Original Article

DOI: 10.7860/JCDR/2025/79987.22166

Insights from Histogram and Flag Analysis in Pseudothrombocytopenia Cases: A Single-centre Cross-sectional Study

Pathology Section

RAJESWARI THIVYA DHANABALAN[1], AARTHI KANNAN[2]

ABSTRACT

Introduction: Pseudothrombocytopenia (PTCP), also known as Spurious Thrombocytopenia (TCP), is a common problem encountered in many laboratories. When stable patients are evaluated for thrombocytopenia, the first step is to exclude PTCP. Most haematology analysers are equipped with software-generated flagging algorithms that identify abnormal platelet distributions, indicating the presence of giant platelets or platelet clumping, which is reflected in the histogram.

Aim: To analyse the White Blood Cell (WBC) and platelet histograms in cases showing falsely low platelet counts.

Materials and Methods: This cross-sectional study was conducted in the Department of Pathology, Chettinad Hospital and Research institute, Tamil Nadu, over a period of six months from January 2021 to June 2021. Haemogram samples were analysed using the Beckman Coulter LH 750 analyser within two hours of sample collection. Samples showing platelet counts of less than one lakh (1,00,000/µL) on the analyser were further evaluated using Leishman-stained peripheral smears to confirm platelet counts. All cases that showed low platelet counts on the analyser but adequate platelet counts on the peripheral smear were included in the study. Demographic details such as age and sex were recorded. Platelet and WBC histograms, along with platelet parameters such as Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW), were analysed. The results were tabulated using Microsoft Excel, and mean and median values were calculated.

Results: In this study, PTCP cases constituted 0.32% of the total cases (18,654) and 1.96% of thrombocytopenic cases (3,060). Among the 60 identified PTCP cases, 44 were males (73%) and 16 were females (27%), giving a male-to-female ratio of 2.75:1. The study population ranged from 19 to 73 years of age, with a mean age of 45±15.11 years. MPV was within normal limits in 40 cases, increased in 15 cases, and decreased in five cases. PDW was increased in 43 cases and normal in 17 cases. Platelet histograms showed changes such as the presence of Multiple Peaks (MP flag) in 41 cases (68.3%). In WBC histograms, platelet clumping was identified by an abnormal curve in front of the lower discriminator in 53 cases (88.3%).

Conclusion: Microscopic blood smear examination in cases of PTCP along with analysis of histogram can prove to be valuable tool to provide the details of the etiology and pathogenesis of PTCP.

Keywords: Giant platelets, Platelet clumps, Platelet flags, Platelet indices

INTRODUCTION

The PTCP, also known as spurious TCP, is a common problem encountered in many clinical laboratories. When stable patients are being evaluated for TCP, the first step is to exclude PTCP [1]. The low platelet count in PTCP is mainly due to in vitro platelet clumping and the presence of giant platelets [2]. Its frequency ranges between 0.09% and 0.11% [3]. Furthermore, its prevalence has been reported to vary between 0.1% and 2% among inpatients [4], and between 15% and 17% among outpatients evaluated for isolated thrombocytopenia [5]. Although PTCP is relatively uncommon, it represents a significant proportion of thrombocytopenia cases that undergo further evaluation [6].

In recent years, automated haematology analysers have revolutionised laboratory practices. In these analysers, platelet counts are estimated either by voltage-pulse counting or by electro-optical counting methods. In 1953, Wallace Coulter first introduced the impedance measurement principle for assessing blood cell size, based on the concept that blood cells cause a measurable increase in electrical resistance as they pass through a small aperture under an applied electrical voltage [7]. This increase in resistance is proportional to the volume of the particle passing through the chamber, thereby allowing simultaneous determination of both cell count and volume. This enables the analyser to differentiate platelets from erythrocytes [8].

The Coulter analyser identifies particles ranging in size from 2-20 fL as platelets. The detection of PTCP largely depends on the analytical technique of the instrument, as each analyser has different sensitivities for identifying platelet aggregates. Techniques such as fluorescence, optical density, and flow cytometry tend to yield higher platelet counts, likely due to partial dissociation of clumps during analysis. In contrast, impedance-based methods are more vulnerable to platelet clumping and thus more likely to show falsely low platelet counts [9]. Depending on their size, platelet clumps may be misclassified as small lymphocytes or giant platelets, resulting in spuriously low platelet counts and, in rare cases, falsely elevated leucocyte counts [10].

