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Postpartum profiling of microRNAs involved in pathogenesis of cardiovascular/cerebrovascular diseases in women exposed to pregnancy-related complications☆

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ABSTRACT

Background and methods: Gestational hypertension (GH), preeclampsia (PE) and fetal growth restriction (FGR) may predispose to later onset of cardiovascular/cerebrovascular diseases. We examined if pregnancy complications induce postpartum alterations in gene expression of cardiovascular/cerebrovascular disease associated microRNAs. 29 microRNAs were tested in peripheral blood of women, compared between groups with a history of GH, PE, FGR and controls, and correlated with the severity of the disease regarding clinical signs, delivery date, and Doppler parameters.

Results: GH was associated with the up-regulation of miR-20a-5p, miR-143-3p, miR-146a-5p, miR-181a-5p, miR-199a-5p, miR-221-3p, and miR-499a-5p. The up-regulation of miR-17-5p, miR-20b-5p, miR-29a-3p, and miR-126-3p was a mutual phenomenon of GH and severe PE. GH and early PE were associated with up-regulation of miR-1-3p and miR-17-5p. GH and late PE showed up-regulation of miR-17-5p, miR-20b-5p, and miR-29a-3p. Severe PE induced up-regulation of miR-133a-3p and down-regulation of miR-130b-3p. MiR-133a-3p up-regulation was also observed in early PE. PE and/or FGR with abnormal Doppler parameters demonstrated up-regulation of miR-100-5p, miR-125b-5p, miR-133a-3p, and miR-145-5p. The combination screening was superior over using individual microRNAs for patients with GH, PE regardless of the severity of the disease, severe PE and early PE. A cardiovascular risk at patients with late PE, PE and/or FGR with abnormal Doppler parameters was identified more accurately using the single microRNA only.

Conclusion: Epigenetic changes characteristic for cardiovascular/cerebrovascular diseases are present in women with a prior exposure to pregnancy complications. Screening of microRNAs may be used to identify patients at a higher risk of later development of cardiovascular/cerebrovascular diseases.

1. Introduction

Preeclampsia (PE) and fetal growth restriction (FGR) are main pregnancy complications affecting 2–10% of women [1,2]. PE develops after 20 weeks and is defined as chronic or gestational hypertension (GH) accompanied by proteinuria [3]. FGR occurs when the foetus lags behind its intrauterine anticipated growth and development as a result of compromise in placental function [4]. Early PE (onset <34 weeks) and early FGR (onset <32 weeks) usually result from improper placentation [5–9]. Late PE (onset >34 weeks) and late FGR (onset >32 weeks) are frequently caused by impaired maternal cardiovascular function and followed by secondary placental dysfunction [6–10]. Since pregnancy complications possibly uncover pre-existing endothelial dysfunction and vascular and metabolic disease [11], the American Heart Association recommends long-term monitoring of women with a prior exposure to GDM, PE, preterm birth, or birth of an infant small for gestational age [12].

Rising evidence suggests a relationship between hypertension in pregnancy and the general risk of diabetes mellitus (DM), kidney, cardiovascular and cerebrovascular diseases later in life [13–18]. Women exposed to PE or eclampsia are at the risk for subsequent onset of hypertension, atherosclerosis, ischemic heart disease, congestive heart failure, stroke, venous thromboembolic events, and metabolic syndrome [18–22]. Augmented risk for hypertension, myocardial infarcts, ischemic heart disease, heart failure, ischemic stroke, obesity, metabolic syndrome, and DM type 2 has also been demonstrated among women

☆ These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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affected with GH [14,19,23]. Women affected with pregnancy complicated by FGR have also been reported at an elevated risk for subsequent development of ischemic heart disease [24–26].

The objective of the study was to examine postpartum expression profile of microRNAs known to play a role in pathogenesis of diverse cardiovascular/cerebrovascular diseases in women with a history of pregnancies complicated by GH, PE and FGR.

miR-1-3p	miR-16-5p	miR-17-5p	miR-20a-5p	miR-20b-5p
miR-21-5p	miR-23a-3p	miR-24-3p	miR-26a-5p	miR-29a-3p
miR-92a-3p	miR-100-5p	miR-103a-3p	miR-125b-5p	miR-126-3p
miR-130b-3p	miR-133a-3p	miR-143-3p	miR-145-5p	miR-146a-5p
miR-155-5p	miR-181a-5p	miR-195-5p	miR-199a-5	miR-210-3p
miR-221-3p	miR-342-3p	miR-499a-5p	miR-574-3p	

MicroRNAs represent small noncoding RNAs responsible for regulation of gene expression either by degrading or blocking translation of target mRNA ([27], e-component 1–5).

A variety of tissues display under pathological events microRNA expression profiles that are different from normal tissues, which may be utilized in clinical diagnostics [28].

We focused mainly on those microRNAs playing a role in pathogenesis of dyslipidaemia [29,30], hypertension [31,32], vascular inflammation [33,34], insulin resistance and diabetes [35], atherosclerosis [36,37], angiogenesis [38,39], coronary artery disease [32,34,38], myocardial infarction and heart failure [31,40,41], stroke [42,43], intracranial aneurysm [44,45], pulmonary arterial hypertension [46,47], and peripartum cardiomyopathy [48,49].

We are not aware of any study on expression profiling of cardiovascular/cerebrovascular disease associated microRNAs in women with a history of GH, PE and FGR.

2. Methods

2.1. Patients

The study was prospective. All women delivering within 2007–2014, whose singleton pregnancies were complicated with pregnancy complications were invited to participate in the study. Finally, the case cohort included Caucasian women with a history of GH ($n = 50$), PE ($n = 101$), FGR ($n = 35$), and women with normal gestation ($n = 89$) that were chosen on the basis of equal age. No exclusion criteria were applied concerning the control group selection. Of the 101 PE patients, 24 had mild PE and 77 severe PE. 36 PE patients required the termination of gestation <34 weeks and 65 patients delivered >34 weeks. Eleven FGR foetuses were delivered <32 weeks and 24 ones >32 weeks. The presence of oligohydramnios/anhydramnios appeared in 41 PE and/or FGR affected foetuses.

