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Immunometabolic activation of neutrophils is associated with tissue factor-positive microparticles in gestational diabetes mellitus

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Key words: immunometabolism, microparticles, neutrophils, pregnancy, tissue factor

Abstract

Introduction: Gestational diabetes mellitus (GDM) is associated with chronic low-grade inflammation and an increased risk of thromboembolic complications. Tissue factor-positive microparticles (TF^+MPs) link inflammatory and coagulation pathways, but their cellular origin and immunometabolic determinants in GDM remain unclear.

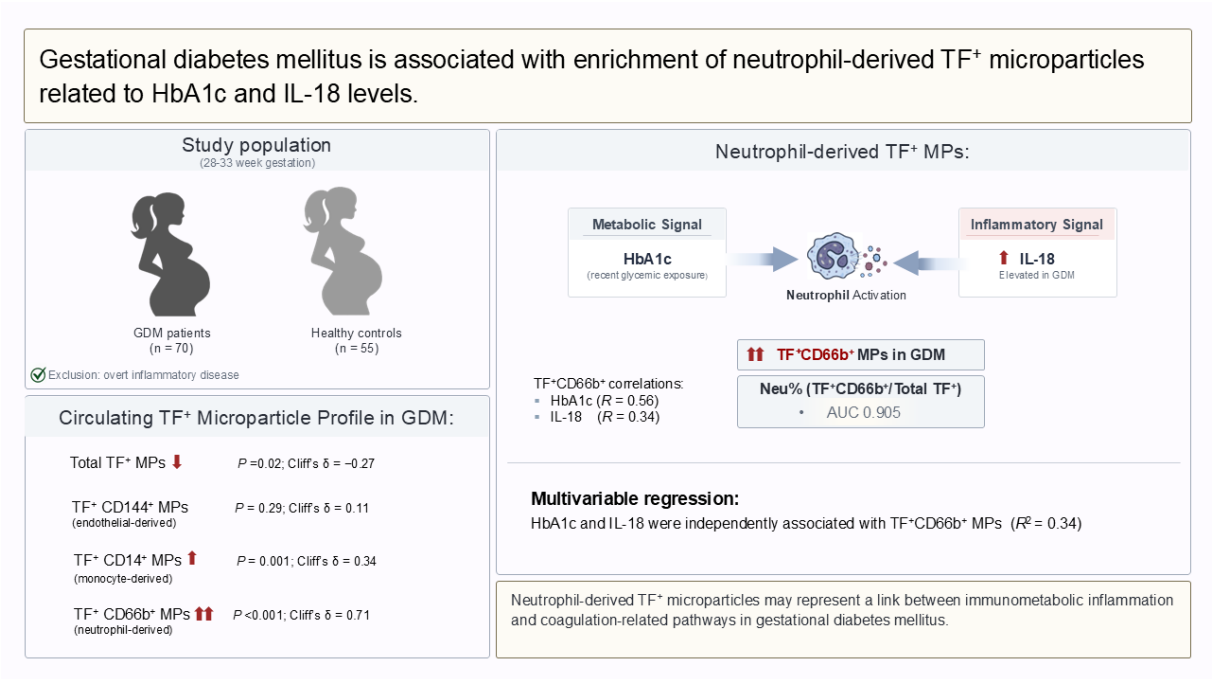
Objectives: To characterize neutrophil-, monocyte-, and endothelial-derived TF^+MPs in GDM and to assess their relationships with metabolic and inflammatory markers.

Patients and methods: We studied 70 women with GDM and 55 healthy pregnant controls at 28-33 weeks' gestation. Neutrophil-derived (TF^+CD66b^+), monocyte-derived (TF^+CD14^+), and endothelial-derived (TF^+CD144^+) MPs were quantified by flow cytometry. Glycated hemoglobin (HbA1c) and interleukin-18 (IL-18) were measured as markers of recent glycemic

exposure and inflammatory response, respectively. Statistical analyses included the Mann-Whitney U test, Cliff's delta, correlation analysis, and receiver operating characteristic (ROC) curve analysis.

Results: Women with GDM exhibited significantly higher counts of TF^+CD66b^+ MPs compared with controls (P <0.001; $\delta = 0.71$). The percentage of TF^+CD66b^+ MPs relative to total TF^+MPs showed marked separation between groups (AUC = 0.905). TF^+CD14^+ MPs were increased ($P = 0.001$; $\delta = 0.34$), whereas TF^+CD144^+ MPs did not differ significantly ($P = 0.29$; $\delta = 0.11$). TF^+CD66b^+ MPs correlated positively with HbA1c ($R = 0.56$, P <0.001) and IL-18 ($R = 0.34$, $P = 0.004$), with no associations observed for maternal BMI, fasting glucose, or glucose-lowering therapy.

Conclusions: GDM is associated with marked enrichment of TF^+CD66b^+ MPs, reflecting immunometabolic neutrophil activation rather than obesity-related effects. These findings identify TF^+CD66b^+ MPs as a downstream marker linking metabolic and inflammatory pathways in GDM.



Introduction

Gestational diabetes mellitus (GDM) is one of the most common metabolic complications of pregnancy and is defined by disturbances in glucose homeostasis [1]. Recent population-based data from Poland have demonstrated substantial national trends in the prevalence and outcomes of GDM, highlighting its clinical and public health relevance [2]. In parallel, current research has increasingly focused on identifying novel predictive biomarkers, including specific lipidome patterns, to enhance early risk assessment and better understand the metabolic heterogeneity of GDM [3]. Increasing evidence, however, indicates that GDM is also accompanied by chronic low-grade systemic inflammation and an increased risk of thromboembolic complications during pregnancy and the postpartum period [4,5]. These observations suggest that inflammatory and procoagulant pathways may contribute to the pathophysiology of GDM beyond hyperglycemia alone.

Circulating microparticles may provide a link between these processes, as they are released during cellular activation, stress, or cell death and can carry surface molecules involved in inflammation and coagulation [6,7]. At the same time, the composition and proportions of individual MP subpopulations, derived from neutrophils, other leukocytes, and platelets, change depending on physiological or pathological conditions, which may reflect the intensity of inflammatory processes and vascular disturbances associated with disease [8].

Among these MP subpopulations, particular interest has focused on MPs expressing tissue factor (TF^+MPs), a potent initiator of the extrinsic coagulation cascade that confers strong procoagulant properties to this MP subpopulation [9]. Elevated levels of TF^+MPs have been reported in a range of inflammatory and thrombotic disorders [10] and are increasingly considered markers of inflammation-associated coagulation activation [11,12].