Most modern haematology analysers are equipped with software-generated flagging algorithms that identify abnormal platelet distributions, indicating the presence of giant platelets or platelet clumping, which are reflected in the histograms [11]. Histograms provide valuable diagnostic information and assist in identifying the underlying pathology. White Blood Cell (WBC) histograms may show abnormalities such as an abnormal curve in front of the lower discriminator, WBC Lower discriminator (WL) flag, WBC Upper discriminator (WU) flag, F1-F3 flags, or an abnormal curve at T2-T2 flag. Platelet histograms may display flags such as abnormal height at the Platelet Lower discriminator (PL), abnormal height at the Platelet Upper discriminator (PU), and Multiple Peaks (MP), indicating platelet anisocytosis [12].

Peripheral smear examination remains the gold standard for the diagnosis of Ethylenediaminetetraacetic Acid (EDTA)-induced PTCP. Identifying PTCP accurately helps prevent unnecessary investigations,

inappropriate treatments, surgical delays, and transfusion-related complications arising from misdiagnosed TCP [13].

In the present study, PTCP cases were confirmed by peripheral smear examination. The WBC and platelet histograms, along with platelet indices such as MPV and PDW, were further analysed.

Study objectives:

- To identify TCP using an automated cell counter and exclude true TCP cases by peripheral smear examination.
- To study the demographic characteristics, including age and sex, in PTCP cases.
- To analyse platelet indices such as MPV and PDW in the study population.
- To evaluate WBC and platelet histograms in cases showing falsely low platelet counts.
- To relate analyser flagging patterns with peripheral smear findings.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Pathology at Chettinad Hospital and Research Institute, Tamil Nadu, India after obtaining ethical clearance from the Institutional Ethics Committee (Ref. No: 748/IHEC/12-19). It was a prospective study carried out over a period of six months, from January 2021 to June 2021.

Inclusion and Exclusion criteria: All blood samples received for haemogram analysis during the study period were considered. Cases showing low platelet count in the analyser but adequate platelet count on peripheral smear were included in the study. Patients who showed low platelet counts in both the analyser and peripheral smear, as well as those diagnosed with haematological malignancies, were excluded.

Study Procedure

A 2 mL blood sample was collected in EDTA vacutainers for haemogram testing and analysed using an automated haematology analyser (Beckman Coulter LH 750) within two hours of collection. Samples showing a platelet count of less than 1,00,000/µL on the analyser were stained using Leishman stain, and the platelet count was confirmed independently by two pathologists.

Demographic details of all patients, including age and gender, were recorded. Smears were prepared and stained with Leishman stain according to the standard operating procedure and examined microscopically to assess platelet count. Platelet clumps, aggregates, blood clots, giant platelets, and WBC and Red Blood Cell (RBC) morphology were evaluated. Platelet indices, such as MPV (reference range: 7.4-10.4 fL) and PDW (reference range: 9-17%), were also studied. The histograms and analyser flags were interpreted, and all observed changes were documented.

STATISTICAL ANALYSIS

All results were tabulated in Microsoft Excel, and mean and median values were calculated.

RESULTS

During the study period, a total of 18,654 haemogram samples were received in the department. Among these, 3,060 cases (16.4%) showed TCP. Of these, 60 cases fulfilled the inclusion criteria and were included in the present study. This represented 0.32% of total samples and 1.96% of TCP cases. The study population ranged in age from 19 to 73 years, with a mean age of 45±15.11 years [Table/Fig-1]. Of the 60 cases, 44 were males (73%) and 16 were females (27%), giving a male-to-female ratio of 2.75:1. PTCP cases were observed in both healthy individuals and patients with various disease conditions. The MPV ranged from 6 to 13.4 fL, with a mean of 9.4 fL and median of 9.3 fL. MPV was normal in 40 cases, increased in 15 cases, and decreased in 5 cases. The PDW ranged from 13.6% to 20%, with a mean of 17.6% and median of 17.7%. PDW was increased in 43 cases and normal in 17 cases. Platelet histograms showed the presence of an MP flag in 41 cases (68.3%).

