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Giovanna Pachele Parizotto

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Birth weight and its relationship with endothelial function and pattern of endothelium-derived microparticles during childhood: New insight about early vascular damage

Giovanna Pachele Parizotto [a]
Livia Victorino de Souza [a,c]
Fernanda Thomazini [a,b]
Mônica Simon Prado [d]
Juan Sebastian Henao Agudelo [e]
Danilo Cândido de Almeida [c]
Maria do Carmo Franco [a, b]

[a] Physiology Department, School of Medicine, Federal University of São Paulo, São Paulo, Brazil.
[b] Program of Translational Medicine, School of Medicine, Federal University of São Paulo, São Paulo, Brazil.
[c] Nephrology Division, School of Medicine, Federal University of São Paulo, São Paulo, Brazil.
[d] Rheumatology Division, School of Medicine, Federal University of São Paulo, São Paulo, Brazil.
[e] Central Unit of Valle del Cauca, Faculty of Health Sciences, Valle del Cauca, Tulua, Colombia.

Corresponding Author

Maria do Carmo Franco

LiTiVasC - Laboratory of Translational Research in Vascular and Molecular Physiology.

School of Medicine. Federal University of São Paulo

Rua Botucatu, 862 – 5° floor - São Paulo, SP, Brazil. Zip Code: 04023-062.

e-mail: maria.franco@unifesp.br

Abstract

Objectives: To investigate whether a specific endothelium-derived microparticles (EMPs) phenotype could be associated with birth weight and microvascular endothelial function in children.

Study design: A total of 95 children, aged 6-14 years, were recruited. Anthropometric measurement, blood pressure, microvascular endothelial function, the biochemical profile was performed. Standardized flow cytometry methods were used to identify and quantify circulating CD144+, CD31+/annexin V+, and CD62E+ EMPs.

Results: We found that the circulating number of CD31+/annexin V+ EMPs and CD144+ EMPs EMP levels were correlated with birth weight, systolic blood pressure, microvascular endothelial function, total cholesterol, and LDLc levels. In the multivariable logistic regression models, we identified strong evidence of higher risk of microvascular endothelial dysfunction among children with low birth weight and increased levels of both CD31+/annexin V+ EMPs and LDLc, while low birth weight and elevated LDLc levels have been identified as independent predictors of the high circulating number of both CD31+/annexin V+ and CD144+ > 75th percentile EMPs.

Conclusion: our data provide evidence that children who had birth weight at the lowest values showed higher levels of the circulating number of CD31+/annexin V+ and CD144+ EMPs. In addition, the LBW and high levels of both CD31+/annexin V+ and LDLc were significant risk factors for the presence of microvascular endothelial dysfunction.

Keyword: birth weight, RHI, endothelium-derived microparticles, LDLc, children

Introduction

Low birth weight (LBW) is a serious public health problem due to the negative impacts detected in the early hours of life and to adverse outcomes observed during childhood and adulthood [1-4]. Global estimates related more than 20.5 million babies are born annually with LBW [5]. It is essential to address and avoid LBW because it is a risk factor for several cardiovascular diseases (CVD) [2-4]. Diverse mechanisms by which LBW leads to the development of CVD have been proposed, and most involve endothelial dysfunction [6-10]. Several clinical studies have established a link between LBW and impaired endothelial function on the regulation of vascular tone in children and young adults [6-10]. Indeed, birth weight showed a significant positive correlation with flow-mediated dilation [6,7]. There is evidence that suggest an association between LBW with dysfunction in the circulating number and functional capacity of endothelial progenitor cells [11-14]. Thus, a better understanding of the mediators of LBW-induced endothelial dysfunction will help us identify new targets that may understand the development of LBW-related CVD.

Endothelium-derived microparticles (EMPs) are submicron anucleated vesicles released in response to apoptotic or activation stimuli [15,16]. Once into the circulation, EMPs bind and fuse with their target cells, thus acting as vectors that could mediate several biological processes with favorable or deleterious effects on vascular homeostasis [17,18]. There is evidence that EMPs play an important role in vascular repair, degenerative processes modulation, endothelial progenitor cells mobilization/differentiation, and a crucial indicator of endothelial damage [19-21].

Currently, the link between EMPs and endothelial function in children with LBW is unclear. To our knowledge, a single study has thus far demonstrated that prepubertal children born preterm have elevated circulating EMPs [22].

