Journal of Rare Cardiovascular Diseases 2015; 2 (3): 77–81
www.jrcd.eu

ORIGINAL PAPER
Rare congenital cardiovascular diseases

Plasma Endothelin-1 in patients with atrial septal defect – the novel diagnostic indicator (RCD code: IV-2B.1)

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Abstract

Background: The study aimed to assess the level of plasma Endothelin-1 (ET-1) in patients before and after transcatheter closure of atrial septal defect (ASD) and to evaluate the usefulness of measuring ET-1 levels for the diagnosis and selection of candidates for ASD closure.
Methods: 21 patients (11 F, 10 M), mean age 40.2 ± 11.9 years with pulmonary artery hypertension were enrolled for an attempt at ASD closure. A group of 19 healthy volunteers, (12 F, 7 M) mean age 39.2 ± 9.15 served as controls. All ASD patients underwent: clinical and echocardiographic study and cardiopulmonary exercise test. ET-1 levels were measured before and after closure. Whole blood was collected from femoral artery and vein and from pulmonary artery during cardiac catheterization.
Results: ET-1 levels at peripheral artery and vein in ASD patients were significantly higher than in the volunteers ($p<0.0001$). The ASD subjects with highest ET-1 level presented the larger area of right ventricle and right atrium and higher pulmonary artery systolic pressure($p<0.05$). The ASD subjects with lower ET-1 level demonstrated longer time of exercise and higher peak oxygen consumption ($p<0.05$). There was a decrease of ET-1 at peripheral artery (5.549 ± 5.32 vs. 1.92 ± 7.2; $p<0.001$) and at peripheral vein (4.012 ± 2.342 vs. 2.15 ± 1.15; $p<0.001$) within 48 hours after ASD closure, as compared to the baseline data. After 6 and 12 months farther drop in ET-1 level was observed.
Conclusions: 1. The level of ET-1 in patients with ASD and pulmonary artery hypertension is elevated in compare to healthy subject. 2. The significant reduction of ET-1 level is observed after percutaneous closure of ASD. 3. Elevated level of ET-1 in patients with ASD is associated with right heart enlargement. 4. Measurements of ET-1 may be a supplemental diagnostic tool and may be helpful in establishing indications for defect closure. JRCD 2015; 2 (3): 77–81

Key words: atrial septal defect, endothelin, congenital heart defect, percutaneous closure

Background

Closure of an atrial septal defect (ASD) in patients with hemodynamically significant shunt has become standard of care in recent years. Correction of ASD prevents the development of pulmonary hypertension, cardiac arrhythmia and heart failure [1–4]. The indications for ASD closure in adults however are ambiguous. The most controversial issue is selection of candidates for ASD closure who have normal pulmonary artery pressure, absent or negligible clinical symptoms and are over 40 years of age [5–9].

In light of divergent opinions regarding ASD correction in all patients irrespective of age and clinical symptoms it appears necessary to look for novel diagnostic and prognostic indicators that may become useful for proper selection of candidates for ASD closure.

Endothelins (ET) comprise a family of three isopeptides: endothelin-1, -2, and -3. ET-1 is released mainly from endothelial cells and cardiomyocytes and is probably the most important isoform in the regulation of cardiovascular function [10,11].

ET-1 is thought to play an important role in the pathogenesis of pulmonary hypertension, both primary and secondary [12–14]. Jia B et al. [14] demonstrated elevated plasma ET-1 concentration in children with ventricular and atrial septal defects that correlated with pulmonary artery pressure. After surgical repair of the defects, plasma ET-1 concentration decreased significantly. In patients with

Conflict of interest: none declared.

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Table 1. Patient characteristics in subgroup G and H with the low and high Endothelin-1 levels, respectively

	Subgroup G	Subgroup H	p
ET-1 - endothelin 1 (fmol/ml)	2.80 ±1.5 (0.90 to 2.77)	9.0 ±9.4 (7.09 to 20.61)	<0,0001

ASD and associated elevated pulmonary artery pressure it seems important to measure ET-1 levels before and after ASD closure, to investigate the potential benefits of ET-1 receptor blockers and to establish the prognostic value of ET-1.

