HEMATOLOGY, TRANSFUSION AND CELL THERAPY

www.htct.com.br

Original article

# Plasma levels of soluble podoplanin are higher in acute promyelocytic leukemia compared to other forms of acute myeloid leukemia

*Carla Roberta Peachazepi Moraes* [a,*], *Camilla Maria de Alencar Saraiva* [a], *Ivanio Teixeira Borba-Junior* [a], *Bruno Kosa Lino Duarte* [a,b], *Paula Melo de Campos* [a,b], *Sara Teresinha Olalla Saad* [a,b], *Erich Vinicius De Paula* [a,b]

[a] *University of Campinas, School of Medical Sciences, Campinas, Brazil*
[b] *University of Campinas, Hematology and Hemotherapy Center, Campinas, Brazil*

ARTICLE INFO

*Article history:*
Received 30 July 2025
Accepted 8 October 2025
Available online 19 December 2025

Editor name: Eduardo Rego

*Keywords:*
Podoplanin
Acute promyelocytic leukemia
ELISA

ABSTRACT

*Background:* Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) marked by a high incidence of coagulopathy. Podoplanin, a glycoprotein involved in platelet activation through interaction with CLEC-2, has recently been identified on leukemic promyelocytes and suggested as a potential contributor to APL coagulopathy. Identification of novel biomarkers and therapeutic targets for APL coagulopathy can potentially improve the outcomes of this condition

*Aim:* To explore whether levels of soluble podoplanin in plasma are different in APL, and to evaluate its association with laboratory and clinical outcomes in these patients

*Methods:* Samples were obtained from consecutive patients with APL at the time of diagnosis in an academic hospital. Biobank samples from 35 patients with non-APL AML matched for age and sex were used as comparators. Circulating podoplanin levels were measured in plasma using a commercial ELISA kit. The study was approved by the institutional ethics committee and all participants provided written informed consent

*Results:* APL patients showed significantly higher plasma soluble podoplanin concentrations compared to non-APL AML. Using the median soluble podoplanin value as a cutoff, a higher proportion of APL patients presented elevated levels. Soluble podoplanin levels correlated with CD40L in APL cases, but not in non-APL AML patients, suggesting a possible interaction with thrombo-inflammatory activation pathways

*Conclusion:* These findings represent a proof-of-concept that measuring soluble podoplanin in plasma samples can contribute to the diagnosis of APL, while also providing novel data on the association of podoplanin with the pathogenesis of APL coagulopathy.

© 2025 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier España, S.L.U. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

* Corresponding author.
E-mail address: cpeachazepi@hotmail.com (C.R.P. Moraes).

https://doi.org/10.1016/j.htct.2025.106227

2531-1379/© 2025 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier España, S.L.U. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

## Introduction

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML) characterized by the accumulation of promyelocytes in the bone marrow and a high risk of severe hemorrhagic complications. Despite significant therapeutic advancements with all-trans retinoic acid (ATRA) and arsenic trioxide (Arsenic Trioxide) [1–3], early mortality remains a major concern, potentially reaching 5–20 %, depending on clinical and geographical factors. The primary cause of early death is coagulopathy, which often leads to fatal intracranial hemorrhages [4]. Therefore, early diagnosis and prompt initiation of treatment are crucial for reducing mortality and improving patient outcomes. In addition, the identification of novel biomarkers and therapeutic targets of APL coagulopathy could bring benefits to these patients.

Podoplanin is a small transmembrane glycoprotein expressed in various cell types, including lymphatic endothelial cells, kidney podocytes, and certain tumor cells [5]. Functionally, podoplanin interacts with the C-type lectin-like receptor 2 (CLEC-2) on platelets, triggering their activation and contributing to thrombotic processes [6]. This mechanism is particularly relevant in cancer-associated thrombosis and inflammatory diseases, where podoplanin overexpression has been linked to prothrombotic states [5–7].