The present study aimed to investigate whether a specific EMPs phenotype could be associated with birth weight and microvascular endothelial function in children. We evaluated EMPs labeled as *a)* $CD144^+$ (vascular endothelial (VE)-cadherin) molecule responsible for the vascular integrity and stability [23]; *b)* $CD62E^+$- indicating inflammatory activation [24]; *c)* $CD31^+$/annexin V^+ released by apoptotic endothelial cells [25].

Methods

From July to December 2019, one hundred and forty-two children, aged 6-14 years, participated in an anthropometric census performed in socioeconomically vulnerable communities located near the campus of the Federal University of São Paulo (UNIFESP, São Paulo, Brazil). To carry out the current study, we excluded thirty-five children (24 males, 11 females) with overweight or obesity (body mass index [BMI]-for-age between > Z-score +1 and < Z-score +3) to avoid the strong confounding effects of nutritional status on endothelial function and EMPs. Further, we also excluded twelve children (5 males, 7 females) without birth weight data. Thus, ninety-five children (54 males and 41 females) remained eligible for the study. None of the children were diagnosed with cardiovascular disease or diabetes mellitus, or under medication. All procedures were conducted in accordance with the Declaration of Helsinki. This study was approved by the Research Ethics Committee of the Federal University of São Paulo (Approval number: 3.408.882). Written informed consent was obtained from all parents or guardians.

Anthropometric Data: Body weight and height were measured in light clothing, without shoes, using standard techniques with a portable digital platform scale and a portable stadiometer, respectively. BMI was calculated using the formula: weight (kg)/height^2 (m^2). Waist circumference was measured using inextensible tape and recorded at a level midway between the lower rib margin and the iliac crest at the end of normal expiration.

Blood Pressure Parameters and Microvascular Endothelial Function Measurement: For these procedures, children were asked to stay in a supine position with spontaneous breathing and minimal body movement for 15 minutes in a temperature-controlled room. The blood pressure levels were measured on the right arm in a seated position using an automated oscillometric device (Omron HBP1100; Omron Healthcare; USA) with an appropriate cuff size. The blood pressure values were recorded as the average of three measurements made at 2 min intervals. A noninvasive technique was performed using an EndoPAT device (Itamar Medical Ltd, Cesareia, Israel) to assess the capacity of the microcirculation to dilate in response to increased blood flow induced by reactive hyperemia test. Data were expressed as reactive hyperemia index (RHI), which was calculated automatically calculated as the ratio of the pulse-wave amplitude (PWA) between 90 and 150 seconds after occlusion and divided by baseline PWA. An RHI value of < 1.67 was used as a cut-off to diagnose of the endothelial dysfunction [26]. All examinations took place during the morning between 8:00 and 9:30 hours. The same researcher evaluated all the children and was blinded to neonatal or clinical data.

Blood Sampling and Biochemical Profile: Antecubital venous blood samples were collected in the morning from all children into six separate vacutainer tubes containing

sodium citrate anticoagulant and one with K2EDTA. All tubes were processed within 1 h for EMPs isolation (sodium citrate tubes: 24 mL) and biochemical parameters (K2EDTA tube: 4 mL). Glucose, total cholesterol, high-density-lipoprotein cholesterol (HDLc), low-density-lipoprotein cholesterol (LDLc), and triglyceride levels were evaluated using routine methods of the clinical laboratory.

Isolation of the Microparticles from Platelet-free Plasma (PFP): Circulating microparticles were isolated from 24 mL citrated venous blood samples. Samples of platelet-free plasma (PFP) were obtained by centrifugation. The first centrifugation (1600 × *g*, 15 min, 21 °C) was performed to separate the plasma from the dense blood components and mononuclear cells. The plasma was further centrifuged (2700 × *g*, 30 min, 21 °C), diluted in ultra-pure phosphate-buffered saline (PBS, pH 7.4), and filtered through a surfactant-free cellulose acetate membrane. The PFP was then ultracentrifuged (30,000 × *g*, 2h, 4 °C). The obtained supernatant was extracted, and the microparticle pellets were resuspended in ultra-pure PBS.

