During treatment protocol for univentricular heart, serum levels of natriuretic peptides decrease

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Abstract

Objective: In children treated for univentricular heart (UVH), prospective evaluation of serum levels of N-terminal proatriopeptide (ANPN) and N-terminal pro-brain natriuretic peptide (NT-proBNP) was performed. Methods: Serum samples were analysed in 19 children before the first operation, before the bi-directional Glenn (BDG) operation, at age 1 year and before total cavopulmonary connection (TCPC). In addition, we performed cross-sectional measurement of peptide levels in 32 children: 22 hypoplastic left ventricle (LV), 10 hypoplastic right ventricle (RV) before; and in 12 children: nine hypoplastic LV, three hypoplastic RV, 2 (range: 0.5—5.3) years after the TCPC operation. Controls comprised 12 children aged less than 6 months and 41 children aged from 6 months to 7 years. Results: Between the first and second operations, peptide levels decreased. Before TCPC, further decreases had occurred. Throughout follow-up, peptide levels were higher than in controls. In the cross-sectional study, before TCPC, median ANPN concentration measured 0.37 (range: 0.18—1.00) nmol l\(^{-1}\) (P = 0.059, compared with controls) and NT-proBNP 155 (range: 13—718) ng l\(^{-1}\) (P < 0.001). After TCPC, levels of NT-proBNP were higher in patients with RV than with LV morphology. Conclusions: Natriuretic peptide levels decreased during treatment protocol for UVH, but NT-proBNP levels remained higher than in controls. These reflect reduction of volume overload of the single ventricle and can prove useful for haemodynamic monitoring.

Keywords: Natriuretic peptide; Univentricular heart; Congenital

1. Introduction

For children with univentricular heart (UVH), surgical palliation is available [1—4]. The aim of the treatment protocol is what is called Fontan circulation. Usually, patients undergo at least two or three operations and, in addition, two to three cardiac catheterisation procedures.

The natriuretic peptide system plays an important role in the regulation of diuresis and natriuresis, blood pressure and cardiomyocyte growth. This system functions to retard the progression of heart failure, the opposite of the effect of neurohumoral systems such as the renin—angiotensin—aldosterone and the sympathetic nervous system [5,6].

Levels of natriuretic peptides have been shown to be higher immediately after birth and to decrease thereafter during the first 2—4 months [7—9].

Cross-sectional studies have reported on levels of natriuretic peptides at different stages of the surgical programme or only before and after bi-directional Glenn (BDG) and total cavopulmonary connection (TCPC) operations [10—12]. To our knowledge, no reports are available on prospective follow-up of peptide levels throughout the UVH treatment protocol.

Our purpose was to study prospectively the influence of treatment for UVH on serum levels of N-terminal proatriopeptide (ANPN) and N-terminal pro-brain natriuretic peptide (NT-proBNP). In addition, we performed a cross-sectional analysis of levels of these natriuretic peptides before and after TCPC.

2. Materials and methods

This follow-up study was carried out at the Hospital for Children and Adolescents, University of Helsinki, Finland.
between February 2003 and February 2006. All parents of the participants agreed to participate in this clinical trial approved by the hospital ethics committee and gave their written informed consent.

2.1. Prospective study

The prospective study group comprised of 19 consecutive patients studied before the first palliative procedure and followed-up during the treatment protocol for UVH until the TCPC. Patients were examined the first time soon after birth, prior to the first operation. The second follow-up was at the age of 1 year and the fourth study point at the time of catheterisation before the TCPC operation. In our hospital, children with hypoplastic left heart syndrome (HLHS) undergo a Norwood operation during the neonatal period. The BDG operation is performed at the age of 2–6 months, and TCPC at the age of 2–4 years.

2.2. Cross-sectional study

In addition, we performed a cross-sectional measurement of peptide levels in 32 consecutive children (22 hypoplastic left ventricle (LV), 10 hypoplastic right ventricle (RV)) studied at the time of preoperative catheterisation before TCPC and in 12 of them (nine hypoplastic LV, three hypoplastic RV) studied at a median 2 (0.5–5.3) years after the TCPC operation.

2.3. Control group

In the control group, there were 12 children aged less than 6 months, and 41 children over 6 months but under 8 years. The control children were examined once. They were asymptomatic and showed no abnormalities in clinical examination, electrocardiogram (ECG) or echocardiography.

Characteristics and levels of NT-proBNP (ng l \(^{-1}\)) and ANPN (nmol l \(^{-1}\)) of patients with univentricular heart and controls are shown in Table 1.

