Serum N-terminal proatrial natriuretic factor in children with congenital heart disease

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An objective and simple method of establishing and grading heart failure in children is needed. The N-terminal of the atrial natriuretic factor prohormone, called proANF, is stable in vitro, relatively easy to measure and has been demonstrated as a clinically useful marker of heart failure in adults.

We measured proANF in 62 children with congenital heart disease and in 62 age-matched controls, in order to examine the relationship of proANF to different clinical and haemodynamic parameters. Echo Doppler cardiology was performed in all children, and 29 also underwent cardiac catheterization. The children were classified for volume and pressure load in each cardiac chamber, for shunt size and for signs of heart failure.

In paediatric patients without cardiac or renal disease, median proANF was 384 pmol. l⁻¹. In children with congenital heart disease, median proANF was 904 (200–5320) pmol. l⁻¹ (P<0.001). The three groups with the highest proANF levels were children with documented high atrial pressure (median proANF 1885 pmol. l⁻¹), a large left to right shunt (median proANF 1565 pmol. l⁻¹) and moderate or severe heart failure (median proANF 1305 pmol. l⁻¹). Furthermore, the proANF level correlated negatively with age and glomerular filtration rate.

We conclude that elevation of the proANF level is related to atrial pressures, heart failure and a high pulmonary to systemic flow ratio. These findings make proANF a potential new diagnostic tool in heart disease in children.

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Key Words: Atrial natriuretic factor, congenital heart disease, children, diagnosis, heart failure, proatrial natriuretic factor.

Introduction

A reliable diagnosis of heart failure can be difficult to establish in children. Important symptoms and signs, such as failure to thrive, respiratory symptoms and liver enlargement are all subjective, semiquantitative and unspecific[1]. Several conditions give rise to differential diagnostic problems[2]. Even echo Doppler cardiology can only detect indirect signs of heart failure. Besides, abnormal anatomy in congenital heart disease often complicates determination of chamber size and assessment of left ventricular function[3]. A regular finding in heart failure is elevation of atrial pressures, but this measurement requires invasive techniques and cardiac catheterization. An objective and simple method of establishing and grading the diagnosis of heart failure, and to evaluate effects of treatment, is needed.

Atrial natriuretic factor (ANF) is a natriuretic peptide, recognized as a regulator of water and electrolyte balance in humans[4]. The major known stimulus for its secretion is atrial wall stretch[5], and increased levels occur in the presence of elevated atrial pressures in heart failure[6]. Plasma ANP levels are increased in proportion to the severity of cardiac dysfunction, and numerous studies have shown significant correlations to cardiac filling pressures[7]. However, because the blood samples require storage at −70 °C and chromatographic extraction prior to measurement, determination of ANF is impractical for clinical purposes[8].

ANF is stored as a prohormone in secretory granules in atrial myocytes. On release, the prohormone is separated into the physiologically active ANF, and the remaining part of the prohormone, called ANF (1–98), N-terminal proANF, or for simplicity, proANF. The latter has a considerably longer half-life in plasma, and up to 50 times higher basal levels than ANF[9]. Thus, a rise in atrial pressures leads to a larger molar increase of proANF as compared to ANF[10]. ProANF is stable to enzymatic degradation, and shows little variation between central and peripheral venous plasma.
Table 1  Main diagnoses in 67 patients with congenital heart disease

<table>
<thead>
<tr>
<th>N*</th>
<th>Main diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Atrial septal defect (ASD)</td>
</tr>
<tr>
<td>13</td>
<td>Ventricular septal defect (VSD)</td>
</tr>
<tr>
<td>6 (2)</td>
<td>Aortic stenosis (Ao St)</td>
</tr>
<tr>
<td>5 (5)</td>
<td>Univentricular heart†</td>
</tr>
<tr>
<td>4</td>
<td>Atrioventricular septal defect (AVSD)</td>
</tr>
<tr>
<td>3 (3)</td>
<td>Patent ductus arteriosus (PDA)</td>
</tr>
<tr>
<td>3 (3)</td>
<td>Tricuspid atresia (TA)</td>
</tr>
<tr>
<td>3 (2)</td>
<td>Transposition of the great arteries (TGA)</td>
</tr>
<tr>
<td>3 (2)</td>
<td>Aortic coarctation (CoA)</td>
</tr>
<tr>
<td>2</td>
<td>ASD and VSD</td>
</tr>
<tr>
<td>2</td>
<td>Aortic insufficiency (AI)</td>
</tr>
<tr>
<td>2 (2)</td>
<td>Tetralogy of Fallot (TOF)</td>
</tr>
<tr>
<td>1 (1)</td>
<td>Mitral insufficiency (MI)</td>
</tr>
<tr>
<td>1 (1)</td>
<td>Pulmonary atresia (PA)</td>
</tr>
<tr>
<td>1 (1)</td>
<td>Double outlet right ventricle (DORV)</td>
</tr>
<tr>
<td>1 (1)</td>
<td>VSD with banding of pulmonary artery (BAP)</td>
</tr>
<tr>
<td>1 (1)</td>
<td>Truncus arteriosus communis (TAC)</td>
</tr>
</tbody>
</table>