In the context of APL, podoplanin expression was first identified in leukemic promyelocytes in 2018, suggesting its potential role as a distinguishing marker for this leukemia subtype [8]. More recently, its expression was validated in a prospective cohort using flow cytometry, reinforcing its diagnostic and pathophysiological relevance in APL [9]. However, the extent to which circulating podoplanin correlates with disease characteristics and coagulation abnormalities remains unexplored.

Soluble forms of membrane proteins can serve as biomarkers, reflecting their biological activity and disease status. For instance, soluble fms-like tyrosine kinase-1 (sFLT1) is well established as a biomarker in preeclampsia and soluble urokinase-type plasminogen activator receptor (suPAR) as a biomarker in different types of cancers [10–13]. Similarly, soluble podoplanin (sPDPN) has been identified as a biomarker in malignancies and inflammatory diseases such as cancer and COVID-19, highlighting its potential and prognostic significance [14–18].

Based on this evidence, this study investigated whether quantification of sPDPN in plasma could be associated with the diagnosis of APL, as well as its relationship with biomarkers involved in APL coagulopathy.

## Methods

### *Study population*

This study analyzed patients diagnosed with acute leukemia between 2014 and 2022 at an academic tertiary hospital. Inclusion criteria were: age ≥18 years and a confirmed diagnosis of APL or AML. Exclusion criteria included: presence of severe infection with hemodynamic instability requiring vasoactive agents at the time of sample collection, and a diagnosis of other forms of acute leukemia. Patients with non-APL AML were used as a comparator group. These patients were selected from the population of consecutive new AML cases during the same study period, matched for age, sex and year of enrollment, before any of the laboratory analyses. The diagnostic workup comprised morphological evaluation, immunophenotyping, cytogenetic analysis, and molecular testing. APL cases were confirmed through molecular detection of the *PML-RARA* fusion gene. Patients with a strong clinical and morphological suspicion of APL, based on peripheral blood smear findings, were promptly initiated on ATRA therapy in accordance with the institutional protocol. This study was approved by the institutional ethics committee (CAAE: 39948520.8.1001.5404) and performed in accordance with the Declaration of Helsinki.

### *Sample collection and processing*

Whole blood samples were collected in EDTA tubes. Plasma was obtained by centrifugation at 2500 g for 15 min at 22 °C within two hours of collection. All samples were aliquoted and stored at −80 °C until analysis.

### *Clinical and laboratory data*

Clinical and laboratory data were retrospectively retrieved from hospital electronic medical records. Laboratory variables included initial bone marrow blast percentage, hemoglobin concentration, white blood cell (WBC) count, platelet count, prothrombin time (TP), activated partial thromboplastin time (aPTT), and fibrinogen concentration, based on the first set of results available upon hospital admission. Clinical outcomes comprised early mortality (defined as death within 30 days of diagnosis) [19] and central nervous system (CNS) and retinal bleeding.

### *Measurement of soluble podoplanin levels*

Plasma levels of podoplanin were measured in duplicate in EDTA plasma using a commercial enzyme-linked immunosorbent assay kit (ELISA) (Sigma Aldrich, Cat. RAB 1632–1KT - Lot: 0325/2117), according to the manufacturer's instructions.

### *Measurement of P-selectin and CD40L*

Plasma concentrations of P-selectin and CD40L were determined using a custom-designed multiplex assay panel (Invitrogen – Thermo Fisher Scientific – Cat. PPX-07-MX323GU – Lot: 283,814–000), following the manufacturer's protocol. All measurements were performed in duplicate, and analyte concentrations were calculated based on standard curves generated using known concentrations of recombinant proteins.

### *Statistical analysis*

Quantitative variables were described using both median with interquartile range and mean with standard deviation (SD). Patients with APL were matched 1:1 to non-APL AML patients, as detailed above. Matching was done prior to any

laboratory analysis. Comparative analyses between groups were performed using parametric and nonparametric statistical methods. The Student's *t*-test was applied for normally distributed data, while the Mann-Whitney *U* test was used for data not meeting parametric assumptions. For the analysis of the association between podoplanin expression with clinical outcomes, APL patients were arbitrarily categorized as podoplanin positive or negative, based on the 50th percentile cut-off. Correlations were evaluated by Spearman's test. A p-value <0.05 was considered statistically significant. Statistical analyses were conducted using IBM SPSS Statistics version 26 and GraphPad Prism version 8.0.