Category	Mean	Median
Age (years)	45	42
MPV (fL)	9.4	9.3
PDW	17.6	17.7

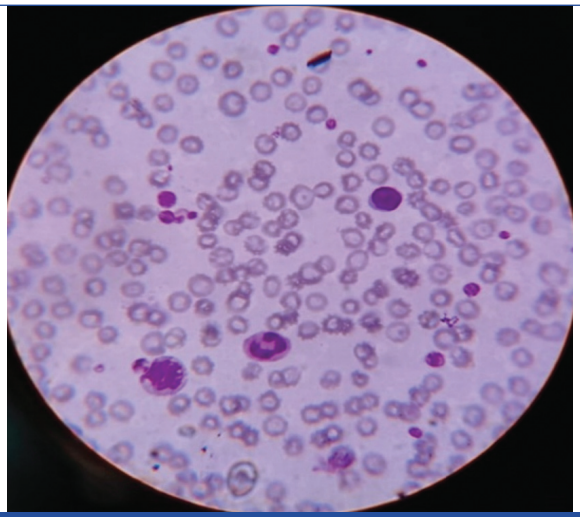
[Table/Fig-1]: Showing mean and median of age, MPV and PDW.

In WBC histograms, platelet clumping was identified by an abnormal curve in front of the lower discriminator in 53 cases (88.3%). Regarding analyser flags, 19 cases showed no flagging, 23 cases showed platelet clumping, 3 cases showed giant platelet flagging, and 15 cases showed both platelet clumping and giant platelet flags [Table/Fig-2].

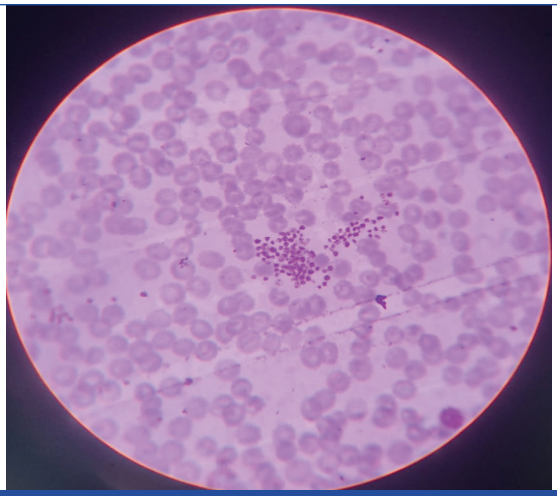
Pattern	Analyser flags	Peripheral Smear Findings
Clumps	23	38
Giant platelet	3	8
Clumps and Giant platelet	15	12
Clot	-	2
No flagging	19	-

[Table/Fig-2]: Showing patterns of analyser flags and peripheral smear findings of platelet distribution.

On peripheral smear examination, 8 cases showed giant platelets, 38 cases showed platelet clumps, 12 cases showed both platelet clumps and giant platelets, and 2 cases showed microclots [Table/Fig-2-4].

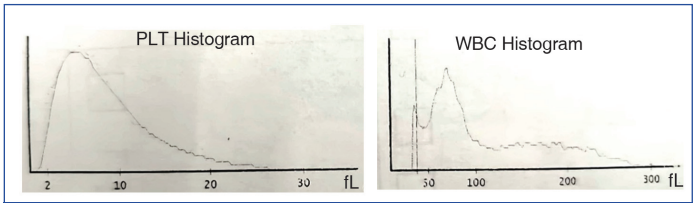


[Table/Fig-3]: Showing peripheral smear with giant platelets (100X).

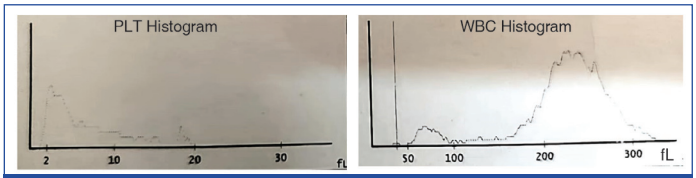


[Table/Fig-4]: Showing peripheral smear with platelet clumps (100X).

