ВОЗМОЖНАЯ РОЛЬ ТРОМБОЦИТАРНО-МОНОЦИТАРНЫХ КОМПЛЕКСОВ В ПАТОГЕНЕЗЕ ПРИВЫЧНОГО НЕВЫНАШИВАНИЯ БЕРЕМЕННОСТИ

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Резюме. Привычное невынашивание беременности представляет собой существенную клиническую проблему, которая затрагивает 1-5% популяции, при этом в более чем половине случаев причина преждевременной потери беременности остается неизвестной. Одной из возможных причин является нарушение баланса в системе гемостаза матери, приводящее к тромбозу маточно-плацентарных сосудов, снижению перфузии плаценты и гипоксии. Изменения морфофункциональных свойств моноцитов и образуемых ими и активированными тромбоцитами агрегатов могут являться факторами, приводящими к различным осложнениям беременности. Однако роль тромбоцитарно-моноцитарных комплексов (ТМК) в патогенезе привычного невынашивания беременности практически не изучена. Целью настоящего исследования было определение количественных изменений в содержании и фенотипических характеристиках ТМК периферической крови при привычном невынашивании беременности, а также оценка влияния тромбоцитов на экспрессию поверхностных белков-моноцитов при физиологическом и патологическом развитии беременности. Исследуемые группы составили женщины с диагнозом «привычный выкидыш», имеющие беременность 6-12 недель, и женщины с неосложненной беременностью (7-12 недель). В общей популяции и субпопуляциях моноцитов периферической крови пациенток методами проточной цитофлуориметрии определяли содержание ТМК и уровни экспрессии поверхностных антигенов: CD62P, CD11b, CD86, CD162, HLA-DR, TREM-1. Установлено, что при привычном невынашивании уровень ТМК повышен (26,5%) в сравнении с беременностью, протекающей без осложнений (15,3%), и это повышение происходит с участием всех трех субпопуляций моноцитов: классических, промежуточных и неклассических. На уровне общей популяции моноцитов в ТМК отмечалось снижение экспрессии HLA-DR и повышение уровня экспрессии CD11b, тогда как экспрессия CD62P, CD162, CD86 и TREM-1 существенно не изменялась. Субпопуляции моноцитов вносили различный вклад в изменение уровней экспрессии активацион-

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ных маркеров моноцитов, связанное с привычным невынашиванием, и эти изменения не всегда проявлялись на уровне всей популяции моноцитов. Сравнение ТМК и свободных моноцитов показало, что изменения поверхностного фенотипа моноцитов в составе ТМК обусловлены как влиянием тромбоцитов, так и другими факторами. При привычном невынашивании наблюдалось индуцированное тромбоцитами усиление адгезионных свойств моноцитов, что проявлялось в повышении уровня экспрессии CD11b. В то же время снижение уровня экспрессии HLA-DR в моноцитах не было вызвано взаимодействием с тромбоцитами. Полученные результаты свидетельствуют о том, что привычное невынашивание беременности сопровождается повышением содержания ТМК в периферической крови и изменениями антигенного фенотипа моноцитов в составе ТМК, демонстрируют иммуномодуляторное влияние тромбоцитов, а также дают обоснования значимости определения паттернов экспрессии поверхностных антигенных маркеров ТМК в диагностических и терапевтических целях.

Ключевые слова: беременность, привычное невынашивание беременности, тромбоциты, моноциты, тромбоцитарно-моноцитарные комплексы, антигенный фенотип

POSSIBLE ROLE OF PLATELET-MONOCYTE COMPLEXES IN THE PATHOGENESIS OF RECURRENT PREGNANCY LOSS

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Abstract. Recurrent pregnancy loss (RPL) is a significant clinical problem that affects 1-5% of population. Moreover, the cause of RPL remains unknown in more than half of cases. Possible reasons include imbalanced maternal hemostasis, thrombosis of uteroplacental vessels, decreased placental perfusion and hypoxia. Changes in morphofunctional features of monocytes and platelet-monocyte aggregates may be the factors causing pregnancy complications. However, role of platelet-monocyte complexes (PMC) in pathogenesis of RPL is unknown. The purpose of our study was to determine quantitative changes in contents and antigenic phenotype of PMCs in peripheral blood of the patients with RPL, and to assess the effect of platelets on the expression of monocyte surface proteins in normal and pathological pregnancy. The study groups consisted of 6 to12-week pregnant women diagnosed with RPL and females with uncomplicated pregnancy (7-12 weeks). PMC content and expression of CD62P, CD11b, CD86, CD162, HLA-DR, TREM-1 were determined in total cell population and subpopulations of peripheral blood monocytes using cytofluorimetry technique. It was found that PMC level was increased in patients with RPL (26.5%) compared to uncomplicated pregnancy (15.3%) with all monocyte subpopulations contributing to this increase. Decrease in HLA-DR expression and increase in CD11b expression was observed in total PMCs, while expression of CD62P, CD162, CD86 and TREM-1 did not change significantly. Monocyte subpopulations were differently involved into the RPL-associated expression of activation markers, while the changes detected in distinct subpopulations were not always evident in total monocyte populations in recurrent pregnancy loss. A comparison of PMC and free monocytes demonstrated that changes in surface phenotype of monocytes were caused by platelets and other exogenous factors. In patients with RPL, we observed platelet-induced increase in adhesive properties of monocytes which manifested as increased CD11b expression. In contrast, decrease in monocyte HLA-DR levels was not caused by platelets. The results obtained suggest that RPL is accompanied by increased level of peripheral blood PMC and changes in antigenic profile of platelet-associated and free monocytes, thus demonstrating the immunomodulatory effect of platelets, and also confirming the importance of determining expression patterns of surface antigenic markers of PMC for diagnostic and therapeutic purposes.

Keywords: pregnancy, recurrent pregnancy loss, platelets, monocytes, platelet-monocyte complexes, antigenic phenotype

Introduction

Recurrent pregnancy loss (recurrent miscarriage) is a significant clinical problem that affects 1-5% of the population, with approximately 80% of pregnancy losses occurring before the 12th week of pregnancy [28]. Despite the progress made in studying this disease, more than half of cases are diagnosed as idiopathic miscarriage, the cause of which is

unknown [8]. In most cases of recurrent miscarriage, including those of unknown etiology, pathological changes in the uteroplacental complex are detected as thrombotic events and signs of inflammation [12]. One of the proposed pathophysiological mechanisms of pregnancy loss is an imbalance in the maternal hemostatic system, leading to thrombosis of the uteroplacental vessels, decreased placental perfusion and hypoxia. Maternal thrombophilia is considered as the cause of these changes, leading to obstetric disorders such as miscarriage, intrauterine growth retardation, intrauterine fetal death, preeclampsia [6, 31, 35]. Although doubts have been expressed about the existence of a cause-and-effect relationship between the thrombophilic condition and recurrent miscarriage [1], thrombophilia continues to be considered a risk factor for this disease [22].

Platelet-leukocyte complexes (PLC) are heterotypic cellular aggregates circulating in the peripheral blood. PLCs are a link between the hemostasis system and the immune system. Activation of platelets causes their degranulation, as a result of which P-selectin molecules (CD62P) are released from alpha granules onto the surface of the plasma membrane, forming a bond with the PSGL-1 ligand (CD162), which is constitutively expressed on the surface of leukocytes [34, 39, 43]. This connection is stabilized and strengthened by the interaction of other molecules on the surface of platelets and leukocytes, including MAC-1 (CD11b/CD18) [10, 36]. The crosstalk between platelets and leukocytes within PLC has a modulating effect on the functions of these cells, affecting their prothrombotic and proinflammatory properties respectively. Circulating PLC produce procoagulant, oxidative and mitogenic factors and can cause both capillary microembolism and arterial thrombosis [19].