Aim of the study

- to assess the level of plasma ET-1 in patients with ASD
- to evaluate the usefulness of measuring ET-1 levels for the diagnosis and selection of candidates for ASD closure

Material and Methods

The study included 21 consecutive adult patients, 11 women (52,3%) and 10 men (47,7%), with ASD and pulmonary hypertension, who underwent percutaneous closure of ASD with an Amplatzer device in the Department of Hemodynamics and Angiography, Institute of Cardiology, Jagiellonian University Medical College. The mean age of the patients was 40.2 ±11.9 (range 18–65). In all the patients the pulmonary hypertension was found (mean pulmonary artery pressure (PAP) 33 ± 9.5 (25–44), systolic PAP 45 ±14.5 (29–55)).

A group of 19 healthy volunteers, 12 women (61.2%) and 7 men (36.8%) with a mean age of 39.2 ±9.15 (range 18–51), matched for age and gender served as controls.

The secundum ASD was diagnosed on the basis of clinical examination and transthoracic echocardiography (TTE) whereas transesophageal echocardiography (TEE) was performed to select candidates for percutaneous ASD closure. Diagnosis was confirmed during cardiac catheterization immediately before the Amplatzer deployment. The following parameters were measured:

- pulmonary artery pressure at systole,
- total pulmonary vascular resistance (TPVR),
- pulmonary blood flow (Qp),
- systemic blood flow (Qs),
- pulmonary/systemic blood flow ratio (Qp/Qs).

Patients were considered for transcatheter closure if they had a single secundum ASD with a diameter measuring less than 30 mm on echocardiography with a rim of tissue of at least 5 mm surrounding the defect located in the central part of the septum and with a hemodynamically significant left-to-right shunt (Qp/Qs > 1.5:1). Two patients had two defects that were so close to each other that it was possible to close them with one device.

All patients underwent preoperative diagnostic procedures:

- Clinical examination including assessment of NYHA class
- TTE to measure right ventricular area at diastole (RV_{area}) and right atrial area at diastole (RA_{area})
- Spirometry at rest to measure forced vital capacity expressed as a percentage of the normal value (FVC%), forced expiratory volume in one second expressed as a percentage of the normal value (FEV_1%)
- Exercise spirometry test to measure duration of exercise in seconds (T), peak oxygen consumption VO_{2peak}(ml/kg/min), time to anaerobic threshold (T_{AT}), oxygen consumption at the anaerobic threshold (VO_{2AT}%), ventilatory equivalent for carbon dioxide (VE/VCO_2)

Measurement of endothelin levels

ET-1 levels were measured immediately before and at 2 days, 6 and 12 months after transcatheter closure.

Whole blood was collected from femoral artery and femoral vein in each ASD patient before the procedure and in volunteers. Additionally, blood samples were collected from pulmonary artery during diagnostic cardiac catheterization. After transcatheter closure blood was sampled from femoral artery and femoral vein.

Whole blood was collected in precooled tubes containing EDTA and aprotinine (1 vol of anticoagulant for 9 vol of blood) and immediately placed on ice. The blood was then centrifuged to the freezing temperature at 3000 rpm for 10 min. The platelet-poor plasma was frozen in tubes and sent to the laboratory. Measurements of ET-1 were done at the Biochemical Laboratory of the John Paul II Hospital in Krakow. ET-1 was measured with a commercial immunoenzymatic ELISA method and results expressed as fmol/ml. The study protocol was approved by the Bioethics Committee of the Jagiellonian University Medical College (KBET/262/B/2002).

Analysis was performed in the whole group and in two subgroups with the high and low plasma ET-1 levels in the pulmonary artery:

- Subgroup G – ET-1 levels from 0.90 to 2.77 fmol/ml
- Subgroup H – ET-1 levels from 7.09 to 20.61 fmol/ml

Patient characteristics in subgroup G and H are summarized in table 1.

To allocate patients to the subgroups ET-1 values were sorted in ascending order to define the median and quartiles. Those who had ET-1 levels in the first quartile were allocated to subgroup G and those with ET-1 levels in the fourth quartile to subgroup H.

