Pediatric RESEARCH

CLINICAL RESEARCH ARTICLE

Increased circulating endothelial progenitor cells (EPCs) in prepubertal children born prematurely: a possible link between prematurity and cardiovascular risk

Panagiota Markopoulou[1], Eleni Papanikolaou[2], Sofia Loukopoulou[3], Paraskevi Galina[4], Aimilia Mantzou[5] and Tania Siahanidou[1]

BACKGROUND: Endothelial progenitor cells (EPCs) ensure vascular integrity and neovascularization. No studies have investigated EPCs in preterm-born children beyond infancy.

METHODS: One hundred and thirty-six prepubertal children were enrolled: 63 preterm and 73 born at term (controls). Circulating CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs were measured in preterm-born children compared to controls. Body mass index (BMI), waist-to-hip ratio (WHR), neck circumference, systolic and diastolic blood pressure (SBP and DBP, respectively), fasting glucose, insulin, lipid profile, common carotid and abdominal aortic intima-media thickness (cIMT and aIMT, respectively), endothelium-dependent brachial artery flow-mediated dilation (FMD), and echocardiographic parameters were also assessed.

RESULTS: Circulating CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs were significantly higher in preterm-born children compared to controls ($p < 0.001$ and $p < 0.001$, respectively). In total study population and in the preterm-born group, EPCs were significantly lower in children born to mothers with gestational diabetes compared to non-diabetic mothers. Prematurity was associated with higher WHR, neck circumference, SBP, DBP, cIMT, aIMT, mean pressure, and velocity of pulmonary artery; the peak velocity of the brachial artery was significantly lower in children born prematurely. In multiple regression analysis, preterm birth and maternal gestational diabetes were recognized as independent predictors of EPCs.

CONCLUSIONS: Circulating EPCs were increased in prepubertal preterm-born children in comparison with peers born full-term. Maternal gestational diabetes was associated with a decrease in EPCs.

Pediatric Research (2021) 90:156–165; https://doi.org/10.1038/s41390-020-01190-y

IMPACT:

- Mounting evidence supports the adverse effect of prematurity on cardiovascular health. However, the underlying mechanisms that could lead to endothelial dysfunction in preterm-born individuals are not fully understood.
- Endothelial progenitor cells (EPCs) ensure vascular integrity, normal endothelial function and neovascularization.
- No studies have investigated the EPCs counts in peripheral blood beyond infancy in children born prematurely.
- Circulating EPCs were significantly higher in preterm-born prepubertal children compared to controls, thus indicating that prematurity is possibly associated with endothelial damage.
- In total study population and in the preterm-born group, maternal gestational diabetes was associated with decreased EPCs concentrations.

INTRODUCTION

Mounting evidence, nowadays, supports the adverse effect of being born preterm on cardiovascular health in the context of the "Developmental Origin of Health and Disease" concept.[1–5] Several studies have shown that preterm-born individuals display cardiovascular and metabolic alterations, including increased arterial blood pressure,[6–8] glucose intolerance and insulin resistance,[8,9] disturbances in lipid levels,[10,11] abnormalities in body composition,[12,13] and also adverse changes in heart shape and function[14–16] compared to individuals born at term. Nevertheless, the relationship between preterm birth and endothelial dysfunction in childhood or later in life is less clear;[8] decreased endothelium-dependent flow-mediated dilation (FMD) of brachial artery[17] and increased pulse wave velocity (PWV)[18] and mean carotid and abdominal aortic intima-media thickness (cIMT and aIMT, respectively)[19–22] have been reported, whereas no association was revealed in other studies.[8,23–26] Furthermore, the underlying mechanisms that could lead to

[1]Neonatal Unit, First Department of Pediatrics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece; [2]Laboratory of Biology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece; [3]Department of Cardiology, "Agia Sofia" Children's Hospital, Athens, Greece; [4]Radiology Department, "Agia Sofia" Children's Hospital, Athens, Greece and [5]Unit of Clinical and Translational Research in Endocrinology, First Department of Pediatrics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece
Correspondence: Tania Siahanidou (siahan@med.uoa.gr)

Received: 8 April 2020 Revised: 17 August 2020 Accepted: 22 September 2020
Published online: 10 October 2020

endothelial dysfunction in individuals born prematurely are not fully understood.

Endothelial progenitor cells (EPCs) are small, immature, bone marrow-derived cells that can be isolated from the peripheral and umbilical cord blood.[27] They proliferate and differentiate into mature endothelial cells, and they have critical roles in vascular repair and remodeling of both existing vessels and new blood vessel growth;[27,28] they restore vascular integrity and they contribute in normal endothelial function, tissue regeneration in ischemia, and physiological neovascularization.[28] The surface markers present in EPCs are mainly CD34, CD133, vascular endothelial growth factor receptor-2 (VEGFR-2), and lack of CD45; CD34(+)/VEGFR-2(+)/CD45(−) cell population has been referred as the most specific for determining EPCs in adults and children,[29,30] while relevant studies suggest that especially the fraction of CD45dim cells may represent the "true" circulating EPCs.[27,31]

In adults, increased number of EPCs is linked to outcomes of cardiovascular disease[32,33] and acute stroke,[34,35] as well as outcomes and progression of cancer.[36–39] In preterm infants, circulating EPCs are present at similar or increased numbers in comparison with term controls.[40] Nevertheless, the preterm EPCs are more vulnerable to hyperoxia-induced oxidative stress.[40] Lower counts of EPCs subpopulations in preterm babies have been associated not only with the development of bronchopulmonary dysplasia (BPD),[40,41] but also with disorders of pregnancy that increase the risk for preterm birth, such as gestational diabetes and preeclampsia.[42,43] Furthermore, EPCs subpopulations dysfunction has been associated with increased systolic blood pressure (SBP) in young adults born preterm.[44] Thus, EPCs impairment could possibly underlie several of the short- or long-term complications of prematurity.[40] To the best of our knowledge, no studies have investigated the EPCs counts in peripheral blood in preterm-born children beyond infancy.

The aims of this study were to evaluate the circulating EPCs levels in prepubertal children born prematurely, in the context of endothelial dysfunction they may exhibit, compared to prepubertal children born at term, to examine the relationship between EPCs and endothelial function parameters, as well as cardiovascular risk factors, and to study possible associations between EPCs and perinatal factors in the preterm group.

METHODS

Study design and population

This cross-sectional observational study enrolled 136 children of prepubertal age (mean age 10.7 ± 1.9 years) who were born between 2007 and 2011; of them, 63 children (25 males and 38 females) were born prematurely (<37 weeks of gestational age). Fifty-two of the preterm-born participants had birth weight appropriate for gestational age (AGA), while 11 were small for gestational age (SGA); AGA was defined as birth weight between 10th and 90th percentiles for gestational age, while SGA was defined as birth weight below 10th percentile for gestational age.[45] The control group consisted of 73 healthy children (37 males and 36 females) born at term (37–42 weeks of gestational age); of them, 61 were born AGA, while 12 were born SGA. All participants were hospitalized as neonates in the Neonatal Unit of the First Department of Pediatrics of National & Kapodistrian University of Athens, at "Agia Sofia" Children's Hospital, Athens, Greece, and were followed up regularly in the neonatal follow-up clinic.

In all participants, medical and family history was obtained, while perinatal and neonatal data were collected from hospital medical records. Pregnancy complications were recorded; maternal gestational diabetes mellitus was defined as any degree of glucose intolerance with onset or first recognition during pregnancy;[46] gestational hypertension was defined as SBP ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg at ≥20 weeks of gestation in the absence of proteinuria or new signs of end-organ dysfunction;[47] preeclampsia was defined as SBP ≥140 mmHg and/or DBP ≥90 mmHg at ≥20 weeks of gestation in the presence of proteinuria or new signs of end-organ dysfunction.[47] Neonatal morbidity was also examined; BPD was defined as supplemental oxygen requirements at 36 weeks of postmenstrual age.[44,48] Exclusion criteria were family history of cardiovascular disease, congenital cardiovascular or pulmonary malformations, active disease, or obesity [defined as body mass index (BMI) ≥95th percentile for age]. Smoking behavior was also assessed; all the participants were non-smokers. The study protocol was approved by the Research and Ethics committee of "Agia Sofia" Children's Hospital, Athens, Greece corresponding to the principles outlined in the Declaration of Helsinki. Informed written consent was obtained from parents and children.