*Number of previously operated patients in parentheses.
†Patients with univentricular hearts were excluded. See text.

Furthermore, proANF can be measured directly in plasma, and samples are proven stable at least 4 days after serum separation.ª

In our centre, proANF measurements have been used for clinical purposes in adults for 2 years. It has proven to be a simple and reliable method to estimate atrial pressures in heart failure, and is also a valuable tool in estimating long-term prognosis in cardiac disease. An important benefit is the feasibility of sending samples by ordinary mail, which allows decentralized follow-up of patients. This experience inspired us to measure the levels of proANF in children with congenital heart disease as compared to other paediatric patients, and to examine its relationship to different clinical and haemodynamic parameters.

Materials and methods

Patient characteristics

ProANF was measured consecutively in 67 children with congenital heart disease, who were admitted for elective cardiac surgery over a period of 4 months. Patient records were studied retrospectively. The main cardiac diagnoses are listed in Table 1. Eleven children also had chromosome defects or syndromes, but none had other important organ involvement. The five children with univentricular hearts were excluded from further analysis because of a complicated haemodynamic situation. In the remaining 62 children the age range was 4—191 months (mean 46.9; median 32). Ten children were below one year of age. The male/female ratio was 30/32. Recorded clinical symptoms and signs included reduced physical performance (n = 31), growth retardation (n = 23), respiratory symptoms (n = 20), feeding difficulties (n = 19), excessive sweating (n = 12) and liver enlargement (n = 5). Thirteen children were on medication for heart failure with digoxin and/or diuretics. All children were evaluated by a paediatric cardiologist the day before operation. The same day all children also had

Table 2  Clinical data for 10 patients with presumed atrial pressure elevation

<table>
<thead>
<tr>
<th>No</th>
<th>Age in months</th>
<th>Diagnosis and indication for surgery</th>
<th>Catheterized</th>
<th>Data supporting presumed atrial pressure elevation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>85</td>
<td>Operated AoSt and VSD. Subvalvular restenosis</td>
<td>Yes</td>
<td>Max. Ao-gradient 74 mmHg. LV edp 15 mmHg</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>Valvular AoSt. MI</td>
<td>No</td>
<td>Max. Ao-gradient 75 mmHg. Moderate mitral insufficiency</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>PDA. ASD 1. MI. (Partial AVSD)</td>
<td>No</td>
<td>Considerable mitral and tricuspid valve insufficiency</td>
</tr>
<tr>
<td>4</td>
<td>124</td>
<td>Corr. TGA. VSD. PS. Complete AV-block. Shunt insufficiency</td>
<td>Yes</td>
<td>RV edp 8 mmHg</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>Supravalvular AoSt. PS</td>
<td>Yes</td>
<td>Max. Ao-gradient 100 mmHg. LV edp 11 mmHg</td>
</tr>
<tr>
<td>6</td>
<td>141</td>
<td>MI. Chronic atrial fibrillation</td>
<td>No</td>
<td>Severe mitral insufficiency</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>ASD 1. VSD. Mitral split. (Partial AVSD)</td>
<td>No</td>
<td>Atrial dilatation. Mitral and tricuspid valve insufficiency. Large shunt</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>ASD 1 and 2. Mitral split. (Partial AVSD)</td>
<td>No</td>
<td>Mitral insufficiency. Large shunt</td>
</tr>
<tr>
<td>9</td>
<td>23</td>
<td>Valvular and subvalvular AoSt.</td>
<td>Yes</td>
<td>Max. gradient 70 mmHg. LV edp 16 mmHg</td>
</tr>
<tr>
<td>10</td>
<td>71</td>
<td>VSD. Primary closure</td>
<td></td>
<td>Slight pulmonary hypertension</td>
</tr>
</tbody>
</table>

Cardiac catheterizations, if performed, were incomplete with respect to atrial pressure measurements. Ao=aorta; St=stenosis; VSD=ventricular septal defect; LV=left ventricle; edp=end-diastolic pressure; MI=mitral insufficiency; ASD=atrial septal defect; PDA=pulmonary ductus arteriosus; AVSD=atrioventricular septal defect; Corr. TGA=congenitally corrected transposition of the great arteries; PS=pulmonary stenosis; AV=atrioventricular; RV=right ventricle.

