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Investigation of platelet–antibody interactions using ultrasound-induced chemiluminescence

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Abstract: Background: Platelet transfusion refractoriness (PTR) presents a significant clinical challenge, often requiring specialized diagnostic and donor-matching strategies. Existing immunoassays detect anti-platelet antibodies but do not assess their functional impact.
Objective: This study aimed to evaluate the feasibility of using ultrasound-induced chemiluminescence (UICL) as a functional assay to detect interactions between platelet concentrates (PCs) and selected antibodies (anti-HLA and ABO antibodies).
Methods: Native and antibody-exposed PCs were analyzed using luminol-based chemiluminescence following high-frequency ultrasound stimulation. The signal intensity, reflecting reactive oxygen species production, was measured and standardized using an activation index. Microscopic, cytometric, and hematologic assessments were performed to validate platelet activation.
Results: Native PCs present low spontaneous chemiluminescence, which increase approximately tenfold upon ultrasound stimulation. The presence of anti-platelet antibodies additionally enhances the chemiluminescence signal following ultrasound activation. Using a quantitative activation index allows for comparison of platelet–antibody interactions and differentiation of compatible versus incompatible PCs. The lower the activation index, the better the antigenic match between the donor platelets and the patient's immune system. The method correlates with conventional HLA matching techniques (e.g., flow cytometry) and may support the selection of compatible PCs for alloimmunized patients. Morphological and volumetric changes confirm platelet activation post-sonication.
Conclusion: UICL is a rapid, sensitive, and functionally informative method for assessing platelet–antibody interactions. It holds promise as a complementary or alternative approach to traditional immunologic testing in the management of immune-mediated PTR and may enhance transfusion decision-making, especially in settings where immunogenetic data are limited or time-sensitive.

Keywords: chemiluminescence, platelet transfusion refractoriness, platelet antibody.

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Introduction

Platelet concentrates are one of the blood components frequently transfused to patients for the prophylaxis or treatment of life-threatening hemorrhages. Unfortunately, in a subset of patients, transfusion of platelet concentrates (PCs) does not result in the expected post-transfusion platelet count increment. Platelet transfusion refractoriness (PTR) represents a significant clinical problem [1].

PTR may have an immunological basis. Among immune-mediated causes, the most relevant are antibodies directed against class I human leukocyte antigens (HLA) present on platelets (accounting for 80–90% of cases) and antibodies against human platelet antigens (HPA), responsible for approximately 10–20% of immune refractoriness. Naturally occurring anti-A and anti-B antibodies directed against ABO antigens, which are also expressed on platelets, may also contribute to this phenomenon [1, 2].

The ability to accurately and efficiently diagnose the causes of platelet refractoriness is crucial for providing effective platelet transfusion support for patients with immune-mediated PTR. Alloantibodies targeting platelet antigens can be detected and/or identified by various methods, including complement-dependent cytotoxicity (CDC) assays, enzyme-linked immunosorbent assays (ELISA), flow cytometry, and microbead-based assays utilizing Luminex technology. The primary differences between these methods lie in their sensitivity and the spectrum of antibody specificities that can be reliably detected [2].

Depending on the availability of these diagnostic techniques, the turnaround time for testing, and the accessible pool of HLA-typed platelet donors, several strategies exist for selecting platelet components for patients with immune-mediated platelet refractoriness. A survey conducted among blood centers and transfusion laboratories revealed a wide variability in available diagnostic options, highlighted areas requiring improvement, and identified common challenges in providing timely and appropriate support for patients with immune-mediated platelet refractoriness [2, 3].

Chemiluminescence is a sensitive and versatile analytical method used to detect light emission resulting from chemical reactions involving reactive oxygen species (ROS). In biological systems, chemiluminescence provides a real-time, non-invasive, and highly sensitive measure of cellular activation and oxidative metabolism. Among various cell types, platelets are of particular interest due to their central role in hemostasis, inflammation, and immune-mediated processes. In platelet research, chemiluminescence has been employed to assess baseline metabolic activity, monitor responses to physiological agonists, and investigate the impact of immune interactions [4–7].

In the context of platelet transfusion refractoriness, the ability to study platelet–antibody interactions using ultrasound-induced chemiluminescence (UICL) offers a promising diagnostic and investigative tool. Antigens expressed on platelets (e.g., ABO, HLA, HPA) can be targeted by specific antibodies. The resulting immune complexes may alter the platelet surface properties and affect their interaction with ultrasound energy, thereby modifying the chemiluminescent response.

This study aimed to evaluate the potential of UICL for investigating interactions between platelets and selected antibodies. The following objectives were addressed:

To evaluate the baseline chemiluminescent activity of native platelets obtained from different types of platelet concentrates.

To investigate the effect of selected antibodies — including naturally occurring anti-A and anti-B antibodies as well as anti-HLA antibodies — on the chemiluminescence signal.

To assess the response of native platelets to high-frequency ultrasound stimulation, in the absence of added antibodies.

To examine how the presence of specific antibodies targeting surface-expressed platelet antigens modulates UICL.

Through this approach, the study explores the feasibility of using UICL as a supplementary or alternative method to traditional immunoassays for the assessment of platelet–antibody interactions in the context of immune-mediated platelet refractoriness.

