World Journal of Cardiology

Submit a Manuscript: https://www.f6publishing.com

World J Cardiol 2024 December 26; 16(12): 720-730

DOI: 10.4330/wjc.v16.i12.720

ISSN 1949-8462 (online)

ORIGINAL ARTICLE

Retrospective Study

Independent prognostic value of lipocalin-2 in congenital heart disease-associated pulmonary artery hypertension

Zhang-Ke Guo, Ping-Gui Chen, Yao-Xuan Li, Hong Jiao, Xiao-Hui Kong, Song Bai, Xiao-Feng Li, Ai-Jun Liu, Guo-Liang Wang

Specialty type: Cardiac and cardiovascular systems

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade C

Novelty: Grade B, Grade B

Creativity or Innovation: Grade B, Grade B

Scientific Significance: Grade B, Grade B

P-Reviewer: He W; Kong MW

Received: April 8, 2024

Revised: September 14, 2024

Accepted: October 22, 2024

Published online: December 26, 2024

Processing time: 232 Days and 2 Hours

Zhang-Ke Guo, Song Bai, Xiao-Feng Li, Department of Cardiac Surgery, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing 100045, China

Ping-Gui Chen, Department of General Surgery, The Third Hospital of Shanxi Medical University, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Taiyuan 030032, Shanxi Province, China

Yao-Xuan Li, Department of Health Statistics, School of Public Health, Shanxi Medical University, Taiyuan 030001, Shanxi Province, China

Hong Jiao, Xiao-Hui Kong, Guo-Liang Wang, Department of Tumor and Immunology, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing 100045, China

Ai-Jun Liu, Department of Pediatric Cardiac Surgery, Beijing Anzhen Hospital, Capital Medical University, Beijing Institute of Heart Lung and Blood Vessel Diseases, Beijing 100029, China

Co-corresponding authors: Guo-Liang Wang and Ai-Jun Liu.

Corresponding author: Guo-Liang Wang, PhD, Research Scientist, Researcher, Department of Tumor and Immunology, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, No. 56 Nanlishi Road, Xicheng District, Beijing 100045, China. wgl163@126.com

Abstract

BACKGROUND

Timely and accurate evaluation of the patient's pulmonary arterial pressure (PAP) is of great significance for the treatment of congenital heart disease. Currently, there is no non-invasive gold standard method for evaluating PAP.

AIM

To assess the prognostic value of lipocalin-2 (LCN2) in relation to PAP in patients with congenital heart disease associated with pulmonary artery hypertension.

METHODS

We conducted a retrospective analysis of 69 pediatric patients diagnosed with

ventricular septal defects. The patients' clinical and laboratory data were collected. The serum LCN2 concentrations were compared between the pulmonary arterial hypertension (PAH) group and the nonPAH group. The correlation of LCN2 concentration with PAH classification was evaluated using binary logistic regression analysis. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic potential of LCN2 for PAH.

RESULTS

Serum LCN2 concentration significantly correlated with patients' mean PAP (r = 0.544, P < 0.001), but not correlated with creatinine (P = 0.446) or blood urea nitrogen (P = 0.747). LCN2 levels were significantly correlated with PAH in both univariate [odds ratio (OR) 1.107, 95%CI: 1.033-1.185, P = 0.004)] and multivariate regression analysis (OR 1.150, 95%CI: 1.027-1.288, P = 0.015). ROC curve analysis revealed an area under the curve of 0.783 for LCN2. At the cutoff value of 19.42 ng/mL, the sensitivity and specificity of LCN2 for diagnosing PAH is 90.19% and 55.56%, respectively. LCN2 concentration also significantly correlated with the post-repair mean PAP in patients with congenital heart disease (r = 0.532, P = 0.009).

CONCLUSION

LCN2 is emerging as a candidate biomarker for assessing PAP in patients with congenital heart disease. Its high sensitivity in diagnosing PAH makes it a valuable tool in patient management.

Key Words: Congenital heart disease; Pulmonary arterial hypertension; Lipocalin-2; Endothelin-1; Biomarker

Core Tip: This retrospective study demonstrates that lipocalin-2 (LCN2) blood levels significantly correlated with the mean pulmonary arterial pressure of patients with congenital heart disease (CHD). In particular, LCN2 was significantly correlated with the post-repair mean pulmonary arterial pressure of CHD patients. LCN2 has emerged as a candidate biomarker for CHD, and its high sensitivity to pulmonary arterial hypertension diagnosis makes it highly valuable in patient management.

Citation: Guo ZK, Chen PG, Li YX, Jiao H, Kong XH, Bai S, Li XF, Liu AJ, Wang GL. Independent prognostic value of lipocalin-2 in congenital heart disease-associated pulmonary artery hypertension. *World J Cardiol* 2024; 16(12): 720-730
URL: https://www.wjgnet.com/1949-8462/full/v16/i12/720.htm
DOI: https://dx.doi.org/10.4330/wjc.v16.i12.720

INTRODUCTION

Pulmonary arterial hypertension (PAH) is characterized by a sustained increase in pulmonary arterial pressure (PAP) and pulmonary vascular resistance[1]. Among the different forms of pediatric PAH, PAH caused by congenital heart disease (PAH-CHD) is particularly significant.

Timely intervention to block abnormal left-to-right shunting in the heart can usually normalize elevated PAP. However, partial patients continue to experience persistent or even worsening PAH, which may be attributed to delayed shunt correction[2,3]. Timely and accurate evaluation of PAP is crucial for the management of congenital heart disease (CHD) patients with left-to-right shunt. At present, evaluations of PAP through echocardiography faces notable limitations. For instance, measurements obtained *via* echocardiography often significantly differ from those obtained through right heart catheterization. Additionally, echocardiography has proven inadequate in predicting mortality in PAH patients, particularly those classified as New York Heart Association functional class III–IV with RV dilation[4,5]. There remains a need for reliable non-invasive gold standard methods to assess PAP.

