Thrombosis Research 178 (2019) 79–84

Contents lists available at ScienceDirect

Thrombosis Research

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

Full Length Article

# Thromboelastography characterized CD36 null subjects as slow clot formation and indicative of hypocoagulability[☆]

Bai-Chin Lee[a,1], Kuan-Hsiao Lin[b,1], Chung-Yi Hu[c,*], Shyh-Chyi Lo[d,e,**]

[a] Department of Medicine, National Taiwan University Hospital, Taipei, Taiwan
[b] Taipei Blood Center, Taipei, Taiwan
[c] Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University, Taipei, Taiwan
[d] Department of Laboratory Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan
[e] Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan

ARTICLE INFO

*Keywords:*
CD36
Thromboelastography (TEG)
Platelet–monocyte aggregate (PMA)

ABSTRACT

*Background:* Platelet CD36 is the receptor for oxidized low-density lipoprotein and collagen. The conventional platelet test cannot distinguish CD36-null subjects from normal expression subjects. Thromboelastography (TEG) testing can analyze global hemostasis. TEG testing data on CD36-null subjects are not available.
*Methods:* Our subjects were 40 apheresis platelet donors, including 8 CD36-null individuals. We grouped the donors according to the platelet CD36 expression levels to assess the effects of platelet CD36 expression levels on TEG measurement variables.
*Results:* The whole blood TEG test revealed that CD36-null subjects had prolonged reaction time of fibrin formation (TEG R time) and a slower rate to build up cross-linked fibrin (TEG α angle). The final maximal amplitudes of clot formation showed little difference between CD36-null individuals and normal expression individuals. Correlation analysis showed that CD36 expression levels were negatively correlated with TEG R time ($r = -0.342$, $p = 0.031$) and positively correlated with the TEG α angle (0.379, $p = 0.016$). TEG testing on apheresis platelet samples with diminished heterocellular interaction did not reveal differences between CD36-null and normal expression individuals. A subanalysis of the data of a group of healthy subjects showed that platelet CD36 levels correlated positively with platelet–monocyte aggregates (PMAs). Low PMA can diminish heterocellular interaction and likely explain the abnormal TEG results observed in CD36-null individuals.
*Conclusion:* TEG distinguishes CD36-null subjects from normal CD36 expression subjects as having a slower rate of fibrin formation and reassessment of TEG-based diagnostic monitoring is necessary for CD36 null subjects.

## 1. Introduction

CD36, also known as platelet glycoprotein IV, is a highly conserved glycosylated transmembrane protein that belongs to the class B family of scavenger receptors [1,2]. CD36 is widely expressed on different cell types, including platelets, monocytes, macrophages, endothelial cells, adipocytes, hepatocytes, myocytes, and some specialized epithelial cells [1,3]. It is a multifunctional receptor that is involved in various biological and pathological processes, such as innate immune responses, uptake of oxidized lipids, thrombosis, inflammation, and atherosclerosis [2,4,5]. Two types of CD36 deficiency exist [6,7]. Type I deficiency is defined as the lack of CD36 on both platelets and monocytes, and type II deficiency is defined as the lack of CD36 on platelets but the presence of CD36 on monocytes ($CD36^+$). The human platelet CD36 deficiency status is approximately 10 times more common in Africans and Asians (3%–7.7%) than in Caucasians and individuals of Caribbean origin (< 0.3%) [6,8]. Among normal CD36 expression individuals, platelet CD36 expression levels vary widely (in the range of

*Abbreviations:* TEG, Thromboelastography; PMA, platelet–monocyte aggregate; LDL, low-density lipoprotein; PRP, platelet-rich plasma; R time, reaction time; CI, coagulation index; MA, maximal amplitude; TMA, time to maximal amplitude

[☆] The authors have disclosed no conflicts of interest.

* Correspondence to: C.-Y. Hu, Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University, No. 1, Chang-Te St., Taipei 10048, Taiwan.
** Correspondence to: S.-C. Lo, Department of Laboratory Medicine, National Taiwan University Hospital, No. 7, Chung-Shan S. Rd., Taipei 100, Taiwan.
*E-mail addresses:* jcyhu@ntu.edu.tw (C.-Y. Hu), losc@ntu.edu.tw (S.-C. Lo).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.thromres.2019.04.006
Received 20 February 2019; Accepted 5 April 2019
Available online 06 April 2019

2000–14,000 molecules per platelet) [9].

Platelet CD36 can bind to collagen, oxidized low-density lipoprotein (LDL), and thrombospondin, which are implicated in platelet physiology [10,11]. However, the role of CD36 in normal hemostasis remains unclear. In the physiological state, CD36-null subjects do not demonstrate noticeable bleeding diathesis. In a study conducted in 1992, Yamamoto et al. reported that CD36-null platelets from healthy donors showed normal platelet aggregation in response to collagen and adenosine diphosphate, epinephrine, arachidonic acid, and thrombin [7]. Only in the pathological status, such as hyperlipidemia, or in the presence of increased endothelial microparticles owing to vascular damage, decreased prothrombotic platelet activation has been observed in CD36-null mice or human subjects [9,12,13]. The subtle clinical phenotype of CD36-null individuals is not robust enough to be detectable, and other redundant systems may compensate for the role of CD36 in normal hemostasis [2].