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Figure 1  N-terminal proANF related to age. Values in children with congenital heart disease are indicated by open circles and a solid regression line. Bold dashes and the dashed regression line represent the control group.
Figure 2 Correlation between glomerular filtration rate (x-axis) and N-terminal proANF (y-axis) in children with congenital heart disease. ProANF values are expressed as natural logarithms.

routine blood sample, and 58 had a chest X-ray. Of these, 23 children had a heart of normal size, and 35 heart enlargement. Other pre-operative investigations were individualized on clinical grounds. All children had normal serum electrolytes. Glomerular filtration rate was estimated from serum creatinine and length. All but two patients had a glomerular filtration rate above 50 ml.min\(^{-1}\), and the mean value was 88 ml.min\(^{-1}\) (range 45–120).

**Haemodynamic classification**

Echo Doppler cardiography was performed in all children, and 29 children also had pre-operative cardiac catheterization. Pulmonary hyperflow was demonstrated in 36 children with echo Doppler cardiography (n=22) and catheterization (n=14). According to the pulmonary to systemic flow ratio, shunt size was considered large (Qp/Qs>3), moderate (Qp/Qs 2–3) or small (Qp/Qs<2). In order to study different clinical and haemodynamic situations all children were classified as to whether or not there was a volume or pressure load in each cardiac chamber at the time of proANF sampling. This assessment was made by a paediatric cardiologist based on all pre- and postoperative information, except for the proANF values.

As regards pressure load of the atria or pulmonary artery, children were divided into three groups according to pressure levels and the degree of diagnostic accuracy. Mean pressures exceeding 5 mmHg in the right atrium, 8 mmHg in the left atrium and 20 mmHg in the pulmonary artery were considered elevated. The first group comprised 14 children with high pressure in atria documented by cardiac catheterization. Eight of these children also had elevation of pulmonary arterial pressure. The second group comprised 10 children with a high atrial pressure suggested from incomplete catheterization data (n=6) and echo Doppler cardiographic findings. These 10 children are presented in Table 2. The third group includes 38 children with normal pressures in the atria and pulmonary artery. This was documented by complete catheterization (n=5) or suggested from incomplete catheterization data (n=5), echo Doppler cardiographic and other clinical information (n=28). Thus, in this group, which mainly consisted of children with atrial and ventricular septal defects, there was a risk of underestimation of pressures. A volume load in one or both atria, without pressure overload, was suggested in 29 children, based on echo Doppler cardiographic and catheterization data.

**Heart failure classification**

All children were divided into three groups from clinical symptoms and signs of heart failure, and haemodynamic, echo-cardiographic and postoperative data.
Figure 3  N-terminal proANF in patients with pulmonary hyperflow. The shunt was considered small if $Q_p/Q_s$ was <2, moderate if $Q_p/Q_s$ was 2–3 and large if $Q_p/Q_s$ was >3. Group median levels are indicated by solid squares, and differences between groups by arrows and $P$-values. (ns = non significant difference).

supporting the diagnosis. This was made by a paediatric cardiologist unaware of the proANF results. In 26 children there was no sign of heart failure, in 24 children there were some signs of heart failure and finally 12 children were considered moderately or severely affected by heart failure.

Control group

With informed consent, proANF was measured in 128 children who had undergone venipuncture for other reasons at a regional paediatric centre. Children with renal impairment, cardiac diseases and electrolyte imbalance were excluded. Sixty-two children were selected to obtain an age-matched control group to the children with congenital heart disease. The proANF serum level in the selected 62 children did not differ significantly from the total control population. Except for children with acute upper airway infection (n=10), samples were collected at the time for routine controls. Other diagnoses were bronchial asthma (n=11), neurological disease (n=8), gastrointestinal disease (n=8), tonsillar hypertrophy (n=7), growth disturbance (n=6), urological disease (n=6), haematological disease (n=4) and congenital, well regulated hypothyreosis (n=2). In the control group, age ranged between 5 and 186 months (mean 47.2; median 32.5). The male/female ratio was 37/25. There was no statistically significant age difference between the study group and the control group, and the mean age difference was 5.3 (range -4 to 5) months. The difference was 3 months or less in all but three pairs.