Lipocalin-2 (LCN2), also known as neutrophil gelatinase-associated lipocalin, is a 25-kDa protein that belongs to the lipocalin family. LCN2 is expressed in various tissues, including the liver, kidney, lung, and adipose tissue, playing pivotal roles in diverse physiological and pathological processes[6,7]. We previously demonstrated that LCN2 is upregulated in PAH-CHD, where it promotes pulmonary artery smooth muscle cell proliferation and helps resist apoptosis [8-10]. LCN2 has also been reported play roles in Kawasaki disease-related PAH[11]. These studies suggest that LCN2 may play an important role in PAH and serve as a potential biomarker for PAH. Furthermore, LCN2 has been identified as a biomarker for acute kidney injury[12,13] and is involved in the development of chronic kidney diseases, including diabetic nephropathy and renal fibrosis[14]. Given the strong physiological and pathological connections between the heart and kidneys[15,16], it is necessary to clarify whether LCN2 upregulation in PAH-CHD patients is a consequence of renal dysfunction.

The aim of the present study was to evaluate the independent predictive value of LCN2 on PAP in a series of consecutive pediatric patients with ventricular septal defect (VSD), for whom right ventricular catheterization pressure, blood cell counts, and biochemical parameters were accessible. Additionally, we compared the predictive values of LCN2 and endothelin-1 (ET-1), a candidate biomarker for PAH[17].

MATERIALS AND METHODS

Subjects

The present study retrospectively collected data from Chinese children with CHD who received treatment at Beijing Children's Hospital over a 4-year period from January 2019 to December 2022. The clinical and laboratory data of the patients were extracted from hospital registry systems by two academic surgeons. This study was approved by the Beijing Children's Hospital Ethics Committee, and all patients or their parents or legal guardians provided written informed consent.

We collected data from pediatric patients who were primarily diagnosed with VSD following cardiac surgery. The inclusion criteria comprised: (1) Surgical repair of VSD; (2) Age less than 72 months; (3) Measurement of PAP *via* intra-operative right ventricular catheterization; and (4) Preoperative serum samples preserved in the biologic sample bank of our hospital. The exclusion criteria included: (1) Cases with abnormal cardiac structures other than VSD or VSD accompanied with atrial septal defect (n = 51); (2) Cases with heart diseases other than CHD (n = 1); (3) Cases with other systemic diseases (n = 2); and (4) Cases that received medications influencing cardiac or renal function during the last 3 months prior to surgery (n = 0). Overall, 69 patients (41 females and 28 males) were included in this study.

ELISA assays

The serum levels of LCN2 and ET-1 were measured using LCN2/NGAL Human ELISA Kit (EHLCN2; Thermo Fisher, Waltham, MA, United States) and ET-1 Human ELISA Kit (EIAET1; Thermo Fisher) following the manufacturer's instructions.

Statistical analysis

The mean PAP (mPAP) was measured through intra-operative right ventricular catheterization. PAH is defined as mPAP > 20 mmHg according to the classification established at the 6th World Symposium of Pulmonary Hypertension[18]. Patients were divided into the PAH group and nonPAH group based on their mPAP values. The diameter of the defect was measured during surgery, and for patients with multiple defects, the defect diameter was calculated based on the total area of all defects.

Continuous variables are expressed as median (Q1, Q3), while categorical variables are expressed as proportion. The Mann-Whitney U test was utilized for comparisons between two independent groups for numerical variables, and the chi-square test was employed for categorical variables, such as the sex of the patients. The Fisher exact test was applied when at least one expected count was less than 5. Pearson correlation was determined between two continuous variables, and Spearman correlation was used when categorical data were included. Odds ratio (OR) and 95%CI were calculated using binary logistic regression analysis. Variables with a P value less than 0.15 in univariate regression were included in the multivariate regression model. The receiver operating characteristic (ROC) curve was employed to evaluate the diagnostic values of the variables. A bilateral P value of < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS 25.0 (IBM Corp., Armonk, NY, United States), Medcalc 19.0 or GraphPad Prism 8.0.

RESULTS

Patients' characteristics

The clinical and laboratory characteristics of the PAH and nonPAH patients are summarized in Table 1. A total of 69 children with CHD participated in this study, including 41 (59.4%) females and 28 (40.6%) males, with a median age of 14.6 months (range 3 months to 55 months). Significant differences were observed between the nonPAH and PAH group in creatinine, blood urea nitrogen (BUN), glucose, mPAP, LCN2 and ET-1. There were no significant differences in sex, age, systolic blood pressure, diastolic blood pressure, ejection fraction, defect diameter, or other laboratory factors between the two groups.

Significant difference in renal function between nonPAH and PAH groups

As shown in Table 1, there were significant differences in median values of creatinine (21.8 μmol/L *vs* 27.8 μmol/L, P < 0.001) and BUN (3.0 mmol/L *vs* 4.2 mmol/L, P = 0.015) between the nonPAH and PAH groups. Pearson correlation analysis demonstrated a significant correlation between mPAP and both creatinine (r = 0.389, P < 0.001) and BUN (r = 0.328, P = 0.006), as shown in Table 2. These results suggest that the PAH group exhibits poorer renal function compared to the nonPAH group among CHD children.

Serum LCN2 concentration significantly correlated with mPAP of VSD patients

According to Table 2, there was a significant correlation between LCN2 and mPAP (r = 0.544, P < 0.001) in VSD children. However, LCN2 does not significantly correlate with creatinine (r = 0.094, P = 0.446) or BUN (r = -0.040, P = 0.747). Even after excluding confounding factors in multivariate regression analysis, LCN2 remained uncorrelated with creatinine or BUN, as shown in Table 3.

To further explore the relationship between LCN2 and PAH, binary logistic regression analysis was conducted. In the univariate regression analysis (Table 4), variables with a P value less than 0.15 (include age, creatinine, white blood cell and LCN2) were included in the multivariate logistic regression model. BUN was excluded in the model due to its significant collinearity with creatinine. The diameter of the defect was also included in the multivariate model based on

Table 1 Baseline characteristics of the nonpulmonary arterial hypertension and pulmonary arterial hypertension patients