Materials and Methods

Biological Materials

The biological materials were provided by the Regional Blood Donation and Treatment Center in Kraków, Poland (RCKiK in Kraków, Poland).

Human Sera

Two types of human sera were used in the study:

- Serum obtained from healthy blood donor with blood group O (containing naturally occurring anti-A and anti-B antibodies) and serum obtained from healthy blood donor group AB (lacking natural antibodies).
- 11 different sera containing anti-HLA antibodies collected from patients for whom HLA antigen-matched PCs had been selected by the HLA laboratory due to immune-mediated platelet refractoriness. Detection of complement-dependent anti-HLA class I antibodies was performed using the lymphocytotoxicity assay, in the HLA laboratory (RCKiK in Kraków, Poland).

Platelet Concentrates

Two types of PCs were analyzed:

- Pooled platelet concentrates (pPCs): Ten units, each prepared by combining buffy coats from five different whole blood donations, and leukoreduction proceed.
- Apheresis platelet concentrates (aPCs): Ten single-donor platelet units obtained by apheresis (Trima Accel™ Automated Blood Collection System, Terumo BCT, Tokyo, Japan).

The ethical committee approval: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Research Ethics Committee of Jagiellonian University Medical College (permission No. 118.0043.1.213.2025), Cracow, Poland.

Reagents

All reagents were of analytical grade and delivered from Merck Life Science (Darmstadt, Germany) unless otherwise stated.

Luminol

A luminol stock solution (200 mM) was prepared by dissolving 35.4 mg of luminol in 1 ml of dimethyl sulfoxide (DMSO). The stock solution was aliquoted and stored at −20°C. Prior to

experimentation, a working solution (20 mM) was prepared by 10-times dilution of the stock in DMSO.

Platelet Additive Solution+ (PAS+, Terumo, Tokyo, Japan)

Platelet Additive Solution+ (PAS+) is routinely applied during platelet concentrate preparation to partially replace plasma in the final product. Following the addition of the working luminol solution, PAS+ served as the reaction medium for chemiluminescence assays. The chemiluminescent reaction mixture was composed of 30 µl of the working luminol solution added to 12 ml of PAS+, resulting in a luminol concentration of 50 µM.

Experimental Procedures

Chemiluminescence Assay Protocol

The study methodology involved incubation of successive platelet concentrate (PC) with appropriate sera. After 15 minutes of incubation, luminol diluted in platelet additive solution (PAS+) was added to each test tube and the intensity of the chemiluminescence signal was measured and recorded. The next step involved additional stimulation of the platelets — both native and antibody-coated — by a physical stimulus, namely high-frequency ultrasound (1 MHz), followed by a second measurement of chemiluminescence.

1. Antibody Coating of Platelets:
   A volume of 10 µl of test serum (containing anti-A and anti-B antibodies, anti-HLA antibodies or control serum — without anti-A and anti-B antibodies) was added to 100 µl of platelet concentrate. The mixture was incubated for 15 minutes without stirring at room temperature to facilitate antibody binding to platelet surface antigens.
2. Preparation of the Reaction Mixture:
   Following incubation, 100 µl of the antibody-treated platelets were combined with 900 µl of PAS+ containing luminol to form the final reaction mixture, with final concentration of luminol 45 µM.
3. Ultrasonic Activation:
   Each sample was exposed to high-frequency ultrasound using a 1 MHz ultrasonic generator (commonly used in humidifiers). Sonication was applied for a standardized duration of 10 seconds using an automatic timer to ensure reproducibility.
4. Measurement of Chemiluminescence:
   Light emission resulting from the oxidation of luminol was measured using a Lumat LB 9507 luminometer (EG&G Berthold, Bad Wildbad, Germany). The device operated in photon-counting mode and was interfaced with software (YAT) for real-time acquisition. Chemiluminescence signal was recorded at 1-second intervals throughout the measurement period.
5. The precision of the method was assessed based on the coefficient of variation (CV), which was 15% for n = 20 control measurements.

Hematological Assessment — Platelet Smears and Microscopic Evaluation

Hematological smears of the investigated platelet samples were prepared both before and after sonication, under conditions with and without antibody coating. The smears were stained using Wright's staining technique, and morphological assessment was performed by light microscopy (Delta Optical, Warsow, Poland).

Analysis of Platelet Size Using an Automated Hematology Analyzer

Platelet size measurements, pre- and post-sonication, were conducted using the Beckman Coulter UniCel DxH 800 (Brea, California, USA) hematology analyzer, ensuring quantitative assessment of platelet morphological alterations induced by the experimental conditions.

Flow Cytometry Analysis of Antibody-Coated Platelets

A total of 50 µL of the test serum was incubated with 50 µL of platelet concentrate (PC) for 30 minutes at 22–24°C without agitation. Following incubation, 500 µL of PAS+ solution was added to the reaction mixture, which was subsequently centrifuged at 1100 x g for 3 minutes at room temperature. The supernatant was carefully removed. The washing step was repeated three times to ensure removal of unbound components. Next, 50 µL of fluorescein isothiocyanate (FITC)-conjugated anti-human IgG secondary antibody (Goat anti-Human IgG F(ab')$_2$, FITC, Cat. No. 31628, Thermo Fisher Scientific, USA), diluted 100-times in distilled water, was added to the cell pellet. The samples were incubated for 30 minutes at room temperature in the dark. Following incubation, the samples were washed three times with PAS+ and finally resuspended in 300 µL of PAS+. The prepared samples were analyzed using flow cytometry (Becton Dickinson Biosciences Immunocytometry Systems, San Jose, CA, USA).

