due to anti-CD36 with a successful treatment outcome*. Immunohematology, 2019. **35**(4): p. 139–144.

Figure Legend

Figure 1. Clinical timeline and therapeutic interventions. The figure delineates the sequential clinical interventions, highlighting the initiation and progression of large-volume blood transfusion therapy, hemodialysis (H/D), and plasma exchange (PE). Each intervention point corresponds precisely with the patient's clinical deterioration and therapeutic escalation during hospitalization. A comprehensive summary of the above interventions and corresponding renal functions (the level of creatinine, BUN) and liver functions (the level of total and direct bilirubin) dynamics was depicted and recorded by dates in the patient's hospitalization.

Figure 2. Comprehensive Timeline of Antibiotic Therapy, Transfusion Interventions, and Hemostatic Dynamics During Hospitalization. (A) A comprehensive summary of antibiotic interventions and corresponding platelet dynamics is depicted and recorded by patient hospitalization dates. **(B)** Transfusion timeline and platelet response during hospitalization. A comprehensive summary of transfusion interventions and corresponding platelet dynamics is depicted, including administration of 44 units of packed red blood cells, 32 apheresis platelet units of which four were specifically CD36-negative, 30 units of fresh frozen plasma, 28 designated for plasma exchange procedures, and six units of frozen plasma, and 1 unit of whole blood. Serial platelet counts are illustrated before and after platelet transfusions, with blood sampling intervals indicated. This figure highlights the temporal relationship between transfusion strategies, particularly CD36-negative platelet transfusions, and the patient's count response amidst ongoing immune-mediated platelet refractoriness and concomitant clinical complexities. **(C)** The corresponding levels of coagulopathy, relevant lab data, such as PT/APTT/INR, hemoglobin, and platelet dynamics, are depicted and recorded by the patient's hospitalization dates.

Figure 3. Detection of anti-CD36 antibody in sera by flow cytometric analysis.

(A) Overlay histograms compare patient serum (purple), CD36(-) control, and CD36(+) control. The patient's curve aligns closely with the CD36(-) control, showing a sharp peak at low fluorescence (FITC-A), with minimal rightward shift. The CD36(+) control demonstrates an apparent change to higher fluorescence, confirming strong CD36 antigen expression. The absence of a comparable rightward change in the patient sample indicates a lack of anti-CD36 antibody binding, consistent with CD36 deficiency. **(B)** Separated histograms clearly show the distribution for each population: patient, CD36(-) control, and CD36(+) control. The patient and CD36(-) control overlap extensively,

reinforcing the finding that the patient's platelets do not express CD36 and do not bind anti-CD36 antibody. The CD36(+) control shows a distinct peak at higher fluorescence, as expected for CD36-expressing cells. (C) PerCP-Cy5.5-A histogram overlays patient (blue), CD36(-) control, and CD36(+) control. The patient's and CD36(-) control's histograms peak at low fluorescence, with no detectable population at higher intensity, while the CD36(+) control displays a broad peak in the high-intensity range. This confirms the absence of CD36 on the patient's platelets, validating the CD36-deficient phenotype both by negative immunoreactivity and by comparison to well-characterized controls.

Summary interpretation: These histograms collectively demonstrate that the patient's platelets lack CD36 antigen expression, as their fluorescence profiles are indistinguishable from CD36(-) controls and distinct from CD36(+) controls. This pattern is diagnostic for type I or type II CD36 deficiency and supports the absence of detectable anti-CD36 antibodies in the patient's serum by flow cytometry. All images have been carefully de-identified to maintain patient confidentiality.

Figure 4. Maternal Type I CD36 Deficiency Confirmed by Flow Cytometry. Flow cytometry showed no CD36 antigen on maternal platelets or monocytes (maternal plots with anti-CD36 overlapped isotype controls, whereas the father's platelets/monocytes (and a standard donor control) were strongly CD36-positive. This pattern indicates a maternal Type I CD36 deficiency, well-recognized in Asian populations, and predisposes to alloimmunization during pregnancy. By contrast, paternal cells express CD36 normally. In NAIT, a CD36-deficient (mother) × CD36-positive (father) pairing is a classic scenario: the mother can form anti-CD36 (Nak) antibodies against the fetal platelets.

Figure 5. Functional Assessment of Platelet and Monocyte CD36 Expression and IgM Binding. Flow cytometric panels depict:

(A) CD36 expression on platelets (PSP-CD36Ab(+)-CD36). Top-left panel: The rectangular gate isolates the platelet population, leveraging platelet-specific CD41a expression and size characteristics to ensure accurate identification. Top-right panel: Platelets -expressing CD41a and CD36 are depicted as red events in the upper-right quadrant, indicating a CD36-positive platelet subpopulation at 0.16% of the gated platelets. Bottom-left panel: Histogram illustrating CD36 fluorescence intensity across the gated platelet population. The mean fluorescence intensity (MFI) threshold for CD36 positivity exceeds 10 (Log scale), aligning with established gating criteria used in quantitative

assays of platelet CD36 expression. This threshold differentiates CD36-deficient from CD36-expressing platelets, as is typical in clinical flow cytometry analyses.

(B) Platelet-bound IgM levels (PSP-CD36Ab(+)-IgM). FITC-conjugated IgM (Isotype Control) is used to set the negative fluorescence intensity and serves as the negative control.