Variables	Total number	nonPAH	PAH	*P* value
Number	69	18	51	-
Sex, number of males	69	7 (38.9)	21 (41.2)	0.866
Age in months	69	8.5 (5.4, 12.0)	11.0 (7.0,19.0)	0.094
BMI	68	15.4 (13.8, 17.4)	15.1 (14.1, 16.6)	0.451
Creatinine in μmol/L	68	21.8 (18.0, 26.5)	27.8 (24.3, 33.6)	< 0.001
BUN in mmol/L	68	3.0 (2.1, 3.7)	4.2 (2.8, 5.1)	0.015
Albumin in g/L	67	42.6 (39.6, 43.7)	43.5 (41.4, 45.3)	0.243
Glucose in mmol/L	65	4.6 (4.1, 5.0)	4.1 (3.8, 4.4)	0.036
Hemoglobin in g/L	69	116 (101, 123)	118 (108, 128)	0.448
Platelets as 10^9/L	69	276 (247, 314)	267 (221, 344)	0.995
White blood cells as 10^9/L	46	8.3 (5.7, 9.7)	8.7 (7.5, 11.0)	0.140
SBP in mmHg	66	93.0 (84.5, 95.0)	95.0 (86.5, 101.5)	0.144
DBP in mmHg	66	55.0 (48.5, 60.0)	55.0 (45.0, 60.0)	0.860
EF as %	67	65.0 (62.0, 66.3)	65.0 (65.0, 69.5)	0.072
Diameter in mm	66	10.0 (7.5, 13.0)	10.0 (8.0, 14.8)	0.219
mPAP in mmHg	69	17.7 (16.7, 18.8)	29.4 (24.6, 40.1)	< 0.001
LCN2 in ng/mL	69	19.3 (16.9, 27.6)	33.4 (23.9, 46.3)	< 0.001
ET1 in μg/mL	69	0.14 (0.07, 0.31)	0.30 (0.10, 0.72)	0.049

Data are *n* (%). Sex is expressed as a proportion; continuous variables are expressed as median (Q1, Q3). Diameter means the diameter of the defect, which was measured during surgery. BMI: Body mass index; BUN: Blood urea nitrogen; DBP: Diastolic blood pressure; EF: Ejection fraction; ET-1: Endothelin-1; LCN2: Lipocalin-2; mPAP: Mean pulmonary artery pressure; PAH: Pulmonary arterial hypertension; SBP: Systolic blood pressure.

its clinical relevance. As shown in Figure 1, after adjusting for confounding variables, LCN2 was significantly correlated with PAH (OR 1.150, 95%CI: 1.027-1.288, $P = 0.015$). LCN2 remained significantly correlated with PAH (OR 1.123, 95%CI: 1.032-1.222, $P = 0.007$) after excluding the white blood cell count from the model, given the considerable missing values for this parameter and its insignificance in relation to PAH.

LCN2 exhibits a higher diagnostic potential for PAH in patients with VSD than ET-1

From Table 2, it is evident that both LCN2 and ET-1 are significantly correlated with mPAP in VSD children. However, in the binary logistic regression analysis, ET-1 did not show a significant correlation with mPAP in either the univariate (Table 4) or multivariate (Table 5) analyses. These results indicate that LCN2 exhibits a strong correlation with mPAP compared to ET-1. The diagnostic values of LCN2 and ET-1 for predicting PAH were evaluated using the ROC curve method. As illustrated in Figure 2A, LCN2 demonstrated a larger area under the curve (AUC) of 0.783 compared to 0.657 for ET-1 when predicting PAH in CHD patients. For LCN2, with a best cutoff value of 19.42 ng/mL, the sensitivity, specificity and Yonden's index for diagnosing PAH were 90.19%, 55.56% and 0.4575, respectively. Conversely, for ET-1, with a best cutoff value of 0.39 μg/mL, the sensitivity, specificity and Yonden's index in diagnosing PAH were 45.10%, 94.44% and 0.3954, respectively (Table 6).

LCN2 concentration significantly correlated with the post-repair mPAP of VSD patients

The post-repair mPAP values (intra-operative) of 23 patients were recorded. All 23 patients exhibited a decrease in post-repair mPAP compared to pre-repair levels. Among these, seven patients were classified as nonPAH and 16 as PAH based on their post-repair mPAP values. Notably, four patients transitioned from pre-repair PAH to post-repair nonPAH. Table 7 shows that correlation analysis indicates a significant relationship between pre-repair mPAP and both post-repair mPAP ($r = 0.471$, $P = 0.023$) and mPAP decrease ($r = 0.883$, $P < 0.001$) following repair surgery. LCN2 was significantly correlated with post-repair mPAP ($r = 0.532$, $P = 0.009$), but it did not show a significant correlation with mPAP decrease ($r = 0.181$, $P = 0.407$). Conversely, ET-1 was not correlated with either post-repair mPAP ($r = 0.140$, $P = 0.523$) or mPAP decrease ($r = 0.298$, $P = 0.167$). From Figure 2B, we can see that the LCN2 concentration in post-repair PAH patients was higher than that in nonPAH patients (31.6 ng/mL *vs* 25.5 ng/mL, median value), although this difference was not statistically significant ($P = 0.154$).

Table 2 Correlations of mean pulmonary artery pressure, lipocalin-2 and endothelin-1 with clinical variables

Variables	mPAP		LCN2		ET-1	
	r	*P* value	*r*	*P* value	*r*	*P* value
Sex	0.087	0.475	0.031	0.800	-0.115	0.347
Age in months	0.298	0.013	0.319	0.008	0.048	0.698
BMI	-0.089	0.483	-0.128	0.310	-0.248	0.046
Creatinine in μmol/L	0.389	0.001	0.094	0.446	-0.156	0.205
BUN in mmol/L	0.328	0.006	-0.040	0.747	-0.070	0.568
Albumin in g/L	0.066	0.594	-0.072	0.563	-0.238	0.052
Glucose in mmol/L	-0.174	0.166	0.021	0.869	-0.004	0.973
Hemoglobin in g/L	0.217	0.074	0.080	0.514	-0.173	0.156
Platelets as 10^9/L	0.007	0.958	-0.128	0.293	-0.098	0.422
White blood cells as 10^9/L	0.345	0.019	0.291	0.050	0.107	0.480
SBP in mmHg	0.135	0.280	0.074	0.554	0.033	0.795
DBP in mmHg	0.097	0.438	0.106	0.399	0.003	0.983
EF as %	-0.107	0.388	-0.176	0.155	-0.021	0.864
Diameter in mm	0.371	0.002	0.081	0.516	0.230	0.063
mPAP in mmHg	-	-	0.544	< 0.001	0.508	< 0.001
LCN2 in ng/mL	0.544	< 0.001	-	-	0.427	< 0.001
ET1 in μg/mL	0.508	< 0.001	0.427	< 0.001	-	-

BMI: Body mass index; BUN: Blood urea nitrogen; DBP: Diastolic blood pressure; EF: Ejection fraction; ET-1: Endothelin-1; LCN2: Lipocalin-2; mPAP: Mean pulmonary artery pressure; SBP: Systolic blood pressure.

