Endothelial Activation and Immune Thrombocytopenia: An Engagement Waiting for Consolidation

Alaa Efat1, Sabry Shoeib1, Aida Nazir2, Essam Abdelmohsen3, Ashraf Dawod4, Hanan M. Bedair5, and Walaa Elgheriany1

Abstract
Immune thrombocytopenia (ITP) appears to be a heterogeneous disease. In some patients, autoimmunity may be associated with an inflammatory process, and in other patients, low platelets may interfere with other aspects of the coagulation system. Either may predispose to thrombosis or bleeding. Further investigation of the interactions of platelets, with inflammatory cytokines and endothelial biomarkers, may help us to better understand the disease, and to recognize those patients at risk of bleeding, or conversely thrombosis. The aim of this work is to estimate von Willebrand factor (vWF) and vascular cellular adhesion molecule (V-CAM) serum levels in adult immune thrombocytopenic patients (ITP) and to decipher their possible clinical correlates. Eighty adults (≥ 18 years) were enrolled in the study; naive newly diagnosed 40 patients with primary ITP (according to the ASH 2019) and 40 sex and age-matched healthy controls, all groups are subjected for complete blood count (CBC), liver, and renal function tests, ESR, CRP, V-CAM, and VWF-Ag by enzyme-linked immunosorbent assay (ELISA). There was a highly statistically significant difference between case and control as regards to the mean level of VWF-Ag and V-CAM. vWF and V-CAM could serve as biomarkers for endothelial alterations and should be investigated as a predictor of thrombocytopenic bleeding and tailor patient management accordingly.

Keywords
immune thrombocytopenia, von Willebrand factor, V-CAM

Date received: 25 July 2021; revised: 29 September 2021; accepted: 2 October 2021.

Introduction
Besides their role in hemostasis, platelets are considered also a key player for preserving endothelial functions and integrity. Platelets continuously support the barrier function of the resting endothelium, and after inflammation by infiltrating leukocytes; they prevent or heal vascular injuries. These vascular protective actions of platelets are apart from their ability to stop bleeding.1

Constitutive release of proangiogenic cytokines and growth factors from platelets maintains vascular integrity. These molecules bind to specific receptors on the surface of endothelial cells (ECs). When platelets numbers decrease dramatically, molecular disassembly of adjacent intercellular endothelial junctions occurs which leads to vascular fragility and liability to bleed.2

In addition, platelets have multiple regulatory functions on endothelial progenitor cell development as well.3

Many patients with immune thrombocytopenia (ITP) have a bleeding time that is disproportionately short for the degree of thrombocytopenia.4

It had been established that the vascular endothelium, rather than being a mere wall between intravascular and subendothelial compartments, is a widely spread organ responsible for many functions like regulation of hemodynamics, angiogenic vascular remodeling, metabolic, synthetic, anti-inflammatory, and anti-thrombogenic processes.5

Endothelial activation is based on its pivotal role in many diseases like coronary artery disease, hypertension,
cerebrovascular disease, and diabetes. And novel therapies aimed at alleviating the morbidity and mortality from these conditions. It may be possible to apply knowledge gained by studying one disease to another disease process.6

Endothelial cell dysfunction (ECD) is a syndrome induced by diverse intrinsic and extrinsic factors that lead to disturbances in the barrier function of the vascular endothelium; in addition to its impaired anti-thrombogenic properties; angiogenic competence; proliferative capacity, disordered regulation of vascular smooth muscle tonicity, and migratory properties; and perturbed synthetic functions and impedance of neutrophils and monocytes from diapedesis.7

Thrombocytopenia per se is associated with ECD, as evidenced by spontaneous leakage of blood at the microvascular. Platelet antibodies may cross-react with EC antigens and cause further endothelial damage, which may increase bleeding.8

Vascular cellular adhesion molecule-1 (VCAM-1) belongs to the immunoglobulin supergene family and is a ligand to the very late antigen-4, a β-integrin found on the surface of mononuclear cells. VCAM-1 expression is restricted to ECs and occasional fusiform cells. VCAM-1 is not expressed on healthy ECs. It has been suggested that VCAM-1 expression may result in endothelial activation.9