Statistical analyses, including t-Student test were performed using STATISTICA version 13.3.

Results

Chemiluminescence values (mean value for 10 platelet samples) for native pooled platelets and for pooled platelets incubated with anti-HLA antibody-containing sera are shown in Fig. 1.

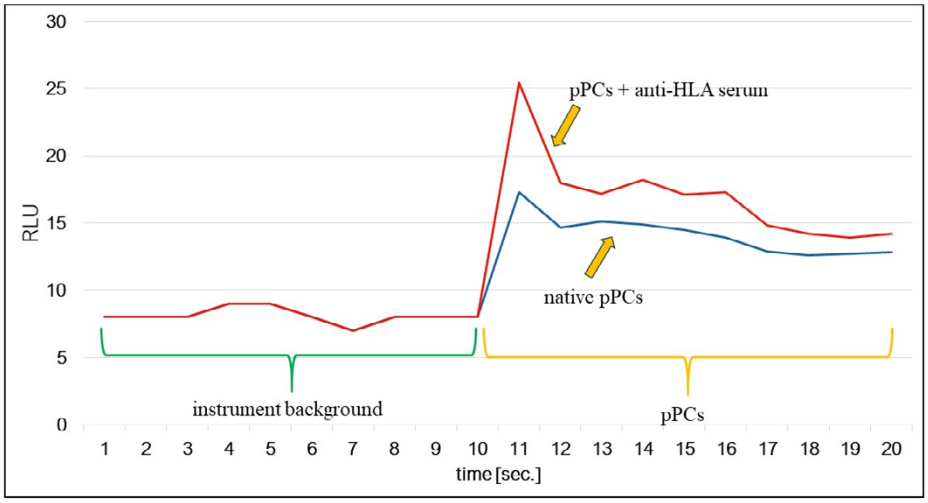

Fig. 1. Chemiluminescence signal of native pooled platelet concentrates (pPCs) and pPCs exposed to anti-HLA antibodies — prior to sonication (mean values for 10 platelet samples). Analyzed mixtures contained luminol at final concentration of 45 µM. RLU — relative light unit.

The analyzed platelet concentrates — both native (i.e., without added antibody-containing sera) and those exposed to sera containing natural anti-A and anti-B antibodies as well as anti-HLA antibodies — showed a low level of spontaneous chemiluminescence, despite the absence of external activating stimuli. This finding indicates a low but detectable metabolic activity of the platelets.

To further stimulate platelet activation, a high-frequency ultrasonic wave (1 MHz) was applied (Fig. 2).

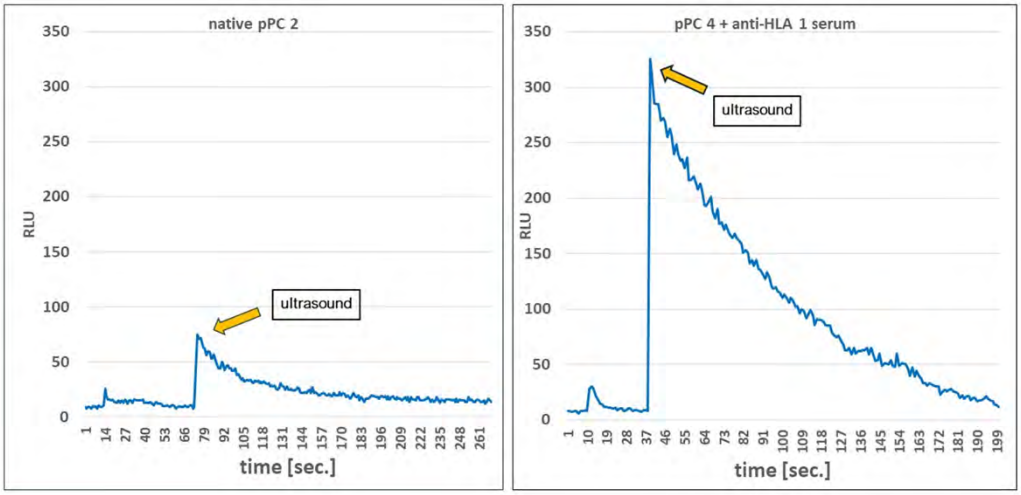

Fig. 2. Time-dependent changes in chemiluminescence signal of a) representative native platelets (pPC 2) and b) representative platelets incubated with serum containing anti-HLA antibodies (pPC 4 + anti-HLA 1 serum) — following exposure to ultrasound. Analyzed mixtures contained luminol at final concentration of 45 μM.

After ultrasound stimulation, a rapid increase in the chemiluminescence signal of the PCs was observed. These results confirm platelet activation following ultrasound exposure. The analysis of signal intensity revealed variability among different platelet concentrates. Individual samples demonstrated differing signal amplitudes and distinct dynamics of changes over time.