Table 3 Multivariate logistic regression analysis to define the correlation between lipocalin-2 and renal function in ventricular septal defect patients

Variables	Creatinine[1]			BUN[1]		
	OR	95%CI	*P* value	OR	95%CI	*P* value
Age in months	1.077	1.006-1.153	0.034	1.117	1.010-1.235	0.031
BMI	1.226	0.913-1.648	0.175	-	-	-
Albumin in g/L	1.089	0.901-1.315	0.378	1.016	0.837-1.232	0.875
Glucose in mmol/L	-	-	-	0.635	0.294-1.371	0.248
Hemoglobin in g/L	-	-	-	1.042	0.992-1.094	0.102
mPAP in mmHg	1.065	0.992-1.142	0.082	1.086	1.008-1.170	0.030
LCN2 in ng/mL	0.962	0.914-1.014	0.147	0.972	0.922-1.025	0.294

[1]Variable was binarized based on its median value.
BMI: Body mass index; BUN: Blood urea nitrogen; LCN2: Lipocalin-2; mPAP: Mean pulmonary artery pressure; OR: Odds ratio.

DISCUSSION

PAH associated with CHD is a subgroup of pulmonary hypertension (PH). While there are established expert guidelines for managing PH, there is a notable lack of evidence specifically addressing the management of PAH in patients with CHD. The advancements in the management of CHD have significantly improved survival rates, allowing many children to reach adulthood. However, a considerable number of these children experience various cardiac sequelae, including persistent or progressive PAH[19]. Therefore, timely and accurate assessment of PAP levels is crucial for the effective management of patients with CHD. Unfortunately, there are currently no robust biomarkers available for evaluating PAP, particularly in the context of CHD patients. This underscores the importance of identifying and assessing new

Table 4 Univariate logistic regression analysis to determine the related factors of pulmonary arterial hypertension and lipocalin-2

Variables	Correlation with PAH			Correlation with LCN2[1]		
	OR	95%CI	*P* value	OR	95%CI	*P* value
Sex, males	1.1	0.366-3.303	0.865	1.212	0.463 ± 3.172	0.696
Age in months	1.05	0.985-1.119	0.137	1.050	1.002 ± 1.101	0.043
BMI	0.868	0.669-1.127	0.289	0.859	0.676 ± 1.091	0.213
Creatinine in μmol/L	1.204	1.066-1.359	0.003	1.021	0.952 ± 1.094	0.566
BUN in mmol/L	1.684	1.089-2.606	0.019	0.967	0.711 ± 1.316	0.832
Albumin in g/L	1.114	0.942-1.317	0.205	1.050	0.930 ± 1.186	0.432
Glucose in mmol/L	0.648	0.346-1.213	0.175	0.763	0.431 ± 1.350	0.353
Hemoglobin in g/L	1.017	0.982-1.053	0.345	1.006	0.977 ± 1.036	0.686
Platelets as 10^9/L	1	0.995-1.006	0.887	0.999	0.995 ± 1.004	0.841
White blood cells as 10^9/L	1.259	0.960-1.651	0.096	1.111	0.908 ± 1.361	0.306
SBP in mmHg	1.036	0.984-1.091	0.176	1.018	0.977 ± 1.060	0.401
DBP in mmHg	0.998	0.955-1.044	0.944	1.027	0.983 ± 1.073	0.239
EF as %	1.099	0.966-1.250	0.152	0.932	0.834 ± 1.040	0.207
Diameter in mm	1.106	0.944-1.296	0.211	1.011	0.883 ± 1.157	0.877
mPAP in mmHg	-	-	-	1.084	1.026 ± 1.145	0.004
LCN2 in ng/mL	1.107	1.033-1.185	0.004	-	-	-
ET-1 in μg/mL	10.028	0.896-112.196	0.061	2.101	0.887 ± 4.979	0.092

[1]Variable was binarized based on its median value.

BMI: Body mass index; BUN: Blood urea nitrogen; DBP: Diastolic blood pressure; EF: Ejection fraction; ET-1: Endothelin-1; LCN2: Lipocalin-2; mPAP: Mean pulmonary artery pressure; OR: Odds ratio; PAH: Pulmonary arterial hypertension; SBP: Systolic blood pressure.

Table 5 Multivariate logistic regression analysis to define the correlation between endothelin-1 concentration and risk for pulmonary arterial hypertension

Variables	OR (95%CI)	*P* value
Age in months	0.994 (0.914-1.081)	0.889
Creatinine in μmol/L	1.232 (1.056-1.436)	0.008
Diameter in mm	0.984 (0.811-1.195)	0.871
ET-1 in μg/mL	9.681 (0.650-144.192)	0.099

ET-1: Endothelin-1; OR: Odds ratio.

biomarkers that could aid in the evaluation of PAP in this population, ultimately enhancing disease management and patient outcomes.

In the present study, we evaluated the predictive value of LCN2 for PAP in VSD patients. Our findings indicate that the blood level of LCN2 significantly correlates with PAH in VSD patients, as demonstrated through both univariate and multivariate regression analyses. ROC curve analysis reveals that LCN2 has a superior diagnostic value compared to ET-1 (with AUC 0.783 *vs* 0.657). Notably, LCN2 exhibits a sensitivity for diagnosing PAH that exceeds 90% at a cutoff value of 19.42 ng/mL, making it a valuable tool for screening patients suspected of having PAH. Therefore, for CHD patients with suspected PAH, LCN2 emerges as a crucial reference for estimating PAP and guiding treatment decisions. Conversely, the diagnostic potential of ET-1 for PAH is inferior to LCN2. There is no significant correlation between blood ET-1 concentration and mPAP. In the ROC curve analysis, however, ET-1 demonstrates a high specificity for diagnosing PAH at 94.44%, indicating that it holds utility as a confirmatory marker in the diagnostic evaluation of PAH.

LCN2 is a multifunctional protein that can be up-regulated in diverse pathological processes, including immunological abnormalities, metabolic diseases, and multiple types of tumors[20-22]. In particular, LCN2 is a marker for kidney injury and renal dysfunction, and its concentration significantly increases during both acute and chronic kidney injuries[12-14].

