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ORIGINAL ARTICLE

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Platelet activation markers in children with congenital heart disease associated with pulmonary arterial hypertension

Timur Mese MD[1] | Baris Guven MD[2] | Murat Muhtar Yilmazer MD[1] | Cem Karadeniz MD[3] | Rahmi Ozdemir MD[1] | Onder Doksoz MD[1]

[1]Medical Faculty, Dr. Behcet Uz Children's Hospital, Department of Pediatric Cardiology, University of Health Sciences, Izmir, Turkey

[2]Medical Faculty, Izmir Tepecik Training Hospital, Department of Pediatric Cardiology, University of Health Sciences, Izmir, Turkey

[3]Medical Faculty, Division of Pediatric Cardiology, Katip Celebi University, Izmir, Turkey

Correspondence
Baris Guven, Izmir Tepecik Training Hospital, Guney M 1140/1 St N: 1, Yenisehir - Konak – Izmir 35180, Turkey.
Email: drbarisguven@yahoo.com

Abstract

Background: Mean platelet volume (MPV), platecrit, and platelet distribution width (PDW) are markers of platelet activation. Previous studies have found that platelet activation occurs in patients with pulmonary arterial hypertension. Platelet indices including MPV, PDW, and platecrit have not been studied in children with congenital heart disease associated pulmonary arterial hypertension (APAH-CHD) who survived and those who died.

Objective: The objective of this study to investigate the value of platelet indices with clinical and hemodynamic indicators predicting the disease severity and survival in children with APAH-CHD.

Methods: This was a nested case-control study. MPV, platecrit, and PDW levels measured in 37 patients with APAH-CHD and 43 healthy subjects at the beginning of the study. Right heart catheterization was performed in all 37 patients. Clinical and hemodynamic data were collected. All patients were followed from the date of laboratory testing. The study was conducted between March 2012–July 2015. The comparison of clinical, hemodynamic data and platelet indices were made between patients with APAH-CHD who died than APAH-CHD patients who survived.

Results: Of 37 patients, after a mean follow-up duration of 67.90 ± 47.90 months, 11 patients died. MPV (12.10 femoliter [fL; 8.20–12.50] vs 8.70 fL [6.40–9.70], $P = .007$), PDW (16.88 ± 1.09% vs 15.75 ± 1.58%, $P = .04$) and platecrit (0.28 ± 0.31 vs 0.22 ± 0.27, $P = .01$) were significantly higher in the patients with APAH-CHD who died than those who survived. Pearson's correlation analysis showed that MPV correlated with mean pulmonary artery pressure ($r = 0.332$, $P = .04$) and correlated negatively with six-minute walking distance ($r = -0.600$. $P = .00$). PDW and platecrit correlated positively with mean pulmonary artery pressure ($r = 0.373$, $P = .02$; $r = 0.389$, $P = .01$, respectively).

Conclusion: Our results showed that MPV, platecrit and PDW were increased in children with APAH-CHD. They might give clue about disease severity.

KEYWORDS

congenital heart disease, mean platelet volume, platelet activation, pulmonary hypertension

1 | INTRODUCTION

Pulmonary arterial hypertension (PAH) is a complex progressive disease with multiple etiologies that has a poor prognosis, particularly in children, if not treated properly.[1,2] Recent registry studies documented that the incidence of idiopathic pulmonary hypertension is lower in children when compared with adults, congenital heart disease associated PAH

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(APAH-CHD) is more common in children, and children with PAH are more likely to present with syncope.[3,4] APAH-CHD is reported as the second commonest subgroup of PAH with an incidence of 2.2 cases per million children.[5] Ziljstra et al found that children with APAH-CHD have favorable survival when compared to those with idiopathic/hereditary PAH.[6] However, there is no established clinical or laboratory parameter to predict disease severity or survival in children with PAH. Although six-minute walking distance (6MWD) has been shown to correlate well with clinical and hemodynamic parameters in adults,[7] 6MWD or exercise tests are not easily applicable and not validated in children. Previous studies showed that serum biomarkers, particularly NT-pro BNP, has been associated with disease severity or survival of PAH in children.[5,6]