Due to the heterogeneity of activation curves, a standardized measure of signal magnitude was introduced — defined as the sum of emitted light impulses within the first 30 seconds post-activation — Σ RLU (RLU — relative light unit) — Fig. 3.

Based on a standardized measure of signal magnitude, the activation of tested native PCs was compared. The mean chemiluminescence value (Σ RLU) for pooled PCs was 1297.75 (SD = 638.78), while for apheresis PCs it was 1298.2 (SD = 280.2).

In the subsequent part of the study, the interaction between individual sera and PCs was assessed — using AB group serum (lacking anti-A and anti-B antibodies), O group serum (containing natural anti-A and anti-B antibodies), and sera with various sets of anti-HLA antibodies.

A representative chemiluminescence signal (sigma RLU) for a pooled PC exposed to anti-A, anti-B, and anti-HLA antibodies is shown in Fig. 4. The activation profiles of a given platelet concentrate varied depending on the series of anti-HLA antibodies used and were determined by the repertoire of antigens expressed on the platelet surface.

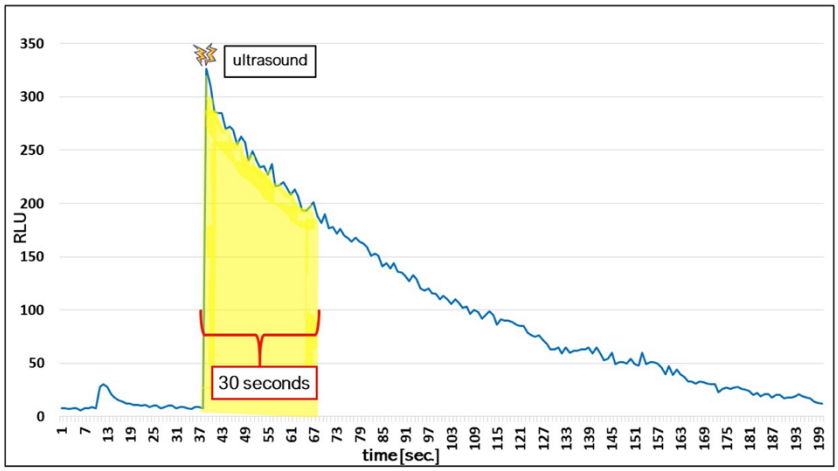

Fig. 3. A standardized measure of chemiluminescence signal magnitude of ultrasound-activated platelets — the sum of emitted light impulses within the first 30 seconds post-ultrasound activation (Σ RLU).

Considering the differences in the baseline activity of individual platelet concentrates, the chemiluminescent values post-antibody exposure were normalized to native platelet values using the formula:

Activation Index = [chemiluminescence signal from PC incubated with serum (Σ RLU) ÷ chemiluminescence signal from native PC (Σ RLU)] — 1.

Fig. 5 presents the calculated activation indices for the examined platelet sample, corresponding to samples presented in Fig. 4.

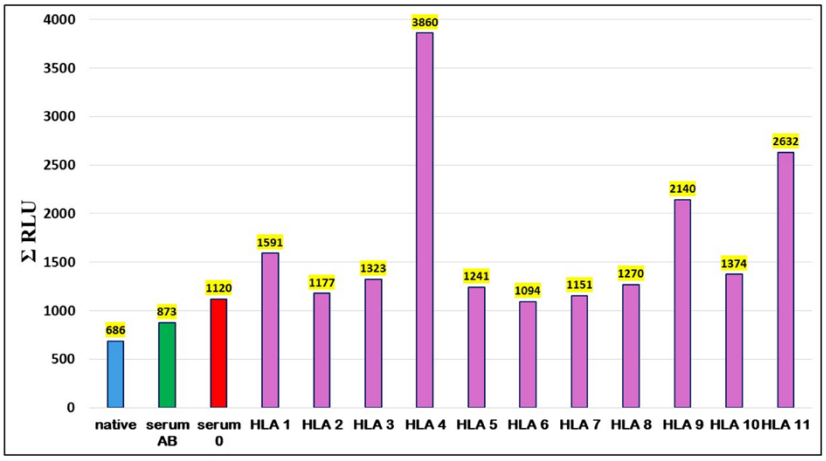

Fig. 4. Chemiluminescence signal values of platelet concentrate (pPC 5) activated by different antibodies — after sonication. The pooled platelet concentrate (pPC 5) was sequentially incubated with AB group serum (lacking natural anti-A and anti-B antibodies), group O serum (containing natural anti-A and anti-B antibodies), and sera containing various sets of anti-HLA antibodies (HLA1–HLA11).