## Results

In total, 35 patients with APL were included in this study, as well as 35 patients with non-APL AML, matched by age, sex and year of diagnosis, used as a comparator group. The clinical and laboratory characteristics of these groups are presented in Table 1. As expected, APL patients presented more frequently with alterations in hemostasis biomarkers, and a lower rate of early mortality. Plasma concentrations of sPDPN ranged from 0.3–113.6 ng/mL. Patients with APL exhibited significantly higher levels of sPDPN (median: 16.4 ng/mL) compared to patients with other AML subtypes (median: 2.2 ng/mL; p-value = 0.003) (Figure 1). To investigate the potential relationship between sPDPN and platelet activation, plasma levels of P-selectin and CD40L, two established markers of platelet degranulation, were measured. Both P-selectin (7194 pg/mL versus 11,220 pg/mL; p-value = 0.003) and CD40L levels (100.4 pg/mL versus 196.6 pg/mL; p-value = 0.002) were significantly lower in APL patients compared to those with other AML subtypes (Figure 2A,B). Interestingly, a significant positive correlation was observed between podoplanin and CD40L levels in APL patients ($R_s$ = 0.60; p-value = 0.0004), but not for other AML subtypes (Figure 2C & D). Podoplanin levels were not associated with predefined clinical outcomes (Table 2).

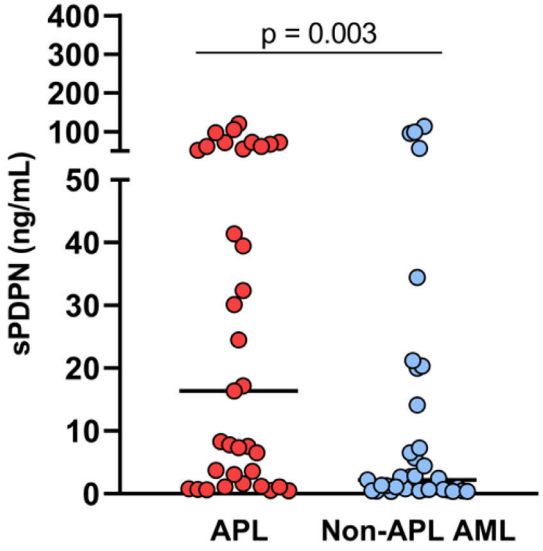


**Figure 1 – Plasma levels of podoplanin of acute promyelocytic leukemia (APL) and non-APL acute myeloid leukemia (AML) patients (p-value: Mann-Whitney test).**

## Discussion

APL coagulopathy still represents a major challenge to the early management of APL, and the availability of biomarkers capable of differentiating APL from other forms of AML, and of providing information about APL coagulopathy could potentially facilitate the management of these patients [20].

**Table 1 – Laboratory and clinical characteristics of study participants.**

| | APL (*n* = 35) | Non-APL AML (*n* = 35) | p-value‡ |
|---|---|---|---|
| Age* | 37.3 (21–61) | 41.4 (18–59) | 0.31 |
| Male:Female | 17:18 | 17:18 | 0.99 |
| Risk group - (low/int/high) | 3/18/14 | 11/11/13 | 0.04 |
| Peripheral blast count, $10^9$/L* | 2.23 (0.01–129.6) | 7.75 (0.1–130.8) | 0.25 |
| Bone marrow blast count - %* | 88.0 (46.0–98.2) | 80.5 (26.5–95.6) | 0.002 |
| Leukocytes - $10^3/\mu$L* | 3.1 (0.32–137.9) | 14.4 (0.91–150.3) | 0.02 |
| Hemoglobin - g/dL** | 8.4 ± 2.39 | 8.1 ± 2.10 | 0.64 |
| Platelets - $10^9$/L* | 22.0 (4.0–89.0) | 27.0 (5.0–230.0) | 0.02 |
| PT (INR)** | 1.29 ± 0.19 | 1.19 ± 0.13 | 0.01 |
| aPTT* | 0.90 (0.75–1.24) | 0.89 (0.67–1.38) | 0.48 |
| Fibrinogen - mg/dL* | 121.7 (26.0–378.5) | 384.5 (58.9–705.8) | <0.0001 |
| *Clinical outcomes* | | | |
| CNS/retinal bleeding - n ( %) | 4 (11.4) | 2 (5.7) | 0.39 |
| 30-day survival - (no/yes) | 5/30 | 12/23 | 0.05 |