Determination of the Circulating Endothelium-derived Microparticles: The EMPs were characterized by flow cytometry using CytoFLEX Nanoparticle Detection (Beckman Coulter; Brea, CA, USA) with the following fluorochrome-coupled antibodies and their corresponding isotypes: phycoerythrin (PE)-conjugated monoclonal antibody against CD144 (VE-cadherin), allophycocyanin (APC)-conjugated monoclonal antibody against CD62E (E-selectin), and APC-Cy7-conjugated monoclonal antibody against CD31 (platelet endothelial cell adhesion molecule [PECAM]-1). They were labeled using brilliant violet 421 (BV421)-Annexin V solution (BD Biosciences; San Jose, CA, USA) in the

presence of 5 mM $CaCl_2$, according to the manufacturer's recommendations. Flow fluorescence calibration microbeads were run to calibrate the microparticle size and distribution (0.02–2 µm diameter) (Flow Cytometry Sub-Micron Size Reference Kit; Thermo Fisher Scientific, Waltham, MA, USA). EMPs are defined as particles less than 1.0 µm in diameter and which express $CD31^+$/annexin V^+ for apoptotic, $CD62E^+$ for inflammatory activation or $CD144^+$ for endothelial barrier impairment [16, 21]. Data were analyzed using CytoFLEX Acquisition and Analysis Software (Beckman Coulter; Brea, CA, USA). Data are reported as count/µL PFP. To remove any bias, the researchers implemented a single-blind analysis technique.

Statistical Analysis

Categorical variables were expressed as frequencies and compared using the chi-square test. Before analysis, data were tested for normality of distribution by the Shapiro-Wilk test. Consequently, birth weight, age, waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), triglycerides, glucose levels, RHI, $CD31^+$/annexin V^+ EMPs, $CD144^+$ EMPs, and $CD62E^+$ EMPs were non-normally distributed. Data were expressed as median with interquartile range or mean with standard deviation. Nonparametric correlations were performed using Spearman's correlation coefficient (*rho*). Logistic regression analyses were performed to establish the degree of association between certain factors with RHI or circulating number of EMPs. For these procedures, cut-off points < 1.67 were adopted for RHI and > 75th percentile for EMPs. Multicollinearity was evaluated using variance inflation factor (VIF). All variables showing $P \leq 0.20$ in the univariable analysis were presented to the multivariate models using the backward method. Variables were retained in the model if the Wald test

yielded $P < 0.05$. BMI was not included in the regression models because of multicollinearity. All statistical tests were two-tailed, and the significance level was set at $P < 0.05$. Statistical analyses were performed using IBM SPSS software (version 22; IBM Corporation; Armonk, NY, USA).

Results

The characteristics of the children are summarized in Table 1. The median birth weight was 3,000 grams (Range: 1,890 – 3,880 grams) with an incidence of LBW (< 2,500 g) of the 19% (n=18) and prematurity rate of the 5.3% (n=5). There were no significant correlations between birth weight and anthropometric data (BMI: *rho* = 0.091, *P* = 0.383; waist circumference: *rho* = 0.041, *P* = 0.692), blood pressure levels (SBP: *rho* = - 0.131, *P* = 0.398; DBP: *rho* = - 0.148, *P* = 0.151), lipide profile (total cholesterol: *rho*= - 0.145, *P* = 0.160; LDLc: *rho* = - 0.145, *P* = 0.161; HDLc: *rho* = - 0.047, *P* = 0.652; triglycerides: *rho* = - 0.163, *P* = 0.114), and glucose levels (*rho* = 0.019, *P* = 0.855).

Endothelial dysfunction, defined as RHI < 1.67, was detected in 23/95 (24.2%). When evaluated the relationship of the birth weight with microvascular endothelial function, was observed a moderate positive correlation between these variables (*rho* = 0.510, *P*< 0.001) (Figure 1B). We found that 50% (9/18) of children with LBW and 18.2% (14/77) without LBW had an RHI < 1.67 (*P*= 0.007). When we compared the RHI values, children with LBW had slightly lower RHI values (median: 1.47, range: 1.00 - 2.25) than subjects without LBW (median: 1.87, range: 1.02 - 2.95) (*P*= 0.012). For the entire cohort, RHI showed an inverse association with LDLc levels (*rho* = - 0.323, *P* = 0.002), while no significant correlation was observed with age, anthropometric parameters, blood pressure levels, other components of lipid profile, and glucose levels (all *P* > 0.05).