Statistical analysis

The discrimination value of selected parameters was determined by comparing two subgroups with the low and high ET-1 levels and ROC analysis was performed to define the cut-off values and calculate sensitivity and specificity. The Peto method was then used to perform meta-analysis ad calculate odds ratios (OR) for individual risk factors. The Peto method also allowed for assessment of multidimensional risk of elevated ET-1 levels. OR were calculated with corresponding 95% confidence intervals (95%CI).

Multivariate analyses such as multiple forward stepwise regression, logistic regression and canonical correlation were used to evaluate the parameters affecting ET-1 levels. Stepwise and logistic

Table 2. Endothelin-1 levels in patients before transcatheter closure of ASD and in healthy volunteers

Group	Vessel	Mean	Minimum	Maximum	SD	*p* ASD vs. healthy
ASD patients before closure n=21	**Peripheral artery**	4,522	0,832	15,621	5,380	< 0,0001
	Peripheral vein	4,012	1,210	11,526	2,342	< 0,0001
	Pulmonary artery	5,549	0,91	22,68	5,320	–
Healthy volunteers n=19	**Peripheral artery**	0,049	0,010	0,098	0,025	< 0,0001
	Peripheral vein	0,054	0,001	0,099	0,027	< 0,0001

ASD – atrial septal defect

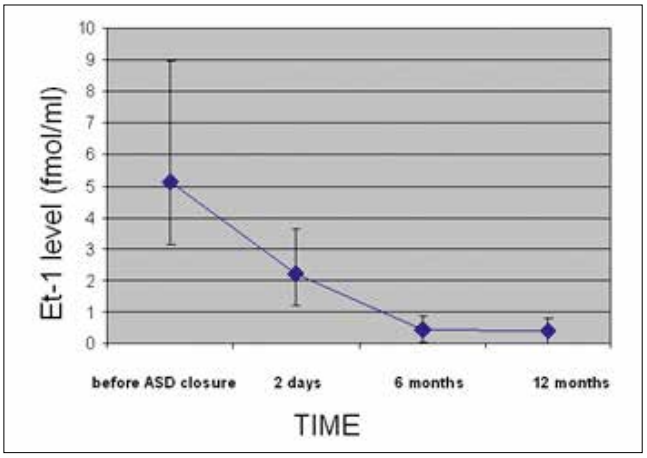

Figure 1. ET-1 levels in the peripheral artery before and after transcatheter closure of ASD. ET-1 – endothelin 1, ASD – atrial septal defect

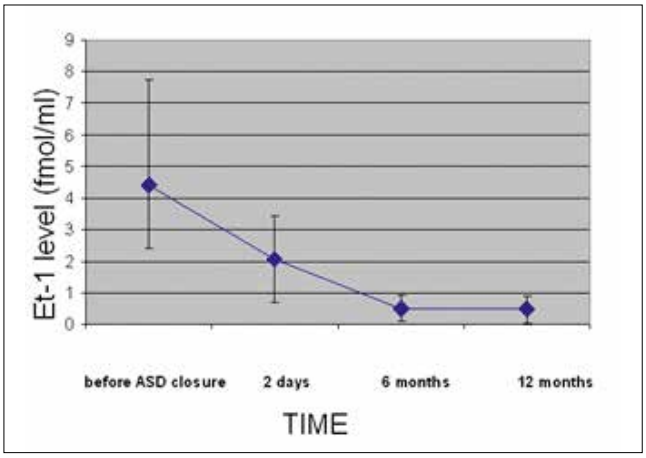

Figure 2. ET-1 levels in the peripheral vein before and after transcatheter closure of ASD. ET-1 – endothelin 1, ASD – atrial septal defect

regression allowed for estimation of the effect of independent variables on the dependent variable i.e. endothelin levels. Statistical significance was set at $\alpha \leq 0.05$. Statistical analyses were performed using Statistica 6.0. Meta-analysis and ROC analysis were performed using StatsDirect 2.1.

