BNP and haematological parameters are markers of severity of Ebstein’s anomaly: correlation with CMR and cardiopulmonary exercise testing

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Aims

Ebstein’s anomaly (EA) involves a displaced and dysplastic tricuspid valve resulting in an atrialized portion of the right ventricle and an enlargement of the functional right ventricle and right atrium. Biomarkers targeting heart failure such as brain natriuretic peptide (BNP) or haematological parameters [haemoglobin (Hb) and haematocrit (Hct)] are upregulated in states of pulmonary hypoperfusion. We hypothesized that decreased pulmonary perfusion dependent on the stage of right heart failure is a possible mechanism in EA, and that it can be correlated with cardiac magnetic resonance (CMR) parameters. The aim of this study was to investigate the relationship between BNP and haematological parameters with functional parameters from CMR and exercise testing in patients with EA.

Methods and results

Twenty-five patients with non-corrected EA were studied prospectively (mean age 26 ± 14 years). BNP level was increased (74 ± 127 ng/L), and in 16% markedly above the heart failure cut-off level of 100 ng/L. Hb and Hct were increased above normal levels in 20 and 24% of patients, respectively. BNP and Hct/Hb correlated with CMR [total right/left (R/L)-Volume-Index, right atrium-end-diastolic volume index (EDVI), functional right ventricle (fRV)-EDVi, fRV-ejection fraction (EF), tricuspid regurgitation, pulmonary artery flow, and left ventricular EF] and exercise testing [workload/kg, oxygen uptake (VO₂), ventilatory response to carbon dioxide production (VE/VCO₂), oxygen (O₂) pulse, and heart rate reserve]. The higher BNP and haematological parameters, the higher was the disease severity and the more limited was the physical exercise capacity.

Conclusion

In this EA cohort, BNP levels and haematological parameters correlated well with functional data from CMR and exercise testing. The total R/L-Volume-Index and BNP, and to some extent hematomal parameters, may be useful as prognostic markers in patients with EA.

Keywords

Cardiovascular magnetic resonance imaging • Ebstein’s anomaly • Brain natriuretic peptide • Cardiopulmonary exercise testing

Introduction

Ebstein’s anomaly (EA) of the tricuspid valve (TV) is a rare congenital cardiac malformation, comprising <0.5% of all congenital heart disease (CHD).¹ The dysplastic TV is offset towards the apex thereby causing an ‘atrialization’ of the right ventricle (RV). Higher grade tricuspid regurgitation (TR), volume overload, altered cardiac muscle morphology² and electrical synchrony, as well as Wolff–Parkinson–White syndrome are associated with the disease.³ Progressive right heart failure and impaired physical exercise
capacity occur in many patients with EA at some stage in their life.\(^6\) Abnormalities of left ventricular (LV) morphology and function have also been described in EA.\(^5,6\) However, it is still unknown how the altered anatomy, haemodynamics, and electrophysiology cause deterioration of overall cardiac function. Some studies have addressed TV replacement or different methods of reconstruction as a feasible therapy, but the optimal timing and long-term success are unknown.\(^7\)

Different approaches to classify the severity of EA have been reported, such as size or volume of right atrium (RA), atrIALIZED RV (aRV), functional RV (fRV), and TV morphology or patency with moderate success.\(^8,9\) Recently, a simple total right/left-Volume-Index (R/L-Volume-Index) from cardiovascular magnetic resonance (CMR) with a good correlation between clinical heart failure indices and functional parameters has been reported by our group.\(^10\)

Serum brain natriuretic peptide (BNP) is a well-studied marker in heart failure and might be of clinical importance in patients with EA due to its proven usefulness in CHD and the simplicity of assessment.\(^11\) BNP is synthesized and released into the circulation by the atrial and ventricular myocytes in response to pressure overload, volume expansion, and increase in myocardial wall stress.\(^12\) BNP levels are increased in most types of cyanotic CHD with RV volume and pressure overload,\(^13,14\) and correlate well with exercise capacity.\(^14,15\) Thus far, its role in EA in conjunction with CMR parameters has not been evaluated.