The role and level of vWF, which is exclusively synthesized and secreted by the ECs, at least in the liver, is not limited to the ECS of the hepatic sinusoid, so many factors affect its level and its role in bleeding and hemostasis is linked to cellular factors and coagulation proteins.10

In an ITP model, thrombocytopenia had led to an alteration in endothelial function, and that the von Willebrand factor (vWF) had served as a marker of EC injury.11

Aim of the Study

The aim is to estimate vWF and vascular cellular adhesion molecule (V-CAM) serum levels (biomarkers of endothelial activation) in adult immune thrombocytopenic patients (ITP) and to decipher their possible clinical correlates.

Subjects and Methods

Patients

Eighty adults (≥18 years) were enrolled in this Case–Control study; 40 treatment naïve patients with primary ITP (according to the ASH 2019 clinical practice guidelines for ITP criteria) and 40 sex and age-matched healthy controls.

Patients were sequentially selected from the hematology unit at the Menoufia University Hospitals in the period from December 2019 to December 2020.

All participants were volunteers, and all of them signed written informed consent with explaining the aim of the study before the study initiation.

Approval of the study protocol was obtained by the local Ethical Scientific Committee of Menoufia university’s institutional review board under number (MNF119/2019).

Patients with a family history of platelets or coagulation defects, pregnant females, acute or chronic inflammatory disorders as well as patients with non-ITP or secondary ITP (eg HCV, autoimmune disease, lymphoproliferative diseases, and thyroid disease, etc.) were excluded.

All participants were subjected to detailed medical history, complete physical examination, and abdominal ultrasound and laboratory investigations including:

1. Complete blood count (CBC) and blood film.
2. Kidney and liver function tests, hepatitis C virus antibodies (HCV-Abs), HBsAg, HBeAb, HIV antibodies, coagulation profiles, antinuclear antibody (ANA), thyroid-stimulating hormone (TSH), H. pylori Ag in stool, CRP, ESR, fasting blood sugar (FBS), HA1C, and finally bone marrow examination (if atypical blood film).
3. Endothelial activation biomarkers (VCAM and vWF) plasma levels using commercially available enzyme-linked immunosorbent assay (ELISA) kits.

Sampling

Ten mL of blood sample were taken by sterile venipuncture, after minimal venous stasis using sterile disposable syringes. The blood samples were distributed as follows:

Four mL of blood were delivered to a vacutainer plain test tube, and then the serum was separated by centrifugation at 3000 rpm for 10 min and half of the separated serum was used for the analysis of liver function tests, renal function tests, TSH, CRP, viral hepatitis markers ANA; the other half was separated, aliquoted, and stored at −80°C for the measurement of VCAM and VWF antigen.

Two mL of blood were delivered to a vacutainer plastic tube containing ethylenediaminetetraacetic acid (EDTA) and used for CBC with film.

Two mL of blood were delivered to a vacutainer plastic tube containing sodium citrate (4:1) (3.8%) for ESR measurement by Westergren method.

Two mL of blood were delivered to a vacutainer plastic tube containing sodium citrate (9:1) (3.2%) for coagulation profile.