Doppler ultrasonography showed an abnormal index of pulsatility (PI) in the umbilical artery (27 cases) and/or in the middle cerebral artery (14 cases). A ratio between the middle cerebral artery and the umbilical artery PIs (the cerebro-placental ratio, CPR) was <5th percentile in 37 cases. Absent or reversed end-diastolic velocity waveforms in the umbilical artery occurred in 5 cases. The mean PI in the uterine artery >95th percentile was identified in 21 cases with the presence of unilateral or bilateral diastolic notch in 20 cases. The examination of ductus venosus (DV) revealed an absence of flow during atrial contraction (deep a wave) in 1 case. Abnormal PI (>1) indicating DV dilatation and poor outcome was detected in 3 cases. The clinical characteristics of patients are outlined in Table 1. The definition of normal and complicated pregnancies was stated in our previous work [50].

All patients provided written informed consent. The study was approved by the Ethics Committees. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (as revised in 2000) as reflected in a priori approval by the responsible committee on human experimentation (institutional and national).

2.2. Processing of samples

For detailed information see the Supplementary Methods [50].

In brief, total RNA enriched for small RNAs was isolated from homogenized cell lysates using a mirVana microRNA Isolation kit (Ambion, Austin, USA). Each microRNA was reverse transcribed into cDNA by a TaqMan MicroRNA Assay (using the first generation chemistry involving miRNA-specific stem loop primer) and TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Branchburg, USA) in a total reaction volume of 10 μL [50]. 3 μL of cDNA were mixed with specific primers and the TaqMan MGB probe and the ingredients of the TaqMan Universal PCR Master Mix (Applied Biosystems, Branchburg, USA) in a total reaction volume of 15 μL [50]. TaqMan PCR conditions were set on 7500 Real-Time PCR System as described in the TaqMan guidelines.

The expression of microRNAs was determined using the comparative Ct method [e-component 6] relative to normalization factor [e-component 7]. Our analyses revealed that RNU58A and RNU38B were optimal for qPCR data normalization in this setting.

2.3. Statistical analysis

MicroRNA levels were compared between groups using the Kruskal-Wallis one-way analysis of variance with post-hoc test for the comparison among multiple groups ($p < 0.05$).

Receivers operating characteristic (ROC) curves were constructed to calculate the area under the curve (AUC) and the best cut-off point for particular microRNA was used in order to calculate the respective sensitivity at 90.0% specificity (MedCalc Software bvba, Ostend, Belgium). To select the optimal combinations of microRNAs logistic regression was used.

Correlation between variables including relative microRNA quantification and Doppler parameters was calculated using the Spearman's rank correlation coefficient (ρ).

3. Results

3.1. Cardiovascular microRNAs are dysregulated postpartum in patients with previous occurrence of pregnancy complications

MicroRNA expression was studied in maternal blood 3 to 11 years postpartum and compared between patients with uncomplicated and complicated pregnancies. Postpartum expression of microRNAs was analysed with regard to the severity of pregnancy complications in relation to clinical signs (mild vs. severe PE, absence vs. presence of oligohydramnios/anhydramnios, absence vs. presence of abnormal Doppler parameters in PE/FGR pregnancies) and delivery dates (< or >34 weeks in case of PE, < or >32 weeks in case of FGR).

The association between microRNA expression and Doppler parameters (PI in the umbilical artery, PI in the middle cerebral artery, the CPR, PI in the uterine artery, PI in the ductus venosus, and the presence of unilateral or bilateral diastolic notch in the uterine artery) was analysed in PE/FGR cases. Only the outcomes that reached a statistical significance or displayed a trend toward a statistical significance in complicated cases are presented below.

3.2. Postpartum overexpression of miR-20a-5p, miR-143-3p, miR-146a-5p, miR-181a-5p, miR-199a-5p, miR-221-3p, and miR-499a-5p is a unique feature of GH

ROC curves analyses showed the diverse expression of miR-20a-5p, miR-143-3p, miR-146a-5p, miR-181a-5p, miR-199a-5p, miR-221-3p, and miR-499a-5p between a control group and a group of women exposed to GH. These microRNAs were up-regulated in GH patients (Fig. S1).

Table 1
The clinical characteristics of women with a history of normal and complicated pregnancies.