Results

An Amplatzer device was implanted without major complications in all eligible patients. The mean duration of the procedure including diagnostic right-heart catheterization was 41.4 ±9.2 (22–65) min and the mean fluoroscopy time 12.9 ±5.42 (5–25) min. The size of Amplatzer devices ranged from 15 to 40 mm (mean 25.2 ±7.9 mm).

ET-1 levels before and after transcatheter closure

Table 2 summarizes ET-1 levels in patients before and after transcatheter closure of ASD and in healthy volunteers.

Patients before transcatheter closure of ASD had significantly higher ET-1 levels both in peripheral artery and in peripheral vein as compared with healthy volunteers ($p<0.0001$). The minimum detectable level of ET-1 in the peripheral artery and vein in ASD patients was higher than the maximum level in healthy volunteers. The highest level of ET-1 was detected in the pulmonary artery in ASD patients (mean 5.549 ±5.32).

After transcatheter closure ET-1 levels significantly decreased both in peripheral artery and vein in all patients. Figures 1 and 2 depict ET-1 levels in the peripheral artery and vein before and after transcatheter closure of ASD.

As early as 48 hours after ASD closure the level of ET-1 was significantly reduced both in the peripheral artery (1.92 ±7.2; $p<0.001$) and vein (2.15 ±1.15; $p<0.001$). At 6 months the levels of ET-1 were further reduced to a mean of 0.59 ±0.37 in the peripheral artery and to 0.62 ±0.12 in the peripheral vein. Selected parameters, including hemodynamics and physical capacity, were compared in two subgroups with the high (subgroup H – ET-1 levels from 7.09 to 20.61 fmol/ml) and low (subgroup G – ET-1 levels from 0.90 to 2.77 fmol/ml) ET-1 levels in the pulmonary artery.

Patients in subgroup H were significantly older than those in subgroup G.

ASD patients with the high ET-1 level had significantly increased RAarea and RVarea area and higher PAPs.

Table 3. Selected parameters in patients with the high (subgroup H) and low (subgroup G) ET-1 levels in the pulmonary artery

	Subgroup G – low ET1 level	Subgroup H – high ET1 level	P
Age	30,1 ±13,43	58,36 ±17,7	<0,001
Defect size	22,1 ±9,18	19,9 ±9,28	NS
RV_{area} (cm^2)	20,1 ±3,01	24 ±2,51	< 0,01
RA_{area} (cm^2)	15,2 ±2.9	20,4 ±1,9	<0,01
PAPs (mm Hg)	22,3 ±16,2	39,6 ±14,9	<0,01
Qp/Qs	2,28 ±1,27	2,39 ±1,34	NS
T (s)	598 ±84,45	436,7 ±93,1	<0,05
VO_{2peak}(ml/kg/min)	23,19 ±4,32	20,1 ±2,92	<0,05

ET-1 – endothelin 1, RVarea – right ventricular area, RAare – right atrium area, PAPs – systolic pulmonary artery pressure, Qp/Qs – left-to-right shunt, T – fluoroscopy time, VO2peak – peak oxygen consumption

ASD patients with the low ET-1 level had higher VO2AT% ($p<0.05$) and TAT was significantly prolonged ($p<0.01$). The VE/VCO2 was significantly lower in subgroup G. Table 3 summarizes the results of the comparative analysis.

The canonical correlation analysis showed that the following parameters had an influence on ET-1 levels in pulmonary artery, peripheral artery and vein: PAPs, Qp/Qs, age, RV_{area}, RA_{area}, size of ASD, VO_{2peak}, FVC%, VO_{2AT}%; $p< 0.05$.

The multiple forward stepwise regression analysis revealed that of all parameters [(PAPs, Qp/Qs, age, RV_{area}, RA_{area}, size of ASD, VO_{2peak}, FVC%, VO_{2AT}%, T_{AT})] PAPs (F5.02)=8.1782; $p< 0.0001$, standard error 2.190) had the strongest influence on ET-1 levels.

ROC curves for selected parameters influencing ET-1 levels are depicted in Figure 3.