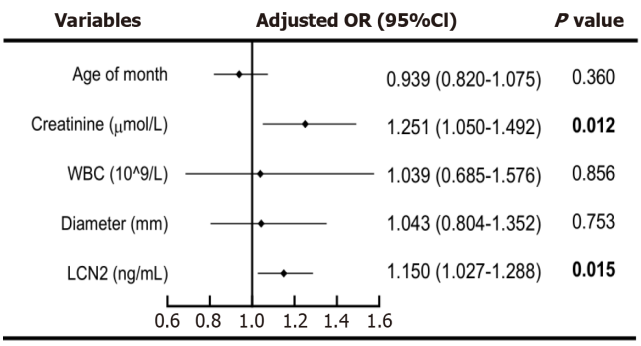

Figure 1 Multivariate logistic regression analysis the correlation between lipocalin-2 concentration and risk for pulmonary arterial hypertension. LCN2: Lipocalin-2; OR: Odds ratio; WBC: White blood cells.

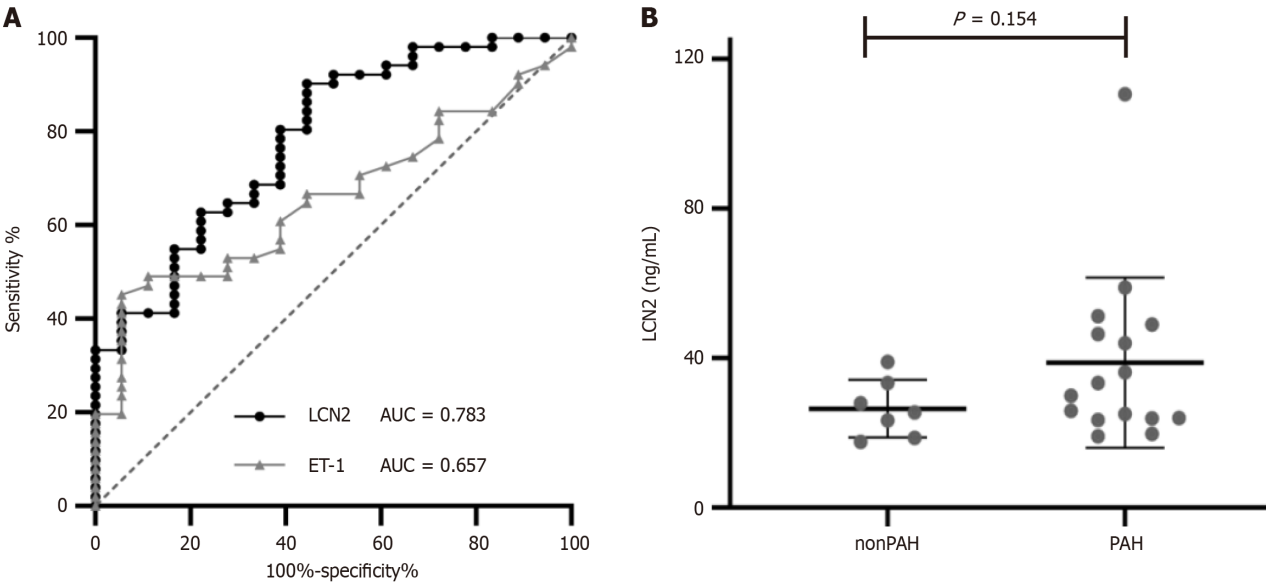

Figure 2 Receiver operating characteristic curve and comparison of lipocalin-2 concentration. A: Receiver operating characteristic curve of blood lipocalin-2 (LCN2) and endothelin-1 as predictors for pulmonary arterial hypertension (PAH); B: Comparison of LCN2 concentration between nonPAH and PAH patients after repair surgery. AUC: Area under the receiver operating characteristic curve; ET-1: Endothelin-1.

Given the close functional connection between the heart and kidneys, many CHD patients also exhibit abnormal renal function[23,24]. In the present study, we observed significant differences in renal function between the nonPAH and PAH groups. Additionally, we found that mPAP is significantly correlated with blood creatinine and BUN levels in VSD children. However, it is crucial to determine whether elevated LCN2 levels are primarily due to renal dysfunction or cardiovascular abnormalities. Despite the presence of abnormal renal function in CHD patients, we did not identify any significant correlation between LCN2 blood levels and creatinine or BUN in either univariate or multivariate regression analyses. This finding suggests that the renal abnormalities present are not sufficient to induce a significant increase in LCN2 levels. Consequently, our results imply that increased LCN2 in patients with CHD is more likely associated with PAH-related pathology rather than renal dysfunction. This underscores the potential role of LCN2 as a biomarker specifically linked to PAH in the context of CHD.

Our previous studies, along with recent reports from others, have demonstrated that LCN2 plays a significant role in promoting pulmonary artery smooth muscle cell proliferation and inhibiting apoptosis[8-11]. In our most recent study [25], we highlighted a critical role for LCN2 in glycolytic regulation, which is central to the metabolic theory in PH progression[26,27]. This association may serve as the molecular basis for the correlation between LCN2 and PAH, independent of renal dysfunction that may be present. The multifaceted roles of LCN2 in various physiological and pathological processes[28-30] render it an intriguing molecule for further research and potential therapeutic applications. Delving deeper into the mechanisms through which LCN2 exerts its effects could yield valuable insights not only for the development of new diagnostic tools for PAH but also for enhancing treatment strategies aimed at this challenging condition.

A recent report by Zhang *et al*[31] evaluated the relationship between LCN2 and PH in patients with CHD[31]. However, the types of CHD included in their study were not classified or specified, and they did not take into account the potential impact of renal function on LCN2 levels. While the authors concluded that LCN2 levels are elevated in patients with CHD, they reported a reference value of LCN2 for predicting PH at just 8.1 pg/mL. This value is substantially lower

Table 6 Predictive values of lipocalin-2 and endothelin-1 for pulmonary arterial hypertension

	AUC	Youden's index	Sensitivity, %	Specificity, %	Cutoff value
LCN2	0.783	0.4575	90.19	55.56	19.42 ng/mL
ET-1	0.657	0.3954	45.10	94.44	0.39 μg/mL

AUC: Area under the receiver operating characteristic curve; ET-1: Endothelin-1; LCN2: Lipocalin-2.