Pregnancy itself is characterized by physiological adaptations that favor coagulation [13]. In the context of GDM, these changes may be further amplified by metabolic stress and immune

activation [14]. Cells of the innate immune system, particularly neutrophils and monocytes, play a central role in inflammatory and procoagulant responses and are capable of expressing tissue factor and releasing TF^+MPs upon activation [15]. This activation is often driven by specific inflammatory mediators, among which interleukin-18 (IL-18) serves as a key link between metabolic stress and innate immune activation and acts as a potent inducer of neutrophil priming [16]. Elevated IL-18 levels have been implicated in the chronic low-grade inflammation characteristic of gestational diabetes mellitus [17]. Therefore, IL-18 was selected as a focused mechanistic marker of innate immune activation rather than as a general screening marker of systemic inflammation.

In contrast, endothelial-derived MPs are commonly considered markers of endothelial injury or dysfunction [18]. Assessing the relative contribution of immune cell-derived versus endothelial-derived TF^+MPs may therefore help clarify whether the procoagulant state observed in GDM primarily reflects immune cell activation or overt endothelial involvement.

Although alterations in circulating MPs have been described in metabolic and inflammatory diseases [8,19], data specifically addressing the cellular origin of TF^+MPs in gestational diabetes mellitus remain limited. In particular, the relationships between chronic glycemic exposure, inflammatory signaling, and TF^+MPs profiles have not been well characterized, nor is it clear whether these associations are independent of maternal adiposity or glucose-lowering therapy.

Therefore, the aim of the present study was to characterize circulating TF^+MPs of neutrophil, monocyte, and endothelial origin in women with GDM during the third trimester of pregnancy. We also examined their relationships with markers of chronic glycemic exposure and inflammation, while accounting for maternal body mass index (BMI) and glucose-lowering treatment. By focusing on TF^+MPs, this study aimed to provide insight into immunometabolic

and procoagulant processes in GDM that are not captured by conventional clinical risk assessment.

Patients and methods

Study population and recruitment

The study included a total of 125 pregnant women, comprising 70 patients diagnosed with gestational diabetes mellitus and 55 healthy pregnant controls. All participants were consecutively recruited during routine or follow-up antenatal visits and examined in the third trimester of pregnancy (28-33 weeks of gestation).

Patients with GDM were recruited from the Diabetes Outpatient Clinic for Pregnant Women at the University Hospital in Krakow, Poland. The control group consisted of healthy pregnant women with normal glucose tolerance, recruited from a private obstetric practice in the same geographical area.

The diagnosis of GDM was established based on a 75-g oral glucose tolerance test (OGTT) performed between 24 and 28 weeks of gestation, in accordance with the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria [1]. GDM was diagnosed if at least one of the following plasma glucose thresholds was met or exceeded: fasting glucose ≥92 mg/dl (5.1 mmol/l), 1-hour glucose ≥180 mg/dl (10.0 mmol/l), or 2-hour glucose ≥153 mg/dl (8.5 mmol/l).

At the time of blood sampling for microparticle (MP) analysis (28-33 weeks of gestation), patients with GDM were undergoing standardized clinical management. Thirty-three women were managed with diet alone, while 37 required insulin therapy to achieve glycemic targets. Glycemic control in the GDM group was routinely monitored according to clinical standards, including measurement of glycated hemoglobin (HbA1c).

To ensure the specificity of circulating MP profiles, the following exclusion criteria were applied to both groups: pre-existing diabetes mellitus (type 1 or type 2), chronic or pregnancy-induced hypertension (including preeclampsia), multiple pregnancy, active infection, chronic inflammatory or autoimmune diseases, and chronic kidney or liver disorders.

The study protocol was approved by the Bioethics Committee of the Jagiellonian University (No. 122.6120.259.2016). All participants provided written informed consent prior to enrollment, in accordance with the Declaration of Helsinki.

Methods

Clinical and Laboratory Assessments

Pre-pregnancy body weight and height were self-reported by the participants at the time of enrollment and were used to calculate pre-pregnancy BMI.

Fasting plasma glucose (FPG) concentration was measured at the Department of Clinical Biochemistry, Jagiellonian University Medical College, using a fully automated biochemical analyzer (Erba Mannheim XL-180, Mannheim, Germany) based on the glucose oxidase enzymatic method. Glycated hemoglobin (HbA1c) was measured only in women with GDM and was used in the present study as an indicator of recent glycemic exposure. HbA1c levels were determined using ion-exchange chromatography on the D-10 Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA, USA). Serum interleukin-18 (IL-18) concentrations were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (SunRed Biotechnology Company, Shanghai, China), according to the manufacturer's instructions.

Blood sampling and microparticle analysis

Venous blood samples were collected into tubes containing 3.2% sodium citrate. Circulating microparticles were isolated by a double centrifugation protocol to remove cells and platelets,

followed by high-speed centrifugation (10,000 × g for 30 minutes at 4°C) to pellet microparticles.

MP analysis was performed by flow cytometry using a BD FACSCanto flow cytometer (Becton Dickinson, San Jose, CA, USA). For MP phenotyping, a no-wash staining protocol was applied. Samples were incubated with fluorochrome-conjugated monoclonal antibodies against tissue factor (TF-142-APC), CD14-FITC, CD66b-Pacific Blue, and CD144-PE (all from BioLegend, San Diego, CA, USA). Phosphate-buffered saline without calcium and magnesium ions (Corning, USA), filtered through a 0.1 μm pore size membrane (Millipore, USA) to eliminate background noise and nanoparticles, was used for sample preparation and as the staining buffer. Appropriate isotype controls (mouse IgG1-PE, mouse IgG1-APC, and mouse IgM-Pacific Blue; BioLegend, USA) were included to define nonspecific binding and confirm negligible background fluorescence across all channels.

The gating strategy used for the identification of TF^+MPs and TF^+MP subpopulations is shown in Supplementary Figures S1 and S2. The microparticle gate was established using size-calibration beads (Megamix-Plus SSC, BioCytex, Marseille, France) and then applied to the analyzed samples. Unstained samples were used to assess the background signal and to define the threshold for TF positivity in the APC channel. TF^+MPs were identified as APC-positive events within the predefined microparticle gate. Within the TF^+MP gate, subpopulations were further identified according to co-expression of CD14, CD66b, or CD144. Representative histograms from stained samples, unstained samples, and isotype controls were used to define marker-positive events and nonspecific fluorescence. Flow cytometry data were analyzed using Kaluza Analysis software, version 1.3.

Both the total number of TF^+MPs and selected TF^+ MP subpopulations were analyzed, including neutrophil-derived TF^+MPs (TF^+CD66b^+), monocyte-derived TF^+MPs (TF^+CD14^+), and endothelial-derived TF^+MPs (TF^+CD144^+).