Right heart failure in EA may be associated with right heart volume overload and decreased pulmonary perfusion. While fRV output is decreased due to diminished RV contraction, a significant portion of the stroke volume is lost due to TR.\(^8\) In cyanotic CHD, erythrocytosis is the result of decreased oxygen saturation, which is in turn caused by decreased pulmonary perfusion.\(^16\) Haematological indices of haemoglobin (Hb) and haematocrit (Hct) are upregulated in states of pulmonary hypoperfusion in cyanotic CHD.\(^17\) This is a physiological response to tissue hypoxia with a resultant increase in serum erythropoietin level, thereby stimulating the bone marrow erythropoiesis and causing an elevated red cell mass, Hct, and whole blood viscosity.\(^18\) A decrease in pulmonary perfusion dependent on the stage of right heart failure is a possible pathophysiological mechanism in patients with EA, but has not been studied so far.

The aim of this study was to evaluate whether BNP and haematological parameters can be used to classify severity of EA in conjunction with CMR and exercise testing parameters.

**Methods**

**Study population**

Fifty-eight living patients with EA were identified from the patient database of the Department of Pediatric and Adult CHD at the University Medical Center Goettingen, Germany. Of these, 4 patients were <10 years, 3 had implantable cardioverter–defibrillator (ICD) or pacemakers, 12 had major corrective surgery (2 Glenn, 2 Fontan, 1 TV replacement, and 7 TV reconstruction), 2 had other associated congenital heart defects (corrected transposition of the great arteries and double aortic arch), 1 suffered from claustrophobia and aborted the CMR scan, 8 were lost to follow-up, and 8 refused to participate in the study. Some patients met multiple exclusion criteria.

Twenty-five of the remaining patients with ‘native’ EA fulfilled the study criteria and gave written informed consent to participate in the study. These 25 patients were examined prospectively within 1 day from January 2013 until July 2013 in our outpatient clinic. Inclusion criteria were EA, the absence of CMR incompatible implants, no TV or major cardiac corrective surgery other than atrial septal defect (ASD) closure, age >10 years, and sufficient compliance. The study protocol was approved by the local ethics committee and complied with the 1975 Declaration of Helsinki.

None of the included patients exhibited rhythm disturbances such as atrial tachycardia at the time of investigation. Seven patients in our cohort had ASDs. However, all but one were post ASD closure (3 interventional and 3 surgical).

**Clinical examination and laboratory testing**

Medical history, clinical examination, laboratory tests for BNP, white and red blood cell count, clinical chemistry, cardiopulmonary exercise testing, and CMR according to the guidelines on CMR in adult CHD by the German Societies for Cardiology and Pediatric Cardiology as well as the European Society for Cardiology\(^19,20\) were conducted on a single day for each patient.

Measurements of transcutaneous oxygen saturation (SaO\(_2\)), blood pressure, heart rate (HR), weight, and height were all part of the clinical examination. For laboratory testing, full blood count, serum electrolytes, creatinine level, and plasma BNP level were measured using standard assays as used by the Institute for Laboratory Medicine, University Medical Center Goettingen, in routine clinical practice. Results for BNP, Hct, and Hb were adjusted to age and gender-specific reference values.

**Cardiopulmonary exercise testing**

For cardiopulmonary exercise testing, a ZAN\(^\circledast\) 600 (nSpire Health GmbH, Oberthulba, Germany) bicycle spirometer using a ramp protocol with a total, stepwise increase of 20 W/min was employed. Respiratory gas flows were measured using a ZAN Variable Impedence Pneumotachograph flow sensor. Data were analysed using the ZAN-Tech Software. Relevant parameters were maximal workload, maximal oxygen uptake (VO\(_2\)), ventilatory response to carbon dioxide production (VE/VO\(_2\)), maximal oxygen (O\(_2\)) pulse, electrocardiographic changes in repolarization, minimal and maximal HR, HR reserve (maximal HR − HR at rest), and minimal and maximal blood pressure.

**Cardiac magnetic resonance**

CMR scans were performed on a 1.5 T Symphony scanner (Siemens Healthcare, Erlangen, Germany). A five-element cardiac coil system was used according to patient’s thorax diameter and length. The sequences comprised the following: stacks of steady-state free precession (SSFP) cine images in transversal and ventricular short-axis orientation with a field-of-view (FOV) including all cardiac structures and great vessels, triplanar half-Fourier acquisition single-shot turbo spin-echo (HASTE) sequences, and 2D through-plane phase-contrast flow measurements with breath-hold in the ascending aorta and pulmonary trunk. The flow measurements were made in an imaging plane perpendicular to the vessel and 1 cm distal to the semilunar valve.