Clinical assessment

The participants attended the outpatient clinic at a similar time of day (7.30–9.30 a.m.) after a 12-h overnight fast and were examined in a temperature-controlled (21–24 °C), dimly lit room.[10] Standing height was measured to the nearest 0.1 cm on bare foot using a Harpenden stadiometer (London, UK) and weight to the nearest 0.1 kg by a scale (Seca 712, UK) with subjects wearing light clothing. Waist and hip circumference were also measured to the nearest 0.1 cm over an unclothed abdomen and at minimal respiration.[10] The waist-to-hip ratio (WHR) and the BMI (weight in kilograms divided by height in meters squared) were calculated. Neck circumference was measured using a flexible tape measure, with the neck in a horizontal plane at the level of the most prominent portion, the thyroid cartilage.[49] All anthropometric measurements were performed twice; the mean was used for analysis. Pubertal stage was assessed according to Tanner criteria.[50] SBP and DBP were measured three times in all participants from the left brachial artery using an automatic oscillometric blood pressure monitor, after a 10-min rest in a sitting position and with an appropriate-sized cuff;[51] the bladder length was 80–100% of the circumference of the arm, and the width was at least 40% of the circumference of the arm.[52] The second and third measurements were averaged for analysis.[19]

Blood biochemistry

Fasting serum glucose and insulin, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were measured in all participants. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as insulin (μU/ml) × blood glucose (mmol/l)/22.5; IR was defined as HOMA-IR >2.5.[53]

Ultrasound studies

Measurement of cIMT and aIMT. Two-dimensional ultrasound examination of left and right common carotid arteries, and abdominal aorta was performed with the Logiq E9 US system (General Electric Healthcare, Wauwatosa, WI, USA) using a 7.5 MHz high-resolution probe following a standardized scanning method.[54,55] The cIMT was defined as the distance between the leading edges of the lumen–intima and the media–adventitia interface of the far wall.[54] Six measurements were performed on each side; the calculated mean of the six measurements provided, respectively, the arithmetic mean left and right cIMT of each subject.[56] The aIMT was defined as the distance from the trailing edge of the intima to the trailing edge of the media–adventitia interface of the far wall.[21,55] Mean aIMT was determined as the average reading of three measurements for each subject.

FMD of the brachial artery. All children were fasted and free of physical exercise for at least 8–12 h before the examination.[51] With

the subject in a supine position for at least 10 min before the scan, the brachial artery was imaged above the antecubital fossa in the longitudinal plane using the Logiq E9 US system (General Electric Healthcare, Wauwatosa, WI, USA) and a 7.5 MHz linear probe, following a standardized scanning protocol.[57] A sphygmomanometer pediatric-size cuff (7 × 20.6 cm) was placed distally to the artery, above the antecubital fossa. A baseline rest image and a pulsed Doppler velocity signal were acquired. Thereafter, arterial occlusion was created by inflating the cuff to a pressure of 200–220 mmHg for 5 min, followed by release.[57] A midartery pulsed Doppler signal was obtained upon immediate cuff release, and the image of the artery was then recorded continuously for 15–180 s after cuff deflation.[57] FMD was calculated using the formula:

FMD = [(maximum dilatation diameter after deflation − baseline diameter)/(baseline diameter)] × 100%.

Echocardiography. Examination was performed in left lateral decubitus position using the Logiq E9 US system (General Electric Healthcare, Wauwatosa, WI, USA) fitted with a 2.5–3.5 MHz probe.[58,59] Left ventricular ejection fraction (EF) and shortening fraction (SF) were evaluated.[58] End-diastolic interventricular septal thickness (IVSd), left ventricular end-systolic (LVIDs) and end-diastolic internal diameters (LVIDd), and end-diastolic left ventricular posterior wall thickness (LVPWd) were measured according to established standardized protocols.[59] Left ventricular mass (LVM) and relative wall thickness (RWT) were calculated;[60] left ventricular mass index (LVMI) was estimated by the formula: LVM/body surface area.[60] The mean pressure and velocity of the pulmonary artery were also examined.

The ultrasound studies of cIMT, aIMT, and FMD of the brachial artery were conducted by a single experienced vascular sonographer, while the echocardiography was performed by a single senior pediatric cardiologist, specialized in cardiac ultrasonography; both were blinded to the gestational age, as well as to the clinical and laboratory characteristics of the study population.

Flow cytometric analysis of circulating EPCs

For detection and quantification of EPCs, blood samples (1 ml) were collected in 3.2% sodium citrate tubes following an overnight fast in an atraumatic fashion. Peripheral blood mononuclear cells (PBMNs) were isolated by Biocoll (Biochrom GmbH, Germany) density-gradient centrifugation (2500 rpm for 25 min at room temperature); recovered cells were washed twice with phosphate-buffered saline (PBS) (Biosera, France), and thereafter they were immediately stored at −80 °C. EPCs were phenotyped and quantified by flow cytometry; 200 µl of each sample were incubated with 5 µl of fluorochrome-labeled antibody specific for CD34 [CD34-fluorescein isothiocyanate (FITC) (ImmunoTools)], 4 µl of fluorochrome-labeled antibody specific for CD45 [CD45-phycoerythrin (PE) (ImmunoTools)], and 10 µl of fluorochrome-labeled antibody specific for CD309 (VEGFR-2/KDR) [CD309 (VEGFR-2/KDR)-APC (Miltenyi Biotec, Germany)] for 20 min at 4 °C in the dark. After staining, samples were diluted with 2 ml of PBS (Biosera, France) before flow cytometric analysis.

For quantification of CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs, initially we gated CD45(−) and CD45dim PBMNs (Fig. 1a). Then the dual expression of CD34 and VEGFR-2 was determined within the CD45(−) and CD45dim populations (Fig. 1b). In addition, unstained and spiked samples for CD34(+) and VEGFR-2(+) were used to assist the set-up of the gates (Supplementary Fig. 1). Further information regarding the gating strategy is given in Supplementary Material.

Samples were analyzed in a fluorescence-activated cell sorter (FACS) Calibur flow cytometer (BD FACSCalibur™, BD Biosciences). The data obtained were analyzed using the Flowing Software version 2.5.1. EPCs values are reported as the percentage of each

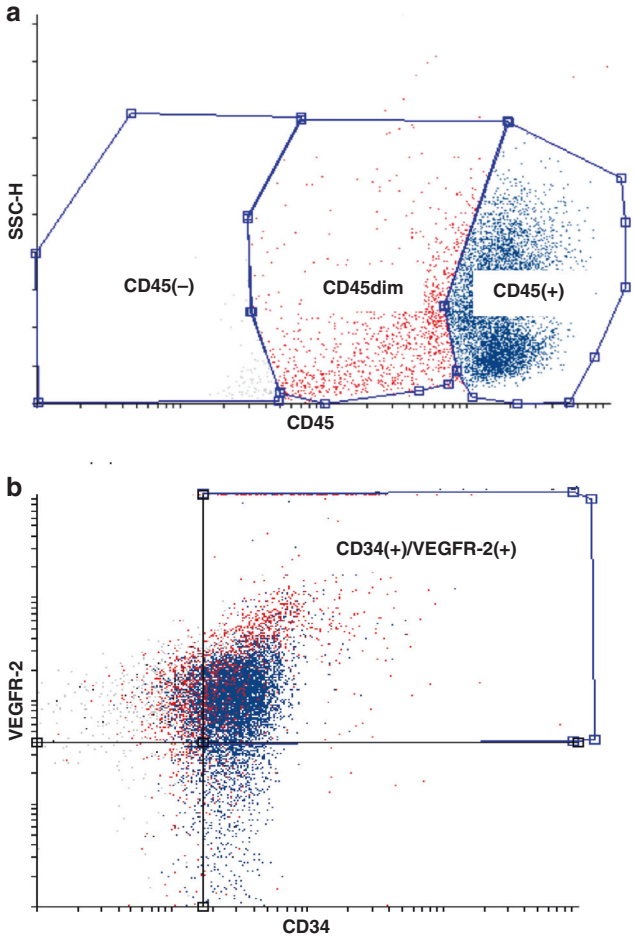

Fig. 1 Gating strategy of endothelial progenitor cells (EPCs). CD45(−) and CD45dim EPCs gating (**a**), and then, the resulting populations from CD45 gating were examined for triple expression of CD34 and VEGFR-2 (**b**). SSC-H side scatter height signal.

EPC subpopulation within the CD45(−) and CD45dim populations. Forward scatter (FSC-H) and side scatter (SSC-H) had a linear gain, while each fluorescent channel had a logarithmic gain. All samples were analyzed in triplicate with excellent repeatability for all EPCs subpopulations [intraclass correlation coefficient (ICC) >0.90 is indicative of excellent reliability[61]]; ICC = 0.97 for CD34(+)/VEGFR-2(+)/CD45(−) and ICC = 0.93 for CD34(+)/VEGFR-2(+)/CD45dim EPCs.