In addition, the percentages of neutrophil-, monocyte-, and endothelial-derived microparticles relative to total TF^+MPs were calculated as follows:

$$\text{Neu\%} = \frac{TF^+CD66b^+}{TF^+MPs} \times 100\%;\ \text{Mono\%} = \frac{TF^+CD14^+}{TF^+MPs} \times 100\%;\quad \text{Endo\%} = \frac{TF^+CD144^+}{TF^+MPs} \times 100\%,$$

Statistical analysis

Statistical analyses were performed using Statistica software, version 14.1 (TIBCO Software Inc., Palo Alto, CA, USA). The distribution of continuous variables was assessed using the Shapiro–Wilk test. As most variables demonstrated a non-normal distribution, data are presented as median with interquartile range (IQR).

Comparisons between two independent groups were performed using the Mann–Whitney U test. Effect size was estimated using Cliff's delta (δ), a nonparametric measure of the magnitude of between-group differences, with values interpreted as negligible ($|\delta| < 0.15$), small ($0.15 \leq |\delta| < 0.33$), medium ($0.33 \leq |\delta| < 0.47$), or large ($|\delta| \geq 0.47$) [20]. Associations between TF^+ MP levels and clinical or biochemical parameters were assessed using Spearman's rank correlation coefficient. All tests were two-tailed, and a *P* value <0.05 was considered statistically significant.

The discriminative performance of selected TF^+MP subpopulation percentages was evaluated using receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) with 95% confidence intervals was calculated. Optimal cut-off values were determined using the Youden index, and corresponding sensitivity and specificity values were calculated at the Youden-derived cut-off.

Linear regression analyses were performed in women with GDM to identify clinical and biochemical determinants of neutrophil-derived TF^+MP levels. Due to skewed distributions, TF^+CD66b^+ microparticle counts and IL-18 concentrations were log-transformed prior to analysis. Univariable linear regression models were initially constructed, followed by

multivariable linear regression including HbA1c and IL-18 as independent variables. Model assumptions were assessed, and standardized regression coefficients (β) were reported.

Results

Study population

The clinical and biochemical characteristics of the study participants are presented in Table 1. The GDM and control groups were comparable in terms of gestational age at sampling and pre-pregnancy BMI. FPG concentrations measured at blood collection did not differ significantly between groups, indicating comparable glycemic status at the time of microparticle analysis. In contrast, circulating IL-18 concentrations differed significantly between the GDM and control groups (P <0.001).

Circulating microparticle profiles

Analysis of circulating TF^+MPs revealed marked differences between women with GDM and healthy pregnant controls (Figures 1-2). Despite a significantly lower total count of circulating TF^+MPs in the GDM group compared with controls [3107 (1780–5053) vs. 4080 (2823-5876) per μl; P = 0.02; Cliff's δ = −0.27], the relative contribution of leukocyte-derived TF^+MP subpopulations was substantially altered.

TF^+CD66b^+ MPs were markedly increased in women with GDM, both in absolute counts [481.00 (173.33-832.00) vs. 86.67 (39.00-130.00) per μl] and as Neu% [16.29 (7.37-25.95)% vs. 1.95 (1.07-2.80)%] (Figures 1-2), with large effect sizes for both comparisons (P <0.001; δ = 0.71 and δ = 0.81, respectively). TF^+CD14^+ MPs were also significantly elevated in GDM, both in absolute counts [270.83 (130.00-606.67) vs. 138.67 (60.67-303.33) per μl] and as Mono% [10.16 (4.25-16.67)% vs. 3.24 (1.89-6.11)%] (Figures 1-2), with medium to large effect sizes (P = 0.001; δ = 0.34 and P <0.001; δ = 0.54, respectively).

In contrast, TF^+CD144^+ MPs did not differ significantly between groups in absolute counts [881.83 (329.33-1525.33) vs. 752.63 (334.29-1144.00) per μl; $P = 0.29$; $\delta = 0.11$]; however, Endo% was significantly higher in the GDM group [29.47 (16.17-38.18)% vs. 17.44 (12.83-23.60)%; $P < 0.001$; $\delta = 0.43$].

Influence of glycemic control strategy

Within the GDM group, TF^+MPs profiles did not differ according to the method of glycemic control. No significant differences were observed in total TF^+MPs or in any TF^+MP subpopulation between women treated with diet alone and those requiring insulin therapy (all $P > 0.05$).

Correlations with clinical and metabolic parameters

No significant correlations were observed between circulating TF^+MP counts or the percentages of TF^+MP subpopulations and pre-pregnancy BMI, FPG, or gestational age (all $R < 0.3$; all $P > 0.05$).

In women with GDM, HbA1c correlated positively with all analyzed TF^+MP counts and percentages, with the strongest association observed for TF^+CD66b^+ MPs. This neutrophil-derived subpopulation also demonstrated a weaker, yet statistically significant, positive correlation with IL-18 (Figure 3). Total TF^+MP levels correlated positively with leukocyte-derived TF^+MP subpopulation counts, whereas the percentages of these subpopulations showed only weak or negligible correlations with total TF^+MP counts (all $R < 0.4$, $P > 0.05$).

Multivariable linear regression analysis

Regression analyses were performed within the GDM group. Both HbA1c and IL-18 were independently associated with log-transformed TF^+CD66b^+ counts. The overall model explained 34% of the variance in TF^+CD66b^+ MP counts ($R^2 = 0.34$), with HbA1c emerging as the stronger predictor (standardized $\beta = 0.49$, $P < 0.001$), while IL-18 also remained a significant contributor (standardized $\beta = 0.23$, $P = 0.03$).

Group separation (ROC analysis)

Receiver operating characteristic (ROC) curve analysis was performed to quantify group separation based on selected TF^+MP subpopulation percentages between women with GDM and healthy pregnant controls (Figure 4). Among the analyzed markers, Neu% demonstrated the highest group separation, followed by Mono%, whereas Endo% showed limited, yet statistically significant, group separation. Detailed ROC characteristics, including AUC values, optimal cut-off points, and corresponding sensitivity and specificity estimates, are presented in Table 2.

Discussion

The present study demonstrates a marked reorganization of the circulating tissue factor-positive microparticle (TF^+MP) pool in women with GDM.

Although the total number of circulating TF^+MPs was significantly lower in GDM compared with healthy pregnant controls (3107 vs 4080 per µl; P =0.02; δ= -0.27), their cellular origin was shifted toward leukocyte-derived subpopulations, most notably neutrophils. This redistribution suggests qualitative rather than quantitative alterations in the TF^+MP compartment.

Both diabetes and pregnancy are recognized prothrombotic states, and numerous studies have reported increased numbers of circulating microparticles or related extracellular vesicles in these conditions [21,22,23,24]. In type 2 diabetes and gestational diabetes, elevated numbers of circulating vesicles have been linked to chronic inflammation, metabolic dysregulation, and vascular alterations [23,25]. However, experimental and clinical evidence indicates that the thrombogenic relevance of circulating microparticles depends not solely on their abundance, but critically on their cellular origin and molecular cargo [9,25].