Interestingly, we found that 20/95 (21.1%) and 25/95 (26.3%) children reached the cut off > 75th percentile for $CD31^+$/annexin V^+ and $CD144^+$ circulating number of EMPs, respectively. Levels of $CD31^+$/annexin V^+ EMPs and $CD144^+$ EMPs were significantly correlated (*rho*= 0.727, *P*< 0.001). Circulating number of $CD31^+$/annexin V^+ EMPs were significantly higher in children with LBW than in those without (median: 545, range: 150 - 870 *vs*. median: 350, range: 50 - 900; *P* = 0.003), as well as levels of $CD144^+$ EMPs (median: 381, range: 113 - 967 *vs*. median: 220, range: 44 - 598; *P* = 0.007). Birth weight was inversely correlated with circulating number of $CD31^+$/annexin V^+ EMPs (*rho*= - 0.400, *P*< 0.001) (Figure 1A), as were with levels of $CD144^+$ EMPs (*rho*= - 0.336, *P*= 0.001) (Figure 2A). Also, $CD31^+$/annexin V^+ EMPs and $CD144^+$ EMPs showed a weak but significant association with SBP levels ($CD31^+$/annexin V^+: *rho* = 0.242, *P* = 0.018; $CD144^+$: *rho* = 0.266, *P* = 0.009), total cholesterol ($CD31^+$/annexin V^+: *rho* = 0.273, *P* = 0.008; $CD144^+$: *rho* = 0.275, *P* = 0.007), and LDLc ($CD31^+$/annexin V^+: *rho* = 0.381, *P* = 0.001; $CD144^+$: *rho* = 0.314, *P* = 0.001), while no correlation could be found between these EMPS and age, anthropometric parameters, DBP, and glucose levels (all *P* > 0.05). Further, no statistically significant association was identified between $CD62E^+$ EMPs and other variables in the study (all *P* > 0.05).

When we evaluated the association between these EMPs with microvascular endothelial function, we found that circulating number of $CD31^+$/annexin V^+ EMPs (*rho* = −0.528, *P* < 0.001) (Figure 1B) and $CD144^+$ EMPs (*rho* = −0.442, *P* <0.001) (Figure 2B) was inverse correlated with RHI. Further, a three-dimensional (3D) scatter plot was performed in order to visualize the correlation between EMPs and RHI by birth weight data, and we found a higher concentration of children with LBW who have high amount of both $CD31^+$/annexin V^+ (Figure 1C) and $CD144^+$ EMPs (Figure 2B) associated with lower RHI

values. On the other hand, we also found a wide variation in RHI and circulating number of EMPs among children without LBW (Figure 1C and 1F).

Considering RHI < 1.67 or EMPs > 75th percentile as a dependent variable, we performed multivariable logistic regression models fit in a backward fashion with all covariates with $P \leq 0.20$ on the univariable model. We identified strong evidence of higher risk of microvascular endothelial dysfunction among children with LBW and increasing levels of both CD31+/annexin V+ EMPs and LDLc (Table 2), while LBW and elevated LDLc levels have been identified as independent predictors of high circulating number of both CD31+/annexin V+ and CD144+ > 75th percentile EMPs (Table 3).

Discussion

This study provides further insights into how birth weight can influence the early development of endothelial damage. The results of the current study indicated that children who had birth weight at the lowest values showed higher levels of the circulating number of $CD31^+$/annexin V^+ and $CD144^+$ EMPs. Moreover, LBW and high levels of LDLc were independent predictors of these EMPs. Of particular interest was that LBW and high levels of both CD31+/annexin V+ and LDLc were significant risk factors for the presence of microvascular endothelial dysfunction in our cohort.

A single study investigating the interconnection between prematurity, birth weight, and circulating number of EMPs was published by Markopoulou et al. [22]. They assessed the abundance of circulating $CD62E^+$, $CD144^+$, and $CD31^+/CD42b^-$ EMPs in children born preterm with very LBW compared to those born at term with normal birth weight. It was found that these EMPs were significantly increased in children born preterm and that the preterm birth was an independent predictor of all EMPs evaluated [22]. Our current

study partially supports these findings, in which an increased circulating number of CD31+/annexin V+ and CD144+ EMPs had been found in children born with the lowest birth weight; however, without a significant correlation between birth weight and CD62E+. Our data also indicate that the risks of having high levels of both CD31+/annexin V+ and CD144+ EMPs were high in children with LBW and elevated LDLc level.

It seems that the negative effects of LBW and LDLc levels create a state of endothelial damage that contributes to EMP release. Impairment of the endothelial function has been described associate with LBW in individuals from three months of age to adulthood [27, 6-10]. Indeed, the LBW-induced endothelial dysfunction is characterized by a decrease in nitric oxide (NO) concentration [28,29], elevated levels of both pro-inflammatory and oxidative stress markers [30,31], reduction in the circulating number and functionality of the endothelial progenitor cells (EPCs) [11,12]. In turn, LDLc is a potentially atherogenic lipoprotein and its high levels also damage the endothelium, leading to cell death through apoptosis and impaired function due to increased oxidative stress and inflammation [32]. Taken together, all these alterations could contribute to endothelial injury process with consequent release of EMPs in our cohort.