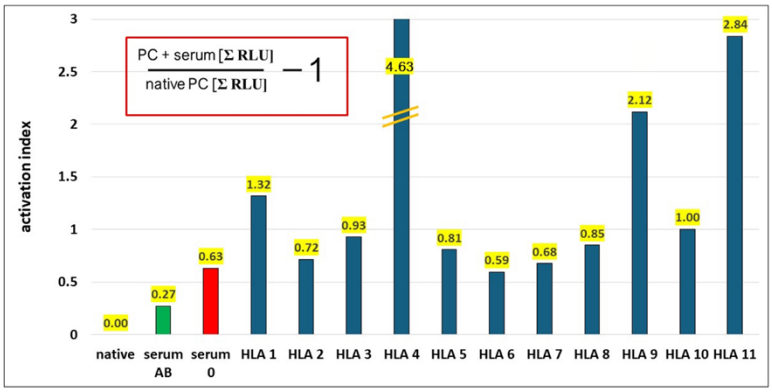

Fig. 5. Activation indices of platelet concentrate (pPC 5) calculated using Σ RLU values from Fig. 4. Activation index was calculated using the formula: [chemiluminescence signal from PC incubated with serum (Σ RLU) ÷ chemiluminescence signal from native PC (Σ RLU)] — 1. The subsequent sera used — from left to right: AB group serum (lacking natural anti-A and anti-B antibodies), group O serum (containing natural anti-A and anti-B antibodies), and sera containing various sets of anti-HLA antibodies (HLA1–HLA11).

Low activation index values indicate the absence of antigen-antibody interaction, suggesting that the platelets lack surface antigens corresponding to the antibodies present in the serum. The lower the activation index, the better the antigenic match between the donor platelets and the patient's immune system.

This was further confirmed by analyzing specific platelet concentrate-serum combinations (Fig. 6). PCs were selected by the HLA laboratory for a highly HLA-immunized patient (Patient P). The platelets were obtained from HLA-compatible donors. Compatibility was further confirmed by

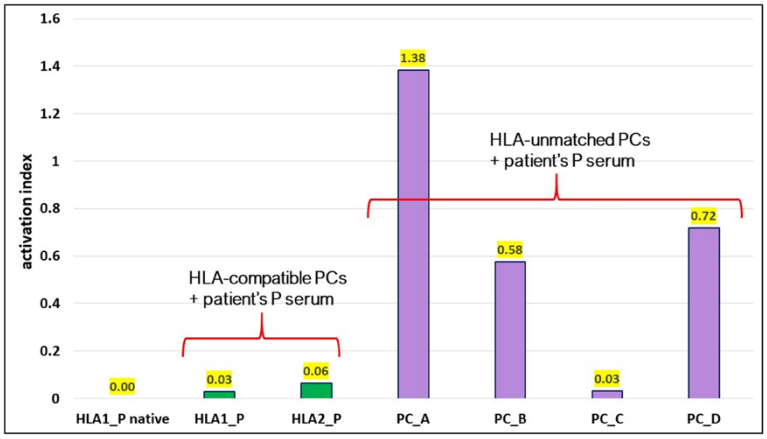

Fig. 6. Activation indices of specific platelet concentrate-serum combinations. aPCs HLA1_P and HLA2_P were selected for a highly HLA-immunized patient (patient P). These aPCs were obtained from HLA-compatible donors. The activation indices derived from chemiluminescence measurements were near zero, indicating high donor-recipient compatibility. Among four randomly selected aPCs (PC_A, PC_B, PC_C, PC_D) incubated with the patient's P serum, PC_C was found to be highly HLA-matched.

a lymphocytotoxicity assay. The activation indices derived from chemiluminescence measurements were near zero, indicating high donor-recipient compatibility. Among four randomly selected PCs incubated with the patient's P serum, only one showed a similarly low activation index, suggesting potential HLA compatibility.

The magnitude of UICL of these specific platelet concentrate-serum combinations (patient's P serum, randomly selected PC_D, HLA-matched PC HLA2_P) was compared with results obtained by flow cytometry analysis. The binding of anti-HLA antibodies to the platelet surface was confirmed in samples with high calculated activation indices (Fig. 7).

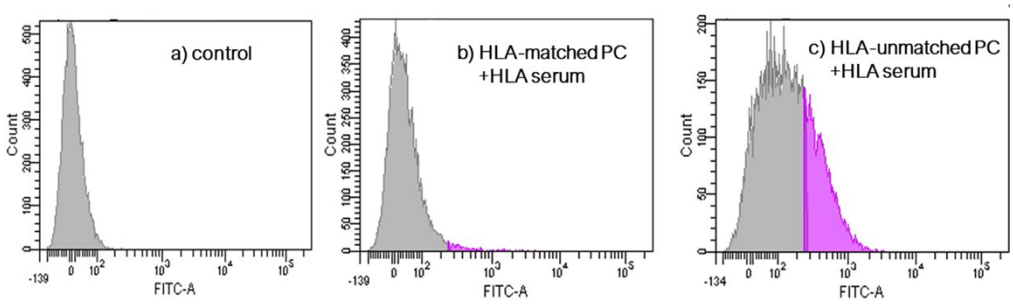

Fig. 7. Flow cytometric analysis of different PCs: a) native PC + secondary antibody FITC (control), b) HLA-matched PC + HLA serum + secondary antibody FITC- correlating with low activation index (0.06), c) HLA-unmatched PC + HLA serum + secondary antibody FITC — corresponding to relatively high activation index (0.72).

To assess the effect of sonication on platelets, hematological smears of the tested PCs were prepared. Fig. 8 shows representative examples of PCs before (Fig. 8a) and after ultrasound exposure (Fig. 8b). Additionally, Fig. 8 presents individual platelets prior to sonication (a) and platelet aggregates formed as a result of ultrasound-induced activation (b).