Characteristics of cases and controls at follow-up	Controls (n = 89)	PE (n = 101)	FGR (n = 35)	GH (n = 50)	p-Value[1]	p-Value[2]	p-Value[3]
Age at follow-up (years)	38 (29–50)	38 (28–52)	38 (26–45)	39 (31–58)	1.000	1.000	1.000
Time elapsed since delivery (years)	5 (3−11)	4 (3–11)	4 (3−10)	4 (3–10)	0.105	0.241	0.122
BMI at follow up (kg/m^2)	22.31 (17.7–39.08)	24.57 (16.22–40.9)	22.31 (17.06–36.76)	25.98 (17.91–46.45)	<**0.001**	1.000	<**0.001**
Waist circumference (cm)	74 (63–117)	80 (64–120)	75 (63–115)	85.5 (65–131)	<**0.001**	1.000	<**0.001**
Glucose status					0.656	0.376	0.100
Normal	85 (95.50%)	95 (94.06%)	32 (91.43%)	44 (88.00%)			
DM/GDM	4 (4.50%)	6 (5.94%)	3 (8.57%)	6 (12.00%)			
Smoking					0.991	0.683	0.821
Non-Smoker	54 (60.67%)	62 (61.39%)	24 (68.57%)	33 (66.00%)			
Ex-Smoker	21 (23.60%)	23 (22.77%)	6 (17.14%)	10 (20.00%)			
Smoker	14 (15.73%)	16 (15.84%)	5 (14.29%)	7 (14.00%)			
Hormonal contraceptive use					0.063	0.270	0.358
No	37 (41.57%)	30 (29.70%)	10 (28.57%)	20 (40.00%)			
In the past	30 (33.71%)	51 (50.50%)	17 (48.57%)	22 (44.00%)			
Yes	22 (24.72%)	20 (19.80%)	8 (22.86%)	8 (16.00%)			
Total number of pregnancies per patient					**0.003**	**0.036**	0.128
1	8 (8.99%)	29 (28.72%)	9 (25.71%)	10 (20.00%)			
2	45 (50.56%)	42 (41.58%)	17 (48.57%)	19 (38.00%)			
3+	36 (40.45%)	30 (29.70%)	9 (25.71%)	21 (42.00%)			
Total parity per patient					<**0.001**	**0.028**	**0.014**
1	13 (14.61%)	40 (39.60%)	12 (34.29%)	18 (36.00%)			
2	62 (69.66%)	51 (50.50%)	21 (60.00%)	27 (54.00%)			
3+	14 (15.73%)	10 (9.90%)	2 (5.71%)	5 (10.00%)			
Characteristics of cases and controls during pregnancy	**Controls (n = 89)**	**PE (n = 101)**	**FGR (n = 35)**	**GH (n = 50)**	**p-Value[1]**	**p-Value[2]**	**p-Value[3]**
Maternal age at delivery (years)	32 (25–43)	32 (21–44)	32 (22–41)	33.5 (28–51)	0.779	0.489	0.078
Gestational age at delivery (weeks)	39.86 (37.71–41.86)	37.14 (26.0–41.71)	36.14 (28.0–41.0)	39 (33.43–41.57)	<**0.001**	<**0.001**	**0.009**
Mode of delivery					<**0.001**	<**0.001**	<**0.001**
Vaginal	82 (92.13%)	18 (17.82%)	7 (20.00%)	23 (46.00%)			
Cesarean section	7 (7.87%)	83 (82.18%)	28 (80.00%)	27 (54.00%)			
Fetal birth weight (g)	3410 (2530–4450)	2510 (610–4490)	2030 (650–3100)	3165 (1940–4440)	<**0.001**	<**0.001**	**0.017**
Fetal sex					0.318	0.890	0.750
Boy	47 (52.81%)	46 (45.54%)	18 (51.43%)	25 (50.00%)			
Girl	42 (47.19%)	55 (54.46%)	17 (48.57%)	25 (50.00%)			
Blood pressure (mmHg)							
Systolic	120 (100–135)	160 (122−201)	125 (90–138)	148 (110–190)	<**0.001**	1.000	<**0.001**
Diastolic	76 (60–85)	100 (62–130)	80 (60–89)	95 (70–120)	<**0.001**	1.000	<**0.001**
Proteinuria	None	Positive	None	None			
Infertility treatment					**0.028**	**0.006**	0.100
Yes	4 (4.49%)	14 (13.86%)	7 (20.00%)	6 (12.00%)			
No	85 (95.51%)	87 (86.14%)	28 (80.00%)	44 (88.00%)			

Data are presented as median (range) for continuous variables and as number (percent) for categorical variables. Statistically significant results are marked in bold. Continuous variables were compared using Kruskal-Wallis test. p-Value[1, 2, 3]: the comparison among normal pregnancies and preeclampsia, fetal growth restriction and gestational hypertension, respectively. Categorical variables were compared using a chi-square test.

3.3. Postpartum overexpression of miR-1-3p, miR-17-5p, miR-20b-5p, miR-29a-3p, and miR-126-3p is a common feature of GH and PE

When compared to the controls, overexpression or a trend to up-regulation of miR-17-5p and miR-29a-3p were observed in both GH (Fig. S2) and PE patients (Fig. 2A). The ROC curve analysis indicated that the accuracy of miR-17-5p and miR-29a-3p was slightly higher for patients with previous occurrence of GH (Fig. S2) when compared with patients with a prior exposure to PE (Fig. 2A).

Besides, higher expression rates or a trend toward higher expression rates of miR-17-5p, miR-20b-5p, miR-29a-3p, and miR-126-3p were detected in patients with previous occurrence of GH (Fig. S2) and severe PE (Fig. S3). At 10.0% FPR, a proportion of patients with GH or severe PE was identified to have up-regulated expression profile of miR-17-5p (20.0% vs. 19.48%), miR-20b-5p (14.0% vs. 18.18%), miR-29a-3p (20.0% vs. 18.18%), and miR-126-3p (12.0% vs. 11.69%) (Figs. S2, S3).

In addition, it was found that the expression of miR-1-3p and miR-17-5p trended to differ between the controls and patients with GH (Fig. S2) or early PE (Fig. 2C). The ROC curve analysis identified up-regulated expression profile of miR-1-3p (16.0% vs. 11.11%) and miR-17-5p (20.0% vs. 16.67%) in GH patients and patients exposed to early PE at 10.0% FPR (Figs. S2, 2C).

Parallel, the difference between the controls and groups of women with a prior exposure to GH (Fig. S2) or late PE (Fig. 2D) was found for miR-17-5p, miR-20b-5p and miR-29a-3p. Postpartum screening of miR-17-5p identified patients with a history of GH and late PE with a sensitivity of 20.0% and 21.54% at 10.0% FPR (Figs. S2, 2D). MiR-20b-5p differentiated between controls and patients with GH or late PE with a sensitivity of 14.0% and 18.46% at 10.0% FPR (Figs. S2, 2D). MiR-29a-3p was up-regulated in 20.0% patients with a history of GH and 13.85% patients exposed to late PE (Figs. S2, 2D).

3.4. Postpartum up-regulation miR-133a-3p and down-regulation of miR-130b-3p are unique features of PE

A trend toward statistical significance for up-regulation of miR-133a-3p was observed for PE patients. The sensitivity of 13.86% at

10.0% FPR for miR-133a-3p was found in PE patients (Fig. 2A). In addition, postpartum overexpression of miR-133a-3p was found in a group exposed to early PE (Fig. 2C). Overexpression of miR-133a-3p was also found in severe PE (Fig. S3). MiR-133a-3p showed a higher performance for patients exposed to severe PE (14.29% sensitivity) (Fig. S3) and/or early PE (16.67% sensitivity) (Fig. 2C) when compared to the group of PE women at 10.0% FPR (Fig. 2A).