In situ thrombosis may contribute to the development of PAH. Sakamaki et al showed that patients with PAH had increased platelet aggregation and activation.[8] Mean platelet volume (MPV), platecrit (PCT), and platelet distribution width (PDW) are markers of platelet activation. Zheng et al found that MPV and PDW were significantly higher in adults with idiopathic PAH.[9] Kaya et al showed that MPV was correlated with systolic pulmonary artery pressure and right ventricular diameter in patients with atrial septal defect.[10] Platelet indices including MPV, PDW, and PCT have not been studied in children with APAH-CHD who survived and those who died. The objective of this study is to investigate the value of platelet indices with clinical and hemodynamic indicators predicting the disease severity and survival in children with APAH-CHD.

2 | MATERIALS-METHODS

2.1 | Study subjects

Patients with APAH-CHD were enrolled in Izmir Dr. Behcet Uz Children's Hospital between March 2012 and July 2015. In this nested case-control study, all pediatric patients with congenital heart disease with following conditions were included: (1) Patients who had group 1 PH according to the Dana classification[11]; (2) Patients who had cardiac catheterization at <18 years of age; (3) Patients who had regular visits between March 2012 and July 2015. We did not include patients if they had a chronic respiratory disease, acute heart failure, pulmonary venous hypertension, left heart disease, platelet disorders and chronic liver or kidney failure. None of the subjects were using antiplatelet or anticoagulant therapy on admission. A standardized follow-up protocol including physical examination, ECG, echocardiography, 6MWD, laboratory tests, and the information about WHO classification about functional status. Forty-three age-sex matched children were recruited from children with normal heart undergoing echocardiography evaluation for murmur and chest pain. The patient group had two cohorts: Those who survived and those who died. The study was conducted in compliance with the Helsinki declaration and was approved by the local ethical committee. All the patient data and informed consent forms signed by parents were collected.

2.2 | Clinical variables

Right heart catheterization was performed only in patients with APAH-CHD at admission. The diagnosis of pulmonary arterial hypertension was defined as mean pulmonary arterial pressure $\geq$ 25 mm Hg, pulmonary capillary wedge pressure $\leq$ 15 mm Hg, and pulmonary vascular resistance indices $\geq$ 3 Wood units $\cdot$ m^2. The acute vasoreactivity test with oxygen or inhaled iloprost was performed in all patients to assess operability. Acute responder definition was based on criteria defined by Barst et al.[12] In all patients, standardized z scores for weight and height were derived from healthy Turkish children based on the studies of Gunoz et al[13] and Gokcay et al.[14] Clinical and hemodynamic variables including right heart failure, syncope, failure to thrive, cardiac index, ratio of mean pulmonary artery pressure to mean arterial pressure (mPAP/MAP), acute vasoreactivity response, mean right atrial pressure, and pulmonary vascular resistance index were collected. Fasting blood samples were obtained at admission. Blood for measurement of mean platelet volume, platelet distribution volume, and platecrit was collected in EDTA potassium tubes. An automated blood counter (Mindray 6800 Auto Hematology Analyser. Mindray Bio-Medical Electronics Co., Ltd. Shenzhen. China) was used for blood tests. Blood samples were analyzed within 15 minutes after collection to avoid possible errors caused by platelet swelling with EDTA.[15,16] Blood analysis for platelet indices was also performed at admission for each subject.

2.3 | Statistical analysis

Descriptive statistics were described using mean $\pm$ standard deviations or median (minimum-maximum) for continuous variables when appropriate and number (%) for categorical variables. Student *t* test was used for normally distributed data and Mann-Whitney *U* test was used for not normally distributed data. Categorical data were analyzed using a $\chi 2$ or Fisher's test. Receiver operating curves (ROC) were analyzed to determine the prognostic value of each platelet indices. *P* values <.05 were considered significant. Statistical analysis was performed by SPSS 17.0 (SPSS. Inc., Chicago, Illinois).