The experimental data obtained indicate that PLC play a role in the pathogenesis of diseases characterized by thrombotic and inflammatory phenomena: cardiovascular diseases [3, 23, 44], ischemic stroke [18, 26, 37], diabetes mellitus [9, 13, 42], chronic obstructive pulmonary disease [2, 25], liver [32] and kidney [5, 11, 41] diseases, bacterial [20, 30, 40] and viral [16, 17, 21, 30] infections, pneumonia and thrombosis caused by the new coronavirus infection COVID-19 [7, 15, 38].

Until now, only one study was known in which changes in PLC content were determined in patients with recurrent pregnancy loss. The authors of this study found an increase in PLC levels in the peripheral blood of non-pregnant women with thrombophilia and previous history of recurrent miscarriage in the first trimester of pregnancy [24].

Platelet-monocyte complexes (PMC) are the most numerous and stable among the aggregates formed by platelets and leukocytes [33], and characterization of these complexes helps to identify their possible role in the development of various diseases, including reproductive pathologies. From this point of view, PMC are of interest both as a diagnostic marker and as a therapeutic target.

Peripheral blood monocytes are divided into three subpopulations: the most numerous subpopulation of classical monocytes ($CD14^{++}CD16^{-}$) and two minor fractions of intermediate ($CD14^{++}CD16^{+}$) and non-classical monocytes ($CD14^{+}CD16^{++}$) [45]. Subpopulations of monocytes differ functionally, exhibit different patterns of expression of surface antigenic markers, cytokines and chemokines, and each subpopulation may have a distinct role under physiological and pathological conditions [27]. It can be assumed that subpopulations of monocytes interact with platelets in various ways, and PMCs formed by different fractions of monocytes play different roles in the pathogenesis of recurrent pregnancy loss.

The interaction of activated platelet with monocytes can result not only in an increase in the number of circulating TMCs, but also in morphofunctional changes affecting all monocytes or individual subpopulations and leading to disturbance of physiological processes. However, these changes may be due to the influence of other physiological (pathophysiological) factors, and it is necessary to distinguish between these ways of modulating monocyte properties and functions.

The purpose of this study was to determine quantitative changes in the content and phenotypic characteristics of PMC in the total population and individual subpopulations of peripheral blood monocytes in patients with recurrent pregnancy loss, as well as a comparative assessment of the effect of platelets on the expression of surface marker proteins of monocytes during the physiological and pathological development of pregnancy.

Materials and methods

Participant groups comprise pregnant women (6-12 weeks of gestation) with a history of recurrent pregnancy loss (RPL group) and women with uncomplicated (normal) 7-12-week pregnancy (NP group). The age of the patients ranged from 24 to 42 years. According to clinical guidelines, a recurrent pregnancy loss was considered to be a history of two or more clinical pregnancy losses within 22 weeks of gestation [29].

Peripheral blood samples were obtained from the antecubital vein by puncture with a 21G needle. To exclude post-traumatic platelet aggregation, the first 3 ml of blood was discarded. Blood was collected into a vacutainer containing 3.8% sodium citrate).

Within 20 minutes of collection, 100 μL of whole blood was incubated with the following fluorochrome-conjugated monoclonal antibodies: anti-CD45-PerCP, anti-CD14-AlexaFluor® 700, anti-CD41a-APC, anti-CD16-PE-Cy7™, anti-CD62P-FITC, anti-CD86-FITC, anti-HLA-DR-FITC, anti-CD162-PE, anti-CD11b-PE, anti-TREM-1-PE

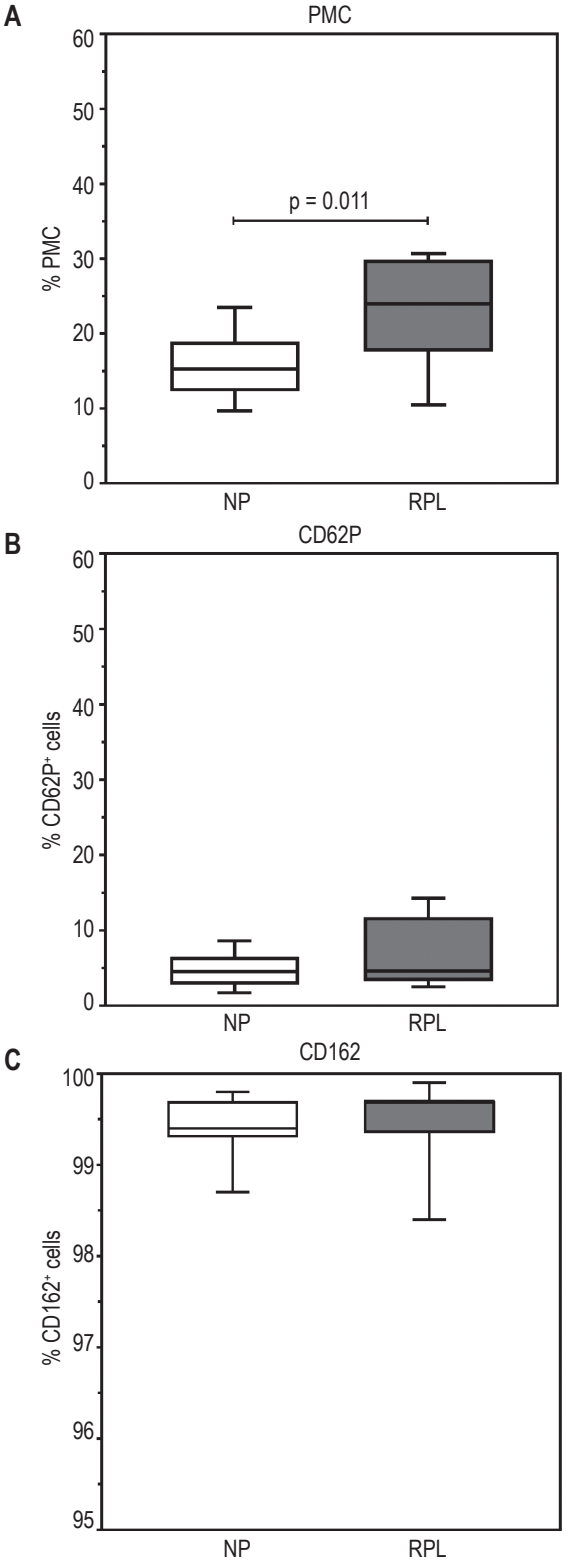

Figure 1. Characteristics of platelet-monocyte complexes in the total monocyte population

Note. Graphs demonstrate the proportion of platelet-monocyte complexes relative to the total monocytes in the peripheral blood (A) and the percentage of platelet-monocyte complexes expressing CD62P (B) and CD162 (C) in patients with normal pregnancy and recurrent pregnancy loss. Plots show the range, median, 25th and 75th percentile. NP, normal pregnancy; RPL, recurrent pregnancy loss.

(all – BD Biosciences, USA). Nonspecific antibodies labeled with appropriate fluorochromes were used as isotype control. The samples were incubated without stirring in the dark at room temperature for 20 minutes.Incubated for 20 min in the dark at room temperature. After incubation, erythrocytes were lysed by adding a tenfold volume of BD FACS™ Lysing Solution (BD Biosciences, USA). The samples were centrifuged (300 g, 5 min), the supernatant was collected, and the cell pellet was resuspended in 200 μL of BD FACS™Lysing Solution.

The samples were analyzed using a FACSCanto II flow cytometer (Becton Dickinson, USA). The obtained data were processed using FACSDiva software (BD Biosciences, USA). On a two-dimensional histogram with SSC/CD45 coordinates, a region corresponding to monocytes was identified, in which 20,000 events were analyzed. To identify free monocytes and PMC, the selected region was analyzed on a two-dimensional histogram with CD14/CD41a coordinates. Events with the $CD14^{+}CD41a^{-}$ phenotype were classified as free monocytes; PMC were defined as events with double positive $CD14^{+}CD41a^{+}$ staining. The expression of surface antigens was determined in each population using specific monoclonal antibodies.