In particular, leukocyte-derived TF^+MPs have been shown to exert a disproportionate procoagulant influence compared with vesicles derived from endothelial cells or platelets [9,11]. Accordingly, a reduced total TF^+MP pool does not contradict a prothrombotic phenotype when TF antigen is selectively redistributed toward leukocyte-derived vesicles [26,27].

More than one mechanism may contribute to the lower total count of circulating TF^+MPs observed in GDM, including selective enrichment of leukocyte-derived vesicles [27], altered clearance [28], and changes in vesiculation dynamics under conditions of metabolic and inflammatory stress [29].

Among the observed changes, enrichment of TF^+CD66b^+ MPs was the most pronounced. TF^+CD66b^+ MPs were markedly increased in women with GDM (P <0.001), with a particularly large effect size observed for Neu% ($\delta = 0.81$). Activated neutrophils can express tissue factor, release TF-bearing microparticles, and amplify thrombin generation through TF-FVIIa-FXa signaling pathways, with downstream activation of protease-activated receptor signaling on endothelial cells and leukocytes [30,31]. Neutrophils are increasingly recognized as central players in immunothrombosis [32], a process in which innate immune activation and coagulation are tightly coupled [33]. Neutrophil-driven prothrombotic features, including elevated markers related to neutrophil extracellular trap formation, have been documented in clinical cardiovascular settings and are linked to enhanced thrombotic potential [34].

Pregnancy itself is associated with physiological neutrophil priming, but GDM appears to exaggerate this response [35]. Prior studies have documented increased neutrophil activation markers, altered neutrophil-to-lymphocyte ratios, and enhanced neutrophil extracellular trap formation in GDM, even in the absence of overt hyperglycemia [35,36]. Our data extend these observations by identifying neutrophil-derived TF^+MPs as a dominant circulating TF compartment in GDM.

In addition to neutrophils, monocytes are a major inducible source of circulating tissue factor under inflammatory conditions [37]. Although TF^+CD14^+ MPs have not been extensively characterized in gestational diabetes, their presence is consistent with the established role of monocytes in linking inflammatory and metabolic signals to coagulation. The medium effect size observed for TF^+CD14^+ MPs in the present study ($\delta = 0.34$) may reflect effective metabolic control in this cohort, rather than the absence of monocyte involvement. Importantly, the increased Mono% (median 10.16 vs 3.24; P <0.001; δ=0.54) suggests that monocytes participate in the qualitative remodeling of the TF^+MP pool in GDM, with a large effect size for the relative contribution of this subpopulation.

Absolute counts of TF^+CD144^+ MPs did not differ significantly between groups (881.83 vs 752.63 per µl; $P = 0.29$; δ=0.11), suggesting that overt endothelial damage is not a dominant feature of GDM at this stage of pregnancy. However, the higher Endo% (29.47 vs 17.44; P <0.001; $\delta = 0.43$) indicates that TF^+CD144^+ MPs make up a greater proportion of the total TF^+MP population in GDM.

This finding is consistent with previous reports showing preserved or only mildly altered endothelial MP levels in well-controlled GDM [23], in contrast to the marked endothelial MP elevations observed in preeclampsia or overt vascular disease [38]. It supports the concept of functional endothelial activation rather than structural injury [39].

Notably, TF^+MP profiles were not associated with BMI or FPG, and did not differ according to the method of glycemic control (all R <0.3; all P >0.05). Similar findings have been reported for inflammatory and coagulation markers in GDM [40,41,42], suggesting that immune-coagulation interactions may persist independently of short-term glycemic control. The lack of differences between diet-treated and insulin-treated women suggests that the observed TF^+MP alterations are related to intrinsic disease biology rather than treatment intensity, and may reflect early aspects of GDM pathophysiology that persist despite metabolic control.

In contrast, TF^+CD66b^+ MP counts were associated with HbA1c and IL-18 concentrations, and multivariable linear regression analysis confirmed that both associations were independent. These findings suggest that cumulative metabolic and inflammatory stress, rather than short-term metabolic fluctuations, is more relevant for neutrophil-driven thromboinflammatory activation in GDM. Chronic hyperglycemia may predispose neutrophils to a prothrombotic and proinflammatory phenotype through oxidative stress [43], altered intracellular signaling [44], and epigenetic reprogramming [45]. In parallel, IL-18 acts as an amplifier of innate immune activation, promoting neutrophil priming and cytokine-driven crosstalk with the coagulation system [16]. These data support a model in which glycemic stress and inflammation act together as complementary drivers of TF^+CD66b^+ MP release in GDM.

Among the analyzed parameters, the Neu% demonstrated marked group separation between women with GDM and healthy controls (AUC = 0.905, 95% CI: 0.847-0.962), reflecting a pronounced shift in the cellular distribution of TF^+MPs. This finding highlights the potential of TF^+MP subpopulation percentages as integrative indicators of thromboinflammatory activation rather than isolated metabolic disturbances. The use of percentages may be particularly advantageous in MP research, as it reduces methodological variability and emphasizes biologically relevant changes in cellular contribution. Although clinical application is premature, further longitudinal and mechanistic studies are needed to clarify the role of TF^+CD66b^+ MPs in GDM.

Limitations

Several limitations of this study should be acknowledged. First, the cross-sectional design and the single time point of sampling preclude conclusions regarding temporal changes in microparticle profiles during pregnancy or their relationship to disease progression. Second, microparticle characterization was based on surface antigen expression and did not include direct assessment of tissue factor activity or other functional properties, limiting insight into the

biological activity of the detected vesicles. Accordingly, further studies incorporating functional assays will be required to determine the pathophysiological relevance of TF^+MPs in gestational diabetes mellitus. Third, inflammatory assessment was limited to IL-18, which was selected a priori because of its mechanistic link with metabolic stress and neutrophil activation. We did not perform broad cytokine profiling; therefore, the relationships between TF^+MPs and other inflammatory pathways require further investigation. Previous pregnancy complications were not systematically analyzed; therefore, we cannot exclude a potential influence of obstetric history on the current MP profile. Finally, the study population was relatively homogeneous, which may limit the generalizability of the findings to other clinical settings or populations with different metabolic or inflammatory profiles.

Conclusions

Gestational diabetes mellitus is associated with a marked qualitative shift in circulating tissue factor-positive microparticles, characterized by enrichment of leukocyte-derived, particularly TF^+CD66b^+ MPs despite reduced total TF^+MP counts. These findings support the concept of GDM as a thromboinflammatory state driven by selective immune cell activation and altered cellular distribution of tissue factor. In this context, TF^+CD66b^+ MPs may represent a link between inflammation and coagulation in GDM and warrant further functional and longitudinal investigation.