Parallel, a trend toward statistical significance for down-regulation of miR-130b-3p was observed for women with severe PE. MiR-130b-3p differentiated between patients with severe PE and the controls with a sensitivity of 10.39% at 10.0% FPR (Fig. S3).

3.5. The association between postpartum microRNA expression and the severity of PE/FGR with regard to Doppler parameters

The PI in the umbilical artery showed a week positive correlation with miR-145-5p expression in PE/FGR women (Fig. S4). That means that women with prior finding of abnormal blood flow velocity waveforms in the umbilical artery showed increased postpartum miR-145-5p levels.

PE and/or FGR patients with abnormal values of flow rate in the middle cerebral artery showed overexpression of miR-125b-5p (14.29% sensitivity at 10.0% FPR) (Fig. S5).

In addition, the impact of CPR on miR-100-5p levels was observed. Centralization of the fetal circulation resulted in increased postpartum miR-100-5p levels in women with previous occurrence of PE/FGR (Fig. S6). At 10.0% FPR, miR-100-5p was up-regulated in 10.81% patients with a prior onset of PE/FGR with a sign of a blood flow centralisation (Fig. S6).

Parallel, ROC analysis revealed up-regulation of miR-125b-5p (19.05% sensitivity), miR-133a-3p (14.29% sensitivity), and miR-145-5p (9.52% sensitivity) at 10.0% FPR in patients with PE/FGR with an abnormal PI in the uterine artery (Fig. 3A).

Moreover, patients with a prior onset of PE/FGR with the presence of unilateral or bilateral diastolic notch in the uterine artery showed up-regulation of miR-125b-5p (20.0% sensitivity) and miR-133a-3p (15.0% sensitivity) at 10.0% FPR (Fig. 3B).

The PI in the DV demonstrated a week positive correlation with miR-145-5p expression in PE/FGR women (Fig. S7). That means that women with prior finding of DV dilatation in foetus indicating poor outcome demonstrated increased postpartum levels of miR-145-5p.

3.6. Postpartum combined screening of microRNAs in the identification of GH patients at an increased cardiovascular risk

Postpartum screening based on the combination of 7/7 unique microRNAs with aberrant expression profile in GH patients only (miR-20a-5p, miR-143-3p, miR-146a-5p, miR-181a-5p, miR-199a-5p, miR-221-3p, and miR-499a-5p) showed the highest accuracy for patients with a prior exposure to GH (Fig. 1A).

In addition, the combination of all five common microRNAs aberrantly expressed in patients with a history of GH or PE (miR-1-3p, miR-17-5p, miR-20b-5p, miR-29a-3p, and miR-126-3p) showed the highest accuracy for patients with a prior onset of GH (Fig. 1B).

There was an additive effect of using combined postpartum screening of both GH unique microRNA biomarkers or GH and PE common biomarkers compared to the single microRNA biomarkers, which showed the best accuracy for GH group (miR-29a-3p and miR-499a-5p) (Figs. S2, S1).

Similarly, the combination of all examined GH unique microRNAs or GH and PE common biomarkers was superior over using the combination of 2 microRNAs (miR-29a-3p and miR-499a-5p), which showed the best accuracy for GH group (Fig. 1C).

3.7. Postpartum combined screening of microRNAs in the identification of various PE patients at an increased cardiovascular risk

The combination of miR-17-5p, miR-29a-3p and miR-133a-3p was superior over using individual miR-17-5p, miR-29a-3p and miR-133a-3p biomarkers up-regulated in a group of PE patients (Fig. 2A).

With regard to patients with prior onset of severe PE, the combination of 5/6 microRNA biomarkers (miR-17-5p, miR-20b-5p, miR-29a-3p, miR-126-3p, and miR-133a-3p) (Fig. 2B) was superior over using individual miR-17-5p, miR-20b-5p, miR-29a-3p, miR-126-3p, miR-130b-3p, and miR-133a-3p biomarkers only (Fig. S3).

Regarding patients previously exposed to early PE, the combination of 3/3 microRNA biomarkers (miR-1-3p, miR-17-5p, and miR-133a-3p) (Fig. 2C) was superior over using individual miR-1-3p, miR-17-5p, and miR-133a-3p biomarkers only (Fig. 2C).

Concerning patients with a prior onset of late PE, the use of various combinations of three microRNA biomarkers up-regulated in a group of patients with late PE (miR-17-5p, miR-20b-5p, and miR-29a-3p) (Fig. 2D) had no advantage over using miR-29a-3p biomarker, which showed the highest accuracy (Fig. 2D).

3.8. Postpartum screening of miR-125b-5p in the identification of PE/FGR patients with abnormal flow rates in the uterine artery at an increased cardiovascular risk

The use of various combinations of three microRNA biomarkers up-regulated in a group of patients with previous occurrence of PE/FGR with an abnormal PI in the uterine artery (miR-125b-5p, miR-133a-3p, and miR-145-5p) (Fig. 3A) had no benefit over using the single miR-125b-5p biomarker, which showed the highest accuracy (Fig. 3A). Moreover, in patients with a prior onset of PE/FGR with the presence of unilateral or bilateral diastolic notch in the uterine artery miR-125b-5p used as a single microRNA biomarker had the best performance (Fig. 3B). Postpartum combined screening based on miR-125b-5p and miR-133a-3p biomarkers showed the lower accuracy for patients with a prior onset of PE/FGR with the presence of unilateral or bilateral diastolic notch in the uterine artery (Fig. 3B).

4. Discussion

Postpartum expression of microRNAs involved in pathogenesis of cardiovascular/cerebrovascular diseases was compared between uncomplicated and complicated pregnancies.

In general, the expression profile of tested microRNAs differed between controls and patients with a history of complicated pregnancies. With respect to particular pregnancy complication subtypes, up-regulation of individual microRNAs was found in patients with a history of GH (miR-1-3p, miR-17-5p, miR-20a-5p, miR-20b-5p, miR-29a-3p, miR-126-3p, miR-143-3p, miR-146a-5p, miR-181a-5p, miR-199a-5p, miR-221-3p and miR-499a-5p) or PE (miR-17-5p, miR-29a-3p and miR-133a-3p).