Table 7 Correlations of post-repair mean pulmonary artery pressure with clinical variables, *n* = 23

Variables	Post-operative mPAP		mPAP decrease	
	r	*P* value	*r*	*P* value
Sex, male	0.404	0.056	0.303	0.161
Age in months	0.347	0.105	0.434	0.039
BMI	-0.141	0.531	-0.050	0.826
Creatinine in μmol/L	-0.150	0.493	0.248	0.255
BUN in mmol/L	0.236	0.278	0.411	0.052
Albumin in g/L	0.313	0.156	0.029	0.897
Glucose in mmol/L	-0.045	0.849	-0.057	0.813
Hemoglobin in g/L	0.223	0.306	0.163	0.458
Platelets as 10^9/L	-0.066	0.764	0.107	0.628
White blood cells as 10^9/L	0.186	0.507	0.623	0.013
SBP in mmHg	0.094	0.677	0.043	0.848
DBP in mmHg	0.361	0.099	0.185	0.410
EF as %	-0.311	0.148	-0.037	0.867
Diameter in mm	0.398	0.060	0.552	0.006
Pre-repair mPAP in mmHg	0.471	0.023	0.883	< 0.001
LCN2 in ng/mL	0.532	0.009	0.181	0.407
ET-1 in μg/mL	0.140	0.523	0.298	0.167

BMI: Body mass index; BUN: Blood urea nitrogen; DBP: Diastolic blood pressure; EF: Ejection fraction; ET-1: Endothelin-1; mPAP: Mean pulmonary artery pressure; SBP: Systolic blood pressure; LCN2: Lipocalin-2.

than our findings, which indicated LCN2 levels of 25 ng/mL[8] in healthy children. Results from other studies reported levels ranging from 0.9 to 5.9 ng/mL or 9.8 to 25.7 ng/mL[32,33]. Compared to the study by Zhang *et al*[31], our research provides more objective and informative results regarding LCN2 Levels in the context of CHD and PH.

In CHD patients with a left-to-right shunt, elevated PAP may not always normalize after the closure of the shunt[1,34]. The reason behind why some CHD patients experience a permanent reversal of PAH following shunt closure, while others continue to exhibit persistent PAH, remain unclear. In our study, we recorded post-repair PAP values for 20 PAH patients; four experienced a reversal of elevated PAP to normal levels, while the remaining patients continued to show signs of PAH. Notably, we found that LCN2 concentration was significantly correlated with post-repair mPAP. Furthermore, patients with post-repair PAH exhibited higher levels of LCN2 compared to non-PAH patients. These findings suggest that blood LCN2 levels could serve as a potential biomarker to indicate whether PAH can be reversed after shunt closure in CHD patients. This highlights the need for future studies with larger sample sizes to further validate LCN2's prognostic value in predicting the outcomes of PAH after surgical intervention. Exploring this relationship could enhance our understanding of PAH in CHD patients and inform clinical decision-making regarding treatment strategies.

Limitations

This study has some limitations. First, it is a single center cross-sectional study with a relatively small number of research subjects. Future prospective and multicenter studies with larger patient populations will be necessary to enhance the generalizability of the findings. Second, this study focused exclusively on patients with VSD or VSD accompanied by

atrial septal defect, which represent relatively simple types of CHD. Given the diversity of CHD, particularly in children with complex conditions, such as those with segmental PH or a combination of group 1 and group 2 PH, the diagnosis and management of PH must be customized to account for the individual patient's unique anatomical and hemodynamic conditions. Therefore, caution should be exercised when interpreting the results. Third, all patients enrolled in our study underwent right ventricular catheterization. However, most CHD patients with simple lesions typically do not require cardiac catheterization. Consequently, patients who were unwilling or did not require right heart catheterization were excluded from this study, introducing a selection bias. Finally, the determination of post-repair PAH in this study relied on intra-operative ventricular catheterization results obtained after shunt closure. It is important to note that with the cessation of anesthesia and the patient's recovery, the final PAP values may change. This potential bias is challenging to avoid and may impact the interpretation of the results. Addressing these limitations in future research will be crucial for validating the findings and enhancing our understanding of LCN2's role in PAH among CHD patients.

CONCLUSION

In conclusion, this retrospective study demonstrated that LCN2 blood levels significantly correlate with mPAP in CHD patients. LCN2 has emerged as a candidate biomarker for CHD patients, and its high sensitivity in diagnosing PAH underscores its potential value in patient management.

FOOTNOTES

Author contributions: Wang GL and Liu AJ conceptualized and designed the research; Guo ZK, Bai S and Li XF screened patients and acquired clinical data; Wang GL, Guo ZK, Chen PG, Jiao H and Kong XH performed data curation, visualization, and interpretation; Wang GL, Li YX and Liu AJ wrote the manuscript; Wang GL and Liu AJ performed reviewing and final editing; All authors have read and agreed to the published version of the manuscript. Both Wang GL and Liu AJ have played important and indispensable roles in the experimental design, data interpretation and manuscript preparation as the co-corresponding authors. Wang GL conceptualized, designed, and supervised the project, including literature collection, manuscript submission and revision, with a focus on the association between lipocalin-2 concentration and risk for pulmonary arterial hypertension. Liu AJ played a crucial role in data re-analysis and re-interpretation, figure plotting, comprehensive literature searching, and preparing the final manuscript submission, focusing specifically on the diagnostic value of lipocalin-2 in PAH. The collaboration between Wang GL and Liu AJ was essential to the publication of this manuscript.

Supported by the Scientific Achievements Transformation Incubation Fund of Beijing Children's Hospital, No. ZHFY3-1-015.

Institutional review board statement: This study was approved by Beijing Children's Hospital Ethics Committee, No. 2022-E-083-Y. This study was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent statement: All patients or their parents (or legal guardians) provided written informed consent.

Conflict-of-interest statement: The authors report no relevant conflicts of interest for this article.

Data sharing statement: No additional data are available.

Country of origin: China

ORCID number: Guo-Liang Wang 0000-0002-9191-6166.