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involved in patient recruitment. BS reviewed the manuscript. All authors approved the final version of the manuscript.

AI statement: Artificial intelligence was not used in the preparation of this manuscript.

References

1 International Association of Diabetes and Pregnancy Study Groups Recommendations on the Diagnosis and Classification of Hyperglycemia in Pregnancy. Diabetes Care. 2010; 33: 676-682.

2 Sibiak R, Iciek R, Gastolek K, et al. Ten-year national trends in the prevalence and outcomes of gestational diabetes mellitus in Poland: a population-based study. Pol Arch Intern Med. 2026;

3 Matutinovic MS, Zeljkovic A, Vekic J, et al. Predictive analysis of lipidome patterns in gestational diabetes mellitus risk assessment. Pol Arch Intern Med. 2025;

4 Xuan Nguyen K, Bui Minh T, Dinh HT, et al. Low-Grade Inflammation in Gestational Diabetes Mellitus and Its Correlation with Maternal Insulin Resistance and Fetal Growth Indices. Int J Gen Med. 2023; Volume 16: 1429-1436.

5 Shehzad S, Shabbir NA, Mumtaz M, et al. Gestational Diabetes and Cardiovascular Risk in Women: A Comprehensive Review of Thrombosis, Hemostasis, and Emerging Insights Into Coagulation and Fibrinolysis. Cureus. 2025;

6 Petroni D, Fabbri C, Babboni S, et al. Extracellular Vesicles and Intercellular Communication: Challenges for In Vivo Molecular Imaging and Tracking. Pharmaceutics. 2023; 15: 1639.

7 Budaj M, Poljak Z, Ďuriš I, et al. Microparticles: a component of various diseases. Pol Arch Intern Med. 2012; 122: 24-29.

8 Pawlica-Gosiewska D, Gawlik K, Owczarek D, et al. Circulating microparticles and platelet-derived growth factor in Crohn's disease: indicators of endothelial damage and disease activity. Pol Arch Intern Med. 2025;

9 Zwicker JI, Trenor CC, Furie BC, Furie B. Tissue Factor–Bearing Microparticles and Thrombus Formation. Arterioscler Thromb Vasc Biol. 2011; 31: 728-733.

10 Key NS. Analysis of tissue factor positive microparticles. Thromb Res. 2010; 125: S42-S45.

11 Owens AP, Mackman N. Microparticles in Hemostasis and Thrombosis. Circ Res. 2011; 108: 1284-1297.

12 Lugo-Gavidia LM, Burger D, Matthews VB, et al. Role of Microparticles in Cardiovascular Disease: Implications for Endothelial Dysfunction, Thrombosis, and Inflammation. Hypertension. 2021; 77: 1825-1844.

13 Vinturache A, Khalil A. Maternal Physiological Changes in Pregnancy. The Global Library of Women's Medicine. 2021;

14 Mittal R, Prasad K, Lemos JRN, et al. Unveiling Gestational Diabetes: An Overview of Pathophysiology and Management. Int J Mol Sci. 2025; 26: 2320.

15 Li H, Shan W, Zhao X, Sun W. Neutrophils: Linking Inflammation to Thrombosis and Unlocking New Treatment Horizons. Int J Mol Sci. 2025; 26: 1965.

16 Leung BP, Culshaw S, Gracie JA, et al. A Role for IL-18 in Neutrophil Activation. The Journal of Immunology. 2001; 167: 2879-2886.

17 Han N, Yuan Z, Zhao H, et al. Relationship between serum NLRP3 along with its effector molecules and pregnancy outcomes in women with hyperglycemia. The Journal of Maternal-Fetal & Neonatal Medicine. 2024; 37.

18 Horstman LL. Endothelial microparticles as markers of endothelial dysfunction. Frontiers in Bioscience. 2004; 9: 1118.

19 Zahran A, Sayed S, Abd El Hafeez HA, et al. <p>Circulating microparticle subpopulation in metabolic syndrome: relation to oxidative stress and coagulation markers. Diabetes Metab Syndr Obes. 2019; Volume 12: 485-493.

20 Meissel K, Yao ES. Using Cliff's Delta as a Non-Parametric Effect Size Measure: An Accessible Web App and R Tutorial . Practical Assessment, Research, and Evaluation. 2024; 29: 2-2.

21 Rodrigues KF, Pietrani NT, Fernandes AP, et al. Circulating microparticles levels are increased in patients with diabetic kidney disease: A case-control research. Clinica Chimica Acta. 2018; 479: 48-55.

22 James-Allan LB, Devaskar SU. Extracellular vesicles and their role in gestational diabetes mellitus. Placenta. 2021; 113: 15-22.

23 Bathla T, Abolbaghaei A, Reyes AB, Burger D. Extracellular vesicles in gestational diabetes mellitus: A scoping review. Diab Vasc Dis Res. 2022; 19.

24 Li S, Wei J, Zhang C, et al. Cell-Derived Microparticles in Patients with Type 2 Diabetes Mellitus: a Systematic Review and Meta-Analysis. Cellular Physiology and Biochemistry. 2016; 39: 2439-2450.

25 Liu Z, Yin R, Tian J. Extracellular vesicles: mechanisms and prospects in type 2 diabetes and its complications. Front Endocrinol (Lausanne). 2025; 15.

26 Mackman N, Bergmeier W, Stouffer GA, Weitz JI. Therapeutic strategies for thrombosis: new targets and approaches. Nat Rev Drug Discov. 2020; 19: 333-352.

27 Zifkos K, Dubois C, Schäfer K. Extracellular Vesicles and Thrombosis: Update on the Clinical and Experimental Evidence. Int J Mol Sci. 2021; 22: 9317.

28 Nakahara A, Nair S, Ormazabal V, et al. Circulating Placental Extracellular Vesicles and Their Potential Roles During Pregnancy. Ochsner Journal. 2020; 20: 439-445.

29 He Y, Wu Q. The Effect of Extracellular Vesicles on Thrombosis. J Cardiovasc Transl Res. 2023; 16: 682-697.

30 Fuchs TA, Brill A, Duerschmied D, et al. Extracellular DNA traps promote thrombosis. Proceedings of the National Academy of Sciences. 2010; 107: 15880-15885.

31 Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. Nat Rev Immunol. 2013; 13: 34-45.

32 De Nardi AC, Coy-Canguçu A, Saito A, et al. Immunothrombosis and its underlying biological mechanisms. Hematol Transfus Cell Ther. 2024; 46: 49-57.

33 Perdomo J, Leung HHL. Immune Thrombosis: Exploring the Significance of Immune Complexes and NETosis. Biology (Basel). 2023; 12: 1332.