Methods

1. CBC using Sysmex XT-1800i automated hematology analyzer (Sysmex Corporation), and then BM aspirate was done if needed.
2. Liver function tests (eg total bilirubin, direct bilirubin, albumin, total protein, AST, ALT), renal function tests, and CRP were analyzed using Cobas 6000 analyzer (c501 module) (Roche).
3. Serum HCV-Abs, HbsAg, and HIV Ab were measured using Cobas e601 auto analyzer (Roche).
4. Prothrombin time and activated partial thromboplastin time were done by Sysmex CS-1600 automated hemostasis testing (Sysmex Corporation).
5. ANA was done by Algeria automated analyzer.
6. Serum levels of endothelial activation biomarkers (VCAM and VWF-Ag) was done by ELISA that is designed to measure the amount of the target that bounded between a matched antibody pair. A target-specific antibody has been pre-coated in the supplied microplate wells. Standards, samples, or controls are then added into these wells and is bind to the immobilized (capture) antibody. The sandwich is then formed by the addition of the second (detector) antibody, and finally, a substrate solution is added that will react with the Enzyme–Antibody–Target complex to produce a measurable signal. The intensity of this signal is directly proportional to the concentration of the present target in the original specimen.
7. VCAM-1 was measured using the human (VCAM-1) ELISA kit (Cusabio) with a detection range from 1.563 ng/mL to 100 ng/mL that measures VWF in less than 5 h according to the manufacturer’s instructions.
8. VWF-Ag measurement: using VWF Human ELISA Kit Catalog # EHVWF (Invitrogen, Thermo Fisher Scientific) with assay range of 0.123 to 30 ng/mL.

Statistical Analysis

Data were collected, tabulated, and statistically analyzed using an IBM personal computer with Statistical Package of Social Science (SPSS) version 22 (SPSS, Inc.).

Descriptive statistics: in which quantitative data were presented in the form of mean, standard deviation (SD), range, and qualitative data were presented in the form of numbers and percentages. Analytical statistics is used to find out the possible association between studied factors and the targeted disease. The used tests of significance included Chi-square test ($\chi^2$), Fischer exact test, and Student’s $t$-test.

Results

Table 1 shows all characteristics of the studied ITP cases ($N = 40$) like demographic distribution and basic laboratory findings and the coagulation profile and bleeding severity in addition to serum von Willebrand and VCAM. Mean age among cases was equal to 46.2 ± 12.7. There were 16 (40%) males and 24 (60%) females among cases.

As regards to bleeding severity (World Health Organization [WHO] bleeding scale); 27 cases (67.5%) suffered from grade 1 bleeding, 10 (25%) suffered from grade 2 bleeding, and 3 (7.5%) suffered from grade 0 bleeding (no bleeding).

Platelets range was 5 to 90 (10^3/mm3) and mean ± SD was 50.3 ± 25.1, hemoglobin (Hb) range was 7.7 to 14 g/dL and its mean was 10.3 ± 1.5 g/dL, white blood cells (WBCs) range was 3.5 to 10 (10^3/mm3) and mean ± SD was 5.7 ± 1.9 (10^3/mm3).

As regards to CRP, 62.5% of the studied newly diagnosed ITP patients had positive CRP. The mean ± SD level of CRP was (10.96 ± 5.4). The mean level of prothrombin concentration (PC) was (85.3% ± 12.6%). The mean level of INR was (1.04 ± 0.06). The mean level of APTT was (30.7 ± 2.2).

The mean level of von Willebrand was (18.1 ± 5.4) in the studied patients and (13.8 ± 2.3) in the control group. The mean level of VCAM was (125.1 ± 42.2).

Comparison between the studied groups regarding age, sex, serum von Willebrand, and VCAM ($N = 80$) results is shown in Table 1.

Table 1. Baseline Characteristics of Studied Cases ($N = 40$).

| Studied Variables | Case ($N = 40$) | Studied Variables | Case ($N = 40$) |
|-------------------|----------------|-------------------|----------------|
| **Age**           |                | **CRP**           |                |
| Mean ± SD         | 46.2 ± 12.7    | Mean ± SD         | 10.96 ± 5.4    |
| Range (years old) | 18 to 60       | Range             | 4 to 21        |
| Sex               |                | ESR               | 30.5 ± 19.7    |
| Female (N, %)     | 24 (60%)       | Mean ± SD         | 5 to 80        |
| Male (N, %)       | 16 (40%)       | Range             | 57 to 100      |
|WHO bleeding scale |                | Prothrombin concen (PC) (%) | 85.3 ± 12.6 |
| ▪ Bleeding grade 0 (N, %) | 3 (7.5%) | Mean ± SD         |               |
| ▪ Bleeding grade 1 (N, %) | 27 (67.5%) | Range             |               |
| ▪ Bleeding grade 2 (N, %) | 10 (25%) | APTT (sec)        |               |
| Hb(g/dL)          |                | Mean ± SD         | 30.7 ± 2.2     |
| Mean ± SD         | 10.3 ± 1.5     | Range             | 26 to 35       |
| Range             | 7 to 14        |                   |               |
| WBCs (10^3/mm^3)  |                |                   |               |
| Mean ± SD         | 5.7 ± 1.9      |                   |               |
| Range             | 3 to 10        |                   |               |
| Platelet count (10^3/mm^3) | 50.3 ± 25.1 |                   |               |
| Mean ± SD         | 5 to 90        |                   |               |