3 | RESULTS

In this study, 37 patients with APAH-CHD and 43 age-sex matched healthy subjects were enrolled. Baseline demographic and clinical data were summarized in Table 1. MPV and PDW were significantly higher in patients with APAH-CHD than the control group. Mean age at right heart catheterization was 63.54 $\pm$ 40.29 (6–142) months. Of these patients, 11 had operable left to right shunt. PAH-targeted therapy was started according to the insurance policy of our country and availability. Height and weight z scores in children with APAH-CHD who died were significantly lower than those of APAH-CHD patients who survived (for height z score; -2.18 ± 1.76 vs -3.16 ± 1.19, respectively, $P = .05$ and for weight z score 2.04 ± 1.25 vs -3.16 ± 0.43, respectively; $P = .00$). Of 37 patients, 11 patients had surgery, four patients who had surgery died, of other 27 patients, 7 patients had died during the follow-up. Mean right atrium pressure, mean pulmonary

TABLE 1 Baseline characteristics and platelet markers of study population and healthy control subjects

Characteristics	APAH-CHD (37)	CONTROL (43)	*P*
Age (months. median)	16–237 (108)	12–228 (72)	.08
Female (%)	22 (59.4%)	21 (48.8%)	.34
Acute responder (%)	22 (59.4%)	–	
Exitus (%)	11 ()	–	
Syncope (%)	1 (2.7%)	–	
WHO-FC	2.65 ± 0.11	–	
MPV (fL)	9.00 (6.4–12.5)	8.10 (6–11.1)	.006[a]
PDW (%)	16.06 ± 1.53	14.42 ± 1.83	<.001
Platecrit (%)	0.24 (0.18-0.41)	0.23 (0.04-0.52)	.39[a]

Data presented as number (%) and mean ± SD.
Abbreviations: MPV, mean platelet volume; PDW, platelet distribution width; WHO-FC, World Health Organization functional classification.
[a]Mann-Whitney U test is used for these comparisons.

artery pressure were significantly higher and cardiac index was significantly lower in the patients with APAH-CHD who died than APAH-CHD patients who survived (Table 2). Comparison of platelet indices were also shown in Table 2. MPV, PDW, and platecrit were significantly higher in the patients with APAH-CHD who died than those who survived. Acute vasodilatory test results revealed that 22 of 37 patients (59%) had acute vasodilator response. None of the patients received Ca channel blocker as PAH monotherapy at the time of enrolment. In this cohort, 19 patients received PAH specific monotherapy, 27.1% of patients treated with bosentan, 21.6% received inhaled prostacyclin analogue, and 13 patients and patients were treated with dual and triple therapy. There was no significant difference regarding distribution of therapeutic agents between patients with APAH-CHD survivors and APAH-CHD nonsurvivors.

TABLE 2 Comparison of clinical hemodynamic parameters and platelet indices in children with APAH-CHD who survives and children with APAH-CHD who died

Characteristics	Surviving (N = 26)	Death (N = 11)	*P*
Age	128.33 ± 65.45	93.90 ± 81.94	.19
RHC months	60.11 ± 37.15	72.80 ± 48.74	.40
Follow-up months	71.77 ± 66.73	67.90 ± 47.90	.86
Height *z* score	−2.18 ± 1.76	−3.16 ± 1.19	.05
Weight *z* score	−2.04 ± 1.25	−3.16 ± 0.43	<.01
6MWT	239.55 ± 120	243.33 ± 32.14	.95
ECHO pPA mmHg	64.45 ± 17.07	85.83 ± 23.32	.009
mRAP mm Hg	5.70 ± 1.45	8.50 ± 5.7	.01
mPA mm Hg	51.67 ± 6.35	57.40 ± 6.15	.019
QP/QS	1.41 ± 1.31	1.66 ± 1.03	0.94
PVRI Wood units · m^2	8.77 ± 8.59	9.00 ± 6.26	.09
CI L /min.m2	4.47 ± 0.94	4.02 ± 1.20	.02
mPA/MAP	0.62 ± 0.20	0.73 ± 0.32	.30
MPV (fL)	8.70 (6.40-9.70)	12.10 (8.20-12.50)	.007[a]
PDW (%)	15.75 ± 1.58	16.88 ± 1.09	.04
Platecrit (%)	0.22 ± 0.27	0.28 ± 0.31	.01