Activated platelets can form complexes with leukocytes, preferentially monocytes, through P-selectin (CD62P) and P-selectin glycoprotein ligand-1 (PSGL-1, CD162) on monocytes. The formation of platelet–monocyte aggregates (PMAs) is regarded as a crucial pathophysiological mechanism underlying thrombosis and inflammation [14]. Platelet–monocyte interaction can activate monocytes and promote coagulation through enhanced monocyte tissue factor expression. CD36 is also implicated in platelet–monocyte interaction. Thrombospondin bridges CD36 molecules on the surface of platelets and monocytes and facilitates PMA formation [10]. Because the P-selectin blocking antibody abolishes PMA formation, CD36–thrombospondin ligation is considered to play only an additive role in PMA formation [14].

CD36 expression levels are highly correlated with platelet reactivity to oxidized LDL, suggesting that high CD36 expression is an indicator of platelet hyperactivity [12]. A positive association between platelet CD36 levels and PMA formation has been reported in treatment-naive asymptomatic human immunodeficiency virus-infected individuals [15]. Although the inflammatory state of the infected subjects may have caused platelet activation and induced PMA formation, high platelet CD36 expression levels may predispose platelets to form complexes with monocytes, facilitating coagulation.

Thromboelastography (TEG), a viscoelastic test, monitors hemostasis in whole blood [16,17]. Unlike routine plasma-based coagulation tests, such as tests based on the prothrombin time and activated partial thromboplastin time, TEG assesses cell-based coagulation. TEG has been widely used in cardiovascular surgery and trauma and is a part of transfusion management strategies [17,18]. Considering the involvement of CD36 in cellular interaction, we hypothesize that TEG may be a more effective indicator of the CD36 null phenotype than platelet aggregometry used previously.

In this paper, we provide evidence that TEG can distinguish CD36-null individuals from CD36 normal expression individuals. Our data demonstrated that the CD36 null status was correlated with longer reaction time of fibrin formation (prolonged TEG R time) and a slower rate to build up cross-linked fibrin (decreased TEG α angle). The final maximal amplitudes (MAs) of clot formation (TEG MAs) were nearly the same between CD36-null individuals and CD36 normal expression individuals. Our data have significant clinical relevance for laboratory diagnosis and transfusion decision algorithms based on TEG monitoring.

## 2. Methods and patients

This study was a subanalysis of two previous studies [19,20]. The first study characterized the frequency and genetic mechanisms of the CD36 null phenotype in Taiwan and assessed the effects of the CD36 deficiency status on coagulation function of whole blood [19]. In the first study, we also used TEG as a tool to analyze the coagulation function of apheresis platelets to investigate how platelet CD36 expression levels affect TEG measurement variables. The second study investigated the association of inflammatory and cellular markers with coronary disease assessed through coronary angiography [20]. In the second study, we analyzed the correlation of CD36 expression levels with PMA formation. The protocols of both studies were approved by the Institutional Review Board of Medical Ethics Review Committee of Taiwan Blood Center and National Taiwan University Hospital. Written informed consents were obtained from all participants.

The first study initially enrolled 640 regular platelet apheresis donors (M/F: 547/73, age: 45 ± 10.6 years) from the Taipei Blood Center to investigate the frequency of CD36 deficiency, genetic variations on *CD36* conferring CD36 deficiency, and coagulation characteristics of platelet products collected from CD36-null donors. In the first study, we identified four donors with type I CD36 deficiency and six donors with type II CD36 deficiency [19]. From this donor pool, we further selected a subset of donors including CD36-null individuals, CD36 low expression individuals, and normal expression individuals, to investigate the effects of platelet CD36 expression levels on TEG variables.

The participants of the second study were 356 healthy subjects (M/F: 243/113, age: 59 ± 9.8 years) who underwent coronary computed tomography angiography as a part of a general health checkup. Participants were examined, and their history was taken by a physician; individuals with overt inflammatory diseases were excluded from the study. Laboratory investigation of markers of monocyte subsets, monocyte and platelet CD36 expression levels, cellular microparticles, and PMA formation was conducted. A part of the study results has been reported elsewhere [20,21].

### 2.1. Measurement of platelet CD36 levels

Platelet CD36 expression was determined within 4 h after blood collection. Blood was collected through venipuncture without stasis by using siliconized vacutainer tubes containing 3.8% trisodium citrate. Whole blood was collected and centrifuged (at 200 ×*g* for 10 min) to obtain platelet-rich plasma (PRP). Ten microliters of PRP was incubated with a saturated amount of FITC-mouse antihuman CD36 and PE-mouse antihuman CD42b (platelet GP Ib) for 15 min at room temperature in the dark.