Statistical analysis

Statistical analysis was performed using SPSS version 23.0 (SPSS Inc., Chicago, IL). Kolmogorov–Smirnov and Shapiro–Wilk procedures were applied for testing normality of distribution. In parameters with normal distribution, results are given as mean ± SD; for parameters not normally distributed, results are expressed as medians (25th–75th percentile). Groups were compared using independent *t* test or Mann–Whitney test, as appropriate, for continuous variables and chi-square test for categorical variables. Linear correlations between variables of interest were calculated using Pearson's or Spearman's correlation coefficient for parameters with normal or skewed distribution, respectively; stepwise multiple regression analysis was further performed to investigate independent determinants of each EPC subpopulation. Due to skewed distribution, EPCs values were log-transformed before applying multiple regression analysis. Statistical significance was accepted if the null hypothesis could be rejected at p value ≤0.05.

RESULTS

The baseline clinical, perinatal, and biochemical characteristics of study participants are shown in Table 1. Mean ± SD gestational age and birth weight of prematurely born children were 31.7 ± 3.2 weeks and 1630 ± 536 g, respectively. Children born prematurely presented with higher waist circumference ($p = 0.02$), WHR ($p = 0.05$), and neck circumference ($p = 0.02$) compared to controls. Moreover, children born prematurely showed significantly higher levels of both SBP ($p = 0.003$) and DBP ($p = 0.02$) compared to children born at term. No significant differences were found between preterm-born children and controls regarding fasting serum glucose, insulin, HOMA-IR, or lipid levels (Table 1).

In the preterm-born group, males presented with higher SBP values compared to females (111.72 ± 10.79 mmHg vs. 105.01 ± 11.76 mmHg, $p = 0.04$). No significant gender-differences were found regarding other clinical and biochemical parameters examined in the preterm-born population.

Vascular and echocardiographic assessment

Higher values of left cIMT ($p < 0.001$), right cIMT ($p < 0.001$), mean cIMT ($p < 0.001$), as well as aIMT ($p = 0.01$), were found in preterm-born children in comparison with controls (Table 2). Furthermore, the peak velocity of the brachial artery was significantly lower in children born prematurely ($p = 0.001$); FMD, baseline and peak diameter, baseline velocity, and time of peak of the brachial artery did not differ significantly between children born prematurely and children born at term. Also, there were no significant differences between children born prematurely and controls regarding EF, SF, IVSd, LVIDs, LVIDd, LVPWd, LVM, LVMI, or RWT (Table 2). However, mean pressure and velocity of pulmonary artery were found significantly higher in preterm-born children in comparison with controls ($p = 0.04$ and $p = 0.02$, respectively).

In the preterm-born group, males presented with greater baseline diameter (3.03 ± 0.32 mm vs. 2.78 ± 0.28 mm, $p = 0.003$) and velocity [77.40 (66.50–83.80) cm/s vs. 66.25 (58.33–76.25) cm/s, $p = 0.01$] and peak diameter (3.49 ± 0.29 mm vs. 3.10 ± 0.34 mm, $p < 0.001$) of the brachial artery, as well as with greater IVSd (8.18 ± 1.71 mm vs. 7.28 ± 1.56 mm, $p = 0.03$) compared to females. No significant gender-differences were found in other vascular or echocardiographic parameters examined in the preterm-born group.

Flow cytometric analysis of EPCs

In preterm-born children, circulating CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs were significantly higher compared to controls [0.45 (0.14–2.55)% vs. 0.15 (0.05–0.42)%, $p < 0.001$ and 8.69 (4.09–22.60)% vs. 3.14 (1.16–7.59)%, $p < 0.001$, respectively] (Fig. 2). In the total study population, CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs were significantly lower in children born to mothers with gestational diabetes compared to those born to non-diabetic mothers [0.10 (0.03–0.25)% vs. 0.28 (0.10–1.55)%, $p = 0.005$ and 2.52 (1.43–5.00)% vs. 5.70 (2.18–13.85)%, $p = 0.02$, respectively]. Similarly, in the preterm-born population, CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs were significantly lower in children born to mothers with gestational diabetes compared to non-diabetic mothers [0.16 (0.12–0.27)% vs. 0.72 (0.16–2.99)%, $p = 0.04$ and 4.13 (2.30–6.36)% vs. 8.60 (4.25–22.36)%, $p = 0.03$, respectively]. In the total study population, as well as in the preterm-born group, EPCs subpopulations studied did not differ either between children born to mothers with gestational hypertension or preeclampsia or mothers who smoke during pregnancy, compared to those born to non-hypertensive, non-preeclamptic, or non-smoking mothers or between preterm-born children who had presented BPD, intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), or patent ductus arteriosus (PDA) in comparison to those with no perinatal morbidity.

Table 1. Clinical and perinatal characteristics and blood biochemistry of preterm-born children and controls.

Variable	Preterm-born children ($n = 63$)	Controls ($n = 73$)	p value
Age (years)	10.9 ± 1.9	10.6 ± 2.0	0.20
Males (*n*)	25	37	0.27
Small for gestational age (SGA)	11	12	0.37
Maternal age at birth (years)	32.8 ± 4.6	32.5 ± 5.1	0.65
Maternal gestational hypertension [*n* (%)]	6 (9.5)	1 (1.4)	**0.01**
Maternal preeclampsia [*n* (%)]	5 (7.9)	1 (1.4)	**0.02**
Maternal gestational diabetes [*n* (%)]	7 (11.1)	7 (9.6)	1.00
Maternal smoking during pregnancy [*n* (%)]	6 (9.5)	1 (1.4)	**0.04**
Cesarean delivery [*n* (%)]	55 (87.3)	30 (41.1)	**<0.001**
Gestational age (weeks)	31.7 ± 3.2	39.0 ± 1.0	**<0.001**
Birth weight (g)	1630 ± 536	3229 ± 452	**<0.001**
BPD [*n* (%)]	11 (17.5)	0 (0)	**<0.001**
IVH [*n* (%)]	14 (22.2)	0 (0)	**<0.001**
ROP [*n* (%)]	18 (28.6)	0 (0)	**<0.001**
PDA [*n* (%)]	8 (12.7)	0 (0)	**<0.001**
Weight (kg)	42.0 ± 11.9	38.4 ± 10.2	0.07
Height (cm)	146.2 ± 12.3	143.5 ± 12.7	0.25
BMI (kg/m^2)	19.3 ± 3.3	18.4 ± 2.8	0.11
Waist circumference (cm)	72.1 ± 9.9	68.3 ± 8.6	**0.02**
Hip circumference (cm)	80.4 ± 10.1	77.7 ± 8.6	0.10
WHR	0.90 ± 0.05	0.88 ± 0.04	**0.05**
Neck circumference (cm)	30.0 ± 2.5	29.0 ± 1.8	**0.02**
SBP (mmHg)	107.7 ± 11.8	101.8 ± 9.0	**0.003**
DBP (mmHg)	66.6 ± 7.4	63.5 ± 7.3	**0.02**
Glucose (mg/dl)	78.6 ± 9.2	79.9 ± 7.0	0.17
Insulin (μUI/ml)	7.0 (5.1–10.1)	6.7 (5.7–8.8)	0.84
HOMA-IR	1.28 (0.94–2.01)	1.29 (1.07–1.82)	0.98
TC (mg/dl)	150.0 (135–176)	156.5 (141.3–168.5)	0.22
HDL-C (mg/dl)	62.2 ± 13.5	63.5 ± 14.0	0.75
LDL-C (mg/dl)	74.0 (60.0–95.0)	80.0 (67.3–93.0)	0.26
TG (mg/dl)	51.0 (40.0–68.0)	55.5 (38.3–69.8)	0.47

Statistically significant p values are in bold ≤ 0.05.
SGA small for gestational age, *BMD* bronchopulmonary dysplasia, *IVH* interventricular hemorrhage, *ROP* retinopathy of prematurity, *PDA* patent ductus arteriosus, *BMI* body mass index, *WHR* waist-to-hip ratio, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HOMA-IR* homeostasis model assessment of insulin resistance index, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglycerides.

Differences remained statistically significant when EPCs were analyzed separately for males and females; circulating CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs were significantly higher in both preterm-born males and females compared to males and females, respectively, born at term [for males: 0.32 (0.16–1.71)% vs. 0.14 (0.05–0.47)%, $p = 0.05$ and 7.20 (3.86–13.13)% vs. 3.65 (1.27–7.02)%, $p = 0.02$, respectively; for females: 0.67 (0.13–5.17)% vs. 0.15 (0.05–0.46)%, $p = 0.004$ and 9.13 (4.14–43.42)% vs. 2.78 (0.99–9.08)%, $p < 0.001$, respectively]. In the preterm group, no differences were found between males and females regarding EPCs subpopulations studied.

Table 2. Vascular assessment and echocardiography in preterm-born children and controls.