Furthermore, we studied the association between postpartum expression of microRNAs and the severity of the disease with regard to the occurrence of clinical signs, delivery date, and Doppler ultrasound examination.

Our study showed that miR-17-5p (19.48%), miR-20b-5p (18.18%), miR-29a-3p (18.18%), miR-126-3p (11.69%), and miR-133a-3p (14.29%) were up-regulated in a proportion of patients with a history of severe PE. Our results also showed that miR-1-3p (11.11%), miR-17-5p (16.67%) and miR-133a-3p (16.67%) were up-regulated in a proportion of patients with previous occurrence of early PE. Otherwise, mothers exposed to late PE showed a higher postpartum expression of miR-17-5p (21.54%), miR-20b-5p (18.46%) and miR-29a-3p (13.85%).

Moreover, the higher postpartum expression of particular microRNAs was found in women with a history of PE/FGR

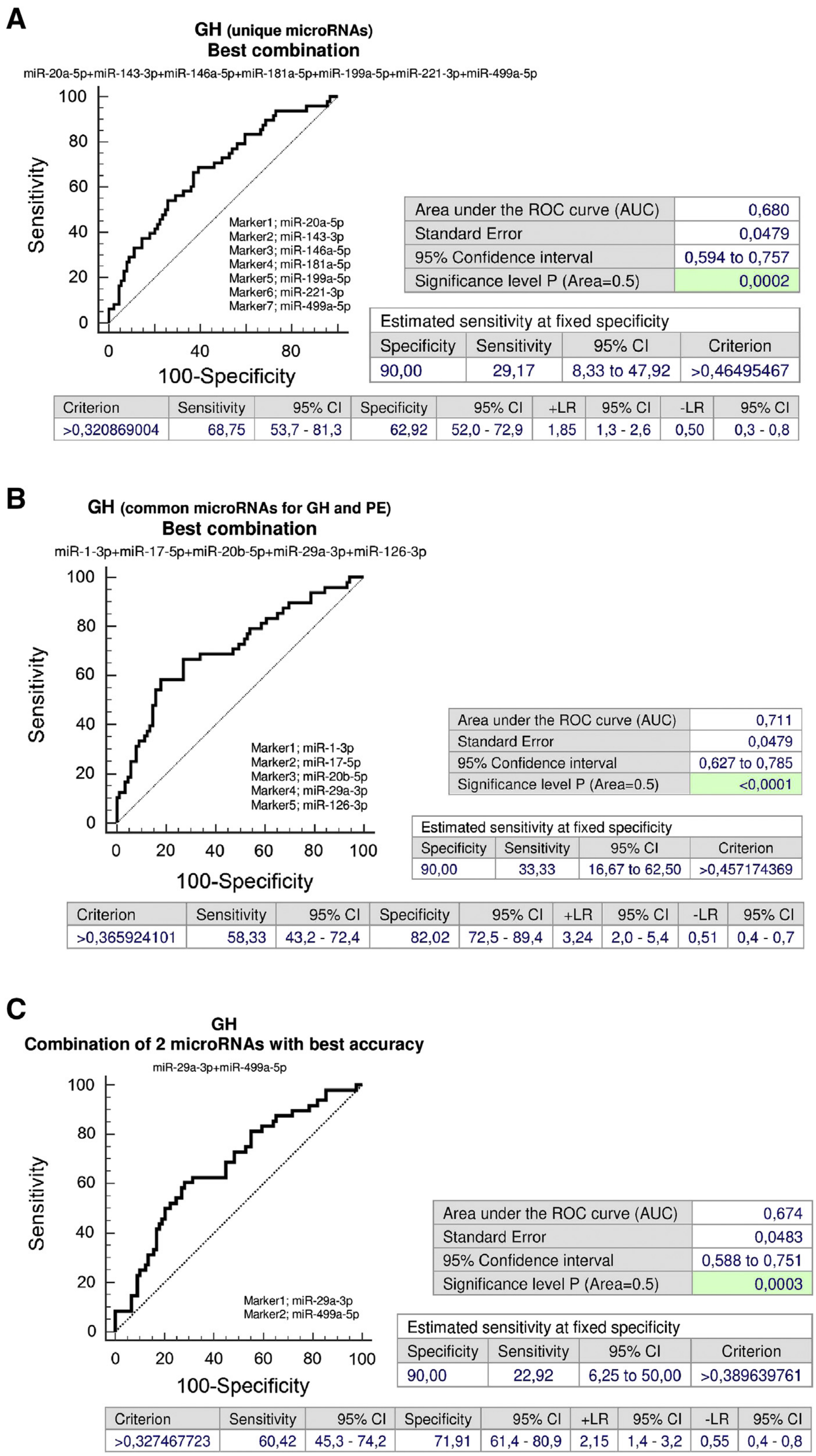


Fig. 1. Combined postpartum screening of microRNAs in the identification of patients with a history of GH at a higher risk of later development of cardiovascular/cerebrovascular diseases. Postpartum screening based on (A) the combination of miR-20a-5p, miR-143-3p, miR-146a-5p, miR-181a-5p, miR-199a-5p, miR-221-3p, and miR-499a-5p or (B) on the combination of miR-1-3p, miR-17-5p, miR-20b-5p, miR-29a-3p, and miR-126-3p showed the highest accuracy for the identification of patients with a history of GH at a higher risk of later development of cardiovascular/cerebrovascular diseases. (C) The combination of all examined GH unique microRNAs or GH and PE common biomarkers was superior over using the combination of 2 microRNAs (miR-29a-3p and miR-499a-5p), which showed the best accuracy for GH group.