To identify monocyte subpopulations, the corresponding region on the two-dimensional histogram SSC/CD45 was projected onto a two-dimensional CD14/CD16 histogram, where three monocyte fractions were identified: classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{++}$). Free monocytes and PMC were determined in each subpopulation by the absence or presence of CD41a, respectively, as well as the expression of surface antigen markers.

Statistical analysis was performed using the GraphPad Prism 8.0.1 statistical software package (Graph Pad Software Inc., San Diego, CA, USA). Variables were assessed for normality using the Shapiro–Wilk test. Parametric and nonparametric (Mann–Whitney) tests were used to assess differences between groups. Paired t-test and non-parametric Wilcoxon test were used to compare the phenotypic characteristics of PMC-associated and free monocytes. Statistical significance was assumed at $p < 0.05$.

Results

In the peripheral blood obtained from RPL group the proportion of PMC reached 26.5%, while in NP group PMC accounted for 15.3% (Figure 1A). PMC are formed primarily through the interaction of P-selectin (CD62), expressed by activated platelets, with the PSGL-1 ligand (CD162) on the surface of the monocyte. We did not find statistically significant changes in the number of PMC expressing CD62P and CD162 in patients with recurrent miscarriage.

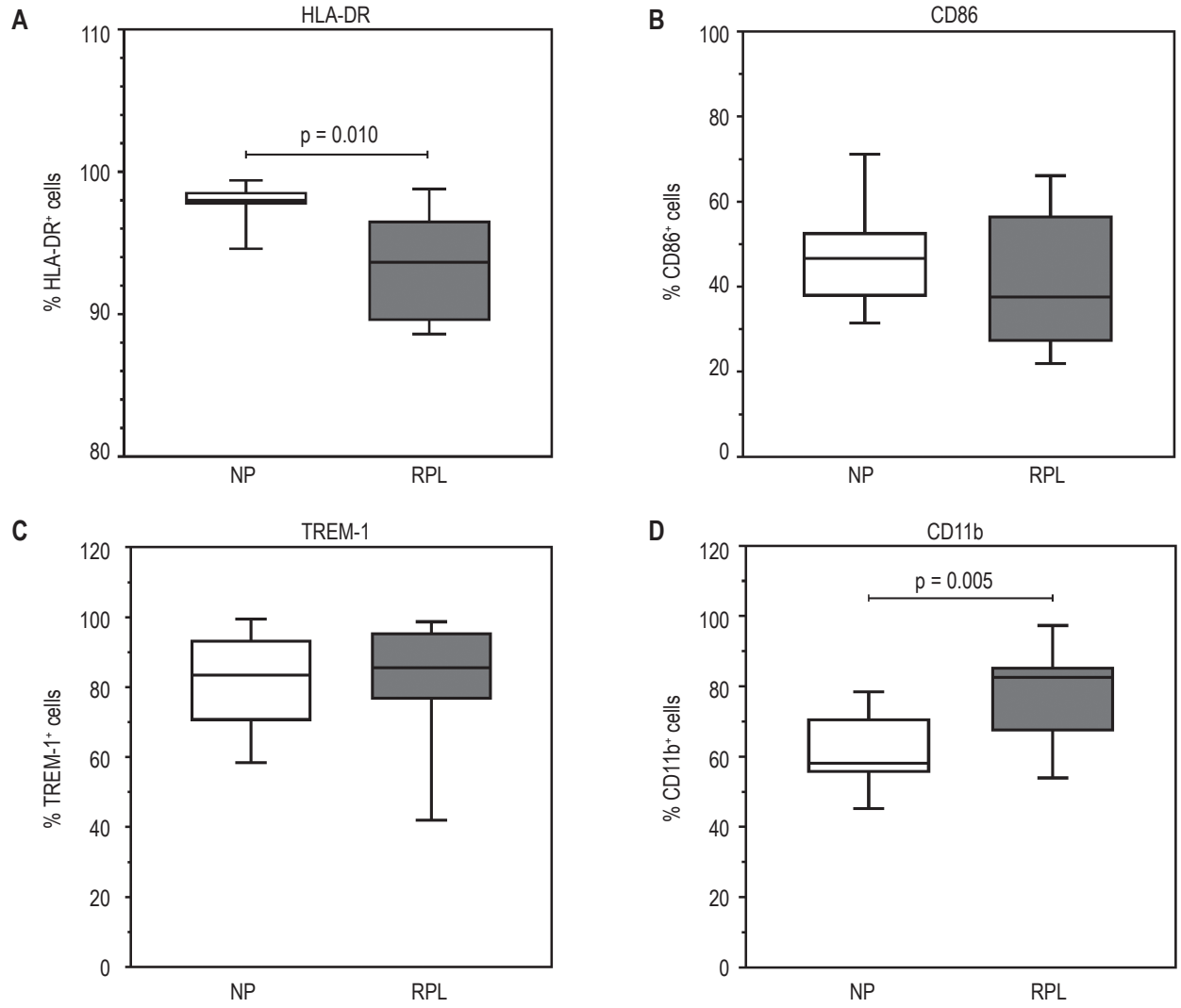

Figure 2. Expression of activation markers of platelet-monocyte complexes in the total monocyte population

Note. Diagrams demonstrate the percentage of platelet-monocyte complexes expressing HLA-DR (A), CD86 (B), TREM-1 (C) and CD11b (D) in the peripheral blood of patients with normal pregnancy and recurrent pregnancy loss. Plots show range, median, 25th and 75th percentile. NP, normal pregnancy; RPL, recurrent pregnancy loss.

The percentage of $CD62P^+$ and $CD162^+$ PMC in both studied groups was about 5% and 99%, respectively (Figure 1B, C).

Increased expression of major histocompatibility complex molecule HLA-DR and costimulatory molecule CD86 are signs of monocyte activation associated with an inflammatory response. In both RPL and NP groups, the vast majority of PMC were positive for HLA-DR, however, in RPL patients there was a small but statistically significant decrease in the amount of $HLA\text{-}DR^+$ PMC (93.7%) compared to NP group (98.0%) (Figure 2A). Staining with anti-CD86 antibodies revealed no differences in the expression of this marker: the proportion of $CD86^+$ TMCs in NP and RPL patients was 44.7% and 37.6%, respectively (Figure 2B).

TREM-1 (triggering receptor expressed on myeloid cells 1) is present on the surface of monocytes. Activation of this receptor stimulates the production of cytokines and chemokines and contributes to the development of the inflammatory response [4]. The interaction of TREM-1 with its ligand ensures the link between leukocytes and platelets [14]. Our study showed that recurrent miscarriage did not accompanied with significant changes in TREM-1 expression: ~85% $TREM\text{-}1^+$ PMC was determined in both groups of patients (Figure 2C).