Variable	Preterm-born children ($n = 63$)	Controls ($n = 73$)	p value
Left cIMT (mm)	0.32 ± 0.05	0.29 ± 0.04	**<0.001**
Right cIMT (mm)	0.33 ± 0.05	0.28 ± 0.03	**<0.001**
Mean cIMT (mm)	0.33 ± 0.04	0.28 ± 0.04	**<0.001**
aIMT (mm)	0.44 ± 0.12	0.39 ± 0.07	**0.01**
Baseline diameter of brachial artery (mm)	2.88 ± 0.32	2.92 ± 0.31	0.54
Baseline velocity of brachial artery (cm/s)	71.50 (62.20–79.80)	67.30 (59.68–90.40)	0.98
Peak diameter of brachial artery (mm)	3.25 ± 0.38	3.26 ± 0.37	0.86
Time of peak of brachial artery (s)	60.0 (45.0–75.0)	60.0 (41.25–75.0)	0.81
Peak velocity of brachial artery (cm/s)	103.70 (91.40–118.45)	119.90 (104.60–127.25)	**0.001**
FMD of brachial artery (%)	12.85 ± 7.84	11.87 ± 5.91	0.44
EF (%)	71.00 (68.30–75.00)	70.26 (68.29–74.00)	0.32
SF (%)	39.88 (38.00–43.18)	39.00 (37.02–42.00)	0.27
IVSd (mm)	7.66 ± 1.67	7.29 ± 1.60	0.21
LVIDs (mm)	22.32 ± 3.31	23.26 ± 4.12	0.17
LVIDd (mm)	37.26 ± 4.87	37.23 ± 6.07	0.98
LVPWd (mm)	6.17 ± 1.96	6.31 ± 1.68	0.66
LVM (g)	64.68 (50.42–85.76)	67.68 (51.97–79.38)	0.97
LVMI (g/m^2)	53.33 ± 14.15	55.41 ± 11.09	0.41
RWT	0.37 (0.31–0.44)	0.37 (0.32–0.42)	0.88
Mean pressure of pulmonary artery (mmHg)	4.68 (3.68–6.26)	3.98 (3.33–5.42)	**0.04**
Mean velocity of pulmonary artery (m/s)	1.09 (0.96–1.27)	1.00 (0.91–1.15)	**0.02**

Statistically significant p values are in bold ≤ 0.05.
cIMT carotid intima-media thickness, *aIMT* abdominal aortic intima-media thickness, *FMD* flow-mediated dilation of the brachial artery, *EF* ejection fraction, *SF* shortening fraction, *IVSd* end-diastolic interventricular septum thickness, *LVIDs* left ventricular end-systolic internal diameter, *LVIDd* left ventricular end-diastolic internal diameter, *LVPWd* end-diastolic left ventricular posterior wall thickness, *LVM* left ventricular mass, *LVMI* left ventricular mass index, *RWT* relative wall thickness.

In the total study population, circulating CD34(+)/VEGFR-2(+)/CD45(−) EPCs were correlated significantly with gestational age, birth weight, SBP, DBP, TC, mean cIMT, and mean pressure and velocity of pulmonary artery; circulating CD34(+)/VEGFR-2(+)/CD45dim EPCs correlated with gestational age, birth weight, SBP, DBP, TC, TG, LDL, mean cIMT, EF, SF, and mean pressure and velocity of pulmonary artery (Table 3). In the preterm-born group, circulating CD34(+)/VEGFR-2(+)/CD45(−) EPCs correlated significantly with SBP, DBP, TC, aIMT, and mean pressure and velocity of pulmonary artery; circulating CD34(+)/VEGFR-2(+)/CD45dim EPCs correlated with age, WHR, SBP, TC, LDL, aIMT, and mean pressure and velocity of pulmonary artery (Table 4).

Multiple linear regression analyses were then applied, with each EPC subpopulation as a dependent variable, in order to examine whether the above associations remained significant. When adjusting for age, sex, as well as for parameters of maternal and neonatal morbidity, preterm birth and maternal gestational diabetes were recognized as independent predictors for each EPC subpopulation studied; more specifically, preterm birth correlated significantly with CD34(+)/VEGFR-2(+)/CD45(−) EPCs ($\beta = 0.33$, $p = 0.003$) and CD34(+)/VEGFR-2(+)/CD45dim EPCs ($\beta = 0.40$, $p < 0.001$), while maternal gestational diabetes correlated significantly with CD34(+)/VEGFR-2(+)/CD45(−) EPCs ($\beta = -0.28$, $p = 0.01$) and CD34(+)/VEGFR-2(+)/CD45dim EPCs ($\beta = -0.21$, $p = 0.04$). Moreover, total cholesterol levels were independently correlated with CD34(+)/VEGFR-2(+)/CD45dim EPCs ($\beta = -0.22$, $p = 0.03$). In preterm-born children, maternal gestational diabetes ($\beta = -0.28$, $p = 0.04$), SBP ($\beta = 0.27$, $p = 0.05$), total cholesterol levels ($\beta = -0.37$, $p = 0.01$) and aIMT ($\beta = -0.40$, $p = 0.004$) were independently correlated with CD34(+)/VEGFR-2(+)/CD45(−) EPCs; maternal gestational diabetes ($\beta = -0.34$, $p = 0.01$), age ($\beta = 0.42$, $p = 0.001$), total cholesterol levels ($\beta = -0.36$, $p = 0.01$), and aIMT ($\beta = -0.39$, $p = 0.002$) correlated independently with CD34(+)/VEGFR-2(+)/CD45dim EPCs.

DISCUSSION

In this study, circulating CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs were significantly higher in prepubertal children born prematurely compared to children born at term, thus indicating that prematurity is possibly associated with endothelial damage and increased EPCs mobilization, which is already evident in childhood. Furthermore, in the total study population, as well as in preterm-born children, circulating CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs were significantly lower in children born to mothers with gestational diabetes compared to those born to non-diabetic mothers. Preterm birth and maternal gestational diabetes were recognized as independent predictors of both EPCs subpopulations studied; moreover, in preterm-born children, maternal gestational diabetes, total cholesterol levels, and aIMT were independently correlated with both CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs. Interestingly, preterm-born children also presented with a number of cardiovascular risk factors, including higher waist circumference, WHR and neck circumference, higher levels of both SBP and DBP, as well as increased cIMT and aIMT values, compared to controls. Furthermore, peak velocity of the brachial artery was lower in preterm-born children compared to controls.

To the best of our knowledge, this is the first study that investigated circulating EPCs in preterm-born individuals in childhood. Our study population was derived from the follow-up outpatient clinic of our Neonatal Unit, whereas healthy