Abbreviations: Hb, hemoglobin; N, number; WBCs, white blood cells; WHO, World Health Organization; %, percentage.
male patients and 5 (55.6%) female patients. Patients with platelet count less than 20,000 had 4 (44.4%) cases but among controls, there were 14 (35%) males and 26 (65%) females. In addition, there is a highly statistically significant difference between the two groups of platelet counts as regards to mean of Hb and WBCs. Mean hemoglobin concentration in group of patients with platelet count less than 20,000 was 10.2 ± 1.5 g/dL and WBCs were 4.9 ± 0.385

There was no statistically significant difference between the two groups of platelet counts as regards to mean of Hb and WBCs. Mean hemoglobin concentration in group of patients with platelet count less than 20,000 was 10.2 ± 1.5 g/dL and WBCs were 4.9 ± 0.385

Table 2. Comparison Between the Studied Groups Regarding Demographic (Sex, Age), Serum Von Willebrand, and VCAM (N = 80).

| Studied Variables | Case (N = 40) | Control (N = 40) | Test of Significance | P value |
|-------------------|--------------|-----------------|---------------------|--------|
| Sex               |              |                 |                     |        |
| Female (N, %)     | 24 (60%)     | 26 (65%)        | χ²                  | .64    |
| Male (N, %)       | 16 (40%)     | 14 (35%)        |                     |        |
| Age               |              |                 |                     |        |
| Mean ± SD         | 46.2 ± 12.7  | 42.8 ± 8.2      | 1.4*                | .17    |
| Range (years old) | 18 to 60     | 28 to 61        |                     |        |
| Von Willbrand (ng/mL) |         |                 |                     |        |
| Mean ± SD         | 18.1 ± 5.4   | 13.8 ± 2.3      | 4.5*                | <.001  |
| Range             | 12.21 to 36.054 | 13.06 to 21.503 |                    |        |
| VCAM (ng/mL)      | 125.1 ± 42.2 | 102.5 ± 53.3    | 2.099*              |        |
| Mean ± SD         | 42.351 to 217.23 | 13.77 to 191.3  |                     |        |

*Student’s t-test, χ², chi-square test. P-value: HS, highly significant (P-value ≤ .001); NS, non-significant (P-value > .05); S, significant (P-value ≤ .05). Abbreviation: VCAM, vascular cellular adhesion molecule.

Table 3. Baseline Characteristics of Cases According to Severity of Thrombocytopenia (N = 40).

| Studied Variables | Platelet count |
|-------------------|----------------|
|                   | <20,000 (N = 29) | 20,000 (N = 11) | Test of Significance | P value |
| Age/years         |               |                |                     |        |
| • Mean ± SD       | 43.7 ± 13.95  | 46.9 ± 12.5    | 0.133*              | .9     |
| Sex               |               |                |                     |        |
| • Male (N, %)     | 12 (41.4%)    | 4 (36.4%)      | FE                  | .08    |
| • Female (N, %)   | 17 (58.6%)    | 7 (63.6%)      |                     | .77    |
| WHO bleeding scale|               |                |                     |        |
| • Bleeding grade 0 (N, %) | 19 (65.5%)    | 11 (100%)      | 2.58                | .02    |
| • Bleeding grade 1 (N, %) | 10 (34.5%)    | 0              |                     |        |
| Hb/g(dL)          | 10.2 ± 1.5    | 10.4 ± 1.5     | 0.0764*             | .94    |
| WBCs (10⁹/mm³)    | 4.9 ± 1.4     | 5.9 ± 2.04     | 0.385*              | .7     |
| • Mean ± SD       |               |                |                     |        |