The subsequent procedures of the two studies involved flow cytometric analyses, which were performed on different flow cytometers at two laboratories. For the health checkup cohort, cytometric analyses were performed on a FACSCalibur (BD Bioscience, Franklin Lakes, NJ, USA). At least 5000 platelets were assessed, and the results were analyzed using Cell Quest software (BD Bioscience). For the apheresis donor cohort, the samples were analyzed on a Beckman Coulter Epics XL (Beckman Coulter, Indianapolis, IN, USA) with at least 5000 platelets assessed and analyzed using EXPO32 ADC software (Beckman Coulter). The mean fluorescence intensity of CD36 staining was used as a parameter of the platelet CD36 level in the following statistical analysis.

### 2.2. TEG using whole blood samples

TEG was performed on a Thromboelastograph Hemostasis analyzer (Haemonetics, Braintree, Massachusetts, USA) following the manufactures' instructions. Citrated whole blood was tested 30 min after collection to allow specimen equilibration at room temperature, and TEG was performed within 3 h after specimen collection. A total of 340 μL of citrated whole blood was added to a prewarmed (37 °C) sample cup that contained 20 μL of calcium chloride, and measurements were made within 30 min.

Three TEG variables are highly related to the cell-based coagulation cascade and are included in most transfusion decision algorithms. TEG R time indicates the time taken for the formation of a clot in minutes. The TEG α angle indicates the rate of fibrin formation and cross-linking (expressed as degree because it is the angle formed by the baseline and tangent to the TEG curve). TEG MA indicates the clot strength (expressed in millimeters because it is measured as the highest point of the TEG curve). Other TEG variables include TEG K time (measurement of the kinetics of clot formation in minutes), TEG time to maximal amplitude (TMA; the time to the maximal clot formation in minutes), TEG ly30 (clot lysis 30 min after MA and is expressed in percentage), and TEG CI (a calculated coagulation index [CI]).

### 2.3. TEG using apheresis platelet products as samples

TEG studies using platelet products were performed as previously reported [22]. We obtained platelet samples using apheresis platelet products collected through the MCS platelet apheresis system (Haemonetics, Braintree Massachusetts, USA). Before testing, each platelet sample was adjusted to 200–300 × $10^3$/μL using pooled platelet-poor plasma prepared from 30 platelet donors (AB blood type). The measurement procedure and analysis were the same as those for whole blood TEG.

### 2.4. Measurement of platelet and monocyte CD36 levels and PMA

A total of 100 μL of citrate anticoagulated blood was incubated with a saturated amount of phycoerythrin-Cy5-conjugated monoclonal antibodies against CD14, PerCP-conjugated anti-CD42a, and FITC-conjugated anti-CD36 or matched isotype antibody pool for 15 min at room temperature in the dark. All the monoclonal antibodies were purchased from BD Bioscience (San Jose, CA, USA). Lysing solution (BD FACS Lyse, Lysing Solution; Becton Dickinson, Germany) was used to lyse erythrocytes and fix leukocytes. Cytometric analyses were performed using the FACSCalibur (BD Bioscience, Franklin Lakes, NJ, USA), and the results were analyzed using Cell Quest software (BD Bioscience). Monocytes were first gated in a forward scatter/side scatter dot plot. The gated monocytes would be displayed as two populations: platelet-coated monocytes (CD14, CD42a double positive) and uncoated monocytes ($CD14^+CD42a^-$ populations). The percentage of PMA was calculated as percentage of platelet-coated monocytes versus total $CD14^+$ monocytes.

### 2.5. Statistical analysis

Data analysis and graphing were performed using Prism 6.01 (GraphPad software Inc. La Jolla, CA, USA). The results are presented as median and interquartile range. Spearman's rank correlation analysis was used to study the correlation between the CD36 expression level and %PMA formation or TEG variables. The Mann–Whitney *U* test was applied to compare TEG variables among the groups with different platelet CD36 levels. A *p* value of < 0.05 was considered as statistically significant.

## 3. Results

A total of 40 participants who were regular apheresis platelet donors at the Taipei Blood Center were enrolled into this study. They were divided into three groups according to the platelet CD36 expression level: the normal expression group (platelet CD36 expression level in the upper three quartiles), the low expression group (platelet CD36 expression level in the lowest quartile), and the CD36-null group. No differences in sex, age, body mass index, platelet count, and cholesterol level were observed among the three groups. The low expression and CD36-null groups had higher rates of detectable CD36 mutations than the normal expression group (Table 1).