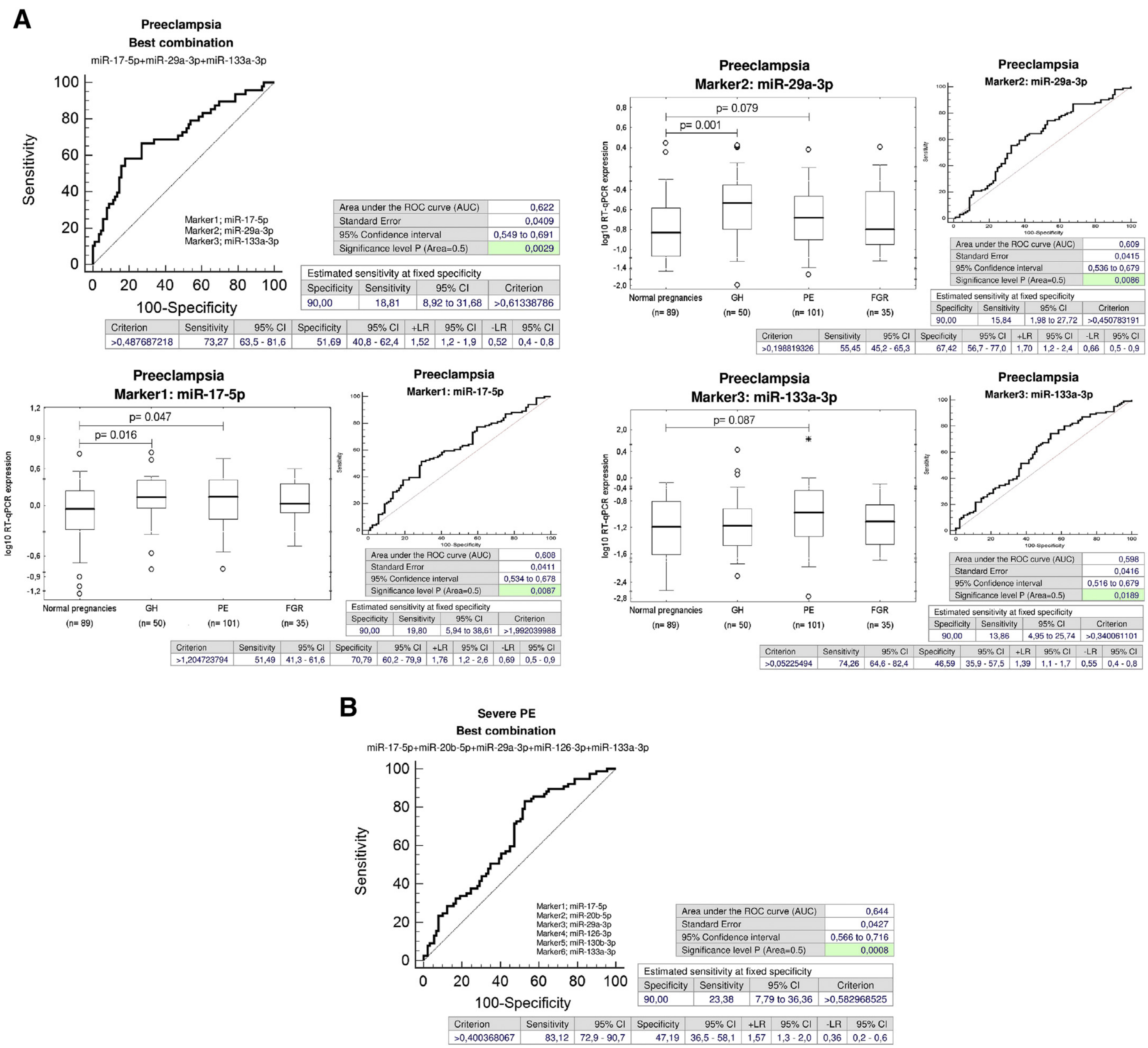


Fig. 2. Combined postpartum screening of microRNAs in the identification of patients with a history of PE at a higher risk of later development of cardiovascular/cerebrovascular diseases. (A) Postpartum screening based on the combination of miR-17-5p, miR-29a-3p and miR-133a-3p showed the highest accuracy for the identification of patients with a history of *PE regardless of the severity of the disease and the delivery date* at a higher risk of later development of cardiovascular/cerebrovascular diseases. (B) Postpartum screening based on the combination of miR-17-5p, miR-20b-5p, miR-29a-3p, miR-126-3p, and miR-133a-3p showed the highest accuracy for the identification of patients with *severe PE* at a higher risk of later development of cardiovascular/cerebrovascular diseases. (C) Postpartum screening based on the combination of miR-1-3p, miR-17-5p, and miR-133a-3p showed the highest accuracy for the identification of patients with *early PE* at a higher risk of later development of cardiovascular/cerebrovascular diseases. (D) Postpartum screening based on various combinations of miR-17-5p, miR-20b-5p, and miR-29a-3p had no advantage over using single miR-29a-3p biomarker for the identification of patients with *late PE* at a higher risk of later development of cardiovascular/cerebrovascular diseases.

pregnancies with aberrant values of flow rates in the arteria umbilicalis (miR-145-5p), the middle cerebral artery (miR-125b-5p), the uterine artery (miR-125b-5p, miR-133a-3p and miR-145-5p) or the ductus venosus (miR-145-5p). Abnormalities in the arteria umbilicalis and middle cerebral artery Doppler waveforms are frequently seen in FGR pregnancies and reflect the underlying increased placental blood flow resistance and fetal hypoxemia, respectively [e-component 8].