While in NP group the proportion of CD11b-expressing PMC was 58.1%, patients with recurrent pregnancy loss demonstrated significant increase up to 82.5% (Figure 2D). CD11b is part of the multifunctional integrin αMβ2 (Mac-1), which, in particular, mediates monocyte functions such as adhesion and phagocytosis. An increase in the

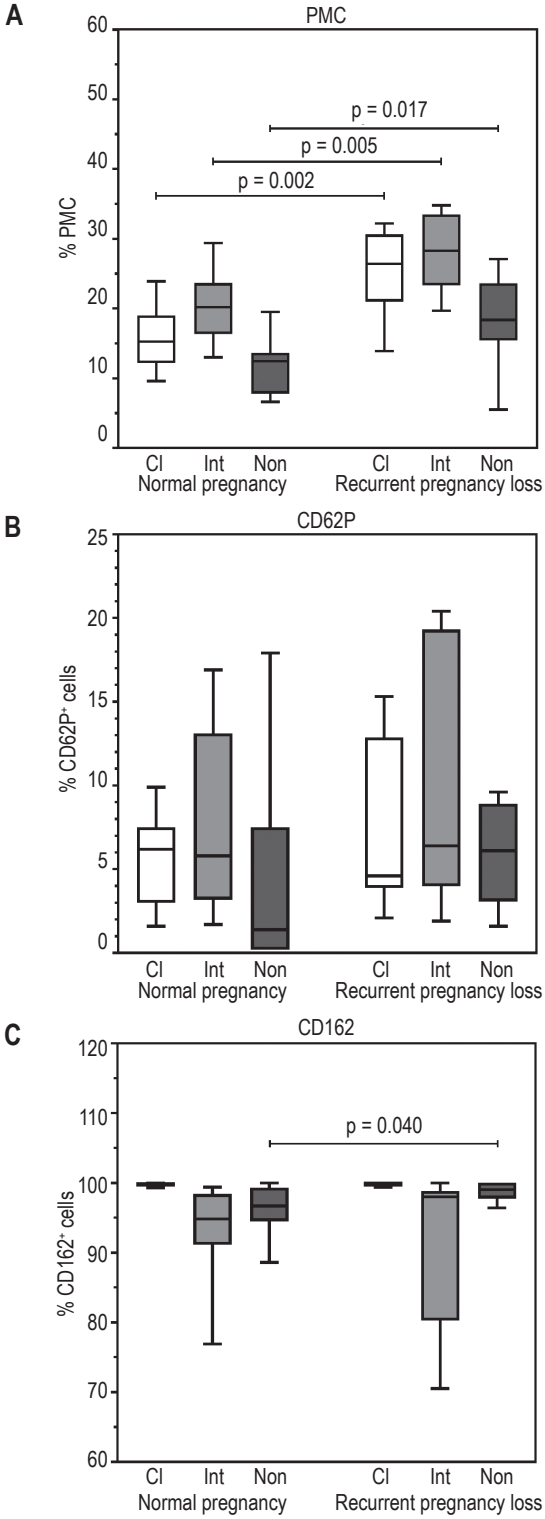

Figure 3. Characteristics of platelet-monocyte complexes in monocyte subsets

Note. Graphs demonstrate the proportion of platelet-monocyte complexes (A) and the percentage of platelet-monocyte complexes expressing CD62P (B) and CD162 (C) in the classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), and non-classical ($CD14^{+}CD16^{++}$) monocyte subsets in the patients with normal pregnancy and recurrent pregnancy loss. Plots show range, median, 25th and 75th percentile. Cl, classical monocytes; Int, intermediate monocytes; Non, non-classical monocytes.

expression of CD11b by monocytes in recurrent miscarriage may indicate an increase in their adhesion potential, resulting in an increased risk of thrombosis and damaging effects on the vascular endothelium.

Analyses of monocyte subpopulations showed that in RPL group all three monocyte fractions contribute to the increase in the number of platelet-monocyte aggregates (Figure 3A). Classical, intermediate and non-classical monocytes accounted for 15.3%, 20.2%, 12.5% PMC, respectively, in NP group and 26.4%, 28.3%, 18.4% PMC, respectively, in RPL group. In both groups, the largest and the lowest proportions of PMC were formed by intermediate and non-classical monocytes, respectively, and this difference was more prominent in RPL group ($p < 0.001$) than in NP group ($p < 0.05$). Similar to the total monocyte population, all three monocyte subsets demonstrated no differences in CD62P expression between the studied groups (Figure 3B). Moreover, in RPL patients, an increase in the expression of CD162 by a minor subpopulation of non-classical monocytes was observed (Figure 3C). Meanwhile, in the classical monocytes decreased $HLA\text{-}DR^{+}$ expression (95.4%) was detected in RPL group when compared to NP group (99.4%) (Figure 4A). In the same fraction, a decrease in $CD86^{+}$ PMC from 52.2% to 38.8%, was observed however, it did not reach statistical significance (Figure 4B). In RPL group no significant changes were found in the expression of HLA-DR and CD86 in PMC formed by intermediate and non-classical monocytes (Figure 4A, B), while expression of TREM-1 was significantly increased in the PMC formed by non-classical monocytes (Figure 4C).

CD11b was the only marker whose expression increased in all three monocyte subsets. In classical, intermediate and non-classical monocyte fractions, the proportions of $CD11b^{+}$ PMC was 86.8%, 29.0% and 30.7%, respectively, in RPL group and 66.4%, 16.3% and 16.0%, respectively, in NP group (Figure 4D).

In order to find out whether the observed phenotypic (and putative functional) changes in PMC-associated monocytes were induced by their direct interaction with platelets or whether these changes were caused by other factors and did not depend on the formation of platelet-monocyte aggregates, we made a comparison of expression patterns of surface antigens between PMC and free monocytes in blood samples obtained from RPL and NP patients.

In NP group, formation of PMC led to a small but statistically significant decrease in the level of CD162 expression in the total monocyte population, while in RPL group this did not occur (Figure 5A). However, analysis of monocyte subpopulations showed that in both studied groups monocyte-platelet interactions attenuated CD162 expression in classical and intermediate monocytes (Figure 5B). In patients with normal pregnancy, the interaction of monocytes with platelets (PMC formation) led to a decrease in