An interrelationship between endothelial function, EMPs, and birth weight was observed in the current study. Children with the lowest birth weight present endothelial dysfunction at the level of small resistance vessels associated with elevated levels of EMPs. Moreover, we found that LBW, elevated circulating number of $CD31^{+}$/annexin V^{+} EMPs, and high levels of LDLc were significant risk factors to microvascular endothelial dysfunction. Our results suggest that these factors can orchestrate the early development of this vascular damage and that the endothelial apoptosis plays a more important role than activation in the worsening of the endothelial function. On the other

hand, previous report has shown that the mean pressure of the pulmonary artery was correlated with $CD62E^+$ EMPs, whereas hyperemic peak velocity of the brachial artery was independently associated with $CD31^+/CD42b^-$ EMPs in children born preterm [22]. Unlike what was observed in the current study, these data indicate that both apoptosis and endothelial activation play important roles in vascular dysfunctions in preterm infants.

The role of EMPs in the progression of endothelial dysfunction is still unclear. They are carriers of biological information to the surrounding milieu involved in cell-cell communication [20]. In vitro studies demonstrated that the EMPs induce pro-inflammatory effects on mature endothelial cells [33,34], impairment in the NO transduction pathway [20,35], and alterations in the vascular repair process by inducing apoptosis and reducing of functional capacity of EPCs [36]. Therefore, the EMPs are more than just a marker of endothelial damage, they could also contribute to this process. In this line, the endothelial dysfunction would produce high levels of EMPs and these, in turn, exacerbate pre-existing vascular damage and release to circulation even more EMPs. However, this link of cause or effect of EMPs release to impairment or injury of the endothelium is still being unraveled. Further research is necessary to obtain a clearer understanding of the complexity of this relationship.

An important limitation was that we performed a cross-sectional study with a limited sample size that does not allow the establishment of causal relationships of the birth weight, cardiovascular parameters, metabolic variables, and circulating number of EMPs. On the other hand, one of the strengths of this study was to have evaluated a cohort of children without overweight or obesity, therefore, excluding the confounding effect of the current nutritional status.

In conclusion, our data provide evidence that children who had birth weight at the lowest values showed higher levels of the circulating number of $CD31^+$/annexin V^+ and $CD144^+$ EMPs. In addition, the LBW and high levels of both $CD31^+$/annexin V^+ and LDLc were significant risk factors for the presence of microvascular endothelial dysfunction. A further understanding of the mechanisms underlying LBW-related endothelial dysfunction and of the role of EMPs on this vascular damage during childhood is of extreme importance for the development of more effective therapies aimed toward restoring vascular function.

Declaration of competing interests: The authors declare no conflicts of interest.

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Data availability: The data might be available on reasonable request. The data are not publicly available due to them containing information that could compromise research participant privacy/consent.

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Table 1: General characteristics of the study population.

Variables	
Birth Weight (g)	3,000 [700.0]
Birth Length (cm)	48.0 [23.0]
Prematurity (%)	
Yes	5.3
No	94.7
Age (years)	9.7 [3]
Sex (%)	
Male	56.8
Female	43.2
BMI (Kg/m^2)	15.9 [2]
Waist circumference (cm)	58.0 [6.5]
SBP (mmHg)	95 [8]
DBP (mmHg)	56 [9]
RHI	
Total Cholesterol (mg / dl)	144.0 [36]
LDLc (mg / dl)	70.0 [24]
HDLc (mg/dl)	53.0 [18]
Triglycerides (mg/dl)	50.8 [22]
Glucose (mg/dl)	88.2 [12]
CD31+/annexin V+ EMPs/ µl PFP	400 [370]
CD62E+ EMPs/ µl PFP	255 [450]
CD144+ EMPs/ µl PFP	230 [274]

Values expressed as Percentage or Median [Interquartile Range]. BMI - Body Mass Index, SPB – Systolic Blood Pressure; DBP – Diastolic Blood Pressure; RHI - Reactive Hyperemia Index; *EMPs - Endothelium-derived microparticles; PFP – platelet-free plasma.*

Table 2: Univariable and Multivariable Logistic Regression Analyses of Factors Associated with Microvascular Endothelial Dysfunction.