*Student’s t-test, χ², chi-square test. P-value: NS = non-significant (P-value > .05), S = significant (P-value ≤ .05). HS = highly significant (P value ≤ .001). Abbreviations: Hb, hemoglobin; WBCs, white blood cells; WHO, World Health Organization.
Table 4. Correlation Coefficient (r) Between von Willebrand and Laboratory Parameters of the Studied Patients (N = 40).

| Parameter                  | Von Willebrand (ng/mL) | r*  | P value |
|----------------------------|------------------------|-----|---------|
| VCAM (ng/mL)               | 0.128                  | .432|         |
| Hb (g/dL)                  | 0.228                  | .156|         |
| WBCs (10^3/mm^3)           | 0.131                  | .42 |         |
| PLTs (10^3/mm^3)           | 0.29                   | .049|         |
| CRP                        | 0.063                  | .764|         |
| ESR                        | 0.044                  | .79 |         |
| INR                        | 0.178                  | .273|         |
| APTT (sec)                 | 0.26                   | .115|         |
| Bleeding score             | 0.136                  | .409|         |

* Spearman’s correlation coefficient.

Abbreviations: Hb, hemoglobin; VCAM, vascular cellular adhesion molecule; WBCs, white blood cells.

Table 5 shows that there was no statistically significant correlation between VCAM and laboratory parameters of the studied patients (P value > .05). The correlation coefficient (r*) of vWF was 0.128, of Hb, was −0.228, of WBCs was −0.131, of platelets was −0.017, of C-reactive protein CRP was 0.063, of ESR was −0.044, of international normalized ratio INR was 0.178, of APTT was −0.26, and of bleeding score was 0.136 (Table 4).

Discussion

Severe thrombocytopenia can result in fatal bleeding. Surprisingly, there is great variability in bleeding manifestations in thrombocytopenic patients, suggesting that factors other than platelet count determine the phenotype. While platelets play a pivotal role in the formation of hemostatic clots at the sites of vascular injury, platelet destruction and/or decreased platelet production. In addition to risks of bleeding, an increased risk of thrombosis has been identified, despite low platelet counts. The reasons behind this remain unclear.

Godfrey and coworkers in 2012 observed elevated levels of VWF and decreased levels of ADAMTS-13 in patients with myocardial infarction and ischemic stroke. Given the interaction of these plasma proteins with platelets, they hypothesized that changes in VWF/ADAMTS-13 levels may contribute to the thrombotic risk in patients with ITP.

In the same study, they investigated whether inflammatory mediators associated with the acute disease could play a role. CRP levels were elevated in many patients with ITP, but this did not correlate with VWF levels.

Garabeta and colleagues in 2020 measured markers of EC activation including intercellular adhesion molecule-1 (ICAM-1), VCAM-1, and thrombomodulin in 21 ITP patients, and E-selectin...
in 18 ITP patients. Higher levels of ICAM-1, thrombomodulin, and H3Cit-DNA were found in ITP patients compared with controls. No differences were found for VCAM-1, E-selectin, or cDNA. This study showed that ITP patients have increased EC activation and NET formation, both of which may contribute to the intrinsic hypercoagulable state of ITP.16

Using the correlation analysis and the linear regression, there was no statistically significant correlation between von Willebrand and VCAM with the laboratory parameters of the studied patients.

In the current study, we didn’t find any statistically significant difference between studied cases and controls as regards to age, sex, and routine laboratory data. ITP patients showed female predominance with a peak in reproductive years. We aimed to examine the near percentage of age, sex, and clinical data in both the studied cases and controls to decrease any differences that could affect the result.