Data presented as mean ± SD.
Abbreviations: 6MWT, six-minute walking test; CI, cardiac index; mPA, mean pulmonary artery pressure; MPV, mean platelet volume; mRAP, mean right atrium pressure; PDW, platelet distribution width; PVRI, pulmonary vascular resistance index; Qp/Qs, ration of pulmonary blood flow to systemic blood flow.
[a]Mann-Whitney U test is used for these comparisons.

Among platelet activation markers, MPV had greatest area under curve (AUC) with 0.94 ($P < .001$; CI, 0.84–1.04) for predicting mortality in children with APAH-CHD. Platecrit had 0.83 of AUC ($P = .002$; CI, 0.71–0.96) and PDW had 0.70 of AUC ($P = .06$; CI, 0.54–0.86). Specificity was 96% and sensitivity was 90% when MPV above 9.70 femtoliter (fL).

Pearson's correlation analysis showed that MPV correlated with mean pulmonary artery pressure ($r = 0.332$, $P = .04$, Figure 1) and correlated negatively with 6MWD ($r = -0.600$. $P = .00$). PDW and platecrit correlated positively with mean pulmonary artery pressure ($r = 0.373$, $P = .02$; $r = 0.389$, $P = .01$, respectively) (Table 3).

4 | DISCUSSION

Previous studies have shown increased platelet activation and aggregation in pulmonary hypertension. However, the role of platelet indices in the evaluation of children with APAH-CHD was not clear, particularly in patients who died during the follow-up. In this study, we studied platelet indices including MPV, RDW, and platecrit in children with APAH-CHD. We found that MPV, PDW, and platecrit were significantly higher in children with APAH-CHD who died. Moreover, we found MPV correlated with mean pulmonary artery pressure and correlated negatively with 6MWD, and PDW and platecrit were correlated with mean pulmonary artery pressure. Mean platelet volume, PDW, and platecrit are simple hematological markers of assessing platelet function and easily available. Mean platelet volume increases during platelet activation and reflects platelet production. Platelet distribution width (PDW) and platecrit also provide information on total platelet mass.[9,16] Increased MPV and PDW have been observed in systemic hypertension,[17] diabetes, myocardial infarction and metabolic syndromes.[18] Larger platelets, which are metabolically and enzymatically more active than smaller ones, might contribute o thrombosis and atheroma formation.[19,20] Antiplatelet therapy is recommended to reduce the risk of many cardiovascular disorders.[21] Increased MPV levels have been found in inflammatory diseases including rheumatoid arthritis and osteoarthritis.[22] Previous studies also investigated the role of MPV as a marker of inflammation and efficacy of anti-inflammatory treatment.[22]

Pulmonary arterial hypertension is complex vasculopathy in which in situ thrombosis, excessive vascular cell growth, and inflammation may play a role.[23] Although our patients had different etiologies, all patients had APAH-CHD (Group 1 of the WHO classification system).