	Univariable Logistic Regression		Multivariable Logistic Regression	
	OR (95% CI)	***P* Value**	**OR (95% CI)**	***p* Value**
RHI < 1.67				
Birth Weight < 2.500g	4.13 (1.40 – 12.20)	0.010	3.61 (1.87 – 10.9)	0.038
Age	1.35 (1.06 – 1.71)	0.014		
Male Sex	2.83 (1.00 – 8.01)	0.049		
BMI	1.03 (0.93 – 1.52)	0.244		
Waist circumference	1.01 (0.90 – 1.21)	0.234		
SBP	1.06 (0.89 – 1.26)	0.308		
DBP	1.02 (0.90 – 1.20)	0.360		
Total Cholesterol	1.02 (0.99 – 1.04)	0.065		
LDLc	1.06 (1.02 – 1.10)	0.003	1.04 (1.02 – 1.08)	0.007
HDLc	0.98 (0.95 – 1.00)	0.815		
Triglycerides	1.00 (0.98 – 1.02)	0.873		
Glucose	1.02 (0.98 – 1.07)	0.398		
> 75th percentile CD31+/annexin V+ EMPs	4.62 (1.65 – 12.91)	0.004	4.07 (2.08 – 10.5)	0.019
> 75th percentile CD144+ EMPs	4.92 (1.79 – 13,54)	0.002		
> 75th percentile CD62E+ EMPs	1.02 (0.91 – 1.46)	0.263		

Data are reported as odds ratio (OR) and 95% Confidence Interval (95% CI). All variables showing a P ≤ 0.20 in the univariable analysis were presented to the multivariable models by using the backward stepwise strategy with Wald criteria. BMI was not included in the regression models due to the multicollinearity issue. BMI - Body Mass Index, SPB – Systolic Blood Pressure; DBP – Diastolic Blood Pressure; EMPs - Endothelium-derived microparticles.

Table 3: Univariable and Multivariable Logistic Regression Analyses of Factors Associated with Circulating levels of $CD31^+$/annexin V^+ and $CD144^+$ EMPs.

	Univariable Logistic Regression		Multivariable Logistic Regression	
	OR (95% CI)	***P* Value**	**OR (95% CI)**	***p* Value**
75th percentile > 550 CD31+/annexin V+ EMPs				
Birth Weight < 2.500g	3.13 (1.92 – 9.60)	0.026	2.71 (1.99 – 10.0)	0.032
Age	1.09 (0.86 – 1.39)	0.468		
Male Sex	2.04 (0.71 – 5.88)	0.186		
BMI	1.05 (0.73 – 1.51)	0.782		
Waist circumference	1.01 (0.93 – 1.11)	0.812		
SBP	1.02 (0.96 – 1.08)	0.580		
DBP	1.00 (0.92 – 1.10)	0.967		
Total Cholesterol	1.01 (0.99 – 1.03)	0.200		
LDLc	1.25 (1.11 – 1.32)	0.014	1.14 (1.09 - 1.29)	0.028
HDLc	0.92 (0.91 – 1.00)	0.484		
Triglycerides	1.02 (0.91 – 1.09)	0.087		
Glucose	1.00 (0.94 – 1.05)	0.999		
75th percentile > 399 CD144+ EMPs				
Birth Weight < 2.500g	3.81 (2.03 – 11.18)	0.015	3.42 (1.77 – 10.4)	0.034
Age	1.22 (0.97 – 1.53)	0.088		
Male Sex	1.50 (0.58 – 3.84)	0.401		
BMI	1.23 (0.87 – 1.72)	0.240		
Waist circumference	1.05 (0.77 – 1.15)	0.208		
SBP	1.04 (0.88 – 1.11)	0.212		
DBP	1.03 (0.92 – 1.14)	0.225		
Total Cholesterol	1.02 (1.00 – 1.04)	0.039		
LDLc	1.35 (1.09 – 1.49)	0.015	1.20 (1.11 – 1.33)	0.034
HDLc	0.90 (0.89 – 0.97)	0.810		
Triglycerides	1.00 (0.98 – 1.03)	0.742		
Glucose	1.00 (0.96 – 1.09)	0.850		