S-Editor: Li L
L-Editor: Filipodia
P-Editor: Wang WB

REFERENCES

1 **van der Feen DE**, Bartelds B, de Boer RA, Berger RMF. Assessment of reversibility in pulmonary arterial hypertension and congenital heart disease. *Heart* 2019; **105**: 276-282 [PMID: 30467194 DOI: 10.1136/heartjnl-2018-314025]

2 **van Riel AC**, Schuuring MJ, van Hessen ID, Zwinderman AH, Cozijnsen L, Reichert CL, Hoorntje JC, Wagenaar LJ, Post MC, van Dijk AP, Hoendermis ES, Mulder BJ, Bouma BJ. Contemporary prevalence of pulmonary arterial hypertension in adult congenital heart disease following the updated clinical classification. *Int J Cardiol* 2014; **174**: 299-305 [PMID: 24794056 DOI: 10.1016/j.ijcard.2014.04.072]

3 **van der Feen DE**, Bartelds B, de Boer RA, Berger RMF. Pulmonary arterial hypertension in congenital heart disease: translational opportunities to study the reversibility of pulmonary vascular disease. *Eur Heart J* 2017; **38**: 2034-2041 [PMID: 28369399 DOI: 10.1093/eurheartj/ehx034]

4 **Brault C**, Marc J, Mercado P, Diouf M, Tribouilloy C, Zerbib Y, Maizel J, Vignon P, Slama M. Estimation of Pulmonary Artery Occlusion Pressure Using Doppler Echocardiography in Mechanically Ventilated Patients. *Crit Care Med* 2020; **48**: e943-e950 [PMID: 32885942 DOI: 10.1097/CCM.0000000000004512]

5 **Farmakis IT**, Demerouti E, Karyofyllis P, Karatasakis G, Stratinaki M, Tsiapras D, Athanassopoulos G, Voudris V, Giannakoulas G. Echocardiography in Pulmonary Arterial Hypertension: Is It Time to Reconsider Its Prognostic Utility? *J Clin Med* 2021; **10**: 2826 [PMID: 34206876 DOI: 10.3390/jcm10132826]

6 **Goetz DH**, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol Cell* 2002; **10**: 1033-1043 [PMID: 12453412 DOI: 10.1016/s1097-2765(02)00708-6]

7 **Sun WX**, Lou K, Chen LJ, Liu SD, Pang SG. Lipocalin-2: a role in hepatic gluconeogenesis *via* AMP-activated protein kinase (AMPK). *J Endocrinol Invest* 2021; **44**: 1753-1765 [PMID: 33423221 DOI: 10.1007/s40618-020-01494-0]

8 **Wang G**, Liu X, Meng L, Liu S, Wang L, Li J, Cui C, Meng J, Hu S, Wei Y. Up-regulated lipocalin-2 in pulmonary hypertension involving in pulmonary artery SMC resistance to apoptosis. *Int J Biol Sci* 2014; **10**: 798-806 [PMID: 25076856 DOI: 10.7150/ijbs.9470]

9 **Wang G**, Ma N, Meng L, Wei Y, Gui J. Activation of the phosphatidylinositol 3-kinase/Akt pathway is involved in lipocalin-2-promoted human pulmonary artery smooth muscle cell proliferation. *Mol Cell Biochem* 2015; **410**: 207-213 [PMID: 26350566 DOI: 10.1007/s11010-015-2553-5]

10 **Wang G**, Liu S, Wang L, Meng L, Cui C, Zhang H, Hu S, Ma N, Wei Y. Lipocalin-2 Promotes Endoplasmic Reticulum Stress and Proliferation by Augmenting Intracellular Iron in Human Pulmonary Arterial Smooth Muscle Cells. *Int J Biol Sci* 2017; **13**: 135-144 [PMID: 28255266 DOI: 10.7150/ijbs.17758]

11 **Shi Z**, Yin Y, Li C, Ding H, Mu N, Wang Y, Jin S, Ma H, Liu M, Zhou J. Lipocalin-2-induced proliferative endoplasmic reticulum stress participates in Kawasaki disease-related pulmonary arterial abnormalities. *Sci China Life Sci* 2021; **64**: 1000-1012 [PMID: 32915407 DOI: 10.1007/s11427-019-1772-8]

12 **Marakala V**. Neutrophil gelatinase-associated lipocalin (NGAL) in kidney injury - A systematic review. *Clin Chim Acta* 2022; **536**: 135-141 [PMID: 36150522 DOI: 10.1016/j.cca.2022.08.029]

13 **Bellos I**, Fitrou G, Daskalakis G, Perrea DN, Pergialiotis V. Neutrophil gelatinase-associated lipocalin as predictor of acute kidney injury in neonates with perinatal asphyxia: a systematic review and meta-analysis. *Eur J Pediatr* 2018; **177**: 1425-1434 [PMID: 30051145 DOI: 10.1007/s00431-018-3221-z]

14 **Huang Z**, Wu L, Zeng M, Wu B, Xu X, Mao H, Yuan Y, Xing C. Value of urine/serum Neutrophil gelatinase-associated lipocalin ratio in distinguishing acute kidney injury from chronic kidney disease. *Clin Chim Acta* 2022; **526**: 23-29 [PMID: 34958753 DOI: 10.1016/j.cca.2021.12.018]

15 **Marassi M**, Fadini GP. The cardio-renal-metabolic connection: a review of the evidence. *Cardiovasc Diabetol* 2023; **22**: 195 [PMID: 37525273 DOI: 10.1186/s12933-023-01937-x]

16 **Kunz M**, Götzinger F, Emrich I, Schwenger V, Böhm M, Mahfoud F. Cardio-renal interaction - Clinical trials update 2022. *Nutr Metab Cardiovasc Dis* 2022; **32**: 2451-2458 [PMID: 36064690 DOI: 10.1016/j.numecd.2022.07.002]

17 **Vizza CD**, Letizia C, Badagliacca R, Poscia R, Pezzuto B, Gambardella C, Nona A, Papa S, Marcon S, Mancone M, Iacoboni C, Riccieri V, Volterrani M, Fedele F. Relationship between baseline ET-1 plasma levels and outcome in patients with idiopathic pulmonary hypertension treated with bosentan. *Int J Cardiol* 2013; **167**: 220-224 [PMID: 22265324 DOI: 10.1016/j.ijcard.2011.12.104]

18 **Simonneau G**, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, Williams PG, Souza R. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J* 2019; **53**: 1801913 [PMID: 30545968 DOI: 10.1183/13993003.01913-2018]