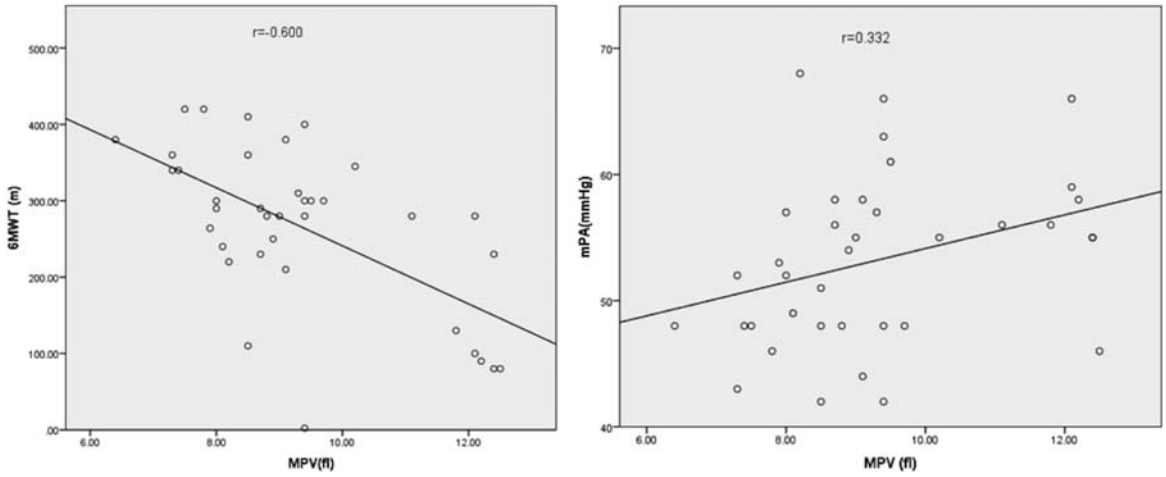

FIGURE 1 Correlations between MPV and six-minute walking test distance and mean pulmonary artery pressure

PAH develop as a consequence of chronic left-to-right shunt and volume overloading of the pulmonary vasculature in patients with APAH-CHD. Histological changes in the intima and media of the pulmonary arteries and arterioles with subsequent remodeling, vasoconstriction, and the increased pulmonary vascular resistance occur in the setting of chronic exposure of the pulmonary vasculature to increased blood flow.[10] Previous studies have shown increased platelet aggregation and activation with PAH with idiopathic and associated with CHD.[9,10] In this study, we found significantly higher MPV, PDW, and platecrit in children with APAH-CHD who died, and MPV was correlated with mean pulmonary artery pressure and negatively correlated with six-minute walking distance. PDW and platecrit were also correlated with pulmonary vascular resistance and mean pulmonary artery pressure as consistent with previous studies. Can et al suggested that platelet activation may directly impact the pathogenesis of PAH by showing the higher MPV levels in patients with PAH.[24] Kaya et al also investigated MPV levels in patients who had transcatheter closure of ASD.[10] They found that MPV levels were correlated with pulmonary artery pressure, and increased MPV levels have been decreased after successful transcatheter ASD closure. Several mechanisms are postulated to explain platelet activation in idiopathic and secondary PAH. Regardless of the etiologies, endothelial dysfunction in the setting of PAH may lead increased procoagulant activity, inappropriate fibrinolysis and platelet activation.[25] Endothelial dysfunction may also result in an imbalance of vasoactive mediators. Proaggregatory thromboxane A_2 levels are increased, whereas NO and prostacyclin (inhibit platelet aggregation) levels are decreased in patients with PAH.[26] Besides, systemic inflammatory cytokines IL-3 and IL-6 were found to be increased in patients with PAH. Increased IL-3 and IL-6 levels may affect megakaryopoiesis, and leading to more large and reactive platelets, therefore, an increase in MPV.[27] Finally, activated the sympathetic nervous system in right ventricular failure might contribute to the activation of platelets. Jafri et al have shown that patients with heart failure and increased sympathetic activity had high plasma concentrations of platelet factor 4 and beta-thromboglobulin, which are markers of platelet activation.[28]

TABLE 3 Correlation analysis between clinical-hemodynamic parameters and platelet indices

Characteristics	Correlation with MPVr *P*		Correlation with PDWr *P*		Correlation with platecritr *P*	
6MWT	−0.600	<.01[a]	−0.270	.10	−0.173	.30
mPA	0.332	.04[b]	0.373	.02[b]	0.389	.01[b]
PVR	0.17	.41	0.19	.06	0.13	.45
Cardiac index	0.210	.24	0.142	.43	0.041	.82

[a]Correlation is significant at the 0.01 level.
[b]Correlation is significant at the 0.05 level.