The cut-off values defined in the meta-analysis and increasing the risk of elevated ET-1 levels in ASD patients are as follows:

- RAarea > 17 mm ($p<0.001$)
- RVarea > 23 mm ($p<0.001$)
- Age > 40 years ($p<0.001$)
- PAPs > 31.0 mm Hg ($p<0.0001$)

Discussion

Elevated circulating ET-1 is observed in patients with left-to-right shunt and pulmonary hypertension indicating that pulmonary vessels are responsible for increased ET-1 synthesis [14].

Endothelin is probably one of the key contributors to the pathogenesis of pulmonary hypertension [12-14].

In the present study ASD patients before transcatheter closure had significantly higher ET-1 levels both in peripheral artery and vein as compared with healthy volunteers. The minimum detectable ET-1 in the peripheral artery and vein in ASD patients was higher than the maximum ET-1 level in healthy volunteers. The highest ET-1 level was detected in the pulmonary artery in ASD patients. The multiple forward stepwise regression analysis revealed that PAP had the strongest influence on ET-1 levels. These findings are concordant with the results obtained by other investigators [15-19]. The high circulating ET-1 as compared with healthy volunteers is accounted for increased pulmonary blood flow, increased pulmonary pressure and increased synthesis of ET-1 [13-19].

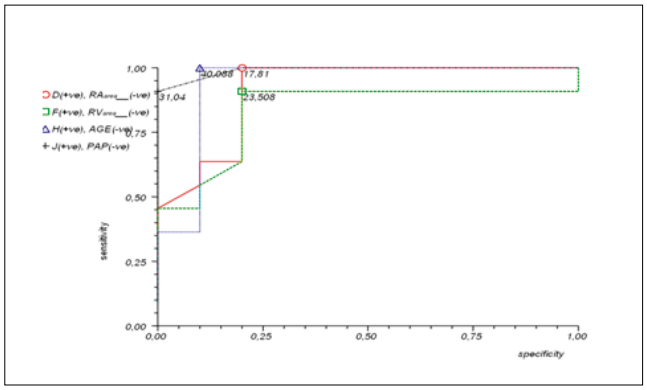

Figure 3. ROC curves for selected parameters: [RVarea (right ventricular area) – green, RAarea (right atrium area) – red, PAPs (systolic pulmonary artery pressure) – black and age – blue] and cut-off values

ET-1 levels decreased significantly both in the peripheral artery and the peripheral vein in all patients as early as 2 days after transcatheter closure. Jia et al. [14] obtained similar results.

In the present study ET-1 levels further decreased at 6 and 12 months, although the rate of decrease was much slower after 6 months. The decrease in ET-1 concentration as early as at 2 days after ASD closure confirms it is a volume response which depends on a significant reduction in pulmonary blood flow. In the present study selected parameters, including hemodynamics and physical capacity, were compared in two subgroups of patients with the high and low ET-1 levels. The high ET-1 levels were correlated with age, right atrial and ventricular enlargement and elevated PAP. Patients with ASD and high ET-1 had lower VO2AT% and shorter TAT corresponding to reduced physical capacity. Patients with low ET-1 were classified as those in the first quartile (0.90 to 2.77 fmol/ml) whereas patients with high ET-1 as those in the fourth quartile (7.09 to 20.61 fmol/ml). In the present study we defined the cut-off values of ET-1 above which we can expect reduced physical capacity and right atrial and right ventricular enlargement. Measurements of ET-1 in ASD patients with borderline shunt ratio may help identify the subjects with elevated PAP who may benefit from ASD closure. Measurements of ET-1 in peripheral blood may be a useful tool for diagnosis and selection of patients with borderline left-to-right shunt ratio for suitable intervention.

Conclusions

1. The level of ET-1 in ASD patients and pulmonary artery hypertension is elevated in compare to healthy subject.
2. The significant reduction of ET-1 level is observed after percutaneous closure of ASD.

3. Elevated level of ET-1 in patients with ASD is associated with right heart enlargement.
4. Measurements of ET-1 may be a supplemental diagnostic tool and may be helpful in establishing indications for defect closure.

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