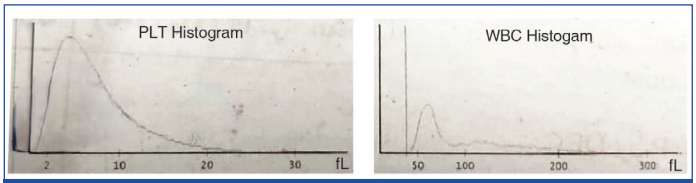
In 38 cases, both WBC and platelet histograms showed changes. In 4 cases, neither histogram showed abnormalities. In 15 cases, only the WBC histogram showed changes, while in 3 cases, only the platelet histogram showed changes [Table/Fig-5-7].



[Table/Fig-5]: Showing normal platelet histogram and WBC histogram showing abnormal curve in front of the lower discriminator.



[Table/Fig-6]: Showing multiple peak flag in platelet histogram and normal WBC histogram.



[Table/Fig-7]: Normal platelet and WBC histogram.

DISCUSSION

Automated cell analysers with 5-part differential counters provide a comprehensive haematological profile of a patient, including histograms and distribution graphs for further analysis. Platelet histograms offer valuable information about platelet size and distribution. It is important to study these histograms, as failure to recognise abnormal patterns may lead to incorrect clinical diagnosis and inappropriate treatment.

Identifying PTCP is essential to avoid unnecessary diagnostic testing and treatment in apparently healthy individuals. Therefore, in patients presenting with TCP, review of histograms, platelet indices, and peripheral smear examination may be necessary to distinguish true TCP from PTCP.

In this study, the cell histograms and platelet indices in PTCP cases were reviewed. The low platelet count was mainly due to EDTA-dependent PTCP, platelet satellitism, giant platelets, and cold agglutinins. In platelet histograms, the most common finding was MP flag, observed in 68.3% of cases, whereas in WBC histograms, an abnormal curve in front of the lower discriminator was seen in 88.3% of cases. MPV was normal in 66.6% of cases, and PDW was increased in 71.6% of cases.

Regarding histogram changes, four types of patterns were observed:

In 38 cases, both WBC and platelet histograms showed abnormalities. In 4 cases, neither WBC nor platelet histograms showed changes. In 15 cases, only the WBC histogram showed changes, while the platelet histogram remained normal. In 3 cases, only the platelet histogram showed changes, while the WBC histogram was normal [Table/Fig-5-7].

Concerning analyser flags, 19 cases did not show any flagging, 23 cases showed platelet clumping, 3 cases showed giant platelet flagging, and 15 cases showed both platelet clumping and giant platelet flags.

Despite these differences, peripheral smear examination revealed either platelet clumps, giant platelets, or both. Therefore, review of peripheral smears is necessary whenever a low platelet count is reported, irrespective of histograms or flagging.

In a study by Meena P and Gupta A, the incidence of PTCP was 0.07% of total haemograms and 4.9% of total TCP cases. In the present study, the incidence was slightly higher at 0.32% of total cases and 1.96% of TCP cases [14].

In this study, out of 60 cases, 44 were male and 16 were female, giving a male-to-female ratio of 2.75:1. The age of the study population ranged from 19 to 73 years, with a mean age of 45±15.11 years. In comparison, Meena P and Gupta A reported a male-to-female ratio of 1:1.3 and patient ages ranging from 3 to 85 years with a mean age of 36.78±20.33 years [14]. In a study by Yildiz A et al., (62 patients), males comprised 37.8% and females 62.2%, whereas the present study showed male predominance with a median age of 52 years (range: 18-94 years) [15]. However, Bizzaro N concluded that PTCP is generally not associated with age or gender [16].

A study by Meena H et al., showed findings similar to this study: MPV was normal in 81% of cases, PDW was increased in 71% of cases, and the MP flag was observed in 44% of cases [17]. The median values of MPV and PDW in this study were similar to those reported by Yildiz A et al., [15], where the median MPV was 9.4 fL (range 6.6-14.8) and the median PDW was 17.6% (range 13.5-20.0).