In agreement with Tzeon-Jye et al.18,17 the prevalence of ITP with a higher incidence in women (60%) than men (40%).

Bleeding events are often unpredictable, and patients with ITP, even in the setting of severe thrombocytopenia, may not exhibit bleeding beyond bruising and petechiae. However, more serious mucosal bleeding may occur, including menorrhagia, epistaxis, gastrointestinal hemorrhage, hematuria, or, rarely, intracranial hemorrhage (ICH). There is a study demonstrated that ICH has been reported in 1.4% of adults and 0.1% to 0.4% of children with ITP. Severe bleeding is reported in 9.5% of adults and 20.2% of children. Adults with ITP have a 1.3- to 2.2-fold higher mortality than the general population due to cardiovascular disease, infection, and bleeding.18

In our study, some studied cases suffered from low Hb which is mostly due to bleeding and low platelets, but our controls have no bleeding.

Muenchrath et al.18,19 reported that patients had anemia due to bleeding secondary to profound thrombocytopenia. This was demonstrated also in Trotter and Hill18,20 study where they identified significant morbidity in patients with ITP, including fatigue, anemia, fear of bleeding which critically affect patients’ health-related quality of life (HRQoL).

The study of Muenchrath et al. in 201818,19 concluded that patients had anemia attributed to sustained slow bleeding secondary to profound thrombocytopenia.

In this study, there was no statistically significant difference between studied patients’ platelet counts as regards to age and sex which was demonstrated also in the study of Bonaccio et al.21

There was no statistically significant difference between studied patients’ platelet counts as regards to Hb and WBCs.

There was a statistically significant difference between studied patients’ platelet count as regards to WHO bleeding scale. This is in agreement with Di Micco and Monreal,22 Hassan and colleagues23 who confirmed that patients with a very low platelet count had a higher rate of major bleeding, 1.2% of these patients had<80,000 platelet/µL and revealed that these patients had increased risk for major bleeding and fatal bleeding than those with normal platelet count.

Slichter in 200424 also demonstrated that in clinically stable patients, major bleeding is unusual unless the platelet count is < 5 x 10(3)/µL. Risk factors for bleeding at higher platelet counts are disseminated intravascular coagulation with contributory clotting factor deficiencies, structural lesions with loss of vascular integrity, and refractoriness to platelet transfusions.

In agreement with Santimone and coworkers in 2011,25 in our study, there was a highly statistically significant difference between case and control as regards to CRP and mean level of ESR. Also in agreement with Kapur and colleagues' study26 in which they revealed a significant and previously unknown association between the CRP and the antiplatelet antibodies in fetal or neonatal alloimmune thrombocytopenia (FNAIT) and ITP.

There is a highly statistically significant difference between case and control as regards to mean level of PC and INR.

In 2017, Elkhalifa and colleagues' study was conducted to evaluate and compare coagulation tests and platelet counts among type 2 diabetes mellitus T2DM versus healthy controls. The mean PT and APTT in T2DM patients showed a statistically significant reduction when compared with healthy individuals.27

This is the first study on the relation between endothelial activation biomarkers (VCAM-1 and vWF) in naive adult ITP patients.

Our study revealed that there was a highly statistically significant difference between Case and Control as regards to mean level of von-Willebrand and a statistically significant difference between Case and Control as regards to mean level of VCAM.

A very interesting study by LeVine et al. in 2019 suggested that there is an association between thrombocytopenia and ultrastructural endothelial abnormalities in a canine model of ITP. The significant decrease in pinocytotic vesicles in thrombocytopenic endothelium shows that endothelial damage occurs in this model and the variability in the degree of alterations of vascular ultrastructure may account for differences in bleeding phenotype in ITP. Plasma vWF could serve as a biomarker for endothelial alterations and should be investigated as a predictor of thrombocytopenic bleeding.8

Endothelial dysfunction has been increasingly recognized in patients with SLE over the past two decades or so. In a Case–Control study by Mak and colleagues in 201728 of SLE patients naïve of cardiovascular disease, worse endothelial function.