All patients and controls underwent clinical cardiovascular examination and echocardiographic examination at the time of blood-test sampling for the measurement of natriuretic peptides.

2.4. Natriuretic peptides

Serum samples were frozen at –20 °C before measurement of concentrations of ANPN by immunofluorometric assay. Reagents were manufactured by Medix Biochemicals (Espoo, Finland) and the instruments by Delfia Research Fluorometer (Wallac, Turku, Finland). Serum concentrations of NT-proBNP were measured by an electrochemilumimometric method. The reagent kit was manufactured by Roche (Mannheim, Germany), and the samples were analysed at Limbach Laboratory in Heidelberg, Germany.

2.5. Echocardiography

We used the Acuson Sequoia C256 echocardiography system (Siemens, Mountain View, CA, USA) for two-dimensional (2D) echocardiography with colour flow Doppler to evaluate cardiac anatomy, ventricular function and insufficiency of atrioventricular and semilunar valves.

2.6. Cardiac catheterisation

Cardiac catheterisation with oximetry, pressure measurements and angiography was performed prior to the Glenn operation and then prior to TCPC. The procedure was performed under general endotracheal anaesthesia, systemic heparinisation and antimicrobial prophylaxis. During catheterisation, all patients were normovolaemic and normotensive.

2.7. Statistical analysis

Analyses were performed with the Statistical Package for Social Science version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). For variables derived from blood samples and catheterisation data, median and range were calculated. Because distribution of parameters tested by Kolmogorov–Smirnov’s goodness-of-fit test was not normal, the Mann–Whitney test was used for statistical analysis between groups, and the Wilcoxon signed-rank test for analysis within groups. Spearman’s correlation coefficient was used for calculating correlations between the echocardiographic and

<table>
<thead>
<tr>
<th>N</th>
<th>Baseline</th>
<th>preGlenn</th>
<th>1 year</th>
<th>preTCPC</th>
<th>Controls &lt;6 months</th>
<th>Controls 6 months to 7 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>m/f</td>
<td>14/5</td>
<td>14/5</td>
<td>9/3</td>
<td>11</td>
<td>8/3</td>
<td>5/7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.01 (0.00–0.07)</td>
<td>0.28 (0.08–0.44)</td>
<td>1.02 (0.70–1.11)</td>
<td>2.05 (1.74–3.50)</td>
<td>0.13 (0.01–0.39)</td>
<td>0.03 (0.58–7.99)</td>
</tr>
<tr>
<td>weight (kg)</td>
<td>3.4 (2.8–3.9)</td>
<td>5.8 (3.7–8.2)</td>
<td>9.8 (6.8–13.3)</td>
<td>12.6 (10.7–15.2)</td>
<td>4.8 (3.1–7.3)</td>
<td>18.0 (6.6–30.0)</td>
</tr>
<tr>
<td>height (cm)</td>
<td>50.0 (47.5–52.0)</td>
<td>62.0 (55.0–65.0)</td>
<td>74.5 (68.0–79.3)</td>
<td>88.0 (81.0–93.5)</td>
<td>55.5 (48.0–68.0)</td>
<td>103.8 (71.0–136.0)</td>
</tr>
<tr>
<td>BSA (m(^2))</td>
<td>0.21 (0.18–0.22)</td>
<td>0.30 (0.23–0.33)</td>
<td>0.45 (0.35–0.50)</td>
<td>0.54 (0.48–0.61)</td>
<td>0.26 (0.19–0.36)</td>
<td>0.71 (0.40–1.07)</td>
</tr>
<tr>
<td>NT-proBNP (ng l (^{-1}))</td>
<td>17.10* (0.73–40.20)</td>
<td>1.80* (0.69–6.10)</td>
<td>0.76* (0.34–10.10)</td>
<td>0.31* (0.26–0.48)</td>
<td>0.88 (0.51–3.70)</td>
<td>0.31 (0.10–0.61)</td>
</tr>
</tbody>
</table>

Table 1: Characteristics and levels of N-terminal proatriopeptide (ANPN) and N-terminal pro-brain natriuretic peptide (NT-proBNP) of patients with univentricular heart and controls aged less than 6 months and controls older than 6 months and younger than 8 years. Values are expressed as median (range).

*P < 0.001; \(\downarrow\)P < 0.05 as compared with controls.

\(\uparrow\)P < 0.001; \(\downarrow\)P < 0.01; \(\downarrow\)P < 0.05 as compared with baseline.

\(\uparrow\)P < 0.01 as compared with preGlenn study.