A proportion of women with the presence of unilateral or bilateral diastolic notch in the uterine artery in gestation complicated

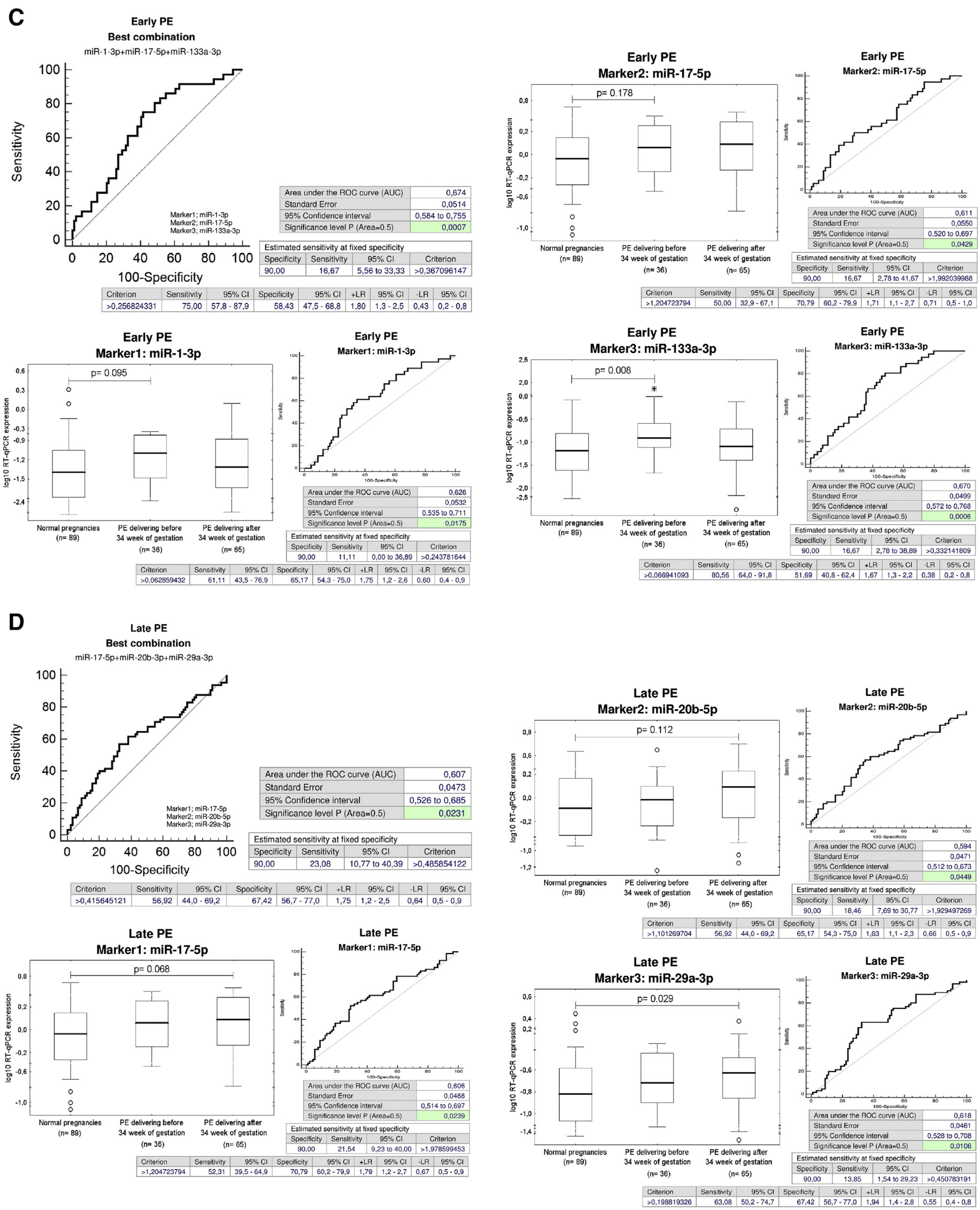
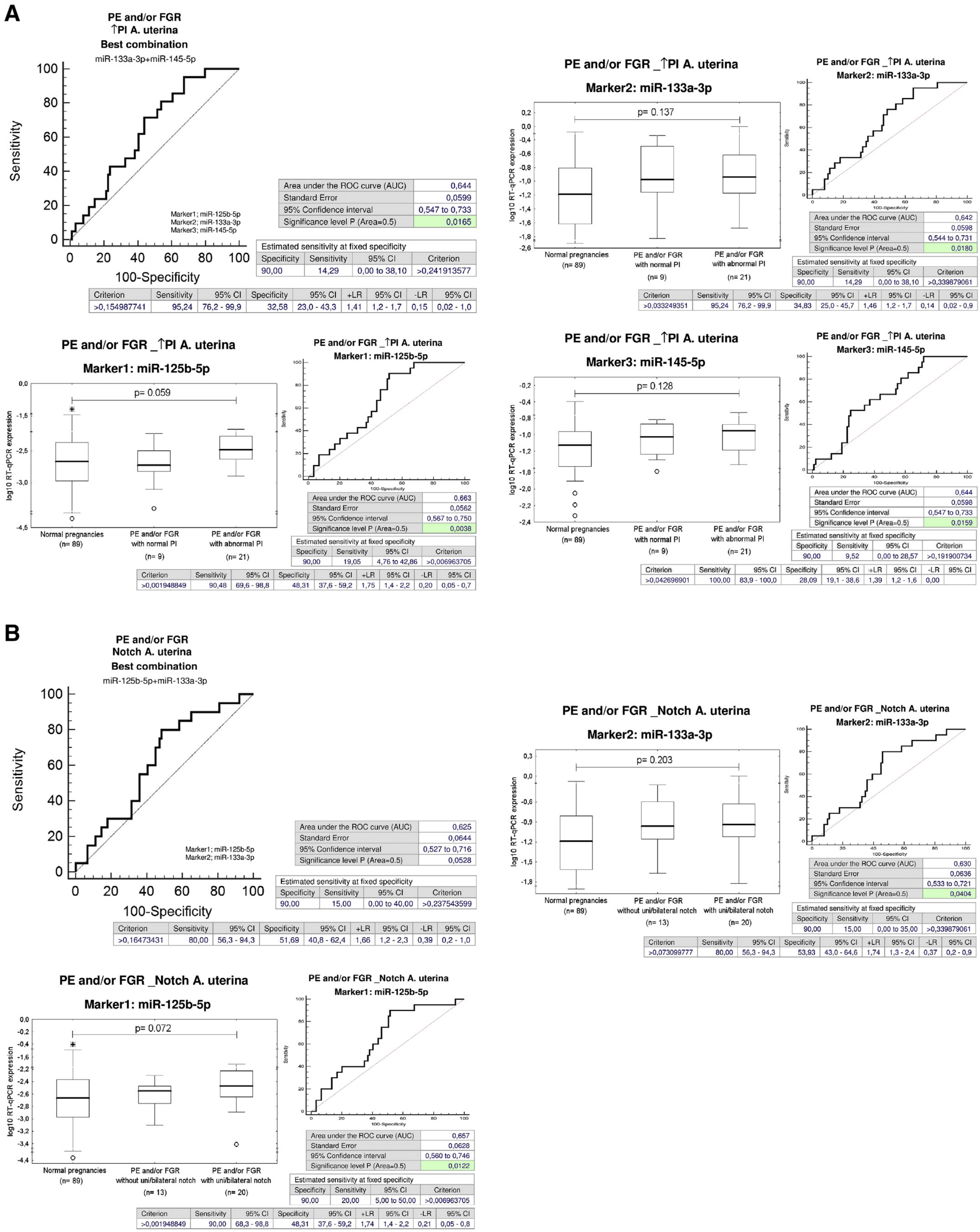