In a study by Cieslik and Hrycek in 2015, pentraxin 3 (PTX3) levels, which is produced by ECs in response to various inflammatory events, and other indicators of endothelial dysfunction (like the soluble form of E-selectin [sE-selectin], VCAM-1 [sVCAM-1], monocyte chemotactic protein-1 [MCP-1], and vWF) were estimated in plasma and serum of 56 women with SLE. These patients had high concentrations of PTX3, vWF, MCP-1, sE-selectin, and sVCAM-1 compared to healthy controls. The expression levels of it were concluded that their concentration may be an indicator of endothelial activation or dysfunction in SLE patients.29
Limitations

The small number of the studied immune thrombocytopenia ITP patients in our work, as from the inclusion criteria we selected newly diagnosed naïve patients only which were limited by that duration.

Detection of vWF and V-CAM only once at diagnosis and it was not repeated after a while of follow-up or after treatment to take a real-time follow-up of it, so we recommend to do this sequential follow up serum level detection to them in future studies.

Conclusion

This pilot observational study depicted the possible association between endothelial activation and ITP, a relationship which needs to be challenged by further large-scale studies with a larger No. of patients with persistent and chronic ITP in addition to comparing it with the newly diagnosed ITP patients. We had taken only limited prototypes of markers, but we recommend other studies in more depth for assessing coagulation proteins, fibrin, inflammatory proteins, and albumin roles on endothelial activation.

Author’s Contributions

Alaa Efat and Walaa Elgheriany wrote the manuscript and analyzed the data. Aida Nazir and Essam Abdelmohsen performed data collection and manuscript preparation. Hanan Bedair and Ashraf Dawood performed the laboratory study. Sabry Shoeb was responsible for the selection and follow-up of patients. All the authors revised the study and reviewed the article.

Data Availability

Data are available upon request by contacting the corresponding author (Dr Alaa Efat).

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Alaa Efat https://orcid.org/0000-0001-8148-3443