MPV and PDW in children with PAH secondary to congenital heart disease were studied in only one study. Arslan et al showed MPV and PDW were lower in children with PAH.[29] The results of our study and adult studies in patients with PAH secondary to CHD did not confirm this study. This discrepancy may in part due to patient characteristics and hemodynamic data. We observed higher MPV levels in patients with APAH-CHD who died. Arslan et al studied 33 children with APAH-CHD, none of the patients died in the study of Arslan et al.[29] In addition, our cohort's Qp/Qs and PVR/SVR ratios were lower than those of this study's cohort. These observations could explain, at least in part, higher MPV observed in our study.

Confounding factors, especially sepsis, must be noted in assessing the result of platelet activation markers in this study. Platelets show diverse characteristics during the clinical course of sepsis. Kim et al[30] demonstrated a greater increase of MPV in patients with sepsis who died. Van der Lelie et al[31] suggested that increase of MPV could reveal the occurrence of septicemia in patients with localized infection. However, in our study, blood analysis was made before right heart catheterization at baseline, we did not repeat blood analyses over study period. There were no active infections in patients with APAH-CHD.

During the last decade, B type natriuretic peptide (BNP) and amino terminal fragment (NTproBNP) have been studied in pediatric patients with PAH. Several studies showed that NTproBNP associated with echocardiographic and exercise data superior than BNP.[32] Along with

NTproBNP, uric acid and norepinephrine were also investigated to predict survival in pediatric PAH.[33] Van Albada et al[33] did not show any significant relation between clinical severity and uric acid-norepinephrine. Despite increasing number of studies about BNP and NTproBNP in relation to pediatric PAH mortality, there are some pitfalls in evaluating these markers in clinical practice of pediatric patients with CHD. Levels of BNP or NTproBNP could easily change with age, gender, type of CHD and severity of left-to-right shunt.[34] Variations of normative levels in these markers limits the usefulness of these markers in the clinical setting.

We found MPV correlated with pulmonary artery pressure and negatively with 6MWD. Although we found significantly higher MPV levels in patients with APAH-CHD who died, we did not study univariate or multivariate analysis to predict mortality using clinical or laboratory parameter. Therefore, we could not say MPV and other platelet indices can be used to predict prognosis in patients with APAH-CHD. However, MPV, PDW and platecrit, at least in part, could reflect disease severity. MPV, platecrit, and PDW are simple platelet activation markers which can be measured by automated hematology analyzer. They are easily accessible and not requiring higher cost and advanced technology. Further investigations are needed to understand the role of platelet activation in patients with APAH-CHD on this perspective.

Certain limitations deserve mention here. Our hospital is a tertiary referral center with patients referred from several regions of Turkey. Therefore, follow-up data were not available in all our patients. As a result, late follow-up data were available in 37/54 (68%). However, we did not include patients whose data were not available during the follow-up period to avoid confusion. In this study, our control group was of normal healthy children. One could argue that it may have been better to use a matched group of patients with congenital heart disease without pulmonary hypertension. Nevertheless, only one study had a matched group of patients with congenital heart disease without pulmonary hypertension. In the current study, we aimed to study platelet indices predicting the disease severity of PAH. Several studies pointed out that nutritional deficiencies such as folic acid and vitamin B12 could influence platelet volume. We did not evaluate the folic acid, vitamin B12 or iron levels in the current study.[35,36] Finally, relatively small sample size due to single-center study is the major limitation.

In conclusion, MPV, PDW, and platecrit were significantly higher in children with APAH-CHD who had a bad prognosis. MPV, PDW, and platecrit were associated with some hemodynamic and clinical variables.

CONFLICT OF INTEREST

We have no conflict of interest and received of no financial support.

AUTHOR CONTRIBUTIONS

Concept/design, drafting article and data collection: *Timur Mese, Murat Muhtar Yilmazer*

Data analysis/interpretation, critical revision of the article and approval of article: *Baris Guven*

Data collection and approval of article: *Cem Karadeniz, Rahmi Ozdemir*

Critical revision of the article and approval of article: *Onder Doksoz*

ORCID

Baris Guven MD http://orcid.org/0000-0002-4520-5574

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