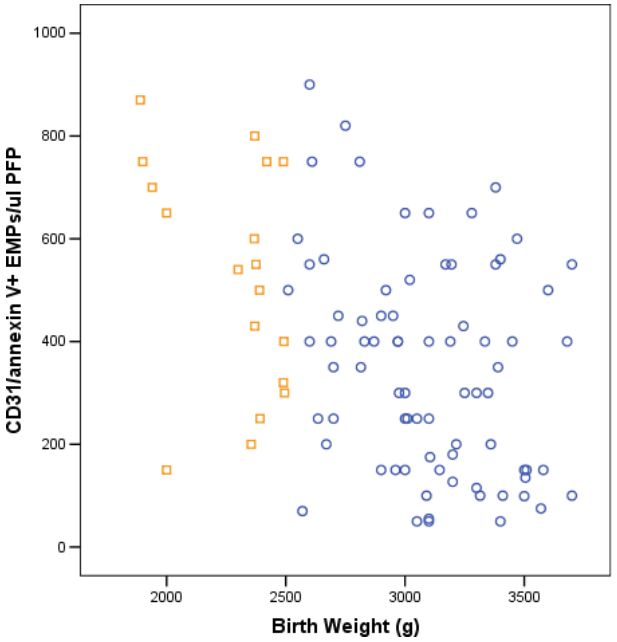
Data are reported as odds ratio (OR) and 95% Confidence Interval (95% CI). All variables showing a P ≤ 0.20 in the univariable analysis were presented to the multivariable models by using the backward stepwise strategy with Wald criteria. BMI was not included in the regression models due to the multicollinearity issue. BMI - Body Mass Index, SPB – Systolic Blood Pressure; DBP – Diastolic Blood Pressure; EMPs - Endothelium-derived microparticles.

Legends

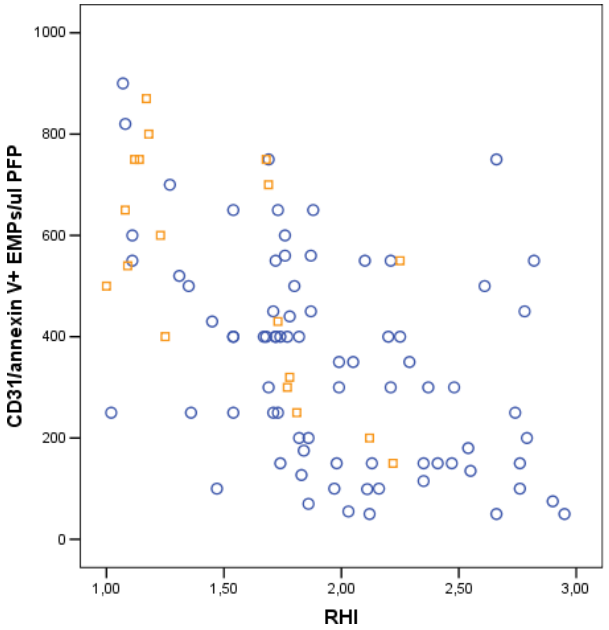
Figure 1. Scatter plots showing the correlation between circulating number of $CD31^+$/annexin V^+ EMPs with **(A)** birth weight, **(B)** RHI, and **(C)** three-dimensional (3D) scatter plot of $CD31^+$/annexin V^+ EMPs, RHI and birth weight in 95 children. Each child occupies a region in the 3D space. Orange squares represent children with low birth weight and blue circles represent children with normal birth weight.

Figure 2. Scatter plots showing the correlation between circulating number of $CD144^+$ EMPs with **(A)** birth weight, **(B)** RHI, and **(C)** three-dimensional (3D) scatter plot of $CD144^+$ EMPs, RHI and birth weight in 95 children. Each child occupies a region in the 3D space. Orange squares represent children with low birth weight and blue circles represent children with normal birth weight.

A


CD31/annexin V+ EMPs/ul PFP
1000
800
600
400
200
0
2000
2500
3000
3500
Birth Weight (g)