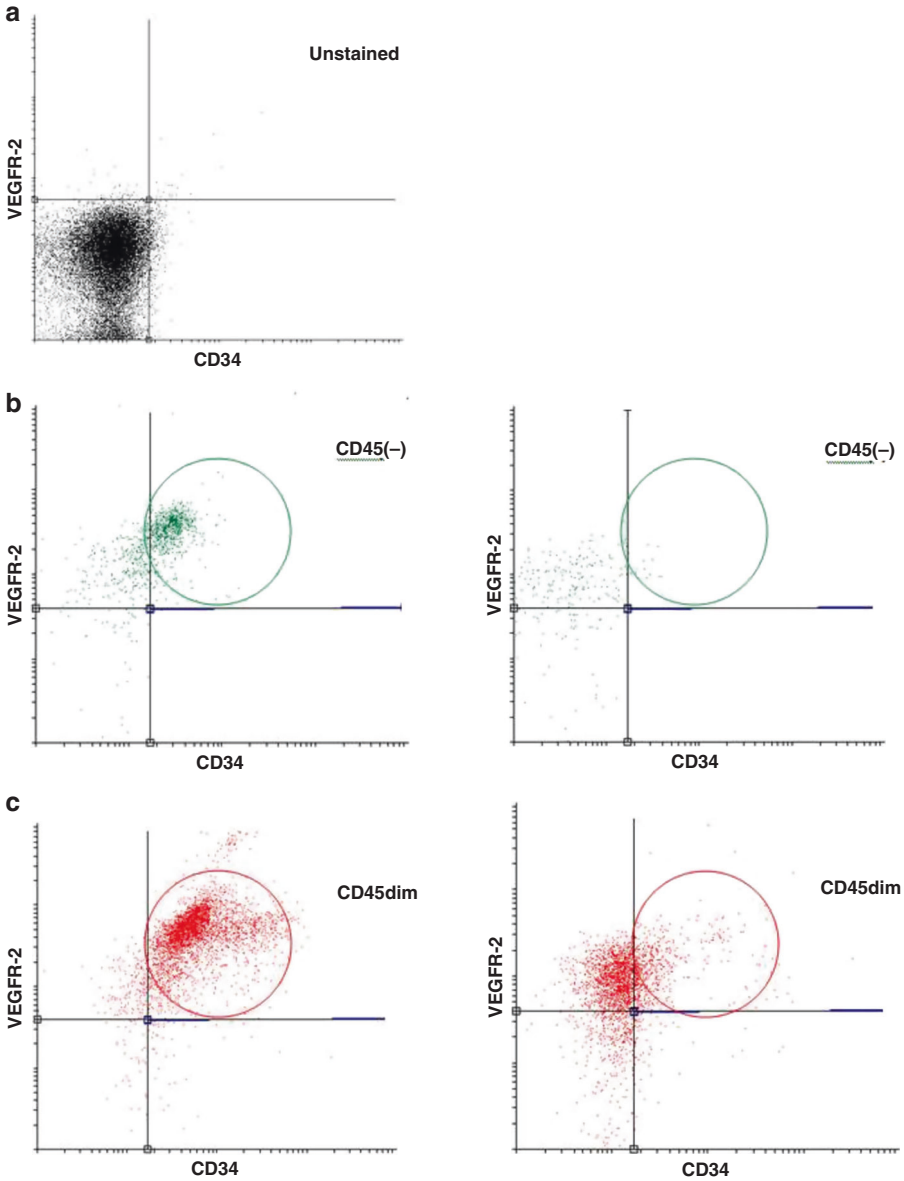

Fig. 2 Representative prints out of flow cytometric determination of endothelial progenitor cells (EPCs) in preterm-born children and in children born at term. Representative prints out of flow cytometric determination of CD34(+)/VEGFR-2(+)/CD45(-) EPCs in a child born prematurely (left) and in a child born at term (right) (**b**). Representatitve prints out of flow cytometric determination of CD34(+)/VEGFR-2 (+)/CD45dim EPCs in a child born prematurely (left) and in a child born at term (right) **(c)**. Unstained sample (**a**).

prepubertal children born either prematurely or after a full-term pregnancy were enrolled; perinatal data and medical records were reliably recorded and easily accessed. It should be acknowledged that EPCs express different cell surface markers and their isolation can be performed by cell culture or by immunofluorometric methods, such as flow cytometry.[62] Flow cytometry, used in this study, is an easy, reliable, and rapid assay for ex vivo quantitative assessment of circulating EPCs subpopulations; small total blood volumes are needed for EPCs enumeration, which is quite important in pediatric populations.[62] Regarding the cell surface markers that have been used to identify EPCs, a combination of an immaturity/stem antigen (CD34 and/or CD133)[62,63] and one endothelial antigen (VEGFR-2) is commonly employed;[62,64] the CD45(−) and, more specifically, the CD45dim population, provide more precision to the characterization of EPCs.[31,62] All the above consisted the main strengths of our study.

EPCs are mobilized from bone marrow to participate in endothelial repair in response to endogenous or exogenous signals;[27] they contribute to vascular integrity, tissue regeneration in ischemia, tissue remodeling, and physiological neovascularization, functions that are extremely important during organogenesis and postnatal development.[27,65] Decreased EPCs counts in preterm infants were predictive for the development of BPD.[44] Furthermore, EPCs dysfunction has been observed in preterm-born adults with a history of BPD, as well as in those with increased SBP into adulthood.[40,44]

EPCs have been examined in several cardiovascular and inflammatory diseases in adults; an increased number of EPCs is often observed in patients with acute coronary syndrome.[32,33,66,67] In contrast, reduced EPCs number and altered EPCs function were found in cardiovascular high-risk patients, including those with diabetes mellitus,[68] systemic hypertension,[69] and chronic kidney

Table 3. Significant correlations of CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs with clinical, biochemical, and vascular/echocardiographic parameters in the total study population.

	Correlation coefficient	*p* value
CD34(+)/VEGFR-2(+)/CD45(−) EPCs		
Gestational age	−0.20	0.03
Birth weight	−0.22	0.01
SBP	0.22	0.02
DBP	0.24	0.01
TC	−0.21	0.03
Mean cIMT	0.24	0.01
Mean pressure of pulmonary artery	0.23	0.01
Mean velocity of pulmonary artery	0.25	0.01
CD34(+)/VEGFR-2(+)/CD45dim EPCs		
Gestational age	−0.28	0.001
Birth weight	−0.30	0.001
SBP	0.18	0.04
DBP	0.21	0.02
TC	−0.20	0.03
TG	−0.22	0.01
LDL	−0.22	0.02
Mean cIMT	0.27	0.03
EF	0.21	0.02
SF	0.24	0.01
Mean pressure of pulmonary artery	0.21	0.02
Mean velocity of pulmonary artery	0.23	0.01

SBP systolic blood pressure, *DBP* diastolic blood pressure, *TC* total cholesterol, *TG* triglycerides, *LDL* low-density lipoprotein cholesterol, *cIMT* carotid intima-media thickness, *EF* ejection fraction, *SF* shortening fraction.

Table 4. Significant correlations of CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs with clinical, biochemical, and vascular/echocardiographic parameters in the preterm-born population.

	Correlation coefficient	p value
CD34(+)/VEGFR-2(+)/CD45(−) EPCs		
SBP	0.31	0.02
DBP	0.25	0.04
TC	−0.32	0.01
aIMT	−0.27	0.04
Mean pressure of pulmonary artery	0.38	0.004
Mean velocity of pulmonary artery	0.39	0.004
CD34(+)/VEGFR-2(+)/CD45dim EPCs		
Age	0.24	0.05
WHR	−0.31	0.01
SBP	0.26	0.04
TC	−0.33	0.01
LDL	−0.26	0.04
aIMT	−0.27	0.03
Mean pressure of pulmonary artery	0.30	0.03
Mean velocity of pulmonary artery	0.32	0.02

SBP systolic blood pressure, *DBP* diastolic blood pressure, *TC* total cholesterol, *TG* triglycerides, *LDL* low-density lipoprotein cholesterol, *cIMT* carotid intima-media thickness, *EF* ejection fraction, *SF* shortening fraction.

disease.[70] Furthermore, increased EPCs counts have been found in early and less severe stages of heart failure, although EPCs numbers seem to decrease with further disease progression.[71] In many inflammatory diseases, such as rheumatoid arthritis,[72,73] systemic lupus erythematosus,[74] and systemic sclerosis,[75,76] the findings on EPCs counts are conflicting; decreased peripheral levels and functionally impaired EPCs, but also increased EPCs counts that correlated with disease activity have been reported. The above conflicting results could not only be attributed to different EPCs subpopulations studied, but also to possible influence of each disease duration and activity on EPCs quantities.[67] Furthermore, EPCs subpopulations were increased in children and adolescents with active systemic vasculitis,[77] transfusion-dependent b-thalassemia,[78] and type 1 diabetes mellitus,[79] while EPCs were found to be reduced and with impaired function in obese children[30] and in children with chronic kidney disease.[80] Normal values of EPCs in children have not been determined yet; however, levels of EPCs observed in controls in the studies mentioned above are similar to EPCs levels of the controls in our study.

Data on EPCs in gestational diabetes mellitus are limited and conflicting.[81–84] In pregnancies complicated with gestational diabetes, cord blood endothelial colony-forming cells (ECFCs)—a specific subpopulation of EPCs—exhibited unique phenotypic alterations and altered functionality,[42] which was hypothesized to contribute to the enhanced cardiometabolic risk of developing hypertension, type 2 diabetes, and obesity in the offspring. Furthermore, women with gestational impaired glucose tolerance or gestational diabetes presented with lower EPCs subpopulations during the third trimester of pregnancy and after delivery compared to women with normal glucose tolerance[42,82,83,85]; reduced EPCs counts in cord blood were also observed.[82] Thus, alterations in glucose tolerance during pregnancy seem to be associated with a decrease in circulating EPCs levels,[83] which was the case in our study. The mechanisms responsible for the above EPCs reduction is less clear; it might have resulted from defective bone marrow EPCs release, increased EPCs apoptosis, and/or increased EPCs utilization in peripheral circulation.[83,85]

In general, it is unanimously accepted that impaired endothelial function is a major regulator in the pathogenesis of atherosclerosis and in the development of cardiovascular disease and/or metabolic syndrome.[86,87] Nevertheless, discrepancy exists among studies in the relationship between preterm birth and endothelial dysfunction.[8,17,19,22,23,88] In our study, prematurity was found to be linked with endothelial dysfunction parameters, such as cIMT and aIMT. cIMT is an indicator of generalized atherosclerosis and a powerful predictor of future cardiovascular events.[89] High cIMT values are strongly associated with cardiovascular risk factors, e.g., increased arterial blood pressure and diabetes mellitus.[90–93] Increased cIMT in preterm-born individuals was considered to be one of the earliest signs of atheromatous plaque formation.[22] Furthermore, aIMT provides an important index of preclinical atherosclerosis, already from childhood, and has significant associations with cardiovascular risk factors, i.e., hypercholesterolemia and diabetes mellitus.[21,55,94] It has been shown that aIMT is superior to cIMT in both feasibility and association with cardiovascular risk factors, and it might provide the best currently available non-invasive marker of preclinical atherosclerosis in children.[55,95] In our preterm-born population, EPCs subpopulations studied correlated inversely and independently with aIMT; these findings are consistent with previous data in adults indicating that EPCs number decreases in the presence of aortic atherosclerotic plaque.[96] Furthermore, several studies have shown an inverse correlation between EPCs and cIMT;[97–99] EPCs decrease was found to correlate with the severity of carotid atherosclerosis and possibly with pathophysiologic mechanisms promoting further structural changes within the arterial wall.[97–99]