(C) CD36 expression on monocytes (WB-CD36Ab(+)-CD36). Top-left panel: The rectangular gate isolates the monocyte population, leveraging CD14 expression and size characteristics for accurate identification. Top-right panel: Monocytes co-expressing CD14 and CD36 are depicted as red events, indicating a CD36-positive monocyte subpopulation at 1.30% of the gated monocytes. Bottom-left panel: Histogram illustrating CD36 fluorescence intensity across the gated monocyte population. The mean fluorescence intensity (MFI) threshold defining CD36 positivity exceeds 100 (Log scale), in accordance with established gating criteria. This enables accurate differentiation between CD36-deficient and CD36-expressing monocytes.

(D) Monocyte-bound IgM levels (WB-CD36Ab(+)-IgM). FITC-conjugated IgM (an Isotype Control is used to set the negative fluorescence intensity, serving as the negative reference). Control.

(E) Molecular Characterization of CD36 Deficiency: Identification of Pathogenic Variants by Sanger Sequencing. This figure illustrates representative gene sequencing chromatograms from patients diagnosed with type I CD36 deficiency, highlighting distinct pathogenic variants across exon 5 of the CD36 gene. The panel displays a 2-base pair deletion (c. 329_330delAC) (rs572295823) in exon 5, observed in a homozygous state.

Figure 6. Validation of CD36 Deficiency and Alloimmunization Mechanisms

Flow cytometric analysis demonstrates:

(A) Platelet CD36 expression (PSP-M2(D1)-CD36). The rectangular gate isolates the platelet population, leveraging platelet-specific CD41a expression and size characteristics to ensure accurate identification. Top-right panel: Platelets co-expressing CD41a and CD36 are depicted as red events in the upper-right quadrant, indicating a CD36-positive platelet subpopulation at 1.89% of the gated platelets. Bottom-left panel: Histogram illustrating CD36 fluorescence intensity across the gated platelet population. The mean fluorescence intensity (MFI) threshold defining CD36 positivity exceeds 10 (Log scale), consistent with established gating criteria used in quantitative assays of platelet CD36 expression. This threshold distinguishes CD36-deficient and CD36-expressing platelets, as commonly applied in clinical flow cytometry analyses.

(B) Platelet IgM binding (PSP-M2(D1)-IgM). FITC-conjugated IgM (Isotype Control) is used to set the negative fluorescence intensity and serves as the negative control.

(C) Monocyte CD36 expression (WB-M2(D1)-CD36). Top-left panel: The rectangular gate isolates the monocyte population, leveraging CD14 expression and size characteristics for accurate identification. Top-right panel: Monocytes co-expressing CD14 and CD36 are depicted as red events, indicating a CD36-positive monocyte subpopulation at 1.54% of the gated monocytes. Bottom-left panel: Histogram illustrating CD36 fluorescence intensity across the gated monocyte population. The MFI threshold defining CD36 positivity exceeds 100 (Log scale), enabling the differentiation of CD36-deficient from CD36-expressing monocytes, consistent with clinical flow cytometry gating standards.

(D) Monocyte IgM binding (WB-M2(D1)-IgM). FITC-conjugated IgM (Isotype Control) is used to set the negative fluorescence intensity and serves as the negative control.

(E) Molecular Characterization of CD36 Deficiency: Identification of Pathogenic Variants by Sanger Sequencing. This figure illustrates representative gene sequencing chromatograms from patients diagnosed with type I CD36 deficiency, highlighting distinct pathogenic variants across exon 5 of the CD36 gene. The panel presents a frameshift mutation (c.329_330delAC) (rs572295823) in exon 5, introducing a premature shift in the reading frame at amino acid 110, predicted to generate a truncated, non-functional protein.

Figure 7. Flow Cytometric Confirmation of Type I CD36 Deficiency and Anti-CD36 Alloantibody Detection. It shows absence of CD36 expression on patient platelets and monocytes, coupled with specific anti-CD36 IgG binding on CD36-positive targets, confirming type I CD36 deficiency and alloimmunization.

(A-C) Flow cytometric analysis of CD36 antigen expression on patient blood cells. Peripheral blood samples were analyzed using PerCP-Cy5.5–conjugated goat anti-human CD36 antibody and matched isotype controls. Flow cytometry shows absent CD36 expression on both platelets and monocytes, confirmed using PerCP-Cy5.5–conjugated anti-CD36 antibody and matched isotype controls. Positive control monocytes from a healthy donor validate assay specificity.

(D-F) Patient and control sera were incubated separately with CD36-negative and CD36-positive platelet targets, followed by FITC-conjugated goat anti-human IgG. **(D) Patient serum + CD36– platelets:** Histogram overlay of FITC fluorescence for CD36– platelets incubated with patient serum (blue) versus negative control serum (purple)

shows only background binding. **(E) Patient serum + CD36+ platelets:** Corresponding overlay for CD36+ platelets revealed a marked rightward shift with patient serum (red), indicating specific anti-CD36 IgG binding; negative control serum (purple) remains at background. **(F) Positive control serum + CD36+ platelets:** FITC fluorescence of CD36+ platelets incubated with patient serum (pink) exhibits a similar rightward shift relative to negative control (purple), confirming assay sensitivity and specificity.

(G-H) Platelet Immunofluorescence Assay (PIFA) Confirms Anti-CD36 Antibody Binding. Immunofluorescence images demonstrate specific binding of anti-CD36 antibodies from patient and positive control sera to CD36-positive platelets, with no reactivity on CD36-negative targets, validating assay specificity. **(I-J) Differential CD36 Expression in Platelets and Monocytes by Flow Cytometry.** The panels demonstrate absent CD36 expression on both patient platelets and monocytes. These findings are consistent with a CD36-deficiency type I phenotype.