Another study by Kiliçaslan PE and Özünal IE reported a median MPV of 12.3 fL and median PDW of 15% [18]. Yavaşoğlu I et al., documented MPV as 7±0.5 fL and PDW as 16.5±0.3% [19].

The anticoagulants EDTA and citrate can themselves lead to an increase in platelet volume and MPV by causing deformation and swelling of platelets. Therefore, it is advisable to measure MPV within two hours of sample collection [20].

The findings of the current study are consistent with those of Nagler M et al., who observed MP flag in platelet histograms and an abnormal curve in front of the lower discriminator in WBC histograms in cases of PTCP [21].

In a case report by Dönmez E and Kaya Z, the platelet histogram displayed a serrated/saw-tooth curve, with the largest platelet aggregates appearing as a distinct peak on the platelet curve, causing a rightward shift in a case of platelet aggregation. Interestingly, the MPV and PDW values were normal [22]. In the present study, graphs demonstrated similar features in cases with platelet aggregation. [Table/Fig-8] compares the findings of the present study with similar studies on PTCP and platelet histograms [14,15,17-19].

In this era of automated diagnostic techniques and improved flagging indicators, the approach to analysing haemograms should include parameters like MPV, PDW, and histograms in the laboratory protocols to aid further work-up.

Study	MPV	PDW	Platelet histogram	WBC histogram	Mean age	Sex ratio (M:F)
Present, 2025 (60)	Median 9.3 (6-13.4)	Median 17.7 (15.4-20)	MP flag (68.3%)	W1 flag abnormal curve in front of the lower Discriminator (88.3.6%)	45±15.11	2.75:1
Meena H et al., (470) 2023 [17]	Normal in 380 cases (81%)	Increased in 340 cases (72%)	MP flag 44%	NA	NA	NA
Meena P and Gupta A (130) 2019 [14]	NA	NA	NA	NA	36.78±20.33	1:1.3
Kiliçaslan PE and Özünal IE (52 cases) 022 [18]	12.3 (9.08-19)	15.3.(10-35.6)	NA	NA	NA	NA

Yildiz A et al., (62 patients) 2020 [15]	9.4 (6.6-14.8)	17.6 (13.5-20.0)	NA	NA	52.0 years	62 males (37.8%) and 102 females (62.2%),
Yavasoglu I et al., (15 cases) 2010 [19]	7±0.5	16.5±0.3	NA	NA	48±4 (years)	(female/male) 9/6

[Table/Fig-8]: Findings of various published studies [14,15,17-19].

Limitation(s)

This is a single-centre study. Further multicentric studies involving different analysers and larger sample sizes are needed for more robust and generalisable findings.

CONCLUSION(S)

This study highlights the importance of studying histogram patterns of both WBCs and platelets in cases of platelet clumping or giant platelets. Microscopic blood smear examination in cases of spurious thrombocytopenia, along with analysis of histograms, can provide valuable insights into the aetiology and pathogenesis of PTCP. Reviewing both slides and histograms is essential to prevent unnecessary platelet transfusions and avoid misdiagnosis.