Measurements of ventricular function and mass were performed using a ‘multislice–multiphase’ technique with fast gradient-echo sequences in expiratory breath-hold and the following parameters: TR = 14 ms, TE = 2.6 ms, flip angle = 20°, slice thickness 3–5 mm, maximal FOV 350, 20–30 phases per heart cycle according to HR, and matrix 128:256 with retrospective gating. Acquisition time for each patient depended on the HR and was ~30–45 min. Imaging was performed during
spontaneous breathing without the need for sedation. Segmentation of individual slices was performed for both transversal and short-axis stacks using the QMass, QFlow, and Visia Software (Medis, Leiden, The Netherlands). Endocardial and epicardial borders were defined manually for all patients. Contours were drawn in end-systole and end-diastole for RA, aRV, fRV, left atrial (LA), and LV volumes (Figure 1); ejection fraction (EF) and mass were calculated. Contours were drawn by the primary investigator (O.H.) and controlled and approved in a blinded and randomized fashion by the senior investigator (M.S.). In case of need for contour adjustment, consensus among O.H., M.S., and J.L. (>10 years of CMR experience) was reached. All imagers were blinded to the laboratory values. RA, aRV, and fRV were defined as previously described and demonstrated in Figure 1. A comparison of transversal and short-axis orientation measurements using the Bland–Altman method formerly showed a significant difference only in the aRV end-diastolic volume index (EDVi). Therefore, only data from the transversal orientation were used for further descriptive and statistical analyses. The R/L-Volume-Index as a measure of severity of EA by CMR ([RA + aRV + fRV]/[LA + LV], volumes in end-diastole) was also derived from transversal orientation. TR was calculated by the formula: \( TR = \frac{fRVSV - PAante}{fRVSV - PAretro} \times 100 \) by CMR flow measurements in the pulmonary trunk.

**Statistical analysis**

Data were expressed as means and standard deviations (SD), or medians and ranges as appropriate. Descriptive analyses were performed and correlation coefficients derived using the Pearson method. A P-value of <0.05 was considered to be significant. Analysis utilized Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and Statistica (Stat Soft, North Melbourne, Australia), in cooperation with the Institute for Medical Statistics, University Medical Center, Goettingen, Germany.

**Results**

The mean age was 26 ± 14 years (range 10–60; 18 males and 7 females). The plasma BNP levels were increased in 16% of patients markedly above the heart failure threshold of 100 ng/L. Increased Hct and red blood cell count were found in 24 and 20% of patients, respectively, exhibited an increased Hb level (BNP, Hct, and Hb all normalized to age and gender-related reference values).

**Table 1** Patient characteristics

<table>
<thead>
<tr>
<th>Study cohort (n = 25)</th>
<th>n (%) or mean ± SD</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26 ± 14</td>
<td>23 (10–60)</td>
</tr>
<tr>
<td>&lt;18 years</td>
<td>10 (40)</td>
<td></td>
</tr>
<tr>
<td>Gender (male)</td>
<td>18 (72)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170 ± 14</td>
<td>172 (145–188)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72 ± 25</td>
<td>77 (31–133)</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.8 ± 0.4</td>
<td>1.9 (1.1–2.6)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 ± 6</td>
<td>25 (15–39)</td>
</tr>
<tr>
<td>SaO₂ (%)</td>
<td>98 ± 3</td>
<td>99 (85–100)</td>
</tr>
<tr>
<td>NYHA classification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>20 (80)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>3 (12)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1 (4)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>1 (4)</td>
<td></td>
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<tr>
<td>Laboratory testing</td>
<td></td>
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</tr>
<tr>
<td>BNP (ng/L)</td>
<td>74 ± 127</td>
<td>28 (10–547)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>15.7 ± 1.5</td>
<td>15.6 (12.8–19.1)</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>46.3 ± 4.3</td>
<td>45.9 (36.8–55.3)</td>
</tr>
<tr>
<td>Erythrocytes (mio/μL)</td>
<td>5.3 ± 0.5</td>
<td>5.1 (4.1–6.2)</td>
</tr>
</tbody>
</table>

BMI, body mass index; BNP, brain natriuretic peptide; BSA, body surface area; Hb, haemoglobin; Hct, haematocrit; NYHA, New York Heart Association; SaO₂, oxygen saturation.