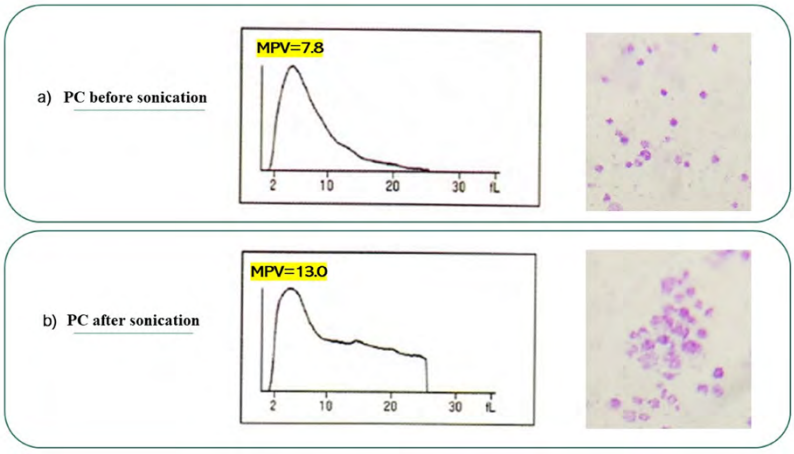

Fig. 8. Representative histograms of platelet size distribution and microscopic images of hematological smears of PCs: a) before sonication (mean platelet volume, MPV = 7.8) and b) after sonication (MPV = 13.0).

In this study, the interactions between the available platelet concentrate units (n = 20) and allogeneic sera (n = 13) were evaluated, resulting in a total of 260 tested combinations. The mean activation index for pPCs was 0.73 (SD = 0.63), whereas for aPCs it was 0.65 (SD = 0.54). Comparative analysis between the two groups (pPCs vs. aPCs) revealed no statistically significant difference (p = 0.46).

Discussion

This study demonstrates the potential utility of UICL as a rapid and functional assay for assessing platelet activation and immune compatibility. The combination of high-frequency ultrasound with luminol-based detection allows for real-time evaluation of platelet activity and their interactions with circulating antibodies. Importantly, this approach captures functional activation processes, which are not always detectable by conventional immunologic methods.

Chemiluminescence is a sensitive analytical technique used to monitor cellular activity through the detection of light emitted during specific chemical reactions, typically involving ROS. In biological systems, chemiluminescence assays are frequently employed to evaluate cellular metabolism, oxidative stress, immune responses, and activation states, particularly in leukocytes, platelets, and phagocytic cells [8, 9].

Recent studies have shown that the ROS generated by platelets are not merely cytotoxic byproducts but also function as secondary messengers in intracellular signaling pathways. ROS production in platelets is closely linked to activation pathways involving arachidonic acid metabolism, NADPH oxidase, the glutathione cycle, and xanthine oxidase activity. These oxidative processes are integral to platelet aggregation, adhesion, and interaction with leukocytes and endothelial cells. Thus, the ability to monitor ROS levels offers a window into the functional and pathological state of platelets [10].

Several clinical studies have demonstrated the value of chemiluminescence-based methods in platelet analysis. In the classic study by Mills *et al.* [11] incubation of human platelets with arachidonic acid led to a marked chemiluminescence response, which was almost totally oxygen-dependent and localized to the particulate fraction. This response was markedly reduced when platelets were incubated with aspirin or came from aspirintreated subjects. Further research confirmed that aspirin inhibits arachidonic acid-induced chemiluminescence via cyclooxygenase inhibition, supporting this as a useful functional assay for monitoring drug efficacy.

In another study, Gabbasov and colleagues [12] demonstrated that approximately 15% of patients in the subacute phase of acute myocardial infarction (8–10 days post-infarction) exhibited a significant increase in spontaneous platelet chemiluminescence, indicating enhanced production of ROS. This phenomenon correlated with the appearance of CD45 on the platelet surface — a marker typically found on leukocytes — suggesting that platelets may acquire immune-like properties and contribute to the post-infarction inflammatory response, potentially leading to endothelial injury through intensified platelet–leukocyte interactions.

Moreover, interactions between platelets and immune cells can amplify oxidative responses. Wetterö *et al.* [13] showed that platelets stimulated by IgG-coated surfaces activate neutrophils via selectin-dependent pathways, leading to enhanced neutrophil adhesion and ROS production. This cross-talk between platelets and neutrophils likely contributes to the amplification of oxidative stress and inflammatory signaling in various clinical settings, including thrombosis, cardiovascular interventions, and biomaterial contact with blood.

The oxidative behavior of native platelets under ultrasound provides key insight into their sensitivity to environmental forces. Sergienko *et al.* [14] showed that exercise-induced mechanical stress similarly elevates platelet chemiluminescence reinforcing the view that physical forces — whether generated internally (e.g., vascular shear stress) or externally (e.g., ultrasound) — can modulate platelet function via redox signaling.

In our study, both native PCs and those exposed to sera containing anti-A, anti-B, or anti-HLA antibodies exhibited low baseline chemiluminescence. However, upon application of high-frequency ultrasound, there was a marked enhancement in the chemiluminescence signal, indicating ultrasound-induced platelet activation. Sonication-induced platelet activation and aggregation was confirmed by microscopy, automated cell sizing and flow cytometry.