Regarding FMD, we did not find a significant difference between children born prematurely and children born at term; this is in concordance with similar studies in the literature in both children and adults.[26,100] The absence of significant difference in FMD values between groups can possibly be explained by the fact that we enrolled healthy children of young age. Age-related endothelial dysfunction has been already highlighted in previous studies in adults.[101] Furthermore, the degree of prematurity,[17] as well as the influence of intrauterine growth restriction (IUGR) on preterm birth, have been associated with critical differences in endothelial function in later life.[19,88] The preterm-born group, in our study, consisted of a limited SGA and/or IUGR fraction, while gestational age had a wide range extending from extremely preterm to late preterm birth. Nevertheless, peak velocity of brachial artery was found to be significantly lower in preterm-born children. In several studies, hyperemic blood-flow velocity of the brachial artery was related to cardiovascular risk factors, while it was also recognized as the main determinant of FMD.[102] Furthermore, peak velocity of the brachial artery, during hyperemia, has been inversely correlated with the Framingham risk score.[103]

Contrary to previous studies demonstrating a unique cardiovascular phenotype of preterm-born adults,[14] no difference was found in cardiac structure and function, including EF, SF, IVSd, LVDIs, LVIDd, LVPWd, LVM, LVMI, and RWT, between our preterm-born children and children born at term. However, modulation of ventricular mass morphology, function, and geometry may appear later in life and not so early in childhood.[14,104] Furthermore, the preterm heart is often exposed to conditions that increase cardiac workload, such as PDA leading to important left-to-right shunting and BPD, with the risk of pulmonary hypertension.[3] The limited sample size in addition to the low prevalence of neonatal comorbidities in our study population may not highlight small-to-moderate differences of cardiac morphology and function parameters between the compared groups. It has been previously shown that the degree of prematurity correlates independently with increased LVM, while very preterm (28–31 weeks of gestation) and extremely preterm (<28 weeks of gestation) individuals seem to be more susceptible to cardiac alterations.[16] Our study indeed showed significantly higher mean pressure and velocity of pulmonary artery, although within normal range, in preterm-born children. Longitudinal cardiac imaging studies in preterm-born individuals are necessary to shed light on the evolution of cardiac remodeling over time in the above population.

Lipid levels did not differ between preterm-born children and children born at term; it remains unclear whether prematurity is associated with alterations in lipid profile, as relevant studies present controversial findings.[8,105] Regarding EPCs and lipid profile, EPCs number was reported to be significantly lower in patients with hypercholesterolemia compared with controls and also inversely correlated with total cholesterol and LDL-C levels;[106,107] results that come in concordance with our findings.

This study has some limitations. First, although we enrolled an adequate number of preterm-born children in order to assure statistically significant results for primary outcomes, this number was not sufficient enough to reveal possible correlations between EPCs and maternal and/or neonatal morbidity related to prematurity, such as maternal gestational hypertension and preeclampsia, BPD, IVH, ROP, etc. Second, another limitation is relevant to the lack of consensus regarding the identity of EPCs by cell surface antigens, while the poor standardization of assays for EPCs isolation remains a major cause of conflicting results as regards EPCs biology in human diseases.[77] In our study, we performed phenotyping EPCs identification without evaluating their functional characteristics or their vasculogenic and clonogenic capacity. However, it is a key point that circulating CD34(+)/VEGFR-2(+)/CD45(−) and CD34(+)/VEGFR-2(+)/CD45dim EPCs were recognized as representing EPCs subpopulations for their role in vascular integrity, tissue remodeling, and physiological neovascularization. Further studies are required to investigate additional associations between EPCs and prematurity, as well as preterm birth complications; the underlying mechanisms leading to impaired EPCs numbers and/or function in preterm-born individuals should be elucidated in order to determine the potential role of EPCs in the development of cardiovascular risk.[44]

In conclusion, circulating levels of EPCs are increased in prepubertal children born prematurely in comparison with their peers born at term, while gestational diabetes mellitus is associated with reduced EPCs levels. Prematurity is associated with indices of endothelial dysfunction and increased cardiovascular risk in children of prepubertal age. As data accumulate, we—as others[16,108]—propose that children born prematurely should be followed up in a regular and prolonged basis for the early detection of abnormalities associated with increased cardiovascular risk. Whether assessment of circulating EPCs is warranted in preterm-born individuals, in clinical practice, as a new tool for non-invasive evaluation of endothelium and risk stratification, or as a potential biomarker for novel therapeutic options remains to be defined in future investigations.

ACKNOWLEDGEMENTS

The authors thank the children and their parents for participating. This work was supported by National and Kapodistrian University of Athens. The funding source played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

AUTHOR CONTRIBUTIONS

All the authors assisted with study design, acquisition, analysis and interpretation of the data; P.M. performed the clinical assessment; P.M. and E.P. were responsible for the flow cytometric analysis of circulating EPCs; P.G. was responsible for the ultrasound study of cIMT, aIMT, and FMD of the brachial artery and S.L. was responsible for cardiology data; A.M. was responsible for blood biochemistry. T.S. supervised the study. P.M. developed the first draft of the manuscript and T.S. critically reviewed it. All the authors edited the manuscript, revised it critically for important intellectual content, and approved the final version to be published.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41390-020-01190-y) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

Informed consent: Informed written consent was obtained from parents and children.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Bavineni, M. et al. Mechanisms linking preterm birth to onset of cardiovascular disease later in adulthood. *Eur. Heart J.* **40**, 1107–1112 (2019).
2. Bassareo, P. P., Namana, V., Fanos, V. & Mercuro, G. Preterm birth and risk of heart failure up to early adulthood. *J. Am. Coll. Cardiol.* **70**, 1943–1944 (2017).
3. Carr, H., Cnattingius, S., Granath, F., Ludvigsson, J. F. & Edstedt Bonamy, A. K. Preterm birth and risk of heart failure up to early adulthood. *J. Am. Coll. Cardiol.* **69**, 2634–2642 (2017).
4. Crump, C. et al. Association of preterm birth with risk of ischemic heart disease in adulthood. *JAMA Pediatr*. https://doi.org/10.1001/jamapediatrics.2019.1327 (2019).
5. Heindel, J. J. et al. Developmental origins of health and disease: integrating environmental influences. *Endocrinology* **156**, 3416–3421 (2015).
6. Lewandowski, A. J. et al. Elevated blood pressure in preterm-born offspring associates with a distinct antiangiogenic state and microvascular abnormalities in adult life. *Hypertension* **65**, 607–614 (2015).