The TEG variables of the three groups are shown in Table 2. TEG normal ranges from other study were used for comparison [23]. The CD36-null group had a significantly prolonged TEG citrated kaolin R time or time to initial clot formation (17.75 min vs. 10.7 min, $p = 0.011$), prolonged K time (kinetics time), time to fixed level of clot strength (4.35 min vs. 2.9 min, $p = 0.003$), decreased α angle, rate of clot formation (38.85° vs. 50.6°, $p = 0.003$), TMA of clot formation

**Table 1**
Demographics of participants grouped according to the platelet CD36 expression level.

| | Normal CD36 expression group (N = 9) | Low CD36 expression group (N = 23) | CD36 null (N = 8) | *p* value |
|---|---|---|---|---|
| Male | 8 | 22 | 8 | 0.49 (low vs. normal)<br>1.00 (null vs. normal) |
| Age | 50 (39.5–54.5) | 46 (35–53) | 44.5 (35–52) | 0.780 |
| BMI[a] | 24.9 (22.75–27.35) | 25.6 (22.8–27.5) | 24.75 (23.5–26.8) | 0.896 |
| Platelet count | 216 (247–201) | 234 (212–283) | 240.5 (226.5–265) | 0.296 |
| cholesterol | 201 (184.5–218.5) | 188 (177–209) | 207 (177–216.3) | 0.435 |
| Cases with detectable CD36 mutation | 0 | 13 | 6 | 0.030 (low vs. normal)<br>0.002 (null vs. normal) |

The results are expressed as number, median, and interquartile range. $p < 0.05$ was considered statistically significant. Kruskal–Wallis test: age, MBI, platelet counts, and cholesterol level. Exact Fisher's test: gender and case number of detectable mutations.

[a] BMI: body mass index.

**Table 2**
Whole blood TEG data.

| | Reported reference range [23] | Normal CD36 expression group | Low CD36 expression group | *p* value (vs. normal) | CD36 null | *p* value (vs. normal) |
|---|---|---|---|---|---|---|
| R (min) | 3.8–9.8 | 10.7 (7.75–13.2) | 14.2 (11.68–16.85) | 0.003 | 17.75 (12.7–23.05) | 0.011 |
| K (min) | 0.7–3.4 | 2.9 (2.6–3.65) | 3.65 (2.9–4.825) | 0.045 | 4.35 (3.78–8.4) | 0.003 |
| Angle (degree) | 47.8–77.7 | 50.6 (45.8–55.05) | 45.1 (37.85–51.8) | 0.045 | 38.85 (30.55–44.33) | 0.003 |
| MA (mm) | 49.7–72.7 | 54.7 (54.25–58.05) | 54.9 (50.98–55.93) | 0.339 | 53.65 (50.53–54.8) | 0.088 |
| TMA (min) | NA[a] | 32.9 (28.85–38.4) | 37 (35.2–41.6) | 0.020 | 40.8 (38.25–57.83) | 0.011 |
| Ly30 (%) | −2.3-5.77 | 0.2 (−0.05–0.85) | 0.0 (0.0–1.0) | 0.825 | 0.0 (0.0–0.275) | 0.678 |
| CI | −5.1-3.6 | 0.4 (0.1–0.85) | −0.4 (−2.1–0.3) | 0.003 | −1.7 (−2.75–0.25) | 0.017 |

The results are shown as median and interquartile range. Mann–Whitney *U* test was used for the assessment of statistical significance, and $p < 0.05$ was considered as statistically significant.

[a] NA: not available.

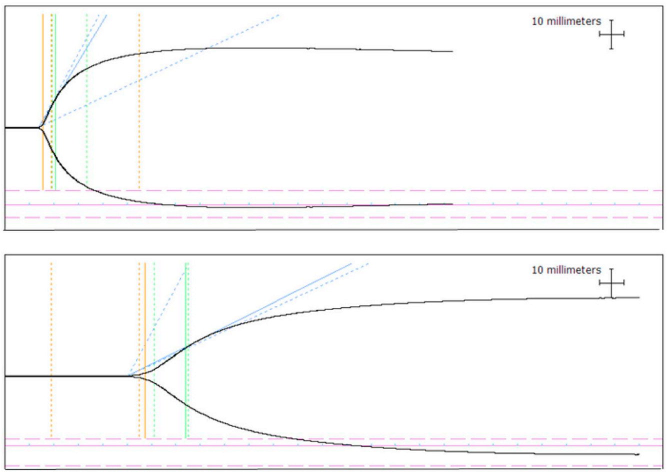


**Fig. 1.** Representative TEG tracing maps drawn based on the data of normal expression and CD36-null individuals. Upper panel: TEG tracing from a normal expression subject. Lower panel: TEG tracing from a CD36-null subject. CD36-null subject has prolonged R time and a small α angle, indicating slow clot formation. The final clot strengths are not different.

(40.8 min vs. 32.9 min), and CI (−1.7 vs. 0.4, $p$ = 0.017; which is a calculated index that measures total coagulation activation) compared with the normal expression group. A trend toward decreased MA (53.65 mm vs. 54.7 mm, $p$ = 0.088) was observed. No difference was observed in lysis function (Ly30) (0% vs. 0.2%, $p$ = 0.678) between the normal expression and CD36-null groups. TEG testing tracing maps drawn based on the data of representative individuals with platelet CD36-null and normal expression are shown in Fig. 1.