REFERENCES

[1] Gauer RL, Whitaker DJ. Thrombocytopenia: Evaluation and management. Am Fam Physician. 2022;106(3):288-98.
[2] Wu W, Guo Y, Zhang L, Cui W, Li W, Zhang S. Clinical utility of automated platelet clump count in the screening for ethylene diamine tetraacetic acid-dependent pseudothrombocytopenia. Chin Med J (Engl). 2011;124(20):3353-57.
[3] Berkman N, Michaeli Y, Or R, Eldor A. EDTA-dependent pseudothrombocytopenia: A clinical study of 18 patients and a review of the literature. Am J Hematol. 1991;36:195-201.
[4] Zandecki M, Genevieve F, Gerard J, Godon A. Spurious counts and spurious results on haematology analysers: A review. Part II: White blood cells, red blood cells, haemoglobin, red cell indices and reticulocytes. Int J Lab Hematol. 2007;29(1):21-41.
[5] Cohen AM, Cycowitz Z, Mittelman M, Lewinski UH, Gardyn J. The incidence of pseudothrombocytopenia in automatic blood analyzers. Haematologia (Budab). 2000;30(2):117-21.
[6] Sane DC, Damaraju LV, Topol EJ, Cabot CF, Mascelli MA, Harrington RA, et al. Occurrence and clinical significance of pseudothrombocytopenia during abciximab therapy. J Am Coll Cardiol. 2000;36(1):75-83.
[7] Graham MD. The Coulter principle: Foundation of an industry. J Assoc Lab Autom. 2003;8:72-81.
[8] Briggs C, Harrison P, Machin SJ. Continuing developments with the automated platelet count. Int J Lab Hematol. 2007;29:77-91.
[9] Lardinois B, Favresse J, Chatelain B, Lippi G, Mullier F. Pseudothrombocytopenia-A review on causes, occurrence and clinical implications. J Clin Med. 2021;10(4):594.
[10] Baccini V, Geneviève F, Jacqmin H, Chatelain B, Girard S, Wuilleme S, et al. Platelet counting: Ugly traps and good advice. Proposals from the French-Speaking Cellular Hematology Group (GFHC). J Clin Med. 2020;9(3):808.
[11] Er TK, Ruiz Ginés MÁ. Interpretation of the flag of platelet clumping from the Coulter LH 750. Platelets. 2009;20(1):68-69.
[12] Lokwani DP. Histograms. In: The ABC of CBC: Interpretation of Complete Blood Count and Histograms. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd.; 2013. p. 87-126.
[13] Momani A, Khasawneh R, Abed R. Anticoagulant-induced pseudothrombocytopenia: a case report. J R Med Serv. 2012;19(3):73-75.
[14] Meena P, Gupta A. A study of EDTA dependent pseudothrombocytopenia. Int J Med Biomed Stud. 2019;3(4).
[15] Yıldız A, Albayrak M, Şahin O, Afacan Öztürk H, Maral S. Analysis of clinical and demographic characteristics for the differential diagnosis of pseudothrombocytopenia. Acta Oncol Turc. 2020;53:342-48.
[16] Bizzaro N. EDTA-dependent pseudothrombocytopenia: a clinical and epidemiological study of 112 cases, with 10-year follow-up. American Journal of Hematology. 1995;50(2):103-09.
[17] Meena H, Sutrakar SK, Usmani RQ, Jain R, Guru A, Gupta R, et al. Utility of automated cell counter histogram in diagnosis of thrombocytopenia and pseudothrombocytopenia. Asian J Med Sci. 2023;14(7):166-70.
[18] Kiliçaslan E, Özünal IE. Diagnostic role of platelet indices and leukocyte count in pseudothrombocytopenia. J DEU Med. 2022;36(2):113-21.
[19] Yavasoglu I, Kaçar M, Kadikoylu G, Bolaman AZ. Platelet aggregation tests are affected in pseudothrombocytopenia. J Lab Med. 2010;41(8):483-85.
[20] Lippi G, Franchini M. Platelets and immunity: the interplay of mean platelet volume in health and disease. Expert Rev Hematol. 2015;8(5):555-57.
[21] Nagler M, Keller P, Siegrist D, Alberio L. A case of EDTA-dependent pseudothrombocytopenia: simple recognition of an underdiagnosed and misleading phenomenon. BMC Clin Pathol. 2014;14:19.
[22] Dönmez E, Kaya Z. Interpretation of pseudothrombocytopenia using platelet histograms and flags in a hematology autoanalyzer in a healthy child: a case report. Turk J Pediatr. 2024;66(5):666-71.

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Pathology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Chennai, Tamil Nadu, India.
2. Physician - Specialist Clinical Pathology, Al Tadawi Medical Centre, Dubai, United Arab Emirates.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Rajeswari Thivya Dhanabalan
Associate Professor, Department of Pathology, Chettinad Hospital and Research Institute Chettinad Academy of Research and Education, Rajiv Gandhi Salai Kelambakkam, Chengalpat District, Chennai-603103, Tamil Nadu, India.
Email: drrthivya@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: May 06, 2025
- Manual Googling: Aug 14, 2025
- iThenticate Software: Aug 16, 2025 (8%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Apr 18, 2025**
Date of Peer Review: **Jun 24, 2025**
Date of Acceptance: **Aug 18, 2025**
Date of Publishing: **Dec 01, 2025**