7. de Jong, F., Monuteaux, M. C., van Elburg, R. M., Gillman, M. W. & Belfort, M. B. Systematic review and meta-analysis of preterm birth and later systolic blood pressure. *Hypertension* **59**, 226–234 (2012).
8. Markopoulou, P., Papanikolaou, E., Analytis, A., Zoumakis, E. & Siahanidou, T. Preterm birth as a risk factor for metabolic syndrome and cardiovascular disease in adult life: a systematic review and meta-Analysis. *J. Pediatr.* **210**, 69–80 (2019).
9. Kajantie, E. et al. Insulin sensitivity and secretory response in adults born preterm: the Helsinki Study of very low birth weight adults. *J. Clin. Endocrinol. Metab.* **100**, 244–250 (2015).
10. Sipola-Leppänen, M. et al. Cardiometabolic risk factors in young adults who were born preterm. *Am. J. Epidemiol.* **181**, 861–873 (2015).
11. Hovi, P. et al. Lipoprotein subclass profiles in young adults born preterm at very low birth weight. *Lipids Health Dis.* **12**, 57 (2013).
12. Breukhoven, P. E., Kerkhof, G. F., Willemsen, R. H. & Hokken-Koelega, A. C. Fat mass and lipid profile in young adults born preterm. *J. Clin. Endocrinol. Metab.* **97**, 1294–1302 (2012).
13. Thomas, E. L. et al. Aberrant adiposity and ectopic lipid deposition characterize the adult phenotype of the preterm infant. *Pediatr. Res.* **70**, 507–512 (2011).
14. Lewandowski, A. J. et al. Preterm heart in adult life: cardiovascular magnetic resonance reveals distinct differences in left ventricular mass, geometry, and function. *Circulation* **127**, 197–206 (2013).
15. Lewandowski, A. J. et al. Right ventricular systolic dysfunction in young adults born preterm. *Circulation* **128**, 713–720 (2013).
16. Lewandowski, A. J. The preterm heart: a unique cardiomyopathy? *Pediatr. Res.* **85**, 738–739 (2019).
17. Bassareo, P. P. et al. Reduced brachial flow-mediated vasodilation in young adult ex extremely low birth weight preterm: a condition predictive of increased cardiovascular risk? *J. Matern. Fetal Neonatal Med.* **23**(Suppl 3), 121–124 (2010).
18. Cheung, Y. F., Wong, K. Y., Lam, B. C. & Tsoi, N. S. Relation of arterial stiffness with gestational age and birth weight. *Arch. Dis. Child.* **89**, 217–221 (2004).
19. Lazdam, M. et al. Elevated blood pressure in offspring born premature to hypertensive pregnancy: is endothelial dysfunction the underlying vascular mechanism? *Hypertension* **56**, 159–165 (2010).
20. Hovi, P. et al. Intima-media thickness and flow-mediated dilatation in the Helsinki study of very low birth weight adults. *Pediatrics* **127**, 304–311 (2011).
21. Shimizu, T. et al. Abdominal aortic intima-media thickness in preschool children born preterm. *Pediatr. Cardiol.* **35**, 121–125 (2014).
22. Lee, H., Dichtl, S., Mormanova, Z., Dalla Pozza, R. & Genzel-Boroviczeny, O. In adolescence, extreme prematurity is associated with significant changes in the microvasculature, elevated blood pressure and increased carotid intima-media thickness. *Arch. Dis. Child.* **99**, 907–911 (2014).
23. Kerkhof, G. F., Breukhoven, P. E., Leunissen, R. W., Willemsen, R. H. & Hokken-Koelega, A. C. Does preterm birth influence cardiovascular risk in early adulthood? *J. Pediatr.* **161**, 390–396 (2012).
24. McEniery, C. M. et al. Cardiovascular consequences of extreme prematurity: the EPICure study. *J. Hypertens.* **29**, 1367–1373 (2011).
25. Bonamy, A. K., Martin, H., Jörneskog, G. & Norman, M. Lower skin capillary density, normal endothelial function and higher blood pressure in children born preterm. *J. Intern. Med.* **262**, 635–642 (2007).
26. Edwards, M. O. et al. Higher systolic blood pressure with normal vascular function measurements in preterm-born children. *Acta Paediatr.* **103**, 904–912 (2014).
27. Martins, S. & Quelhas-Santos, J. Circulating endothelial progenitor cells: a review of definition, characterization and identification. *Res. Rev. Res. J. Biol.* **4**, 42–49 (2016).
28. Burger, D. & Touyz, R. M. Cellular biomarkers of endothelial health: microparticles, endothelial progenitor cells, and circulating endothelial cells. *J. Am. Soc. Hypertens.* **6**, 85–99 (2012).
29. Bui, K. C. et al. Circulating hematopoietic and endothelial progenitor cells in newborn infants: effects of gestational age, postnatal age and clinical stress in the first 3 weeks of life. *Early Hum. Dev.* **89**, 411–418 (2013).
30. Bruyndonckx, L. et al. Endothelial progenitor cells and endothelial microparticles are independent predictors of endothelial function. *J. Pediatr.* **165**, 300–305 (2014).
31. Schmidt-Lucke, C. et al. Quantification of circulating endothelial progenitor cells using the modified ISHAGE protocol. *PLoS ONE* **5**, e13790 (2010).
32. Massa, M. et al. Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction. *Blood* **105**, 199–206 (2005).
33. Shintani, S. et al. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation* **103**, 2776–2779 (2001).
34. Chu, K. et al. Circulating endothelial progenitor cells as a new marker of endothelial dysfunction or repair in acute stroke. *Stroke* **39**, 1441–1447 (2008).
35. Yip, H. K. et al. Level and value of circulating endothelial progenitor cells in patients after acute ischemic stroke. *Stroke* **39**, 69–74 (2008).
36. Zhang, H. et al. Circulating endothelial progenitor cells in multiple myeloma: implications and significance. *Blood* **105**, 3286–3294 (2005).
37. Zahran, A. M., Aly, S. S., Altayeb, H. A. & Ali, A. M. Circulating endothelial cells and their progenitors in acute myeloid leukemia. *Oncol. Lett.* **12**, 1965–1970 (2016).
38. Rhone, P. et al. Increased number of endothelial progenitors in peripheral blood as a possible early marker of tumour growth in post-menopausal breast cancer patients. *J. Physiol. Pharmacol.* **68**, 139–148 (2017).
39. Yu, P. et al. Identification and significance of mobilized endothelial progenitor cells in tumor neovascularization of renal cell carcinoma. *Tumour Biol.* **35**, 9331–9341 (2014).
40. Bertagnolli, M., Nuyt, A. M., Thébaud, B. & Luu, T. M. Endothelial progenitor cells as prognostic markers of preterm birth-associated complications. *Stem Cells Transl. Med.* **6**, 7–13 (2017).
41. Baker, C. D. et al. Cord blood angiogenic progenitor cells are decreased in bronchopulmonary dysplasia. *Eur. Respir. J.* **40**, 1516–1522 (2012).
42. Blue, E. K. et al. Gestational diabetes induces alterations in the function of neonatal endothelial colony-forming cells. *Pediatr. Res.* **75**, 266–272 (2014).
43. Muñoz-Hernandez, R. et al. Decreased level of cord blood circulating endothelial colony-forming cells in preeclampsia. *Hypertension* **64**, 165–171 (2014).
44. Bertagnolli, M. et al. Endothelial colony-forming cells in young adults born preterm: a novel link between neonatal complications and adult risks for cardiovascular disease. *J. Am. Heart Assoc.* **7**, e009720 (2018).
45. Fenton, T. R. & Kim, J. H. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr.* **13**, 59 (2013).
46. Committee on Practice Bulletins—Obstetrics. ACOG Practice Bulletin No. 190: gestational diabetes mellitus. *Obstet. Gynecol.* **131**, e49–e64 (2018).
47. ACOG Practice Bulletin No. 202: gestational hypertension and preeclampsia. *Obstet. Gynecol.* **133**, e1–e25 (2019).
48. Higgins, R. D. et al. Bronchopulmonary dysplasia: executive summary of a workshop. *J. Pediatr.* **197**, 300–308 (2018).
49. Yashoda, H. T., Swetha, B. & Goutham, A. S. Neck circumference measurement as a screening tool for. *Int. J. Contemp. Pediatr.* **4**, 426–430 (2017).
50. Tanner, J. M. & Whitehouse, R. H. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch. Dis. Child.* **51**, 170–179 (1976).
51. Faienza, M. F. et al. Vascular function and myocardial performance indices in children born small for gestational age. *Circ. J.* **80**, 958–963 (2016).
52. Flynn, T. J. et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics* **140**, e20171904 (2017).
53. Cutfield, W. S., Jefferies, C. A., Jackson, W. E., Robinson, E. M. & Hofman, P. L. Evaluation of HOMA and QUICKI as measures of insulin sensitivity in prepubertal children. *Pediatr. Diabetes* **4**, 119–125 (2003).
54. Dalla Pozza, R. et al. Intima media thickness measurement in children: a statement from the Association for European Paediatric Cardiology (AEPC) Working Group on Cardiovascular Prevention endorsed by the Association for European Paediatric Cardiology. *Atherosclerosis* **238**, 380–387 (2015).
55. Järvisalo, M. J. et al. Increased aortic intima-media thickness: a marker of preclinical atherosclerosis in high-risk children. *Circulation* **104**, 2943–2947 (2001).
56. Bots, M. L., Hoes, A. W., Koudstaal, P. J., Hofman, A. & Grobbee, D. E. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation* **96**, 1432–1437 (1997).
57. Corretti, M. C. et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J. Am. Coll. Cardiol.* **39**, 257–265 (2002).
58. Tissot, C., Singh, Y. & Sekarski, N. Echocardiographic evaluation of ventricular function-for the neonatologist and pediatric intensivist. *Front. Pediatr.* **6**, 79 (2018).
59. Lai, W. W. et al. Guidelines and standards for performance of a pediatric echocardiogram: a report from the Task Force of the Pediatric Council of the American Society of Echocardiography. *J. Am. Soc. Echocardiogr.* **19**, 1413–1430 (2006).
60. Foster, B. J. et al. A novel method of expressing left ventricular mass relative to body size in children. *Circulation* **117**, 2769–2775 (2008).
61. Koo, T. K. & Li, M. Y. A Guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J. Chiropr. Med.* **15**, 155–163 (2016).
62. Paviotti, G. et al. Endothelial progenitor cells, bronchopulmonary dysplasia and other short-term outcomes of extremely preterm birth. *Early Hum. Dev.* **87**, 461–465 (2011).
63. Peichev, M. et al. Expression of VEGFR-2 and AC133 by circulating human CD34 (+) cells identifies a population of functional endothelial precursors. *Blood* **95**, 952–958 (2000).