Statistically significant differences were observed in R time, K time, α angle, TMA, and CI between the low expression and normal expression groups (Table 2). We repeated TEG using apheresis platelets as testing samples. Platelet products are devoid of red cells and contain residual white blood cells, thus diminishing heterocellular interactions occurring in whole blood. In TEG using platelet products, no differences were observed in all TEG variables among the groups (Table 3).

To precisely characterize the relationship between platelet CD36 expression levels and TEG variables, we performed a series of linear regression analyses. Platelet CD36 levels were negatively correlated with R time, K time, and TMA and were positively correlated with the α angle. No correlations were noted between platelet CD36 levels and MA and lysis function (Ly30) (Table 4). According to data obtained from TEG tests using platelet products, no correlation was observed between platelet CD36 levels and all TEG varialbes (Table 5).

Given the significant relationship between platelet CD36 expression levels and impaired clot formation revealed by TEG, we hypothesized that platelet–leukocyte interaction may partially explain the hypocoagulopathy of CD36-null individuals demonstrated by TEG. The low platelet CD36 expression level entails low PMA and therefore results in a hypocoagulative state characterized by slow clot formation.

This explanation was supported by a subanalysis from our previous study. The study investigated the association of inflammatory markers and cellular markers with coronary disease assessed through coronary computed tomography angiography. A total of 356 healthy subjects (M/F: 243/113, age: 59 ± 9.8 years) who underwent coronary computed tomography angiography were enrolled for the correlative study between the platelet CD36 levels and PMA formation. In this cohort of apparently healthy subjects, platelet CD36 levels were positively correlated with PMA formation (r = 0.44, $p < 0.0001$). Monocyte CD36 levels were also positively correlated (although weaker correlation) with PMA formation (r = 0.24, $p < 0.0001$) (Fig. 2).

## 4. Discussion

In the current study, we demonstrated that the viscoelastic assay (TEG) could distinguish CD36-null subjects from CD36 normal expression subjects. CD36-null subjects had prolonged TEG R time and a smaller TEG α angle compared with normal subjects, with a minimal change in TEG MA (final fibrin strength).

Although TEG demonstrated that CD36-null individuals had a slow clot formation rate, their final fibrin clot strength (TEG MA) was nearly the same as that of CD36 normal expression individuals. This may explain why CD36-null individuals had no clinical bleeding phenotype under normal physiological conditions because clot strength is a major determinant in hemostasis, and the abundance of clotting factors can compensate for the moderate delay in thrombus formation. However, whether CD36-null individuals are more vulnerable to bleeding complications under conditions that decrease clotting factor levels, such as oral anticoagulant therapy or sepsis, than normal expression individuals is unclear and requires further study.

TEG has been widely used in the transfusion decision algorithm [16,17]. Most guidelines suggest that prolonged TEG R time may be interpreted as a low clotting factor level, and a small TEG α angle may be interpreted as an insufficient fibrinogen level. Transfusion with plasma components (such as fresh frozen plasma or cryoprecipitate) is recommended in both situations [17,18]. In the present study, whole blood TEG revealed prolonged TEG R time and reduced TEG α angle in CD36-null or low expression individuals, which may be misinterpreted and possibly lead to unnecessary transfusion. Additional studies should redefine the normal ranges of TEG variables, especially in Asian and African populations, in which CD36 deficiency and CD36 low expression are more prevalent. The TEG-based transfusion algorithm requires reassessment accordingly.

Previous studies on the role of CD36 have focused on pathophysiological conditions such as oxidative stress, hyperlipidemia, or increased cell-derived microparticles generated under vascular injury [9,24]. These prothrombotic effects are attributed to CD36 as receptors for oxidized LDL and apoptotic ligands. Although CD36 is among the major surface proteins expressed on platelets, whether CD36

**Table 3**
Platelet products TEG data.

| | Normal CD36 expression group | Low CD36 expression group | $p$ value (vs. normal) | CD36 null | $p$ value (vs. normal) |
|---|---|---|---|---|---|
| R (min) | 16.2 (12.8–17.7) | 16.4 (14.0–19.65) | 0.495 | 15.15 (11.53–19.45) | 0.832 |
| K (min) | 2.5 (1.85–3.05) | 2.35 (1.925–3.375) | 0.651 | 2.7 (2.25–3.95) | 0.382 |
| Angle (degree) | 59.3 (55.15–63.5) | 61.05 (49.08–64.38) | 0.910 | 54.85 (48.53–61.1) | 0.246 |
| MA (mm) | 69.5 (66.65–70.4) | 66.55 (65.08–67.3) | 0.159 | 66.9 (62.88–68.3) | 0.087 |
| TMA (min) | 34.4 (29.9–39.6) | 32.6 (28.65–41.08) | 0.791 | 32.5 (28.13–40.95) | 0.909 |
| Ly30(%) | 0.2 (−0.05–1.55) | 0.7 (0.775–3.275) | 0.436 | 0.65 (−0.175–5.350) | 0.725 |
| CI | 1.2 (0.9–1.75) | 32.6 (28.65–41.08) | 0.051 | 32.5 (28.13–40.95) | 0.791 |

The results are shown as median and interquartile range. Mann–Whitney $U$ test was used for the assessment of statistical significance, and $p < 0.05$ was considered as statistically significant.