34 Stępień K, Natorska J, Ząbczyk M, et al. High-dose atorvastatin and rosuvastatin reduce neutrophil extracellular traps-related proteins in coronary artery disease: association with prothrombotic state. Pol Arch Intern Med. 2024;

35 Pace NP, Vassallo J. Association Between Neutrophil-Lymphocyte Ratio and Gestational Diabetes—A Systematic Review and Meta-Analysis. J Endocr Soc. 2021; 5.

36 Stoikou M, Grimolizzi F, Giaglis S, et al. Gestational Diabetes Mellitus Is Associated with Altered Neutrophil Activity. Front Immunol. 2017; 8.

37 Østerud B, Bjørklid E. Sources of Tissue Factor. Semin Thromb Hemost. 2006; 32: 011-023.

38 González-Quintero VH, Smarkusky LP, Jiménez JJ, et al. Elevated plasma endothelial microparticles: Preeclampsia versus gestational hypertension. Am J Obstet Gynecol. 2004; 191: 1418-1424.

39 McElwain CJ, Tuboly E, McCarthy FP, McCarthy CM. Mechanisms of Endothelial Dysfunction in Pre-eclampsia and Gestational Diabetes Mellitus: Windows Into Future Cardiometabolic Health? Front Endocrinol (Lausanne). 2020; 11.

40 Wolf M, Sandler L, Hsu K, et al. First-Trimester C-Reactive Protein and Subsequent Gestational Diabetes. Diabetes Care. 2003; 26: 819-824.

41 Levent Cirakli Z, Gulec N. Evaluation of White Blood-cell-based Inflammatory Markers in Gestational Diabetes Mellitus. Bakirkoy Tip Dergisi / Medical Journal of Bakirkoy. 2022; 18: 157-163.

42 Gorar S, Alioglu B, Ademoglu E, et al. Is There a Tendency for Thrombosis in Gestational Diabetes Mellitus? J Lab Physicians. 2016; 8: 101-105.

43 Wang L, Zhou X, Yin Y, et al. Hyperglycemia Induces Neutrophil Extracellular Traps Formation Through an NADPH Oxidase-Dependent Pathway in Diabetic Retinopathy. Front Immunol. 2019; 9.

44 Omori K, Ohira T, Uchida Y, et al. Priming of neutrophil oxidative burst in diabetes requires preassembly of the NADPH oxidase. J Leukoc Biol. 2008; 84: 292-301.

45 Shrestha S, Lee Y-B, Lee H, et al. Diabetes Primes Neutrophils for Neutrophil Extracellular Trap Formation through Trained Immunity. Research. 2024; 7.

Table 1. Clinical and biochemical characteristics of the study population.

Variable	**GDM (n=70)**	**Controls (n =55)**	***P* value**
Gestational age (weeks)	31 (29-33)	31 (30-32)	0.45
Pre-pregnancy BMI (kg/m²)	24 (21-28)	23 (20-26)	0.07
FPG (mmol/l)	4.56 (4.29-4.85)	4.58 (4.33-4.74)	0.89
HbA1c (mmol/mol)	32 (26-36)	NA	—
IL-18 (ng/l)	175 (93-211)	88 (57-139)	<0.001

Data are presented as median (interquartile range). *P* values were derived using the Mann-Whitney U test. BMI, body mass index; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; HbA1c, glycated hemoglobin; IL-18, interleukin-18; NA, not available.

Table 2. ROC curve analysis of TF^+MPs subpopulation percentages for the discrimination of GDM from healthy controls.

Parameter	**AUC (95% CI)**	***P* value**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**
Neu%	0.905 (0.847-0.962)	<0.001	4.85	84.3	94.5
Mono%	0.758 (0.674-0.843)	<0.001	8.01	58.6	87.3
Endo%	0.707 (0.615-0.799)	<0.001	26.6	57.1	89.1

Optimal cut-off values were determined using the Youden index. Sensitivity and specificity were calculated at the Youden-derived cut-off. AUC, area under the curve; CI, confidence interval; Endo%, percentage of endothelial-derived TF^+MPs; GDM, gestational diabetes mellitus; Mono%, percentage of monocyte-derived TF^+MPs; Neu%, percentage of neutrophil-derived TF^+MPs; ROC, receiver operating characteristic; TF^+MPs, tissue factor-positive microparticles.

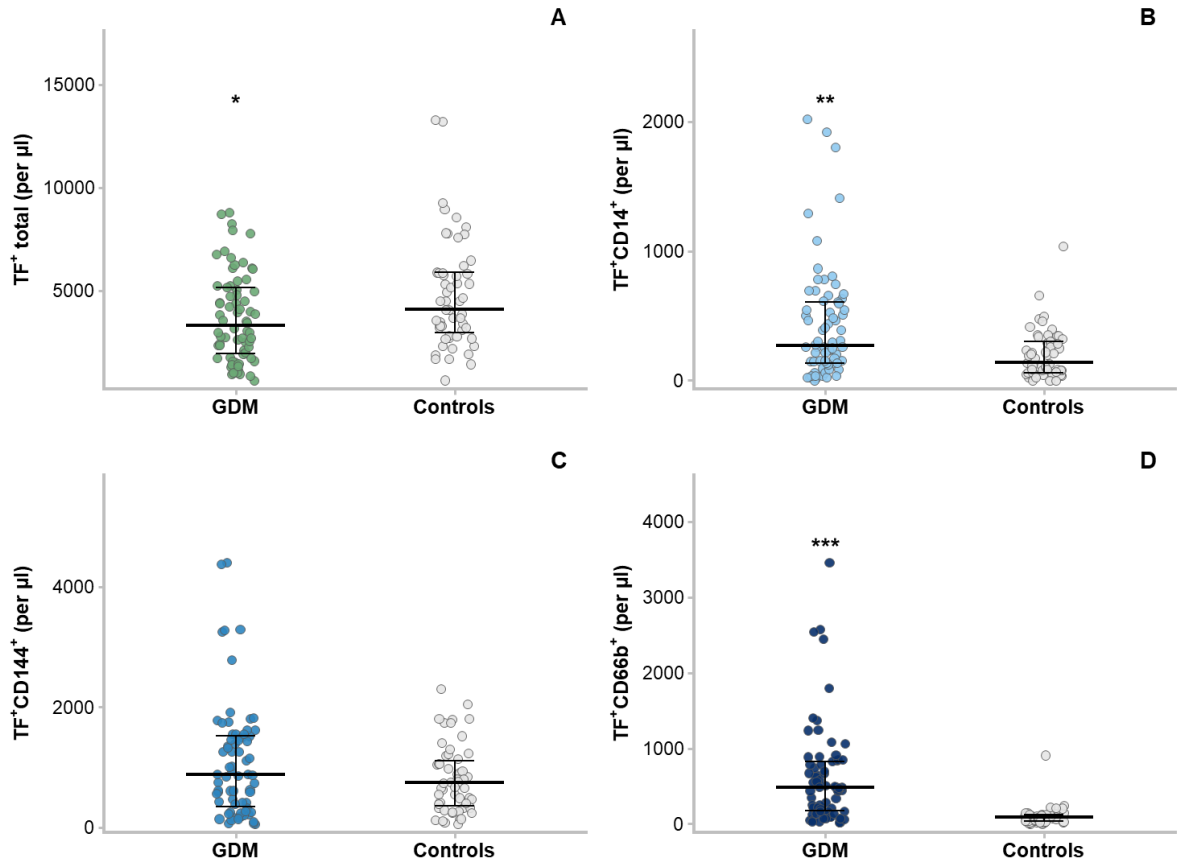

Figure 1. Absolute counts of circulating TF$^+$ microparticles in women with GDM and healthy pregnant controls