19 **Jone PN**, Ivy DD, Hauck A, Karamlou T, Truong U, Coleman RD, Sandoval JP, Del Cerro Marín MJ, Eghtesady P, Tillman K, Krishnan US. Pulmonary Hypertension in Congenital Heart Disease: A Scientific Statement From the American Heart Association. *Circ Heart Fail* 2023; **16**: e00080 [PMID: 37357777 DOI: 10.1161/HHF.0000000000000080]

20 **Jung BK**, Park Y, Yoon B, Bae JS, Han SW, Heo JE, Kim DE, Ryu KY. Reduced secretion of LCN2 (lipocalin 2) from reactive astrocytes through autophagic and proteasomal regulation alleviates inflammatory stress and neuronal damage. *Autophagy* 2023; **19**: 2296-2317 [PMID: 36781380 DOI: 10.1080/15548627.2023.2180202]

21 **Wen X**, Su B, Gao M, Chen J, Zhou D, You H, Li N, Chang S, Cheng X, Qian C, Gao J, Yang P, Qu S, Bu L. Obesity-associated up-regulation of lipocalin 2 protects gastric mucosa cells from apoptotic cell death by reducing endoplasmic reticulum stress. *Cell Death Dis* 2021; **12**: 221 [PMID: 33637683 DOI: 10.1038/s41419-021-03512-2]

22 **Rahimi S**, Roushandeh AM, Ahmadzadeh E, Jahanian-Najafabadi A, Roudkenar MH. Implication and role of neutrophil gelatinase-associated lipocalin in cancer: lipocalin-2 as a potential novel emerging comprehensive therapeutic target for a variety of cancer types. *Mol Biol Rep* 2020; **47**: 2327-2346 [PMID: 31970626 DOI: 10.1007/s11033-020-05261-5]

23 **Gist KM**, Kwiatkowski DM, Cooper DS. Acute kidney injury in congenital heart disease. *Curr Opin Cardiol* 2018; **33**: 101-107 [PMID: 29028633 DOI: 10.1097/HCO.0000000000000473]

24 **Hongsawong N**, Khamdee P, Silvilairat S, Chartapisak W. Prevalence and associated factors of renal dysfunction and proteinuria in cyanotic congenital heart disease. *Pediatr Nephrol* 2018; **33**: 493-501 [PMID: 28971258 DOI: 10.1007/s00467-017-3804-3]

25 **Wang G**, Liu S, Kong X, Jiao H, Tong F, Guo Z, Zhang M, Guan X, Ren N, Li W, Qi L, Wei Y. Lipocalin-2 induced LDHA expression promotes vascular remodelling in pulmonary hypertension. *Cell Prolif* 2024; e13717 [PMID: 39021353 DOI: 10.1111/cpr.13717]

26 **Paulin R**, Michelakis ED. The metabolic theory of pulmonary arterial hypertension. *Circ Res* 2014; **115**: 148-164 [PMID: 24951764 DOI: 10.1161/CIRCRESAHA.115.301130]

27 **Li M**, Riddle S, Zhang H, D'Alessandro A, Flockton A, Serkova NJ, Hansen KC, Moldovan R, McKeon BA, Frid M, Kumar S, Li H, Liu H, Caánovas A, Medrano JF, Thomas MG, Iloska D, Plecitá-Hlavatá L, Ježek P, Pullamsetti S, Fini MA, El Kasmi KC, Zhang Q, Stenmark KR. Metabolic Reprogramming Regulates the Proliferative and Inflammatory Phenotype of Adventitial Fibroblasts in Pulmonary Hypertension Through the Transcriptional Corepressor C-Terminal Binding Protein-1. *Circulation* 2016; **134**: 1105-1121 [PMID: 27562971 DOI: 10.1161/CIRCULATIONAHA.116.023171]

28 **Zhao RY**, Wei PJ, Sun X, Zhang DH, He QY, Liu J, Chang JL, Yang Y, Guo ZN. Role of lipocalin 2 in stroke. *Neurobiol Dis* 2023; **179**:

106044 [PMID: 36804285 DOI: 10.1016/j.nbd.2023.106044]

29 **Jaberi SA**, Cohen A, D'Souza C, Abdulrazzaq YM, Ojha S, Bastaki S, Adeghate EA. Lipocalin-2: Structure, function, distribution and role in metabolic disorders. *Biomed Pharmacother* 2021; **142**: 112002 [PMID: 34463264 DOI: 10.1016/j.biopha.2021.112002]

30 **Kim KE**, Lee J, Shin HJ, Jeong EA, Jang HM, Ahn YJ, An HS, Lee JY, Shin MC, Kim SK, Yoo WG, Kim WH, Roh GS. Lipocalin-2 activates hepatic stellate cells and promotes nonalcoholic steatohepatitis in high-fat diet-fed Ob/Ob mice. *Hepatology* 2023; **77**: 888-901 [PMID: 35560370 DOI: 10.1002/hep.32569]

31 **Zhang H**, Sun T, Yang J, Sun Y, Liu G, Krittanawong C, El-Am EA, Chaaya RGB, Xu L, Ye Z, Li Z, Ma N. Lipocalin 2: could it be a new biomarker in pediatric pulmonary hypertension associated with congenital heart disease? *Rev Cardiovasc Med* 2021; **22**: 531-536 [PMID: 34258922 DOI: 10.31083/j.rcm2202061]

32 **van Donge T**, Staub E, Atkinson A, Gotta V, van den Anker J, Risch L, Welzel T, Pfister M. Age appropriate reference intervals for eight kidney function and injury markers in infants, children and adolescents. *Clin Chem Lab Med* 2020; **59**: 373-382 [PMID: 32759403 DOI: 10.1515/cclm-2020-0781]

33 **Takaya J**, Tanabe Y, Kaneko K. Increased lipocalin 2 levels in adolescents with type 2 diabetes mellitus. *J Pediatr Endocrinol Metab* 2021; **34**: 979-985 [PMID: 34118796 DOI: 10.1515/jpem-2021-0216]

34 **Lammers AE**, Bauer LJ, Diller GP, Helm PC, Abdul-Khaliq H, Bauer UMM, Baumgartner H; German Competence Network for Congenital Heart Defects Investigators. Pulmonary hypertension after shunt closure in patients with simple congenital heart defects. *Int J Cardiol* 2020; **308**: 28-32 [PMID: 31926641 DOI: 10.1016/j.ijcard.2019.12.070]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: office@baishideng.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com