Physical stressors such as ultrasound have been shown to influence oxidative responses in blood cells. In their review of sonodynamic therapy (SDT), Rosenthal *et al.* [15] described how the combination of ultrasound with sensitizing agents can synergistically increase ROS production, contributing to cellular damage or apoptosis in targeted therapies. Although SDT is primarily investigated in the context of oncology, the underlying mechanisms of ROS generation under physical stimulation are also relevant for understanding platelet activation and chemiluminescence responses under non-therapeutic stress conditions.

The effect of a mechanical stimulus on blood platelets was also studied by Otto *et al.* [16]. To examine the impact of ultrasound on platelet aggregation in vitro, they designed a standardized ultrasound apparatus. Their findings demonstrated a strong correlation between platelet aggregation triggered by ultrasound and that induced by ADP, indicating that both types of stimulation may activate similar pro-aggregatory mechanisms.

The study by Poliachid *et al.* [17] also demonstrated that high-intensity focused ultrasound at 1.1 MHz is capable of stimulating platelet activation, aggregation, and adhesion to a collagen-coated surface in a controlled in vitro environment using platelet-rich plasma.

Miller *et al.* [18] provide a broad overview of therapeutic ultrasound modalities, emphasizing both their clinical applications and safety concerns. While primarily used in diagnostic and therapeutic contexts (e.g., tumor ablation, drug delivery), ultrasound can also affect blood components, particularly when high-intensity or focused waveforms are applied. These effects have dual implications: on one hand, they raise safety considerations for off-target platelet activation; on the other, they open avenues for therapeutic exploitation.

Indeed, ultrasound has also shown promise as a modulator of engineered platelet-based therapies. Nandi *et al.* [19] developed an ultrasound-responsive synthetic platelet system capable of enhancing wound healing. Their findings highlight a growing area of interest: the use of ultrasound not just as a diagnostic or physical stressor, but as a precise trigger for platelet-like therapeutic agents. This approach demonstrates how ROS and platelet-derived factors can be harnessed in a controlled manner for tissue repair and regeneration.

In sum, the integration of chemiluminescence and ultrasound research has broadened the understanding of platelet biology. It underscores both the vulnerability and adaptability of platelets to external stimuli, with potential implications for thrombosis, inflammation, and regenerative medicine.

Antibodies-platelet interactions are not merely of theoretical interest but hold significant clinical implications for platelet transfusion strategies in immune-sensitized patients.

PTR is a significant clinical issue, particularly in patients requiring repeated platelet transfusions, such as those undergoing treatment for hematologic malignancies. PTR is characterized by

the failure to achieve a sufficient rise in platelet count following transfusion. This condition can adversely affect patient outcomes, increasing the risk of bleeding and complicating overall treatment strategies [20].

PTR can be broadly categorized into immune and non-immune causes. Non-immune factors — such as fever, infection, splenomegaly, disseminated intravascular coagulation, and certain medications — are the most common and lead to platelet destruction or consumption regardless of immunologic compatibility .

In contrast, immune-mediated PTR typically results from alloimmunization against human leukocyte antigens or less commonly, human platelet antigens. The presence of anti-HLA class I antibodies is the most frequently implicated cause of immune PTR and often necessitates the use of specially selected platelet products [21].

In patients with immune-mediated PTR, particularly those with anti-HLA or anti-HPA antibodies, identifying compatible platelet units is essential. Several strategies have been developed to address this challenge.

The first option is the transfusion of HLA-matched platelets. This requires prior identification of the patient's HLA Class I genotype. Platelet matching is limited to the HLA-A and HLA-B loci. Unfortunately, fully HLA-matched platelets are difficult to obtain, particularly in patients with rare HLA alleles or in those belonging to ethnic minority groups underrepresented in the blood donor population. In such cases, partially HLA-matched platelets, which have been shown in numerous studies to achieve a sufficient post-transfusion platelet count increment and clinical improvement, are used [2, 3].

The second approach involves transfusing antigen-negative platelets, meaning platelets that lack the specific HLA antigens against which the patient has circulating antibodies, a method referred to as "virtual crossmatching". In this scenario, only the patient's anti-HLA antibody profile needs to be determined, without the necessity of HLA typing. This strategy increases the likelihood of finding compatible platelet units compared to full HLA matching [2, 22].

Various techniques are used for the detection and identification of antibodies, each differing in sensitivity, specificity, and clinical utility. The classical serological method for detecting anti-HLA antibodies is the complement-dependent cytotoxicity assay, which involves incubating the patient's serum with a panel of lymphocytes in the presence of complement. If antibodies are present, they induce cell lysis, which can be assessed microscopically. While relatively simple, this method is time-consuming, has low sensitivity, and fails to detect non-complement-fixing antibodies [3, 23].

Another commonly used technique is the enzyme-linked immunosorbent assay in which HLA antigens are immobilized on a test plate. Antibodies from the patient's serum bind to the antigens, and detection is performed using enzyme-conjugated secondary antibodies. ELISA is more sensitive than CDC and allows for the identification of both class I and class II anti-HLA antibodies. However, it has limited specificity for rare alleles and offers lower resolution compared to the Luminex method [2].