\(\downarrow\)P < 0.01 as compared with 1-year follow-up.
catheterisation measurements and serum concentrations of ANPN and NT-proBNP. The level of significance chosen was at $P < 0.05$.

3. Results

Clinical examination and blood-test sampling for the analysis of natriuretic peptides were performed on 19 children before the first operation and on two additional children before the BDG operation. Two patients died before the second phase; so a total of 19 children were examined before the BDG operation; 12 children were examined at the age of 1 year, and 11 before the TCPC operation.

3.1. Baseline

At baseline, 19 children were studied at a median age of 5 (range: 1—27) days. Of these 19, 16 had hypoplastic LV and three had systemic ventricle of LV morphology. In serum levels of natriuretic peptides, no differences emerged between these two groups. Serum levels of both ANPN and NT-proBNP were higher than in controls aged less than 6 months (Table 1, Figs. 1 and 2).

The first operation was the Norwood operation with either a modified 3.5- to 4-mm Blalock—Taussig shunt (six patients) or a 6-mm shunt from RV to pulmonary artery (10 patients). Two children with RV hypoplasia also needed a Blalock—Taussig shunt. A patient with tricuspid valve atresia underwent a pulmonary artery banding procedure at the age of 1 month. In echocardiography, systemic ventricular function was moderately impaired in two patients. Only one patient had moderate ativoventricular valve regurgitation. No correlation existed at baseline between serum levels of natriuretic peptides and ventricular function or degree of ativoventricular valve regurgitation.

Two children with HLHS died before the second operation. Both had undergone a Norwood operation with 3.5-mm Blalock—Taussig shunts. One of them died during the early postoperative phase. The other died suddenly at home due to shunt thrombosis. Peptide levels were among the highest in both of them: serum concentrations of ANPN measured 25.2 and 5.5 nmol l$^{-1}$, and those of NT-pro-BNP 22 681 and 14 408 ng l$^{-1}$.

3.2. First follow-up before bi-directional Glenn operation

Prior to the second palliative operation, that is, the BDG operation, patients underwent haemodynamic catheterisation at a median age of 3.4 (range: 1.7—5.3) months. After the first operation, serum levels of both natriuretic peptides had decreased but were still higher than in controls aged less than 6 months (Table 1, Figs. 1 and 2). Natriuretic peptide levels did not correlate with the shunt size. In echocardiography, 12 patients had ventricular systolic function scored as good and seven as moderately decreased. No regurgitation of the ativoventricular valve occurred in three patients, trivial regurgitation in 10 and moderate in six. In catheterisation, the end-diastolic pressure of the systemic ventricle measured a median 5 (range: 3—10) mm Hg. Oxygen saturation measured a median 72% (range: 66—86) in the artery and 43% (range: 29—61) in the superior caval vein. No correlation appeared between oxygen saturation, arterial saturation and levels of natriuretic peptides. Venous saturation correlated with levels of ANPN ($r = -0.69$, $P = 0.003$) but not with those of NT-proBNP ($r = -0.48$, $P = 0.06$).

Ten patients were treated with diuretics and two of them also with angiotensin-converting enzyme inhibitors. One patient was treated only with enalapril. No difference emerged in serum levels of natriuretic peptides between the groups.
patients treated with and without diuretics or angiotensin-converting enzyme inhibitors.

### 3.3. Second follow-up at age 1 year

All patients had undergone a BDG operation at a median age of 4.9 (1.8—10.4) months. In addition to superior caval-vein-to-pulmonary-artery anastomosis, reconstruction of the pulmonary artery had been performed in 12 patients, aortic arch reconstruction in three, balloon angioplasty for aortic recoarctation, tricuspid valve repair and correction of partially anomalous pulmonary venous drainage in one patient each.

A follow-up examination was performed for each patient at the age of 1 year. By that time, after BDG, peptide levels had remained unchanged, and were higher than in controls aged from 6 months to 8 years (Table 1, Figs. 1 and 2). In echocardiography, for all patients except one, ventricular systolic function was evaluated as good. Atrioventricular valve regurgitation was graded as none or trivial in eight and moderate in three. Two of these three had already, before the BDG operation, suffered a moderate AV-valve regurgitation, and one of them had undergone tricuspid valve repair.

### 3.4. Third follow-up before TCPC

The third follow-up took place before the TCPC operation, at a median age of 2.1 (range: 1.7—3.5) years. The median age of the patients at the time of TCPC was 2.9 (range: 1.7—3.8) years. Significant reduction had taken place in levels of both ANPN and NT-proBNP since the BDG operation and the 1-year follow-up. Serum levels of ANPN did not differ from those of control children aged from 6 months to 8 years, but those of NT-proBNP remained higher than in controls (Table 1, Figs. 1 and 2).