* Median (interquartile range).
** Mean ± SD.
‡ Mann-Whitney test, *t*-test or chi-square test.
APL: acute promyelocytic leukemia; AML: acute myeloid leukemia; CNS: central nervous system; PT: prothrombin time; aPTT: activated partial thromboplastin time; INR: International Normalized Ratio

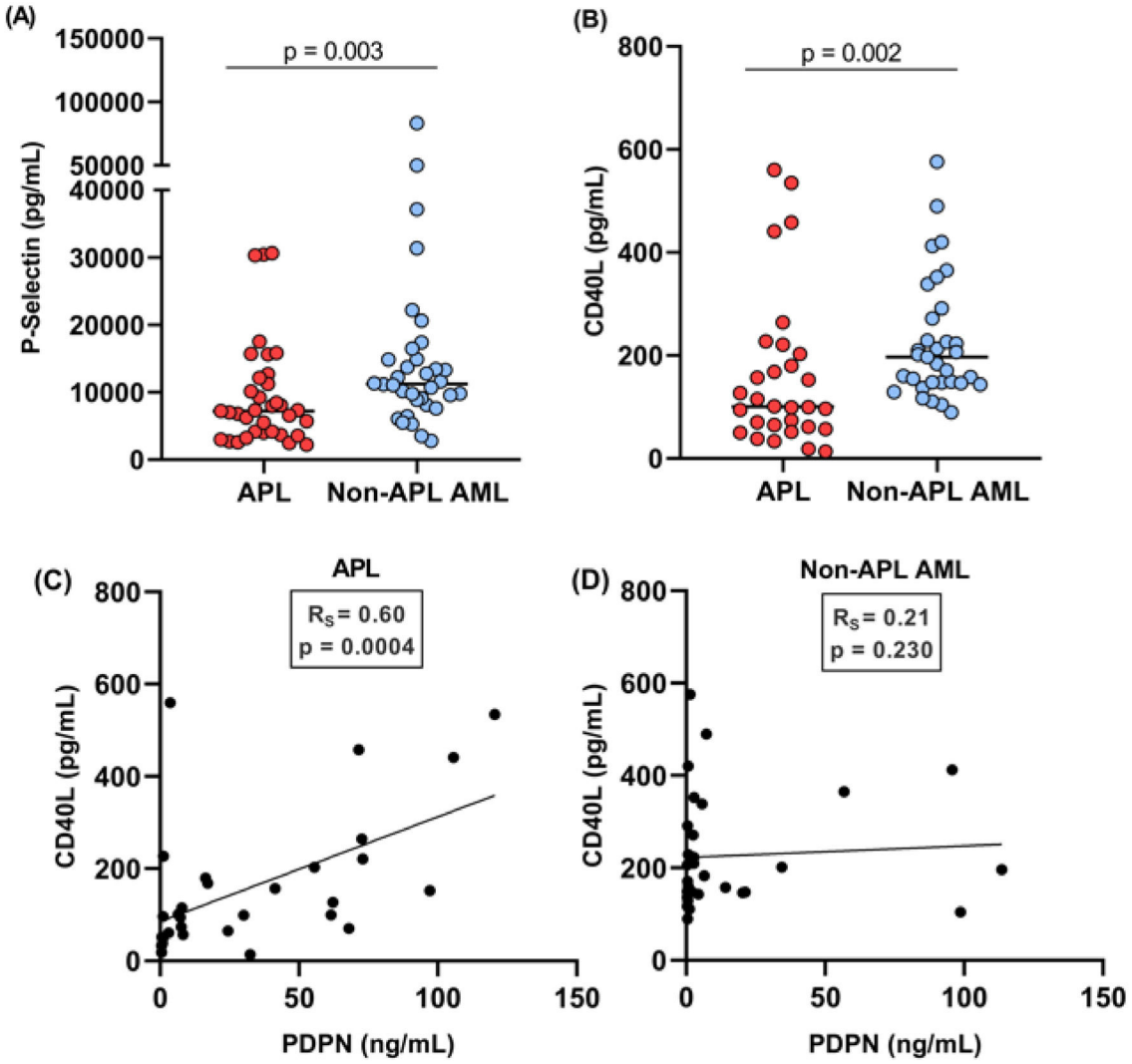


**Figure 2 – (A) Plasma levels of P-selectin were significantly lower in acute promyelocytic leukemia (APL) patients compared to non-APL acute myeloid leukemia (AML) patients (Mann-Whitney test; p-value = 0.003). (B) Plasma levels of CD40L were significantly lower in APL patients compared to non-APL AML patients (Mann-Whitney test; p-value = 0.002). (C) A significant positive correlation between podoplanin and CD40L was observed in APL patients (Spearman's correlation; p-value = 0.0004). (D) No significant correlation between podoplanin and CD40L was found in non-APL AML patients.**

**Table 2 – Association of podoplanin expression with clinical outcomes in acute promyelocytic leukemia.**

| Clinical outcome | Podoplanin expression category* | | p-value** |
|---|---|---|---|
| | Podoplanin + | Podoplanin - | |
| Early mortality (30-day) | | | 0.46 |
| **Yes** | 4 | 1 | |
| **No** | 19 | 11 | |
| CNS/ retinal bleeding | | | 0.67 |
| **Yes** | 3 | 1 | |
| **No** | 20 | 11 | |

CNS: Central nervous system.

* For the purpose of association analyses, acute promyelocytic leukemia patients were arbitrarily categorized as podoplanin positive or negative, based on the 50th percentile cutoff.

** Statistical comparison was performed using the chi-square test.

The main contribution of the present study was the demonstration of sPDPN as a potential biomarker. Its expression is markedly higher in APL compared to other forms of AML, and it correlates with an important thrombo-inflammatory mediator. This suggests that podoplanin expression may be a unique biological feature contributing to the well-known predisposition of these patients to hemorrhagic and thrombotic complications.