Absolute counts of circulating TF$^+$ microparticles are shown for (A) total TF$^+$MPs, (B) TF$^+$CD14$^+$ MPs, (C) TF$^+$CD144$^+$ MPs, and (D) TF$^+$CD66b$^+$ MPs in women with GDM and healthy pregnant controls. Dots represent individual study participants; horizontal bars indicate medians with interquartile ranges. Controls are shown in grey, whereas GDM is shown in green for total TF$^+$MPs and in blue tones for TF$^+$MP subpopulations. *P <0.05, **P <0.01, ***P <0.001.

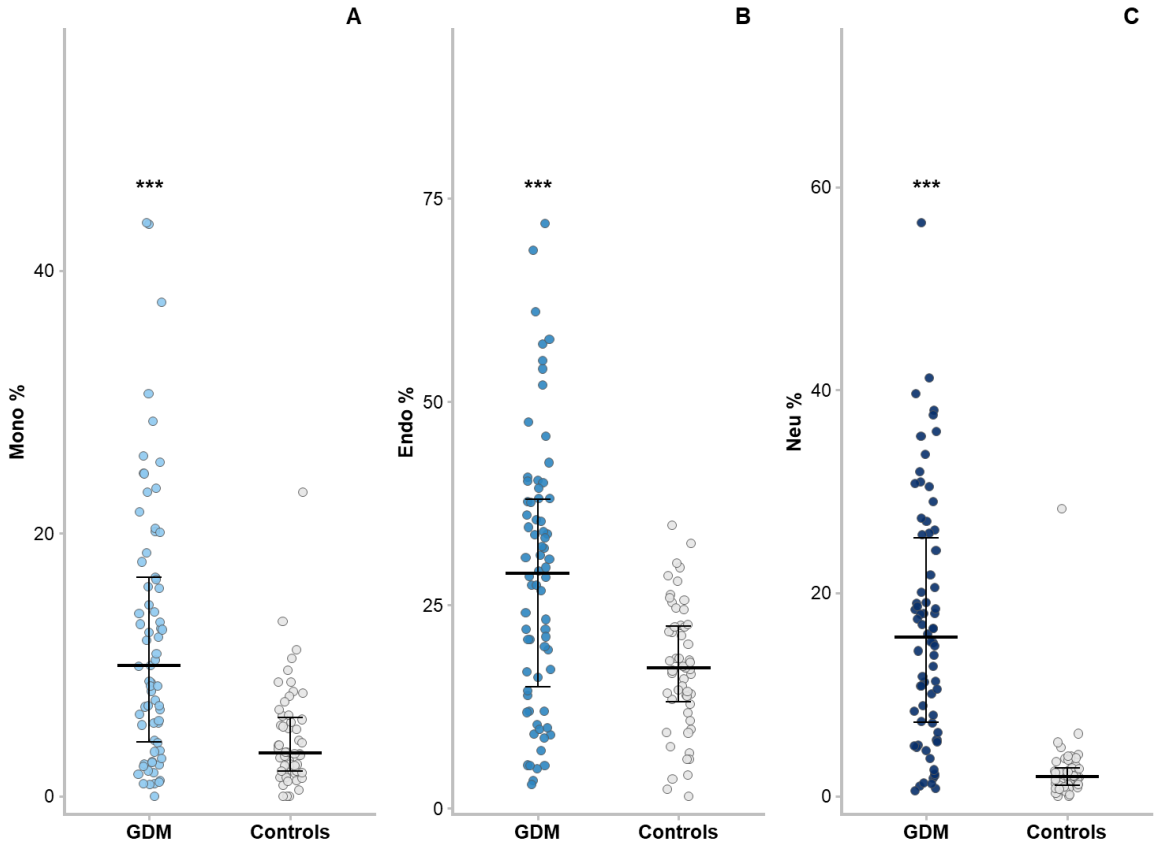

Figure 2. Percentages of circulating TF$^+$MP subpopulations in women with GDM and healthy pregnant controls

Percentages of circulating TF$^+$MP subpopulations relative to total TF$^+$MPs are shown for (A) TF$^+$CD14$^+$ MPs (Mono%), (B) TF$^+$CD144$^+$ MPs (Endo%), and (C) TF$^+$CD66b$^+$ MPs (Neu%) in women with GDM and healthy pregnant controls. Dots represent individual study participants; horizontal bars indicate medians with interquartile ranges. Controls are shown in grey, whereas GDM subpopulations are shown in blue tones. ***P <0.001.