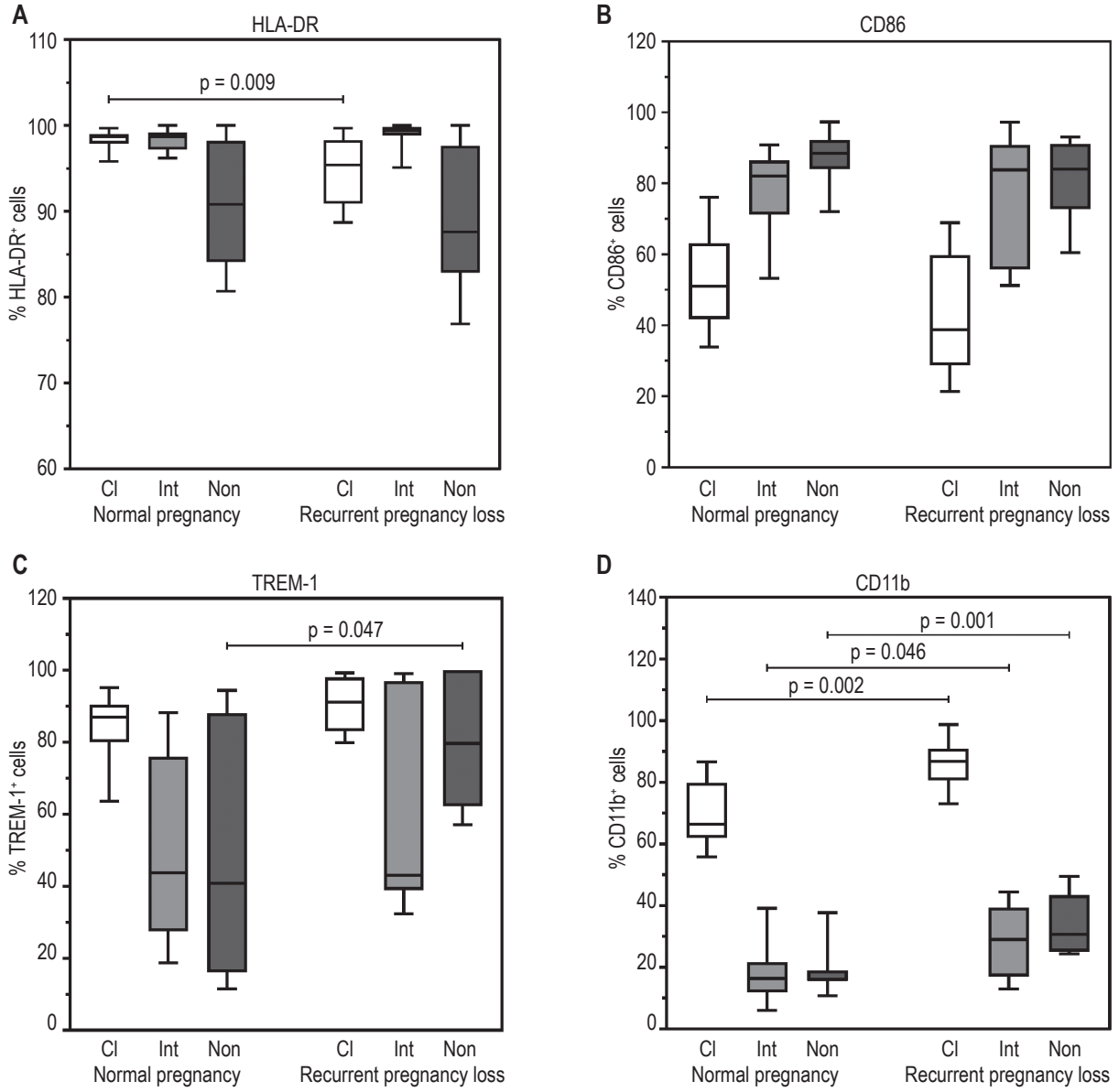

Figure 4. Expression of activation markers of platelet-monocyte complexes in monocyte subsets

Note. Diagrams demonstrate the percentage of platelet-monocyte complexes expressing HLA-DR (A), CD86 (B), TREM-1 (C) and CD11b (D) in the classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), and non-classical ($CD14^{+}CD16^{++}$) monocyte subsets in the patients with normal pregnancy and recurrent pregnancy loss. Plots show range, median, 25th and 75th percentile. Cl, classical monocytes; Int, intermediate monocytes; Non, non-classical monocytes.

the number of cells expressing activation markers HLA-DR and CD86, while in patients with recurrent miscarriage this change affected only CD86, but not HLA-DR (Figure 6A, 7A). A decrease in HLA-DR expression was observed in all three subpopulations of monocytes in NP group, while in RPL group HLA-DR expression decreased in non-classical monocytes only, and this change did not manifest itself at the level of total monocyte population (Figure 6B). In NP group, the number of $CD86^{+}$ cells decreased in all subpopulations, and in RPL group the changes occurred in the fractions of classical and non-classical monocytes (Figure 7B). Platelets had a stimulating effect on the expression of TREM-1 by monocytes: in both groups of patients, the proportion of TREM-1 positive PMC increased (Figure 8A). This increase was ensured by classical monocytes, while multidirectional changes in TREM-1 expression was observed in subpopulations of intermediate and non-classical monocytes (Figure 8B). In the total monocyte population, formation of PMC did not significantly affected CD11b expression (Figure 9A). The absence of changes was observed in all monocyte subpopulations, with one exception: in RPL group, the proportion of $CD11b^{+}$ non-classical monocytes significantly increased from 20% to 28% (Figure 9B).

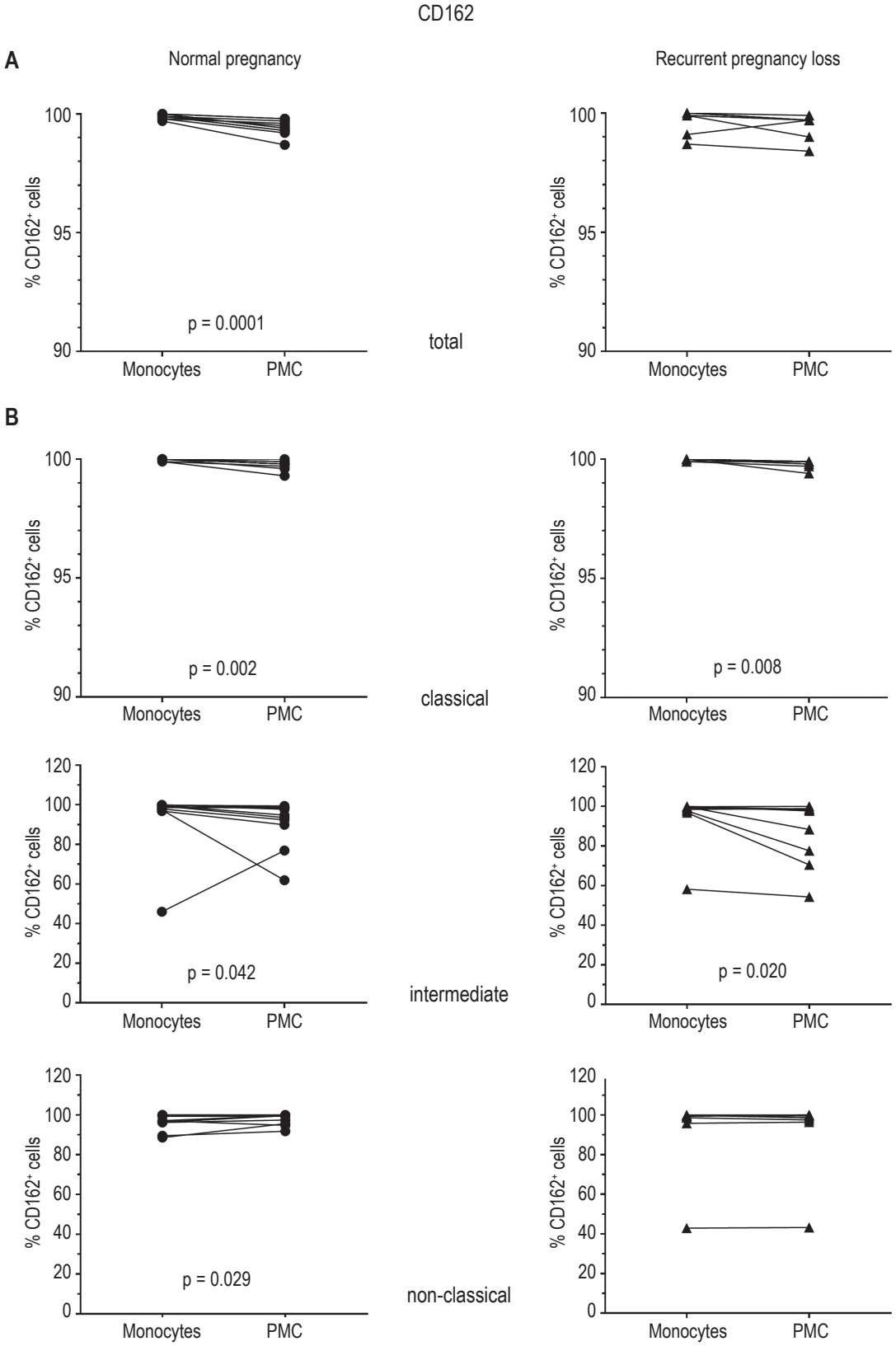

Figure 5. CD162 expression in monocytes and platelet-monocyte complexes

Note. Graphs demonstrate pairwise comparison of CD162 expression levels (percentage of $CD162^+$ cells) on the surface of free and platelet-associated monocytes in the patients with normal pregnancy and recurrent pregnancy loss. A, total monocytes; B, monocyte subsets.