Luminex technology is currently considered the gold standard for antibody detection and identification. Depending on the variant used, it enables rapid screening for the presence of anti-HLA antibodies without defining their specificity (panel-reactive antibody — PRA screen), determination of a patient's sensitization level (PRA) or — using the most precise method for identifying anti-HLA antibody specificities — Single Antigen Bead (SAB) assay, allows for high-resolution mapping of antibody specificities [24].

The advantages of Luminex method include very high sensitivity and specificity and the ability to detect low-titer antibodies. However, it is a costly method and can yield false-positive results due to phenomena such as the prozone effect or interference from serum components. A major challenge is the lack of standardized thresholds for interpreting mean fluorescence intensity (MFI); different laboratories use cutoffs ranging from 1,000 to 5,000 MFI, and not all detected antibodies are clinically relevant [25, 26].

The third approach in PRT is transfusion of crossmatch-compatible platelets. This method does not require knowledge of the patient's HLA type or the specificity of their antibodies. HLA crossmatching involves evaluating whether the patient's serum reacts with the platelets intended for transfusion. If the crossmatch test is compatible, an immune response following transfusion is unlikely. An advantage of this method is that it accounts for antibodies other than anti-HLA, particularly anti-HPA antibodies. This technique can be applied while awaiting HLA typing results or in patients with a low panel-reactive antibody percentage, where testing 10–20 platelet units may yield a compatible match. However, in highly sensitized patients, identifying compatible platelets may require testing a much larger number of units. Direct platelet crossmatching to identify compatible components can be performed using various techniques, including solid-phase red cell adherence (SPRCA), modified antigen capture ELISA (MACE), and flow cytometry [27].

Each method used for selecting compatible platelet units has its own advantages and limitations. The choice of strategy depends on several factors, including the availability of HLA-typed donors, the degree of patient sensitization (as reflected by values such as calculated Panel Reactive Antibody, or cPRA), available laboratory resources, turnaround time, and the clinical urgency of the transfusion. In many clinical settings, a combined approach — utilizing more than one method — is often implemented to enhance compatibility, improve transfusion efficacy, and optimize patient outcomes.

It is important to emphasize, as previously mentioned, that highly sensitive antibody detection methods — such as Luminex — may detect antibodies that do not have clinical relevance [3, 27]. In their study Jackman *et al.* [28] suggest that not all HLA antibodies detected by Luminex or bead-based assays are predictive of PTR. For instance, cytotoxicity-positive antibodies are more strongly associated with clinical refractoriness than those detected only by highly sensitive platforms. This highlights the importance of interpreting antibody data within the clinical context and adjusting assay thresholds to reduce overdiagnosis.

In contrast, UICL used in our study enables functional, real-time evaluation of antibody–platelet interactions by quantifying ROS production following ultrasonic stimulation. UICL differentiates between native and antibody-coated platelets by comparing their chemiluminescent responses.

Our study demonstrated that the absolute values of chemiluminescence signals from native platelets after ultrasound activation differed between various platelet concentrate units. Similar results were obtained in the study by Mills *et al.* [11], where the intensity of light emission following the addition of arachidonic acid to human platelet suspensions varied significantly among donors. To address this issue, we proposed the introduction of a standardized activation index, which enables normalized comparison across units and mitigates baseline variability in activation-related chemiluminescence signals.

When compared to established diagnostic methods previously mentioned UICL offers several notable advantages. Most importantly, it is rapid, does not require prior HLA genotyping or

extensive antibody profiling, and can be applied directly to evaluate functional compatibility of donor platelets with patient sera. Our results confirm that antibody binding to platelets leads to a pronounced increase in ultrasound-activated chemiluminescence — in comparison with native platelets. These attributes make UICL especially appealing in urgent clinical scenarios or when comprehensive immunogenetic data are unavailable. Another notable strength of this method is its ability to detect responses not only to anti-HLA antibodies but also to naturally occurring anti-A and anti-B antibodies.

This expands the scope of the assay beyond classical HLA alloimmunization and raises the possibility of assessing other clinically significant antibody specificities, such as anti-HPA antibodies.

Nevertheless, the method has some limitations. The potential influence of serum proteins, platelet storage conditions, and ultrasonic parameters (e.g., duration, intensity) warrants further standardization to ensure reproducibility.

In conclusion, UICL offers a novel, rapid, and functionally informative technique for assessing platelet–antibody interactions and transfusion compatibility. It holds promise as a complementary tool in the management of immune-mediated PTR and may improve the precision and timeliness of transfusion support in sensitized patients. The potential clinical applications of the method are broad: from screening compatible platelet donors in PTR, to real-time monitoring of antiplatelet therapy, and even early detection of thromboinflammatory complications.

Author contributions

Conceptualization, A.L.; methodology, A.L.; software, A.L., W.W. and W.K.; validation, A.L., W.W. and W.K.; formal analysis, A.L., W.W., W.K. and M.P.; investigation, A.L., W.W. and M.P.; resources, A.L. and J.M.; data curation, A.L., W.W., W.K. and M.P.; writing—original draft preparation, A.L.; writing—review and editing, A.L., W.K., M.P. and J.M.; visualization, A.L. and W.K.; supervision, W.K. All authors have read and agreed to the published version of the manuscript.

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Informed consent statement

Informed consent statement was not required as the serum samples were obtained from blood donors and patients following routine diagnostic procedures and were used anonymously.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

None declared.

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