**Table 4**
Linear regression of platelet CD36 levels vs. whole blood TEG variables.

| | Coefficient | 95% confidence interval | r | *p* value |
|---|---|---|---|---|
| R | −0.332 | −0.530 to −0.135 | −0.342 | 0.031 |
| K | −0.093 | −0.156 to −0.030 | −0.379 | 0.016 |
| Angle | 0.651 | 0.263–1.038 | 0.379 | 0.016 |
| MA | 0.663 | −0.130–0.254 | 0.064 | 0.693 |
| TMA | −0.624 | −0.992–0.255 | −0.403 | 0.010 |
| Ly30 | 0.110 | 0.008–0.211 | 0.040 | 0.804 |
| CI | 0.080 | 0.013–0.147 | 0.256 | 0.112 |

**Table 5**
Linear regression of platelet CD36 levels vs. platelet product–TEG variables.

| | Coefficient | 95% confidence interval | r | *p* value |
|---|---|---|---|---|
| R | −0.007 | −0.201–0.187 | 0.077 | 0.710 |
| K | −0.029 | −0.074–0.015 | −0.240 | 0.238 |
| Angle | 0.206 | −0.157–0.569 | 0.225 | 0.268 |
| MA | 0.170 | −0.002–0.342 | 0.383 | 0.059 |
| TMA | 0.066 | −0.323–0.456 | 0.140 | 0.505 |
| Ly30 | −0.056 | −0.224–0.118 | −0.083 | 0.693 |
| CI | 0.024 | −0.029–0.078 | 0.137 | 0.515 |

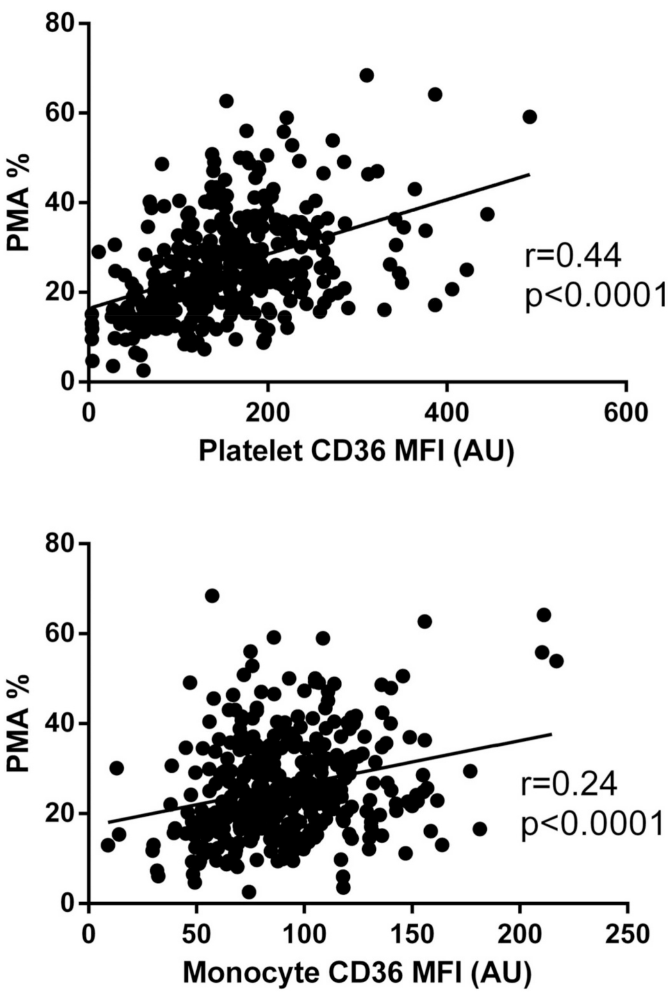


**Fig. 2.** Both platelet and monocyte CD36 expression levels are positively correlated with platelet–monocyte aggregate (PMA) formation. The study enrolled 356 healthy individuals with no apparent acute illness who underwent health examination.

participates in hemostasis is unclear. No bleeding diathesis under physiological conditions has been reported for CD36-null individuals [7]. Routine laboratory tests showed no impaired platelet function in individuals with CD36 deficiency [7].