In echocardiography, ventricular function was good in all patients except for one with severe dysfunction. In that patient, serum concentration of ANPN measured 8.1 nmol l\(^{-1}\) and that of NT-proBNP 22.137 ng l\(^{-1}\). She had HLHS and later underwent cardiac transplantation. One had a moderate and the others trivial atrioventricular valve regurgitation or none.

### 3.5. Cross-sectional study before TCPC

Because the number of children with prospective follow-up before and after TCPC was small, we performed a cross-sectional study in a total of 32 patients (22 hypoplastic LV and 10 hypoplastic RV) examined before TCPC. This group comprised 11 patients followed-up prospectively and 21 additional patients. Of them, 12 children (nine hypoplastic LV and three hypoplastic RV) were also examined at a median age of 2.0 (range: 0.5—5.30) years after TCPC. In the group of 32 children examined prior to TCPC, median serum levels of ANPN measured 0.37 (range: 0.18—1.00) nmol l\(^{-1}\) (P = 0.059 as compared with controls) and those of NT-proBNP 155 (range: 13—705) ng l\(^{-1}\) (P < 0.001 as compared with controls). After TCPC, serum concentration of ANPN measured 0.39 (range: 0.09—0.98) nmol l\(^{-1}\) (P = 0.122 as compared with controls) and of NT-proBNP 201 (range: 76—1406) ng l\(^{-1}\) (P < 0.001 as compared with controls). These levels did not differ from those measured before TCPC.

In cardiac catheterisation performed on 28 of the 32 children prior to TCPC operation, oxygen saturation measured a median 62% (range: 57—73) in the superior caval vein and 83% (range: 75—93) in the artery. No correlation was evident between arterial saturation and levels of natriuretic peptides. The ventricular end-diastolic pressures had a median of 6 (range: 1—18) mm Hg. Pulmonary vascular resistance was calculated a median 2.0 (range: 1.1—3.1) Wood’s units m\(^{-2}\). In echocardiography, atrioventricular valve regurgitation was graded as none or trivial in 29 children and moderate in three children with HLHS. Ventricular function was good or fairly good in all except one child with HLHS.

### 3.6. Ventricular morphology

In the prospective study, of 19 patients, only three had predominant left ventricular morphology. Because of this, it was impossible to perform any analysis of the influence of ventricular morphology on the levels of natriuretic peptides.

In the cross-sectional study, prior to TCPC, serum concentration of NT-proBNP in patients with hypoplastic LV was higher. It measured a median 200 (range: 63—718) ng l\(^{-1}\) in those with hypoplastic LV and 79 (range: 13—355) ng l\(^{-1}\) in those with hypoplastic RV (P = 0.002). Serum concentration of ANPN measured a median 0.38 (range: 0.18—0.89) nmol l\(^{-1}\) in patients with hypoplastic LV, and 0.29 (range: 0.19—1.00) nmol l\(^{-1}\) in those with hypoplastic RV (P = 0.072). In patients with hypoplastic LV, levels of both ANPN and NT-proBNP were higher than in controls (P = 0.017 and P < 0.001), but were similar to controls in patients with hypoplastic RV.

After TCPC, serum levels of ANPN and NT-proBNP measured a median 0.35 (0.09—0.98) nmol l\(^{-1}\) (P = ns as compared with controls), 190 (range: 76—1406) ng l\(^{-1}\) (P < 0.001 as compared with controls) in patients with LV hypoplasia, 0.46 (range: 0.34—0.50) nmol l\(^{-1}\) (P = ns as compared with controls) and 211 (77—409) ng l\(^{-1}\) (P = 0.022 as compared with controls), respectively, in patients with RV hypoplasia.

### 4. Discussion

In this study, children with UVH were followed-up prospectively after diagnosis throughout their surgical management. During follow-up, serum levels of natriuretic peptides decreased, but even prior to TCPC, levels of NT-proBNP were higher than in controls. Similarly, in the cross-sectional study, NT-proBNP levels were higher than in controls before and after TCPC.