The first demonstration that podoplanin expression is a hallmark of APL was published in 2018 and included data obtained by flow cytometry and RNA sequencing [8]. That study also presented evidence linking podoplanin expression to platelet activation in vitro. While the diagnosis of APL using flow cytometry and molecular techniques is well established, reliance on methods that are not usually available outside specialized cancer centers may delay diagnosis and jeopardize

patient outcomes [20]. Immune-based methods such as ELISA are widely available and have the advantage of being potentially performed with diagnostic kits in almost every setting. Based on the demonstration that circulating levels of podoplanin is capable of identifying discrete subgroups of patients with other conditions [14–18], and that soluble levels of membrane proteins can serve as useful disease biomarkers [10–13], we hypothesized that sPDPN levels might contribute to the differential diagnosis of APL from other forms of AML. In fact, this study demonstrates a significant difference in sPDPN levels between these two patient subgroups, although a subgroup of patients with non-APL AML also presents higher sPDPN values.

In addition, a positive correlation between sPDPN and CD40L levels was found in APL patients, suggesting a biological interaction between these two markers. CD40L is a transmembrane protein expressed on activated platelets that has been implicated in platelet-leukocyte crosstalk and inflammatory signaling [21]. CD40L is also expressed by other hematopoietic cells such as lymphocytes, neutrophils, dendritic cells, monocytes and macrophages, in response to cytokines [22], which are key elements of thrombo-inflammatory pathways [23]. So, the correlation described in the current study could represent either evidence of the participation of podoplanin in platelet activation, in alignment with Lavallé et al. [8], who demonstrated that podoplanin-expressing APL blasts can activate platelets, or a yet unknown association between podoplanin and CD40L in another hematopoietic compartment. In the present dataset, sPDPN levels were not associated with clinical outcomes, although this study was not powered for this type of analysis.