Blood samples and radioimmunoassay

All blood samples were drawn from a peripheral vein into plastic syringes containing coagulation activating silica particles. N-terminal proANF was determined in unextracted serum by radioimmunoassay. The method is modified from that published by Sundsfjord et al. and has a detection limit of $32 \text{ pmol} \cdot \text{L}^{-1}$. The inter-assay coefficient of variation is 5.1–5.6% at different concentrations, with a corresponding intra-assay coefficient of variation of 4.5–5.7%.

Statistical analysis

All comparisons between groups were performed as a two-sided Wilcoxon rank sum test for the difference between two population medians, with corresponding point estimates and confidence intervals (Minitab;
Results

In the control group, mean proANF was 423 (± 193) pmol.1⁻¹ with a median of 384 and range 200–859 pmol.1⁻¹. There was a strong negative correlation to age (r = -0.75, P<0.01), thus younger children tended to have higher values. The results did not differ significantly between boys and girls.

For all 62 children with congenital heart disease, values ranged from 200–5320 pmol.1⁻¹ with a mean of 1065 and a median level of 904 pmol.1⁻¹ (Fig. 1). In this group proANF also had a negative correlation to age (r = -0.62, P<0.01). The estimated glomerular filtration rate was age-dependent, with a positive correlation coefficient of 0.76 (P<0.01). ProANF values correlated to glomerular filtration rate, as demonstrated in Fig. 2. Median proANF levels for the five most common diagnoses were all significantly higher as compared to the control group, but ranged widely. Results in pmol.1⁻¹ were: ventricular septal defect 539 (range 298–5310), atrial septal defect 626 (200–1570), aortic stenosis 833 (360–1630), patent ductus arteriosus 934 (772–1360) and for atrioventricular septal defect 2515 (1180–5320).

In children with pulmonary hyperflow, the proANF levels varied with shunt size, as shown in Fig. 3. The median proANF in children with a large shunt was 1565 pmol.1⁻¹ (range 460–5320), a moderately large shunt 938 pmol.1⁻¹ (200–2010) and in children with a small shunt 600 pmol.1⁻¹ (350–2270). In children with atrial volume load, median proANF was 596 pmol.1⁻¹ (200–1570), and this was not significantly different from children without volume load, whose median proANF level was 743 pmol.1⁻¹ (418–1630). Figure 4 presents proANF results related to heart failure. Children with moderate or severe heart failure had a median proANF of 1305 pmol.1⁻¹ (range 642–5320), children with some sign of heart failure 1055 pmol.1⁻¹ (261–5310) and children without any sign of heart failure 579 pmol.1⁻¹ (200–1560). The relationship between heart size on chest X-ray and proANF is illustrated in Fig. 5. The median proANF was 1125 pmol.1⁻¹ (298–5320) in children with heart enlargement, and 772 pmol.1⁻¹ (200–1630) in children with a normal heart size. Finally, Fig. 6 demonstrates the variation of proANF values in patients with atrial
pressure elevation. In children with a documented pressure elevation the median proANF was 1885 pmol.1⁻¹ (1020–5320), in children with a presumed pressure elevation 1100 pmol.1⁻¹ (743–1630) and in children without suspected pressure elevation 608 pmol.1⁻¹ (200–1570). Altogether, the three groups with the highest proANF levels were children with documented high atrial pressure, a large left to right shunt and moderate or severe heart failure. In these groups median proANF was four to six times higher than in the control group.

Discussion

The main finding in this study was that the proANF level was considerably higher in children with congenital heart disease, as compared to other paediatric patients. The most important determinant was atrial pressure, but proANF values also corresponded to age, glomerular filtration rate, shunt size, clinical signs of heart failure and heart size on chest X-ray.

After a rise in the neonatal period ANF decreases to adult levels during the subsequent months. In this study, proANF seems to persist at a high level through the first year of life, and maybe even longer. This was appreciable both in the control group and the study group, although it was most prominent in children with congenital heart disease. The reason for this is unclear. One possible explanation might be that children selected for early heart surgery in general have a more serious degree of heart failure. However, this was not true for all children with high proANF values in our study, and it does not explain the higher level in the first year of life in the control group. Another hypothesis might be perceived in the relationship between glomerular filtration rate, age and proANF (Fig. 2). The metabolism of atrial peptides is dependent on receptor uptake, degradation of enzymes and renal elimination. The relative contribution of each pathway will differ depending on the affinities of the receptors or enzymes for each peptide. Because proANF is stable to enzymatic degradatio, and the significance of receptors is still not defined, proANF clearance might be mainly determined by renal elimination. Consequently, a reason for persistent high proANF values could be the renal immaturity and physiologically low glomerular filtration rate during early infancy. Likewise, it is possible that low glomerular filtration rate amplifies a rise in the proANF level. However, the method of estimating glomerular filtration rate from plasma creatinine is not fully reliable, and further studies on proANF, renal function and other age-dependent factors are needed to clarify these connections.