Fig. 2 (*continued*).



by PE/FGR also showed postpartum overexpression of miR-125b-5p (20.0%) and miR-133a-3p (15.0%). It was well documented that increased resistance in the uterine artery without or with the presence of unilateral or bilateral diastolic notch identifies pregnancies with a risk of placental failure [e-component 9–11].

In addition, the overexpression of miR-100-5p was found in a proportion of women with a prior exposure to PE/FGR with the centralization of the fetal circulation (10.81%), which ensures redistribution of the circulation into the vitally important organs [e-component 12, 13].

Both individual and combined screening of microRNA biomarkers showed the highest accuracy for identification of patients with previous occurrence of pregnancy complications at a higher risk of later development of cardiovascular/cerebrovascular diseases. The postpartum combination screening was superior over using individual microRNA biomarkers for patients with a prior exposure to GH (the combination of biomarkers: miR-1-3p, miR-17-5p, miR-20b-5p, miR-29a-3p, and miR-126-3p or the combination of other biomarkers: miR-20a-5p, miR-143-3p, miR-146a-5p, miR-181a-5p, miR-199a-5p, miR-221-3p, and miR-499a-5p), PE (miR-17-5p, miR-29a-3p, and miR-133a-3p), severe PE (miR-17-5p, miR-20b-5p, miR-29a-3p, miR-126-3p, and miR-133a-3p), and early PE (miR-1-3p, miR-17-5p, and miR-133a-3p). Nevertheless, a cardiovascular risk at patients with late PE (miR-29a-3p), PE/FGR with an abnormal PI in the uterine artery (miR-125b-5p) or the presence of unilateral or bilateral diastolic notch the uterine artery (miR-125b-5p) was identified more accurately using the single microRNA biomarker only.

This study demonstrated that epigenetic profile of microRNAs has been changing with advancing time by force of various circumstances, since several microRNAs which were up-regulated postpartum in women exposed to GH (miR-1-3p, miR-20a-5p, miR-20b-5p, miR-29a-3p, miR-143-3p, and miR-181a-5p), PE and/or FGR (miR-1-3p, miR-20b-5p, miR-29a-3p, miR-133a-3p, and miR-145-5p) were not observed to be dysregulated during the clinical manifestation of pregnancy complications [e-component 14]. Nevertheless, miR-1-3p and miR-133a-3p were overexpressed in placenta and/or umbilical cord blood samples of women with PE and/or FGR with an abnormal PI in the arteria umbilicalis or the presence of centralization of the fetal circulation ([50], e-component 15). That is why the postpartum overexpression of miR-1-3p and miR-133a-3p present in circulation of patients, whose pregnancies were affected with GH, PE and/or FGR, may also be associated with previous occurrence of these pregnancy disorders.

Parallel, several microRNAs which were up-regulated postpartum in women exposed to GH (miR-17-5p, miR-126-3p, miR-146a-5p, miR-199a-5p, miR-221-3p, and miR-499a-5p), PE and/or FGR (miR-17-5p, miR-100-5p, miR-125b-5p, and miR-126-3p) were observed to be down-regulated in mothers during the clinical manifestation of pregnancy complications ([50], e-component 10).

At present, there are no data on the postpartum expression profile of microRNAs associated with cardiovascular/cerebrovascular diseases in patients with a history of pregnancy complications. However, current data support the involvement of these microRNAs in the pathogenesis of cardiovascular/cerebrovascular diseases (Supplementary Material).

4.1. Study limitations

Consecutive multiple large scale studies are needed to verify the findings resulting from this pilot study.

5. Conclusion

In conclusion, epigenetic changes characteristic for cardiovascular/cerebrovascular diseases are also present in women exposed to pregnancy complications. Previous occurrence of GH, PE and/or FGR may predispose to later development of cardiovascular/cerebrovascular diseases.

Both individual and combined screening of microRNA biomarkers showed the highest accuracy for identification of patients with previous occurrence of pregnancy complications at a higher risk of later development of cardiovascular/cerebrovascular diseases.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.05.036.

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Declaration of competing interest

All authors declare there are no financial or non-financial competing interests and no relationships with industry.

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Fig. 3. Combined postpartum screening of microRNAs in the identification of patients with a history of PE and/or FGR with abnormal values of flow rates in the uterine artery at a higher risk of later development of cardiovascular/cerebrovascular diseases. (A) Postpartum screening based on various combinations of miR-125b-5p, miR-133a-3p, and miR-145-5p had no advantage over using single miR-125b-5p biomarker for the identification of patients with PE and/or FGR with *an abnormal PI in the uterine artery* at a higher risk of later development of cardiovascular/cerebrovascular diseases. (B) Postpartum screening based on the combination of miR-125b-5p and miR-133a-3p had no advantage over using single miR-125b-5p biomarker for the identification of patients with PE and/or FGR with *the presence of unilateral or bilateral diastolic notch in the uterine artery* at a higher risk of later development of cardiovascular/cerebrovascular diseases.

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