Surgical palliation for HLHS consists of three stages: the Norwood operation, BDG and TCPC [13,14]. For other types of UVH, the first operation may be pulmonary artery banding, a modified Blalock—Taussig shunt or BDG [15]. In our study, serum levels of NT-proBNP were higher than in controls throughout the follow-up. Levels of ANPN were higher than in controls at the time of diagnosis and at the first two follow-up stages. The systemic ventricle is unloaded during each step after the first palliation. Desaturation during the course of a treatment protocol for univentricular heart may have an
influence on cardiac function and on release of natriuretic peptides. In an adult population, an inverse relationship has been evident between levels of ANP and resting arterial oxygen saturation [16]. In a study on paediatric patients, no correlation was evident between natriuretic peptides and arterial oxygen saturation [17]. We could find no correlation between arterial saturation and levels of natriuretic peptides at baseline or at the fourth follow-up.

In this study, we did not exclude patients with atrioventricular valve regurgitation. This differs from one cross-sectional study [10], in which only patients with trivial or mild regurgitation and good ventricular function were examined. In our study, however, the number of children with greater than trivial regurgitation was originally small and decreased further after BDG, with reduction of systemic ventricular volume load. With conversion of the Blalock–Taussig shunt to BDG, the aim is to avoid any excessive volume overload caused by a shunt [18].

Atrial natriuretic peptide is produced primarily in atrial myocytes and in smaller amounts in ventricular myocytes. It appears in foetal ventricular tissue and in hypertrophied ventricles [6,19]. In healthy individuals, the main site of BNP gene expression is the atria, but in disease states, ventricular gene expression is the atria, but in disease states, ventricular gene expression is up-regulated [20,21]. Ventricular wall stress stimulates synthesis and release of both atrial and brain natriuretic peptides, concentrations of which increase in heart failure [5,19]. Changes in wall stress may explain our differences in levels of natriuretic peptides during the treatment protocol in our study.

Our study is in concordance with a study in which, after BDG and TCPC operations, levels of natriuretic peptides were higher than in controls [11]. In some cross-sectional studies, however, levels of natriuretic peptides have been higher in children after the first palliative operation than in controls, but similar to controls after the second or third palliation [10,22]. Levels of natriuretic peptides have been higher in patients treated with furosemide. No difference has emerged between patients treated with and without ACE inhibitors [10]. In our patients with and without medication, levels of natriuretic peptides were similar. After BDG, levels of natriuretic peptides remained unchanged until the 1-year follow-up but decreased thereafter, until the follow-up performed prior to TCPC.

After the Norwood operation, two of our patients died. No other deaths occurred during follow-up. One child with severe ventricular dysfunction underwent heart transplantation. In this patient, levels of natriuretic peptides increased from the time of BDG to the 1-year follow-up. Before BDG, cardiac function was evaluated as good, but tricuspid valve regurgitation was moderate. Koch has shown that elevated and increasing levels of BNP are associated with severity of atrioventricular regurgitation, morbidity and mortality [23].

In our cross-sectional study on serum levels of natriuretic peptides in children before and after TCPC, levels of both natriuretic peptides were higher in patients before TCPC than in controls. Serum levels of NT-proBNP were higher also after TCPC than in controls. In the study by Koch et al., NT-proBNP concentration was normal in most patients after TCPC [23], but, in these studies, the systemic ventricular morphology significantly differs. In our study, 22 of 32 patients had hypoplastic LV and in the study by Koch et al., only two of 67 patients had HLHS. Ventricular morphology seems to have an influence on peptide levels. Before TCPC, levels of NT-proBNP were higher in patients with hypoplastic LV than in hypoplastic RV, in concordance with earlier findings [10]. In our study, serum levels of ANP and NT-proBNP in patients with LV hypoplasia were higher than in controls at the time of catheterisation before TCPC, with no differences between patients with RV hypoplasia and controls. Atrial stretch decreases during the treatment protocol for UVH. Serum levels of ANP decreased during the protocol and did not decrease at the time of third follow-up. Levels of NT-proBNP were higher in children with UVH than in controls and did not decrease significantly after TCPC.

The levels of natriuretic peptides have been shown to be higher immediately after birth and to decrease thereafter, during the first 2–4 months [7–9]. Therefore, we divided controls in two groups, those aged less than 6 months and those aged from 6 months to 8 years. The age range in control groups was the same as in patients. The number of patients and control children was small, which is a limitation to this study.

5. Conclusion

In this prospective study, reduction in serum levels of natriuretic peptides during the treatment protocol for UVH was evident. Even after the TCPC operation, however, levels of NT-proBNP were still higher than those of controls. They, thus, can serve as a part of haemodynamic monitoring during the treatment protocol for this group of patients with very complicated heart defects; this has recently become standard practice in our unit.

References