Based on our study results, we propose a model explaining how CD36 contributes to normal hemostasis. Under the physiological state, CD36 facilitates platelets to form complexes with monocytes. Higher CD36 expression predisposes subjects to higher baseline PMA formation. The proximity of platelets and monocytes facilitates accelerated thrombus formation, which can be detected using cell-based viscoelastic tests. Because the final strength of the fibrin formed depends on clotting factors, fibrinogen levels, and the ability of platelet glycoprotein IIb/IIIa cross-linking with fibrin, the strength of the final clot (TEG MA) is CD36 independent. Our model suggests that the CD36 expression level is correlated with baseline PMA formation and thus may play prothrombotic and proinflammatory roles in normal hemostasis.

Thrombospondin can facilitate the formation of platelet–monocyte complexes by binding to CD36 on both cells [10]. CD36 also potentiates other ligand–receptor interactions involved in PMA formation. CD36 ligation can induce platelet activation and CD62 (P-selectin) expression on platelet surface; this may enhance PMA formation through interaction with CD162 (PSGL-1) on monocytes [25]. Previous reports have revealed that CD36 is spatially associated with glycoprotein IIb/IIIa, and ligation of CD36 leads to GP IIb/IIIa-dependent activation [26]. All these mechanisms may underlie CD36-augumented PMA and subsequent thrombus formation.

In the present study, we did not measure P-selectin expression on platelets. P-selectin is detectable after platelet activation and is shed into circulation or endocytosed for reuse; therefore, it is more relevant to the setting of acute reaction [27]. CD36 is constitutionally expressed on platelets and monocytes, and CD36-augumented PMA formation observed under physiological condition is of more clinical relevance in the setting of chronic diseases. We enrolled only two female participants in the TEG study because of the apheresis donor pool mainly comprised male donors. The CD36 genetic variants conferring CD36 deficiency found in Taiwan are different from those in other populations. For example, the most frequent CD36 variant (CD36 Pro90Ser) in the Japanese population and other well-studied single nucleotide polymorphisms in African Americans or Caucasians were lacking in our population [28,29]. Sex-specific aspects and genetic variants may have different effects on TEG variables, and further study is warranted to clarify these issues before generalizing our results.

In conclusion, TEG distinguishes CD36-null subjects from normal CD36 expression subjects as having a slower rate of fibrin formation, and TEG indicates that CD36-null subjects exhibit hypocoagulability. Reassessment of TEG-based diagnostic monitoring and transfusion algorithm is necessary for CD36 null subjects.

## Author contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

## Research funding

The study was supported by grants from the National Taiwan University Hospital (NTUH.100-S1611) and from Taiwan Blood Services Foundation (PM-98-TP-046).

## Employment or leadership

None declared.

## Honorarium

None declared.

## Competing interests

The funding organization(s) played no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