The results also demonstrate that a significant proportion of AML patients present detectable sPDPN levels, while some APL patients present low sPDPN levels. This is in contrast with data from flow cytometry and reverse transcription polymerase chain reaction (RT-PCR) results from our lab that show less overlap between APL and other forms of AML (data not shown). Additional studies are warranted to explore and explain this lower accuracy of sPDPN ELISA for the segregation of APL from other forms of AML compared to flow cytometry.

This study has limitations that should be acknowledged. Although APL is a relatively rare condition and the present sample size was sufficient to demonstrate relevant biological differences in sPDPN levels, this study was not powered to analyze clinical outcomes. Another limitation lies in the absence of additional hemostatic biomarkers, which might have enhanced the interpretation of coagulation-related mechanisms. However, these markers are not routinely assessed in the standard clinical workflow of the hospital, and citrate plasma samples were no longer available. Future studies involving larger cohorts and broader biomarker panels are important to further investigate the potential diagnostic and clinical relevance of sPDPN in APL.

In conclusion, the results of this study represent proof-of-concept that the immunodetection (in this case, using ELISA) of circulating levels of sPDPN can serve as a complementary tool in the differential diagnosis of APL. The data also adds support to the concept that podoplanin expression is a distinct hallmark of APL and contributes to the pathogenesis of the unique thrombo-inflammatory complications seen in these patients.

## Data availability

All reported data are available for sharing upon a reasonable request to the corresponding author.

## Ethics statement

The study was performed in accordance with the Declaration of Helsinki and approved by the local institutional research board (protocol CAAE: 39948520.8.1001.5404).

## Author contributions statement

CRPM: performed all assays; revised medical records; contributed to data analysis and drafted the manuscript; revised and approved the manuscript. CMAS: revised medical records; contributed to data analysis; revised and approved the manuscript. ITBJ: contributed to data analysis; revised and approved the manuscript. BKLD: contribute to study design; revised and approved the manuscript. PMC: provided laboratory support and infra-structure; revised and approved the manuscript. STOS: provided laboratory support and infra-structure; revised and approved the manuscript. EVDP: designed the study, oversaw and provided resources and infra-structure for ELISA analysis, contributed to data analysis; drafted the manuscript; revised and approved the manuscript.

## Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Acknowledgements

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by the Sao Paulo Research Foundation (FAPESP), grants 2022/13216–0 and 2023/03765–0. FAEPEX-Unicamp grant 2633/23; Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior – Brasil (CAPES), finance code 001.

REFERENCES

1. Meng-er H, Yu-chen Y, Shu-rong C, Jin-ren C, Jia-Xiang L, Lin Z. et al. Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. Blood. 1988;72:567–72.
2. Chen G, Zhu J, Shi X, Ni J, Zhong H, Si G, et al. In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia: As2O3 induces NB4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. Blood. 1996;88:1052–61.