64. Fadini, G. P. & Avogaro, A. Cell-based methods for ex vivo evaluation of human endothelial biology. *Cardiovasc. Res.* **87**, 12–21 (2010).
65. Asahara, T. et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ. Res.* **85**, 221–228 (1999).
66. Güven, H., Shepherd, R. M., Bach, R. G., Capoccia, B. J. & Link, D. C. The number of endothelial progenitor cell colonies in the blood is increased in patients with angiographically significant coronary artery disease. *J. Am. Coll. Cardiol.* **48**, 1579–1587 (2006).
67. Grisar, J. C., Haddad, F., Gomari, F. A. & Wu, J. C. Endothelial progenitor cells in cardiovascular disease and chronic inflammation: from biomarker to therapeutic agent. *Biomark. Med.* **5**, 731–744 (2011).
68. Tepper, O. M. et al. Human endothelial progenitor cells from Type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* **106**, 2781–2786 (2002).
69. Umemura, T. et al. Aging and hypertension are independent risk factors for reduced number of circulating endothelial progenitor cells. *Am. J. Hypertens.* **21**, 1203–1209 (2008).
70. Lorenzen, J. et al. Endothelial progenitor cells and cardiovascular events in patients with chronic kidney disease: a prospective follow-up study. *PLoS ONE* **5**, e11477 (2010).
71. Valgimigli, M. et al. CD34+ and endothelial progenitor cells in patients with various degrees of congestive heart failure. *Circulation* **110**, 1209–1212 (2004).
72. Grisar, J. et al. Depletion of endothelial progenitor cells in the peripheral blood of patients with rheumatoid arthritis. *Circulation* **111**, 204–211 (2005).
73. Rüger, B. et al. Endothelial precursor cells in the synovial tissue of patients with rheumatoid arthritis and osteoarthritis. *Arthritis Rheum.* **50**, 2157–2166 (2004).
74. Ebner, P. et al. Accumulation of VEGFR-2+/CD133+ cells and decreased number and impaired functionality of CD34+/VEGFR-2+ cells in patients with SLE. *Rheumatology* **49**, 63–72 (2010).
75. Kuwana, M., Okazaki, Y., Yasuoka, H., Kawakami, Y. & Ikeda, Y. Defective vasculogenesis in systemic sclerosis. *Lancet* **364**, 603–610 (2004).
76. Allanore, Y. et al. Levels of circulating endothelial progenitor cells in systemic sclerosis. *Clin. Exp. Rheumatol.* **25**, 60–66 (2007).
77. Hong, Y., Eleftheriou, D., Klein, N. J. & Brogan, P. A. Impaired function of endothelial progenitor cells in children with primary systemic vasculitis. *Arthritis Res. Ther.* **17**, 292 (2015).
78. Adly, A. A. et al. Vascular dysfunction in patients with young β-thalassemia: relation to cardiovascular complications and subclinical atherosclerosis. *Clin. Appl. Thromb. Hemost.* **21**, 733–744 (2015).
79. Głowińska-Olszewska, B. et al. Relationship between circulating endothelial progenitor cells and endothelial dysfunction in children with type 1 diabetes: a novel paradigm of early atherosclerosis in high-risk young patients. *Eur. J. Endocrinol.* **168**, 153–161 (2013).
80. Jie, K. E. et al. Reduced endothelial progenitor cells in children with hemodialysis but not predialysis chronic kidney disease. *Pediatrics* **126**, e990–e993 (2010).
81. Robb, A. O., Mills, N. L., Newby, D. E. & Denison, F. C. Endothelial progenitor cells in pregnancy. *Reproduction* **133**, 1–9 (2007).
82. Acosta, J. C. et al. Gestational diabetes mellitus alters maternal and neonatal circulating endothelial progenitor cell subsets. *Am. J. Obstet. Gynecol.* **204**, 254 (2011).
83. Penno, G. et al. Circulating endothelial progenitor cells in women with gestational alterations of glucose tolerance. *Diab. Vasc. Dis. Res.* **8**, 202–210 (2011).
84. Buemi, M. et al. Concentration of circulating endothelial progenitor cells (EPC) in normal pregnancy and in pregnant women with diabetes and hypertension. *Am. J. Obstet. Gynecol.* **196**, 68 (2007).
85. Mordwinkin, N. M. et al. Alteration of endothelial function markers in women with gestational diabetes and their fetuses. *J. Matern. Fetal Neonatal Med.* **26**, 507–512 (2013).
86. Visentin, S. et al. Early origins of adult disease: low birth weight and vascular remodelling. *Atherosclerosis* **237**, 391–399 (2014).
87. Paudel, K. R., Panth, N. & Kim, D. W. Circulating endothelial microparticles: A key hallmark of atherosclerosis progression. *Science* **2016**, 8514056 (2016).
88. Skilton, M. R. et al. Fetal growth and preterm birth influence cardiovascular risk factors and arterial health in young adults: the Cardiovascular Risk in Young Finns Study. *Arterioscler. Thromb. Vasc. Biol.* **31**, 2975–2981 (2011).
89. Lorenz, M. W., Markus, H. S., Bots, M. L., Rosvall, M. & Sitzer, M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation* **115**, 459–467 (2007).
90. Lim, S. M. et al. Association between blood pressure and carotid intima-media thickness. *J. Pediatr.* **154**, 667–671 (2009).
91. Páll, D. et al. Increased common carotid artery intima media thickness in adolescent hypertension: results from the Debrecen Hypertension study. *Cerebrovasc. Dis.* **15**, 167–172 (2003).
92. Dalla Pozza, R. et al. Age of onset of type 1 diabetes in children and carotid intima medial thickness. *J. Clin. Endocrinol. Metab.* **92**, 2053–2057 (2007).
93. Järvisalo, M. J. et al. Carotid artery intima-media thickness in children with type 1 diabetes. *Diabetes* **51**, 493–498 (2002).
94. Dawson, J. D., Sonka, M., Blecha, M. B., Lin, W. & Davis, P. H. Risk factors associated with aortic and carotid intima-media thickness in adolescents and young adults: the Muscatine Offspring Study. *J. Am. Coll. Cardiol.* **53**, 2273–2279 (2009).
95. McCloskey, K. et al. Reproducibility of aortic intima-media thickness in infants using edge-detection software and manual caliper measurements. *Cardiovasc. Ultrasound* **12**, 18 (2014).
96. Chironi, G. et al. Decreased number of circulating CD34+KDR+ cells in asymptomatic subjects with preclinical atherosclerosis. *Atherosclerosis* **191**, 115–120 (2007).
97. Bogdanski, P. et al. Plasma total homocysteine is a determinant of carotid intima-media thickness and circulating endothelial progenitor cells in patients with newly diagnosed hypertension. *Clin. Chem. Lab. Med.* **50**, 1107–1113 (2012).
98. Palombo, C. et al. Circulating endothelial progenitor cells and large artery structure and function in young subjects with uncomplicated type 1 diabetes. *Cardiovasc. Diabetol.* **8**, 88 (2011).
99. Bitterli, L. et al. Endothelial progenitor cells as a biological marker of peripheral artery disease. *Vasc. Med.* **21**, 3–11 (2016).
100. Evensen, K. A. et al. Effects of preterm birth and fetal growth retardation on cardiovascular risk factors in young adulthood. *Early Hum. Dev.* **85**, 239–245 (2009).
101. Seals, D. R., Jablonski, K. L. & Donato, A. J. Aging and vascular endothelial function in humans. *Clin. Sci.* **120**, 357–375 (2011).
102. Järhult, S. J., Sundström, J. & Lind, L. Brachial artery hyperaemic blood flow velocity and left ventricular geometry. *J. Hum. Hypertens.* **26**, 242–246 (2012).
103. Järhult, S. J., Hall, J. & Lind, L. Hyperaemic blood-flow velocities in systole and diastole relate to coronary risk in divergent ways. *Clin. Physiol. Funct. Imaging* **28**, 189–195 (2008).
104. Burchert, H. & Lewandowski, A. J. Preterm birth is a novel, independent risk factor for altered cardiac remodeling and early heart failure: is it time for a new cardiomyopathy? *Curr. Treat. Options Cardiovasc. Med.* **21**, 8 (2019).
105. Inomata, S. et al. Effect of preterm birth on growth and cardiovascular disease risk at school age. *Pediatr. Int.* **57**, 1126–1130 (2015).
106. Chen, J. Z. et al. Number and activity of endothelial progenitor cells from peripheral blood in patients with hypercholesterolaemia. *Clin. Sci.* **107**, 273–280 (2004).
107. Werner, N. et al. Circulating endothelial progenitor cells and cardiovascular outcomes. *N. Engl. J. Med.* **353**, 999–1007 (2005).
108. Bassareo, P. P., Fanos, V. & Mercuro, G. Response to 'In adolescence, extreme prematurity is associated with significant changes in the microvasculature, elevated blood pressure and increased carotid intima-media thickness'. *Arch. Dis. Child.* **100**, 508–509 (2015).