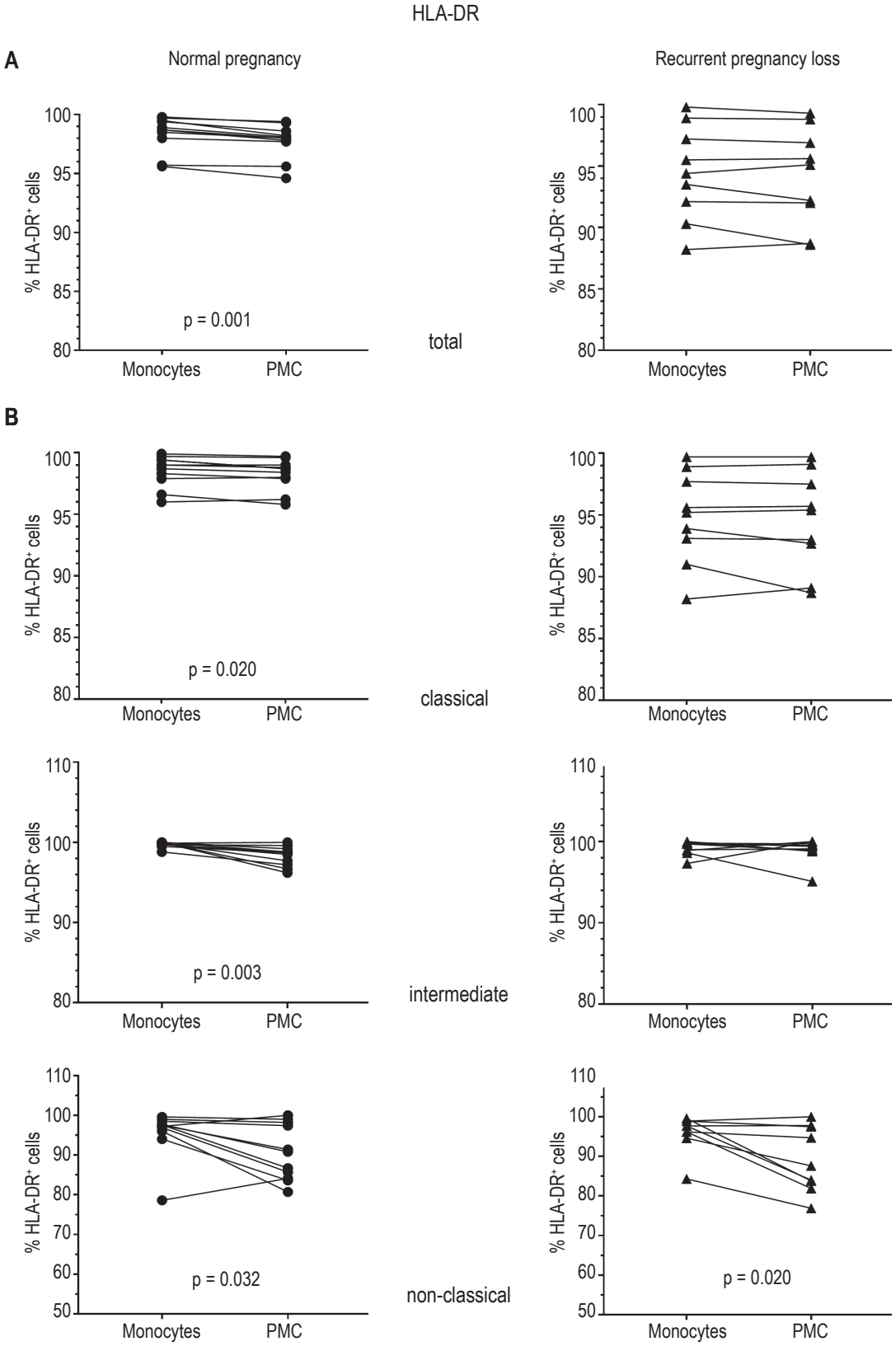

Figure 6. HLA-DR expression in monocytes and platelet-monocyte complexes

Note. Graphs demonstrate pairwise comparison of HLA-DR expression levels (percentage of HLA-DR+ cells) on the surface of free and platelet-associated monocytes in the patients with normal pregnancy and recurrent pregnancy loss. A, total monocytes; B, monocyte subsets.

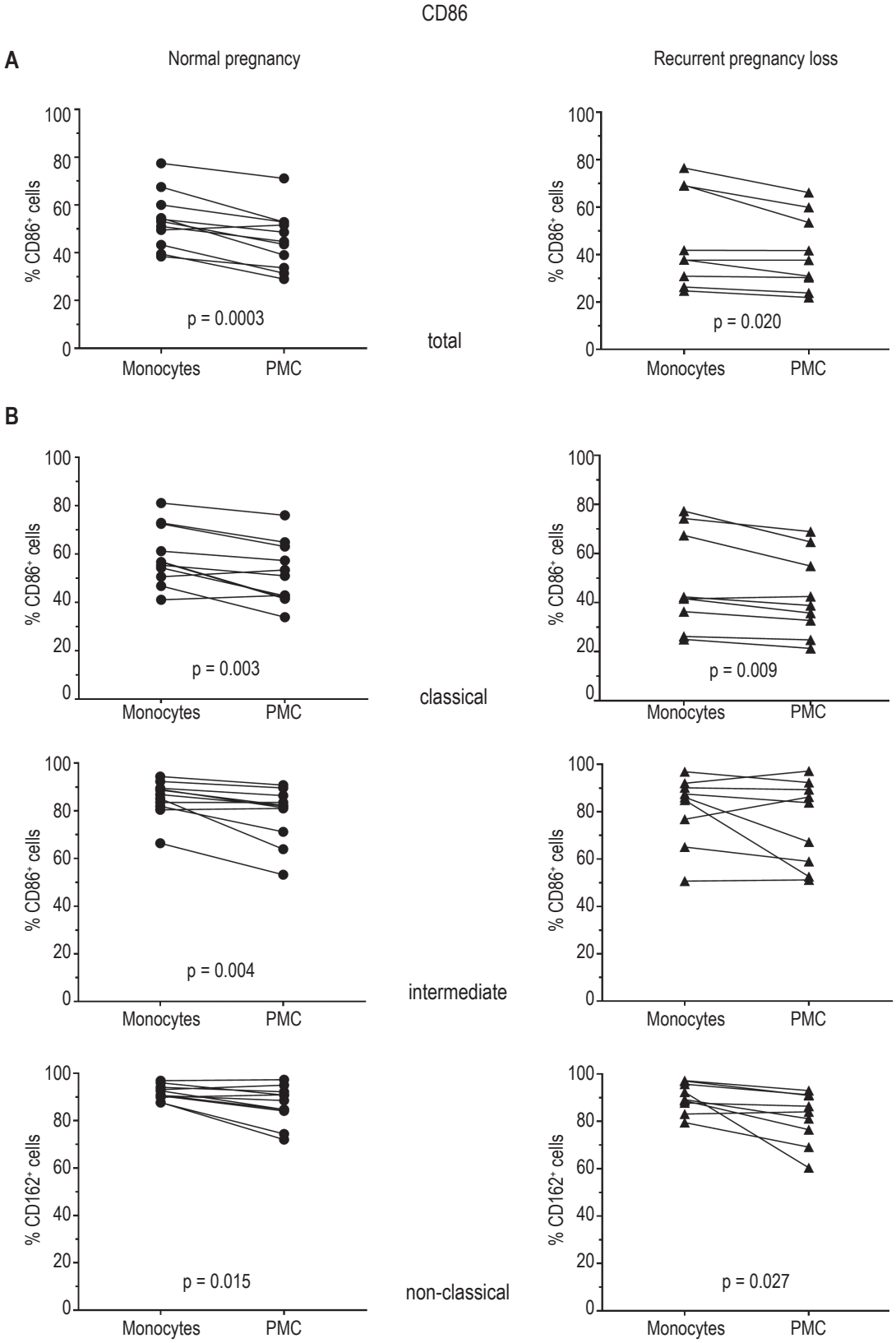

Figure 7. CD86 expression in monocytes and platelet-monocyte complexes

Note. Graphs demonstrate pairwise comparison of CD86 expression levels (percentage of CD86+ cells) on the surface of free and platelet-associated monocytes in the patients with normal pregnancy and recurrent pregnancy loss. A, total monocytes; B, monocyte subsets.