B


CD31/annexin V+ EMPs/ul PFP
1000
800
600
400
200
0
1,00
1,50
2,00
2,50
3,00
RHI

C

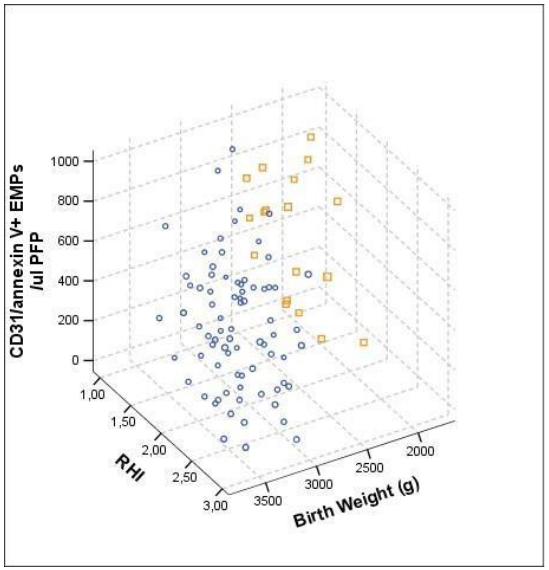

CD31/annexin V+ EMPs /ul PFP
1000
800
600
400
200
0
1,00
1,50
2,00
2,50
3,00
RHI
2000
2500
3000
3500
Birth Weight (g)

Figure 1

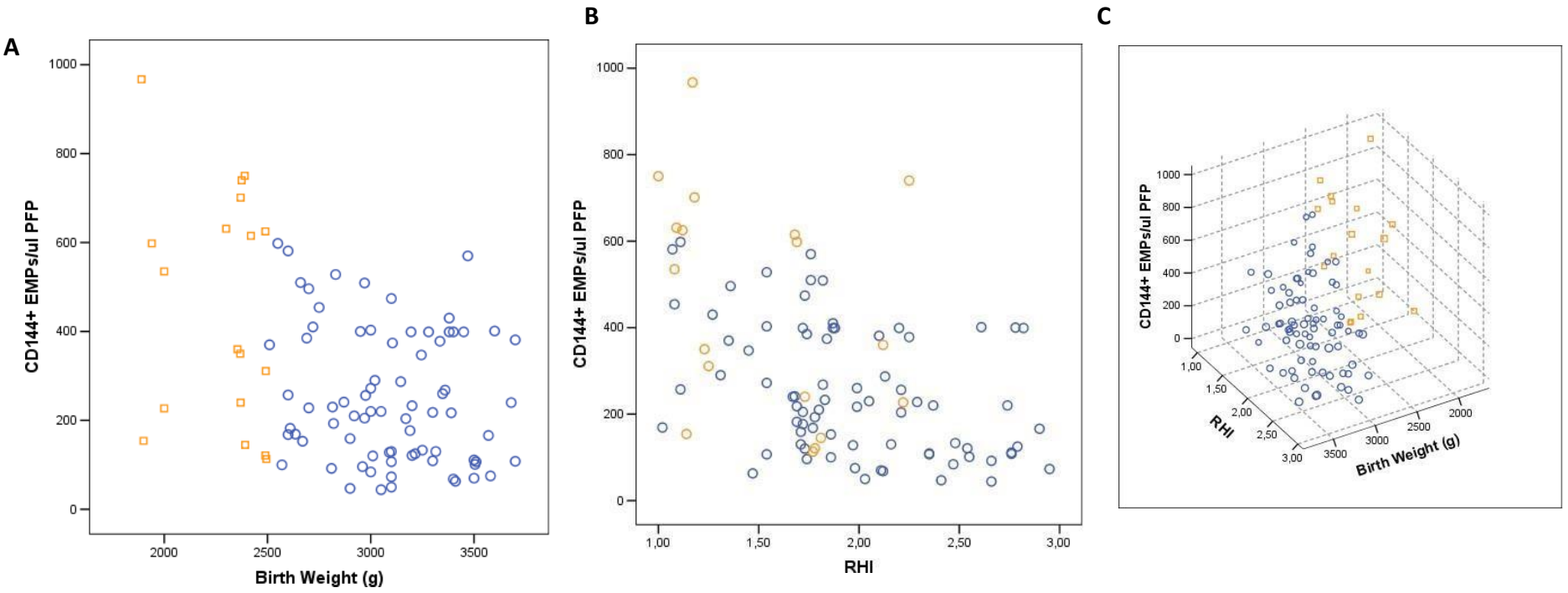

A
CD144+ EMPs/ul PFP
1000
800
600
400
200
0
2000
2500
3000
3500
Birth Weight (g)
B
CD144+ EMPs/ul PFP
1000
800
600
400
200
0
1,00
1,50
2,00
2,50
3,00
RHI
C
CD144+ EMPs/ul PFP
1000
800
600
400
200
0
1,00
1,50
2,00
2,50
3,00
RHI
2000
2500
3000
3500
Birth Weight (g)

Figure 2