3. Sanz MA, Fenaux P, Tallman MS, Estey EH, Löwenberg B, Naoe T, et al. Management of acute promyelocytic leukemia: Updated recommendations from an expert panel of the European LeukemiaNet. Blood. 2019;133:1630–43.
4. Park JH, Qiao B, Panageas KS, Schymura MJ, Jurcic JG, Rosenblat TL, et al. Early death rate in acute promyelocytic leukemia remains high despite all-trans retinoic acid. Blood. 2011;118:1248–54.
5. Suzuki-Inoue K, Osada M, Ozaki Y. Physiologic and pathophysiologic roles of interaction between C-type lectin-like receptor 2 and podoplanin: partners from in utero to adulthood. Journal of Thrombosis and Haemostasis. 2017;15: 219–29.
6. Mir Seyed Nazari P, Riedl J, Pabinger I, Ay C. The role of podoplanin in cancer-associated thrombosis. Thromb Res. 2018;164:S34–9.
7. Tawil N, Bassawon R, Meehan B, Nehme A, Montermini L, Gayden T, et al. Glioblastoma cell populations with distinct oncogenic programs release podoplanin as procoagulant extracellular vesicles. Blood Adv. 2021;5:1682–94.
8. Lavallée VP, Chagraoui J, MacRae T, Marquis M, Bonnefoy A, Krosl J, et al. Transcriptomic landscape of acute promyelocytic leukemia reveals aberrant surface expression of the platelet aggregation agonist Podoplanin. Leukemia. 2018;32:1349–57.
9. Saraiva CM de A, Moraes CRP, Duarte BKL, Oliveira GB, Sales Filho HLA, de Melo Campos P, et al. Prospective validation of podoplanin expression as a diagnostic biomarker of acute promyelocytic leukemia. Cytometry B Clin Cytom. 2025: 1–6.
10. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, et al. Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia. New England Journal of Medicine. 2016;374:13–22.
11. Ng KW, Chaturvedi N, Coté GL, Fisher SA, Mabbott S. Biomarkers and point of care screening approaches for the management of preeclampsia. Communications Medicine. Springer Nature. 2024;4:1–15.
12. Hao W, Friedman A. Serum uPAR as biomarker in breast cancer recurrence: A mathematical model. PLoS One. 2016;11:1–24.
13. Blomberg K, Hansen TF, Brasen CL, Madsen JB, Jensen LH, Thomsen CB. The soluble urokinase-type plasminogen activator receptor as a biomarker for survival and early treatment effect in metastatic colorectal cancer. Cancers (Basel). 2021;13: 1–11.
14. Zhao X, Pan Y, Ren W, Shen F, Xu M, Yu M, et al. Plasma soluble podoplanin is a novel marker for the diagnosis of tumor occurrence and metastasis. Cancer Sci. 2018;109:403–11.
15. Zhu X, Xu M, Zhao X, Shen F, Ruan C, Zhao Y. The detection of plasma soluble podoplanin of patients with breast cancer and its clinical signification. Cancer Manag Res. 2020;12:13207–14.
16. Takiguchi K, Shoda K, Nakayama T, Takahashi K, Saito R, Yamamoto A, et al. Soluble podoplanin as a biomarker in diffusetype gastric cancer. Oncol Rep. 2022;47:1–7.
17. Zhang Q, Peng K, Wang L, Zhang RY, Xin LH, Huang JY, et al. Plasma Soluble Podoplanin as a Biomarker of Hypercoagulability and Cellular Immunity Status in Patients With Non-small Cell Lung Cancer. Clinical and Applied Thrombosis/ Hemostasis. 2023;29:1–8.
18. IT Borba-Junior, Lima F, Sidarta-Oliveira D, Moraes CRP, Annichino-Bizzacchi JM, Bombassaro B, et al. Podoplanin and CLEC-2 levels in patients with COVID-19. Res Pract Thromb Haemost. 2023;7:1–9.
19. Mantha S, Goldman DA, Devlin SM, Lee JW, Zannino D, Collins M, et al. Determinants of fatal bleeding during induction therapy for acute promyelocytic leukemia in the ATRA era. Blood. 2017;129:1763–7.
20. Odetola O, Tallman MS. How to avoid early mortality in acute promyelocytic leukemia. Hematology Am Soc Hematol Educ Program. 2023: 248–53.
21. Gerdes N, Seijkens T, Lievens D, Kuijpers MJE, Winkels H, Projahn D, et al. Platelet CD40 exacerbates atherosclerosis by transcellular activation of endothelial cells and leukocytes. Arterioscler Thromb Vasc Biol. 2016;36:482–90.
22. Van Kooten G, Banchereau J. CD40-CD40 ligand. Journal of Leukocyte Biology. 2000;67:2–17.
23. Cognasse F, Duchez AC, Audoux E, Ebermeyer T, Arthaud CA, Prier A, et al. Platelets as Key Factors in Inflammation: focus on CD40L/CD40. Front Immunol. 2022;13:1–11.