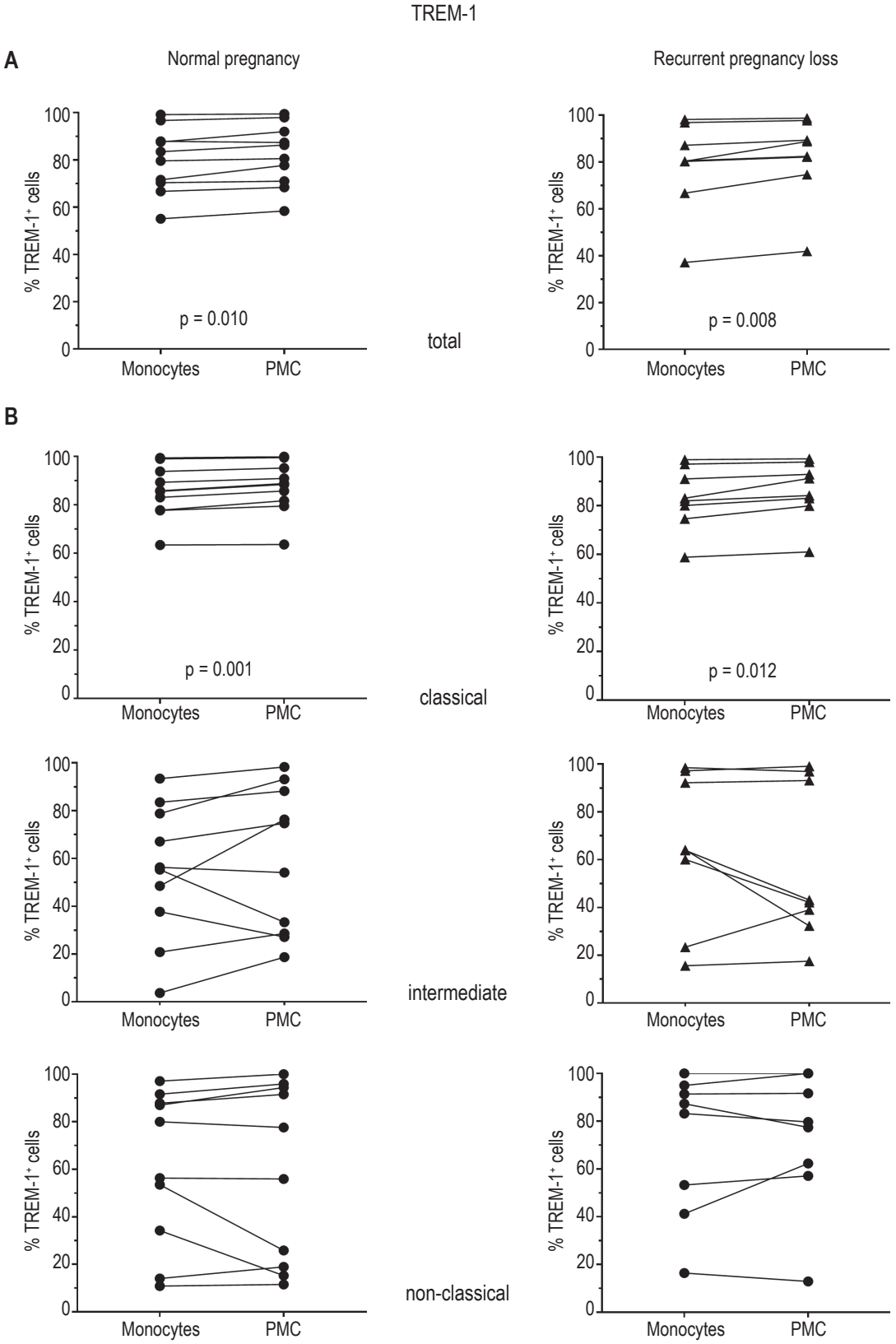

Figure 8. TREM-1 expression in monocytes and platelet-monocyte complexes

Note. Graphs demonstrate pairwise comparison of TREM-1 expression levels (percentage of TREM-1$^+$ cells) on the surface of free and platelet-associated monocytes in the patients with normal pregnancy and recurrent pregnancy loss. A, total monocytes; B, monocyte subsets.

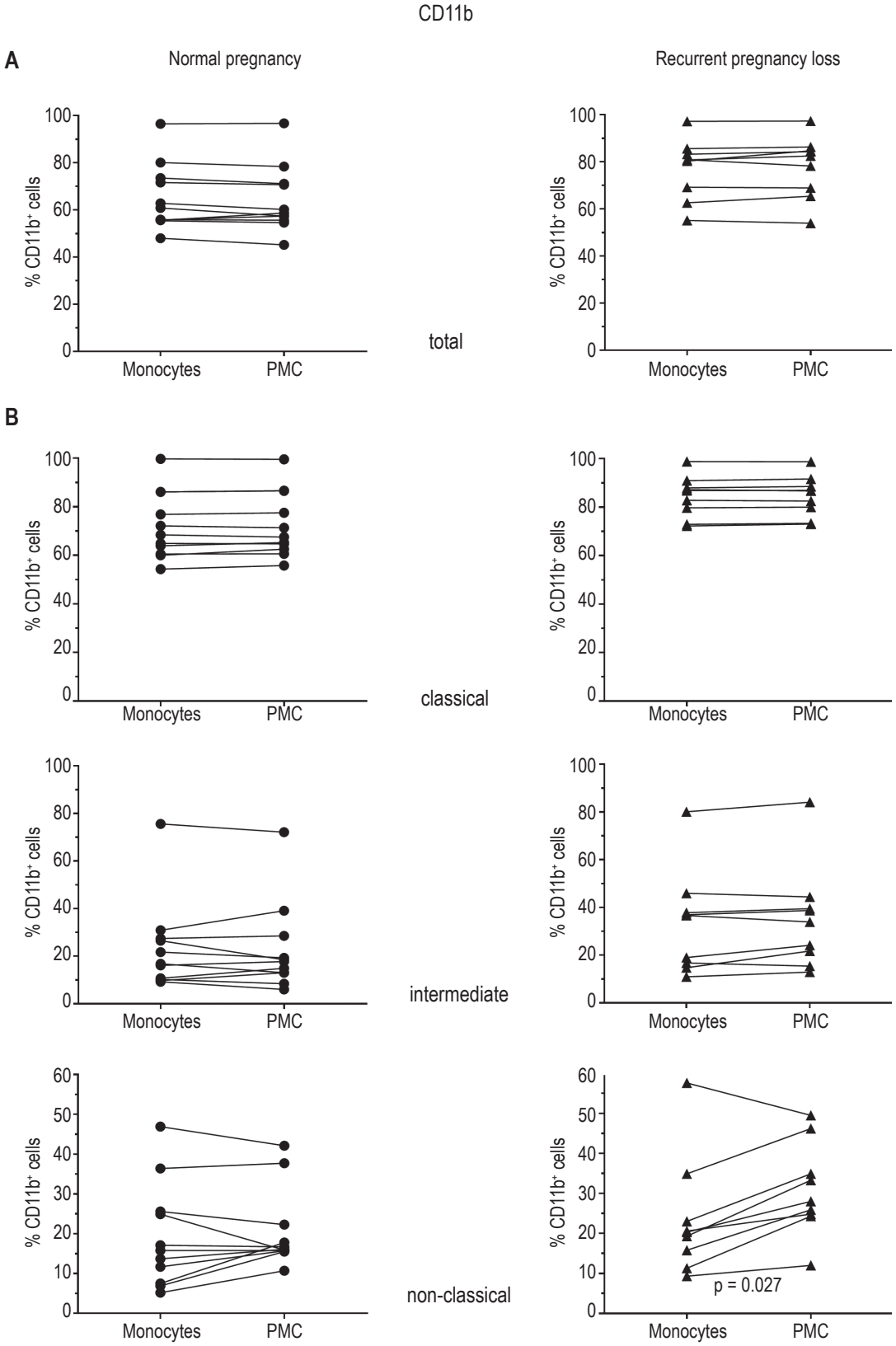

Figure 9. CD11b expression in monocytes and platelet-monocyte complexes

Note. Graphs demonstrate pairwise comparison of CD11b expression levels (percentage of $CD11b^+$ cells) on the surface of free and platelet-associated monocytes in the patients with normal pregnancy and recurrent pregnancy loss. A, total monocytes; B, monocyte subsets.