## References

[1] M. Febbraio, D.P. Hajjar, R.L. Silverstein, CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism, J. Clin. Invest. 108 (2001) 785–791.
[2] R.L. Silverstein, Inflammation, atherosclerosis, and arterial thrombosis: role of the scavenger receptor CD36, Cleve. Clin. J. Med. 76 (Suppl. 2) (2009) S27–S30.
[3] R.L. Silverstein, M. Febbraio, CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior, Sci. Signal. 2 (2009) re3.
[4] R.L. Silverstein, Platelet CD36 links not only alpha-granule-derived proteins to thrombus stability but also metabolic and oxidant stress to a prothrombotic phenotype, Arterioscler. Thromb. Vasc. Biol. 34 (2014) 1120–1121.
[5] R.L. Silverstein, W. Li, Y.M. Park, S.O. Rahaman, Mechanisms of cell signaling by the scavenger receptor CD36: implications in atherosclerosis and thrombosis, Trans. Am. Clin. Climatol. Assoc. 121 (2010) 206–220.
[6] B.R. Curtis, R.H. Aster, Incidence of the Nak(a)-negative platelet phenotype in African Americans is similar to that of Asians, Transfusion 36 (1996) 331–334.
[7] N. Yamamoto, N. Akamatsu, H. Yamazaki, K. Tanoue, Normal aggregations of glycoprotein IV (CD36)-deficient platelets from seven healthy Japanese donors, Br. J. Haematol. 81 (1992) 86–92.
[8] K. Lee, P. Gane, F. Roudot-Thoraval, B. Godeau, D. Bachir, F. Bernaudin, et al., The nonexpression of CD36 on reticulocytes and mature red blood cells does not modify the clinical course of patients with sickle cell anemia, Blood 98 (2001) 966–971.
[9] A. Ghosh, G. Murugesan, K. Chen, L. Zhang, Q. Wang, M. Febbraio, et al., Platelet CD36 surface expression levels affect functional responses to oxidized LDL and are associated with inheritance of specific genetic polymorphisms, Blood 117 (2011) 6355–6366.
[10] R.L. Silverstein, R.L. Nachman, Thrombospondin binds to monocytes-macrophages and mediates platelet-monocyte adhesion, J. Clin. Invest. 79 (1987) 867–874.
[11] S.O. Rahaman, D.J. Lennon, M. Febbraio, E.A. Podrez, S.L. Hazen, R.L. Silverstein, A CD36-dependent signaling cascade is necessary for macrophage foam cell formation, Cell Metab. 4 (2006) 211–221.
[12] L. Love-Gregory, R. Sherva, L. Sun, J. Wasson, T. Schappe, A. Doria, et al., Variants in the CD36 gene associate with the metabolic syndrome and high-density lipoprotein cholesterol, Hum. Mol. Genet. 17 (2008) 1695–1704.
[13] E.A. Podrez, T.V. Byzova, M. Febbraio, R.G. Salomon, Y. Ma, M. Valiyaveettil, et al., Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype, Nat. Med. 13 (2007) 1086–1095.
[14] E. Shantsila, G.Y. Lip, The role of monocytes in thrombotic disorders. Insights from tissue factor, monocyte-platelet aggregates and novel mechanisms, Thromb. Haemost. 102 (2009) 916–924.
[15] B.B. Nkambule, G. Davison, H. Ipp, Platelet leukocyte aggregates and markers of platelet aggregation, immune activation and disease progression in HIV infected treatment naive asymptomatic individuals, J. Thromb. Thrombolysis 40 (2015) 458–467.
[16] D. Whiting, J.A. DiNardo, TEG and ROTEM: technology and clinical applications, Am. J. Hematol. 89 (2014) 228–232.
[17] L.T. Da Luz, B. Nascimento, A.K. Shankarakutty, S. Rizoli, N.K. Adhikari, Effect of thromboelastography (TEG(R)) and rotational thromboelastometry (ROTEM(R)) on diagnosis of coagulopathy, transfusion guidance and mortality in trauma: descriptive systematic review, Crit. Care 18 (2014) 518.
[18] D. Bolliger, M.D. Seeberger, K.A. Tanaka, Principles and practice of thromboelastography in clinical coagulation management and transfusion practice, Transfus. Med. Rev. 26 (2012) 1–13.
[19] S.C. Lo, K.H. Lin, H.H. Hsieh, D.T. Lin, C.Y. Hu, Genetic variations of CD36 and low platelet CD36 expression - a risk factor for lipemic plasma donation in Taiwanese apheresis donors, Vox Sang. 110 (2016) 236–243.
[20] S.C. Lo, W.J. Lee, C.Y. Chen, B.C. Lee, Intermediate CD14(++)CD16(+) monocyte predicts severe coronary stenosis and extensive plaque involvement in asymptomatic individuals, Int J Cardiovasc Imaging 33 (2017) 1223–1236.
[21] B.C. Lee, W.J. Lee, S.C. Lo, H.C. Hsu, K.L. Chien, Y.C. Chang, et al., The ratio of epicardial to body fat improves the prediction of coronary artery disease beyond calcium and Framingham risk scores, Int J Cardiovasc Imaging 32 (Suppl. 1) (2016) 117–127.
[22] I.J. Bontekoe, P.F. van der Meer, D. de Korte, Determination of thromboelastographic responsiveness in stored single-donor platelet concentrates, Transfusion 54 (2014) 1610–1618.
[23] S. Scarpelini, S.G. Rhind, B. Nascimento, H. Tien, P.N. Shek, H.T. Peng, et al., Normal range values for thromboelastography in healthy adult volunteers, Braz. J. Med. Biol. Res. 42 (2009) 1210–1217.
[24] S. Badrnya, W.C. Schrottmaier, J.B. Kral, K.C. Yaiw, I. Volf, G. Schabbauer, et al., Platelets mediate oxidized low-density lipoprotein-induced monocyte extravasation and foam cell formation, Arterioscler. Thromb. Vasc. Biol. 34 (2014) 571–580.
[25] C. Ouk, C. Jayat-Vignoles, M. Donnard, J. Feuillard, Both CD62 and CD162 antibodies prevent formation of CD36-dependent platelets, rosettes, and artefactual pseudoexpression of platelet markers on white blood cells: a study with ImageStream(R), Cytometry A 79 (2011) 477–484.
[26] D.J. Dorahy, M.C. Berndt, D.R. Shafren, G.F. Burns, CD36 is spatially associated with glycoprotein IIb-IIIa (alpha IIb beta 3) on the surface of resting platelets, Biochem. Biophys. Res. Commun. 218 (1996) 575–581.
[27] P. Andre, P-selectin in haemostasis, Br. J. Haematol. 126 (2004) 298–306.
[28] Y. Masuda, S. Tamura, K. Matsuno, A. Nagasawa, K. Hayasaka, C. Shimizu, et al., Diverse CD36 expression among Japanese population: defective CD36 mutations cause platelet and monocyte CD36 reductions in not only deficient but also normal phenotype subjects, Thromb. Res. 135 (2015) 951–957.
[29] L. Love-Gregory, R. Sherva, T. Schappe, J.S. Qi, J. McCrea, S. Klein, et al., Common CD36 SNPs reduce protein expression and may contribute to a protective atherogenic profile, Hum. Mol. Genet. 20 (2011) 193–201.