References

1. Ho-Tin-Noé B, Demers M, Wagner DD. How platelets safeguard vascular integrity. J Thromb Haemost.2011;9(Suppl 1): 56-65.
2. Nachman RL, Rafii S. Platelets, petechiae, and preservation of the vascular wall. N Engl J Med. 2008;359(12):1261-1270.
3. Baimukanova G, Miyazawa B, Potter DR, et al. Platelets regulate vascular endothelial stability: assessing the storage lesion and donor variability of apheresis platelets. Transfusion 2016;56(Suppl 1): S65-S75.
4. Giltay JC, Brinkman HJM, Viekke A, Kiefel V, van Mourik JA, von dem Borne AE. The platelet glycoprotein IIdia associated Br-alloantigen system is expressed by cultured endothelial cells. Br J Haematol 1990;75(4):557-60.
5. Goligorsky MS. Endothelial cell dysfunction: can’t live with it, how to live without it. Am J Physiol Renal Physiol 2013;288: F871-F880.
6. Chirayath HH. An overview of endothelial dysfunction in diabetes. Vascul Dis Ther.2016;1(1): 1-3.
7. Vita J, Keaney J. Endothelial function: a barometer for cardiovascular risk. Circulation 2002;106(6):640-2.
8. LeVine DN, Cianciolo RE, Linder KE, et al. Endothelial alterations in a canine model of immune thrombocytopenia. Platelets 2019;309(1): 88-97.
9. David A., Oliver D. How does endothelial cell injury start? The role of endothelin in systemic sclerosis. Arthritis Res Ther 2007;9(Suppl 2): S2.
10. Ramadori G. Albumin infusion in critically Ill COVID-19 patients: hemodilution and anticoagulation. Int J Mol Sci 2021;22(13):7126.
11. Hoffman G, Calabrese L. Vasculitis: determinants of disease patterns,” nature reviews. Rheumatol 2014, 10; 8: 454-462.
12. Watson R, Buck J, Levin L, et al., Endothelial CD99 signals through soluble adenylyl cyclase and PKA to regulate leukocyte transendothelial migration. J Exp Med 2015;212 (7):1021-1041.
13. McFadyen J, Peter K. Chapter 11 platelets in the pathogenesis of vascular disease and their role as a therapeutic target. Atherothrombosis and vascular biology, baker heart and diabetes institute, melbourne, VIC; Australia 2020.
14. Sadler J. New concepts in von willebrand disease. Annu Rev Med 2005, 56(1):173-191.
15. Godfrey C, Terrinoni I, Laffan M, Crawley J, Cooper N. Elevated plasma Von willebrand factor and decreased ADAMTS13 antigen levels in patients with immune thrombocytopenia (ITP). Blood 2012;120 (21): 1096.
16. Garabeta L, Henrikssonc C, Lozanoe M, et al. Markers of endothelial cell activation and neutrophil extracellular traps are trapped in immune thrombocytopenia but are not enhanced by thrombopoietin receptor agonists. Thromb Res 2020;185: 119-124.
17. Tzeon-Jye Chiou Liang-Tsai, Hsiao Wang-Fang Tzeng. “The epidemiology of immune thrombocytopenia in Taiwan: a retrospective analysis of data from the national health database”. American Research Journal of Hematology 2018;3(1): 1-15.
18. Neunert C, Terrell D, Arnold D, et al. American Society of hematology 2019 guidelines for immune thrombocytopenia. blood Adv, 2019, 3(23): 3829-3866.
19. Muenchrath M, Aribindi K, Farnie M, DaSilva-DeAbreu A. Acute myocardial infarction and renal dysfunction due to chronic extreme anemia (hemoglobin 2.5 g/dL) in immune thrombocytopenia. Proc (Bayl Univ Med Cent) 2018;31(4): 508-510.
20. Trotter P, Hill A. Immune thrombocytopenia: improving quality of life and patient outcomes. Patient Relat Outcome Meas, 2018, 9: 369-384.
21. Bonaccio M, Castelnuovo A, Costanzo S, et al. Age-sex–specific ranges of platelet count and all-cause mortality: prospective
findings from the MOLI-SANI study. *Blood*, 2016;127(12):1614-1616.

22. Di Micco P, Monreal M. Platelet count and bleeding in patients receiving anticoagulant therapy for venous thromboembolism: lesson from the RIETE registry. *J Blood Med* 2019;10: 453-456.

23. Hassan AE, Shoeib S, Abdelmohsen E, et al. Toll-Like receptor 9 (TLR9) gene C/T (rs352140) polymorphisms in adult primary immune thrombocytopenia. *Clin Appl Thromb Hemost*. 2020;26:1-7.

24. Slichter S. Relationship between platelet count and bleeding risk in thrombocytopenic patients. *Transfus Med Rev* 2004;18(3):153-167.

25. Santimone L, Di Castelnuovo A, De Curtis A, et al. White blood cell count, sex and age are major determinants of heterogeneity of platelet indices in an adult general population: results from the MOLI-SANI project. *Haematologica*. 2011;96(8):1180-1188.

26. Kapur R, Heitink-Pollé KM, Porcelijn L, et al. C-reactive protein enhances IgG-mediated phagocyte responses and thrombocytopenia. *Blood*. 2015;125(11):1793-1802.

27. Elkhalifa A, Abozer Y, Yassin N, et al. Estimation of coagulation profile and platelet counts in type 2 diabetic patients original article estimation of coagulation profile and platelet counts in type 2 diabetic patients. *Aljouf University Medical Journal (AUMJ)*, 2017; 1;4[4]: 27-31.

28. Mak A, Kow N, Schwarz H, Gong L, Tay S, Ling L. Endothelial dysfunction in systemic lupus erythematosus – a case-control study and an updated meta-analysis and meta-regression. *Sci Rep* 2017(1);7: 7320.

29. Cieslik P, Hrycek A. Pentraxin 3 as a biomarker of local inflammatory response to vascular injury in systemic lupus erythematosus. *Autoimmunity* 2015;48; 4: 242-250.