Discussion

Our results show that in first trimester pregnant women diagnosed with recurrent pregnancy loss, the content of platelet-monocyte complexes in the peripheral blood was increased in comparison with women of the same gestational age who had an uncomplicated pregnancy. It is known that normal pregnancy is characterized by increased coagulation capacity and activation of components of the immune system. Increased complex formation reflects platelet activation, which, along with a change in the activation status of monocytes as a result of their interaction with activated platelets, may represent an additional pathogenetic factor causing disturbances in the physiological course of pregnancy.

To our knowledge, this is the first study of platelet-monocyte complexes in recurrent pregnancy loss. In the literature, there is only one publication on a similar topic, but its authors evaluated complexes formed by all leukocyte cells, without differentiation between types of leukocytes, and the studied groups consisted of non-pregnant women [24]. This work demonstrated an increase in the level of platelet-leukocyte aggregates in patients with recurrent miscarriage. Our study not only quantified PMC, but also determined their surface antigenic markers, both in the total population and in individual subpopulations of monocytes. Based on the results of a subpopulation analysis, we concluded that the fraction of non-classical monocytes has a lower ability to form complexes with platelets than the fractions of classical and intermediate monocytes.

According to the results obtained, in patients with recurrent pregnancy loss the number of PMC increased in all subpopulations of monocytes. At the same time, in the fractions of classical and intermediate monocytes, the expression of CD162 decreased due to the formation of PMC, thus suggested the participation of this molecule in the connections between monocytes and platelets. In contrast, the expression of CD162 by PMC-associated non-classical monocytes increased in patients with physiological pregnancy and did not change in patients with recurrent miscarriage. Moreover, it was the fraction of non-classical monocytes that formed the smallest number of PMC compared to the other monocyte subpopulations and only this subpopulation demonstrated increased CD162 level in RPL group when compared to NP group.

The patterns of expression of surface markers of monocytes suggested that recurrent miscarriage was not accompanied by inflammatory activation of monocytes. Along with unchanged expression of the costimulatory molecule CD86 both in total population and in individual subpopulations of monocytes, in patients with recurrent pregnancy loss there was a decrease in the expression level of the activation marker HLA-DR ensured by classical monocytes. In total monocytes, the expression of another activation marker, TREM-1, also did not undergo significant changes due to recurrent miscarriage, although the number of TREM-1$^+$ PMC increased in non-classical monocytes. In contrast to these antigens, which are characteristic of the "inflammatory" phenotype of monocytes, increased level of CD11b expression in RPL patients suggested enhancement of adhesive properties of PMC-associated monocytes that may represent one of the possible pathophysiological mechanisms of spontaneous misscarriage.

Our study shows the reasonability of identifying monocyte subpopulations when quantifying and phenotyping PMC, since the results obtained from studying total population of monocytes do not necessarily reflect changes occurring in individual subpopulations.

Another aspect of our study was to reveal whether phenotypic characteristics of monocytes were affected by their interaction with platelets in consequence of formation of heterotypic aggregates (PMC) in normal pregnancy and in recurrent pregnancy loss, since changes in the morphofunctional properties of monocytes may be a pathogenetic factor for the disease.

To this end, we compared the expression levels of surface antigen markers between PMC-bound and free monocytes. Analysis revealed some patterns of changes in the antigenic phenotype of monocytes in patients with normal pregnancy and recurrent miscarriage. Unexpectedly, it turned out that formation of platelet-monocyte aggregates resulted in decreased expression of HLA-DR and CD86. Nevertheless, in patients with normal pregnancy these changes occurred in all subpopulations of monocytes, whereas in women with recurrent pregnancy loss only one or two subpopulations, respectively, were involved. It would follow that the weakening of HLA-DR expression by monocytes in RPL group was not a result of platelet-monocyte interaction, but was caused by other factors. Moreover, platelets induced comparable changes in the expression of CD86 in both studied groups, which did not lead to significant differences between them. Similarly, increased expression of TREM-1 by classical monocytes upon interaction with platelets was observed in both groups, but no difference was found in the expression of TREM-1 between NP and RPL patients. In our study, CD11b was the only marker whose expression increased as a result of platelet interactions with nonclassical monocytes, and this appeared to contribute to the increase in the number of CD11b$^+$ PMC in women with recurrent pregnancy loss.

We hypothesize that development of inflammation and damaging effects on the vascular endothelium would underline the pathophysiological effects of PMC. However, our study did not reveal the signs of inflammatory changes in the antigenic phenotype of PMC in patients with recurrent pregnancy loss. It is possible that observed attenuation of the pro-inflammatory properties of PMC, which is expressed

as reduced expression of HLA-DR, in some way contributes to the development of this reproductive pathology; however, no theoretical explanation has yet been found. Nevertheless, the increased expression of CD11b in RPL group that we discovered may lead to increased adhesion of monocytes to the surface of endothelial cells and damage to the vascular endothelium. Obviously, we should continue to search for antigenic markers, the changes in expression of which may serve as a clue to understanding the mechanisms of PMC participation in the pathogenesis of recurrent miscarriage.

Unfortunately, it is not possible to compare our data with the results of other studies due to the lack of relevant information in the available literature. We can only refer to the results of our previous work which determined and characterized PMC in patients with another reproductive pathology – preeclampsia. As in the present study, in preeclampsia we observed an increased content of TMC in the blood; however, in addition to an increase in the expression level of CD11b, there was an increase in the expression of pro-inflammatory markers: HLA-DR, CD86 and TREM-1 (unpublished data). Moreover, we found that in patients with preeclampsia, increased expression of TREM-1 and CD11b in PMC-associated monocytes was induced by platelets, while changes in the expression of HLA-DR and CD86 were caused by other factors.

This comparison led us to the following conclusion. Obviously, an increased level of PMC in the circulation is a general phenomenon, as it is observed in various diseases that are thromboinflammatory in nature, and from this point of view, determining the surface antigenic phenotype of PMC-associated monocytes seems to be more specific and more informative, since we assume that each disease is characterized by a particular pattern of expression of antigenic markers, which is formed under the influence of a unique combination of various factors, including platelets. Determining these patterns can help improve methods for diagnosing reproductive disorders, including recurrent miscarriage, reveal underlying mechanisms and develop new treatment approach.

Conclusion

In patients with recurrent pregnancy loss, the level of platelet-monocyte complexes in the peripheral blood increases compared to patients with uncomplicated pregnancy. All three monocyte subpopulations participate in this increase. Changes in the surface antigenic phenotype of PMC-associated monocytes reflect weakening of the inflammatory features and strengthening of adhesive properties of these cells. Monocyte subpopulations differently contribute to the changes in the expression of activation markers associated with recurrent miscarriage.

The immunomodulatory effect of platelets is manifested in stimulation of the expression of the CD11b molecule, which characterizes the adhesion phenotype of monocytes, while decrease in the expression of another activation marker HLA-DR is apparently caused by other factors.

Determining specific expression patterns of surface antigenic markers of platelet-monocyte complexes may have diagnostic value and also help improve approaches to the treatment of recurrent pregnancy loss.

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