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The major known stimulus for ANF release is atrial wall stretch. Most stimuli evoking ANF secretion in humans can be linked to changes in atrial pressures and/or heart rate. It is unknown whether a volume load of the atrium can give rise to wall stretch with subsequent elevation of the ANF in children, independent of intra-atrial pressure. Some authors have found a good correlation between atrial size and plasma ANF, irrespective of pressure levels. Others recorded a normal ANF level in children with atrial septal defect and an enlarged right atrium or a large volume load. Matsuoka et al. found that a volume load of the left atrium led to a greater increase in plasma ANF than a volume load of the right atrium. We did not measure atrial size, but in our study, volume load alone seemed to have only a marginal influence on the proANF level. Several studies in children have shown a correlation between ANF and other clinical and haemodynamic parameters, such as atrial or pulmonary arterial pressures, pulmonary to systemic flow ratio, size of septal defects, and heart failure. Thus, ANF could be considered a general index of cardiac disease in children. However, findings have been varying and partly contradictory. This can be explained by methodological problems, for example in determining atrial size, left atrial pressure or the degree of heart failure. Besides, ANF is unstable in vitro, levels vary over time and differ between central and peripheral venous plasma.

In our centre, the upper reference level of (N-terminal) proANF in young adults is 800 pmol . l$^{-1}$. Only 1/24 children with a documented or presumed high atrial pressure had a value below 800 pmol . l$^{-1}$. Applying this level, proANF in this study had a negative predictive value of 96% for atrial pressure elevation in children with congenital heart disease, and a positive predictive value of 66%. The corresponding sensitivity was 96%, with a specificity of 68%. However, only 5/28 patients with atrial or ventricular septal defects had pre-operative cardiac catheterization, and only in three of these children was there a proven elevation of atrial pressures. The other children with septal defects were described as having a pure volume load of the right and left side, respectively. Since high pressures can sometimes be demonstrated, but never excluded by echo Doppler cardiography, there was a risk of underestimation of atrial pressures in our classification. This could explain the high proANF values in the group of children with estimated normal atrial pressures, leading to a relatively low positive predictive value in this study.

We found a significantly higher proANF level in all diagnostic groups as compared to controls. However, this should be interpreted with caution, because patients were selected for cardiac surgery, and represent the more serious degrees of disease. The proANF values had a wide range, and low values were recorded in all diagnoses, except for atrioventricular septal defect. This probably reflects individually varying haemodynamic situations within the groups. Consequently, proANF had little value in discriminating children with congenital heart disease from healthy children, unless there was an elevation of atrial pressures.

Five children with atrial septal defects had a marked elevation of proANF, without obvious clinical
signs of heart failure. Parents to the five children reported few symptoms, but interestingly, four of these children were the only ones in the atrial septal defect group with growth retardation. There was no correlation between shunt size and proANF values in children with atrial septal defects, which seems to contradict an influence of volume load alone on the proANF level. Echo Doppler cardiological data did not indicate atrial pressure elevation, but these children had no pre-operative catheterization, and their actual pressure levels are unknown. The number of patients in this study is too small to clarify whether this observation reflects true pathophysiology. If a rise in serum proANF can be used to disclose a subclinical atrial pressure load, it might become a tool in selecting patients for cardiac surgery, and in the timing of the operation.

In adults, proANF is closely related to atrial pressures corresponding to atrial wall stretch [23]. Our study indicates that atrial pressures also in children is the best clinical correlate for the proANF level. High levels in heart failure and pulmonary hyperflow probably reflect the same relationship. Our findings suggest that the advantages of proANF as a diagnostic marker demonstrated in adults could also be useful in children. To clarify this, further studies of natriuretic peptides in different haemodynamic situations are required.

**Conclusion**

Because of its stable and high basal plasma concentration and ease of measurement, (N-terminal) proANF has a potential as a diagnostic tool in heart disease in children. Elevation of the proANF level is related to atrial and pulmonary arterial pressures, heart failure and a high pulmonary to systemic flow ratio. The study indicates that proANF in children, as in adults, can be considered a biochemical marker for atrial pressure elevation. This could be used to diagnose heart failure in children, to indicate optimal timing of surgery and patient follow-up. Further studies of proANF are needed, especially with respect to different haemodynamic situations, glomerular filtration rate and reference limits in children of all ages.

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**References**