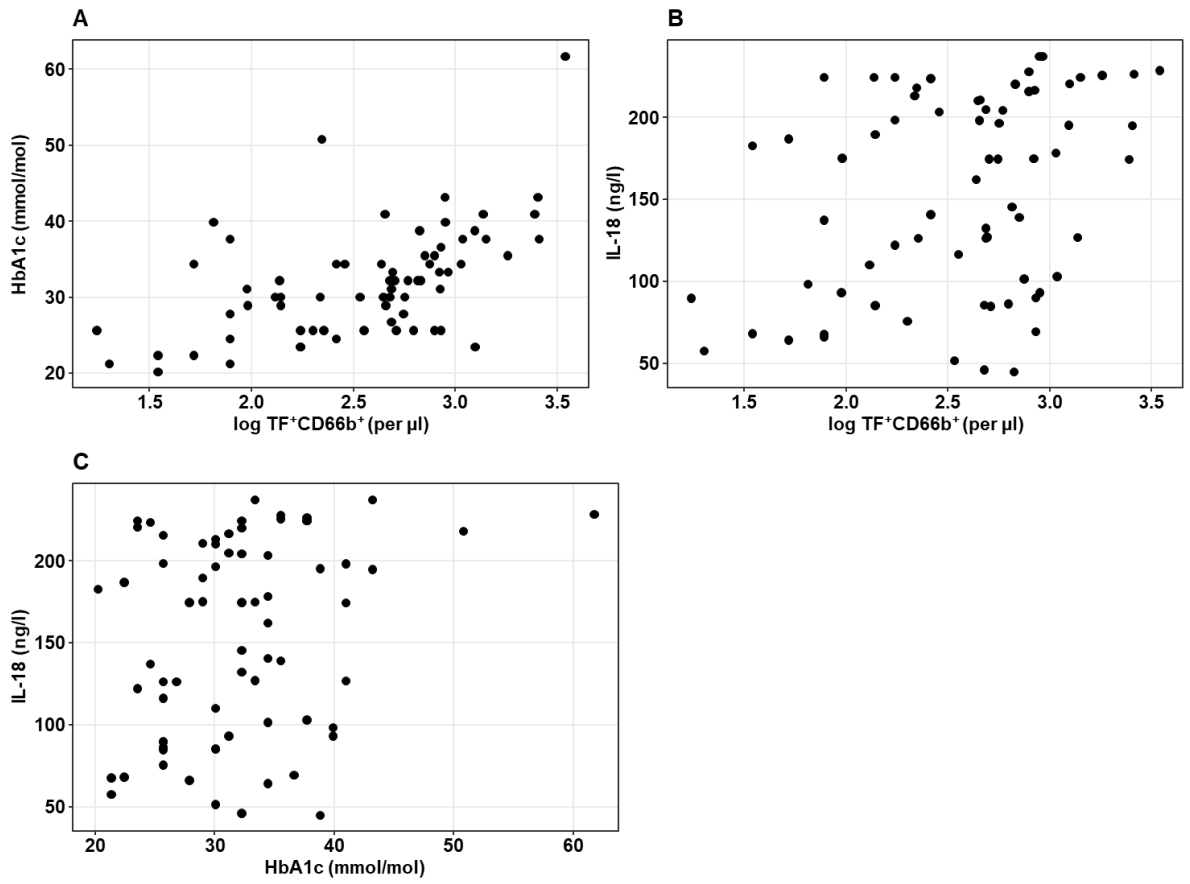

Figure 3. Correlations between neutrophil-derived TF$^+$MPs, HbA1c, and IL-18 in women with gestational diabetes mellitus

Scatter plots illustrating correlations between neutrophil-derived TF$^+$MPs (TF$^+$CD66b$^+$) and (A) glycated hemoglobin (HbA1c), (B) interleukin-18 (IL-18), and (C) the relationship between HbA1c and IL-18. All analyses were performed in women with GDM. TF$^+$CD66b$^+$ MP counts are presented on a logarithmic ($\log_{10}$) scale for visualization purposes only. Correlations were assessed using Spearman's rank correlation coefficient. Spearman's correlation coefficients and *P* values were as follows: (A) $R = 0.56$, $P < 0.001$; (B) $R = 0.34$, $P = 0.004$; (C) $R = 0.25$, $P = 0.04$.

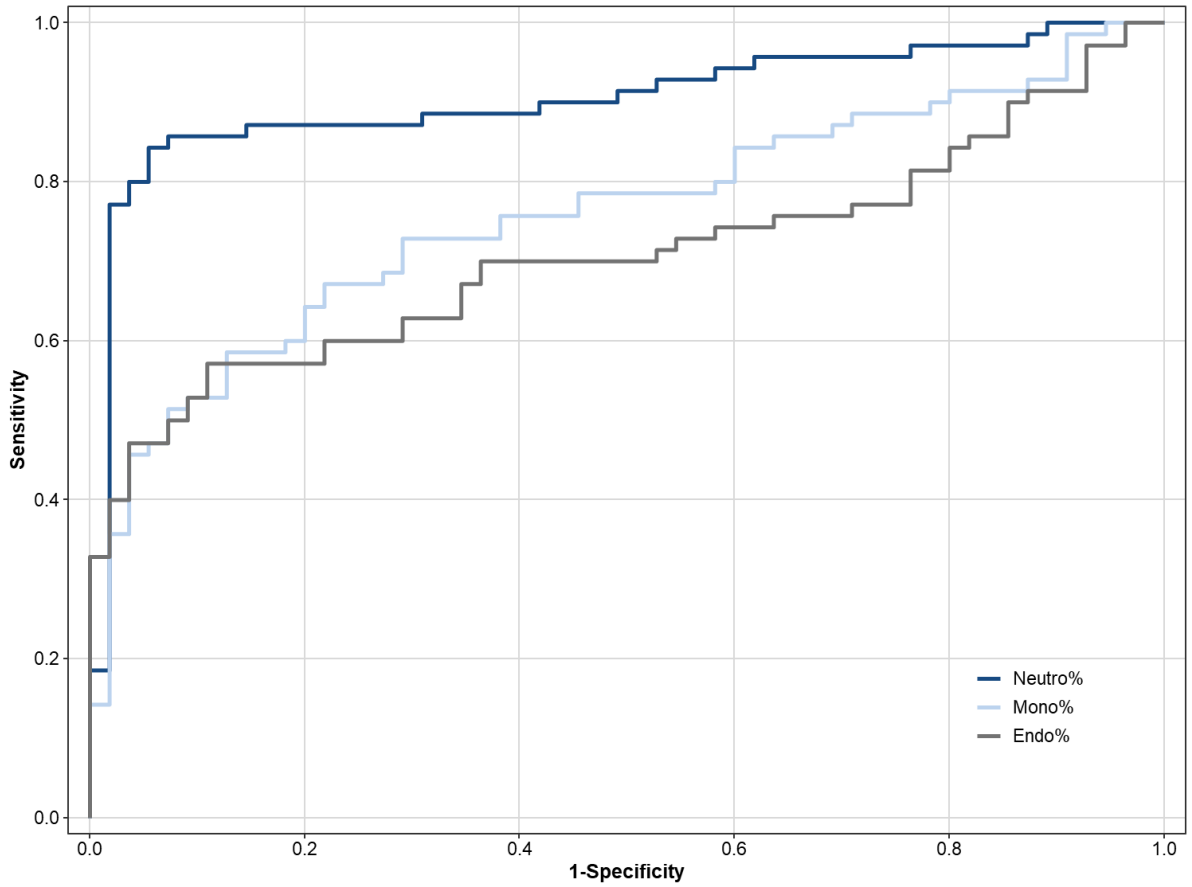

Figure 4. Receiver operating characteristic (ROC) curves illustrating the separation between women with GDM and healthy pregnant controls based on TF^{+}MP subpopulation percentages

Receiver operating characteristic (ROC) curves illustrating group separation based on selected TF^{+}MPs subpopulation percentages in women with GDM and healthy pregnant controls. ROC curves are shown for Neu%, Mono%, and Endo%. The diagonal line represents the reference line corresponding to no discrimination (AUC = 0.5). Corresponding AUC values are provided in Table 2.

Short title: Neutrophil-derived TF-positive microparticles in GDM