Transcutaneous oxygen saturation was normal (>96%) in all but one patient. Patient demographics and laboratory data are summarized in Table 1.

CMR and cardiopulmonary exercise testing parameters are summarized in Table 2. The indexed IRV and RA end-diastolic volumes (EDVs) were increased to 118 ± 57 and 107 ± 56 mL/m² respectively, whereas IRV EF decreased to 47 ± 8%. Pulmonary blood flow measured by phase-contrast imaging was 2.7 ± 0.8 L/min × m² and aortic blood flow 2.5 ± 0.7 L/min × m².

Correlations for BNP and Hct against CMR and cardiopulmonary exercise data are demonstrated in Table 2. There was fair correlation
Table 2 CMR and exercise data and correlations with BNP and Hct

<table>
<thead>
<tr>
<th>Study cohort (n = 25)</th>
<th>Mean ± SD</th>
<th>Correlations with BNP</th>
<th>Correlations with Hct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r</td>
<td>P-value</td>
</tr>
<tr>
<td>Cardiopulmonary exercise testing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Watt/kg (% predicted)</td>
<td>90 ± 27</td>
<td>-0.443</td>
<td>0.030</td>
</tr>
<tr>
<td>Peak VO2 (% predicted)</td>
<td>67 ± 22</td>
<td>-0.406</td>
<td>0.055</td>
</tr>
<tr>
<td>VE/VCO2 (% predicted)</td>
<td>118 ± 36</td>
<td>0.474</td>
<td>0.022</td>
</tr>
<tr>
<td>Peak O2 pulse (% predicted)</td>
<td>79 ± 21</td>
<td>-0.429</td>
<td>0.041</td>
</tr>
<tr>
<td>Peak HR (% predicted)</td>
<td>94 ± 9</td>
<td>-0.581</td>
<td>0.003</td>
</tr>
<tr>
<td>Peak HR reserve</td>
<td>30 ± 16</td>
<td>0.512</td>
<td>0.011</td>
</tr>
<tr>
<td>CMR data (derived from transversal planes)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total R/L-Volume-Index*</td>
<td>2.6 ± 1.7</td>
<td>0.691</td>
<td>&lt;0.0003</td>
</tr>
<tr>
<td>RA EDVi (mL/m²)</td>
<td>107 ± 56</td>
<td>0.834</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>aRV EDVi (mL/m²)</td>
<td>52 ± 36</td>
<td>0.359</td>
<td>0.085</td>
</tr>
<tr>
<td>fRV EDVi (mL/m²)</td>
<td>118 ± 57</td>
<td>0.834</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>fRV EF (%)</td>
<td>47 ± 8</td>
<td>-0.262</td>
<td>0.228</td>
</tr>
<tr>
<td>LV EDVi (mL/m²)</td>
<td>73 ± 11</td>
<td>-0.273</td>
<td>0.198</td>
</tr>
<tr>
<td>LV EF (%)</td>
<td>57 ± 8</td>
<td>-0.670</td>
<td>0.0003</td>
</tr>
<tr>
<td>TR (%)</td>
<td>27 ± 24</td>
<td>0.804</td>
<td>0.0009</td>
</tr>
<tr>
<td>Qs (L/min × m²)</td>
<td>2.5 ± 0.7</td>
<td>0.536</td>
<td>0.059</td>
</tr>
<tr>
<td>Qp (L/min × m²)</td>
<td>2.7 ± 0.8</td>
<td>-0.743</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Bold values are significant with P < 0.05.

*The index is defined as volumes of [(RA + aRV + fRV)/(LA + LV)] in end-diastole.10
*Calculated by the formula: TR = [(fRVSV − PAante)/(fRVSV − PAretro) × 100] in CMR flow.8

(r > ± 0.4) of BNP and Hct levels with cardiopulmonary exercise testing indices—peak workload/kg, peak VO2, peak VE/VC02, peak HR, peak HR reserve, and peak O2 pulse. Strong correlations (r > ± 0.6) were found for CMR parameters: total R/L-Volume-Index as well as right heart parameters (RA and fRV EDVi), LVEF, pulmonary blood flow and TR (as measures of heart failure and pulmonary perfusion) with BNP, and fair correlation with Hct.

The higher the BNP level and Hct were, the higher was the severity of EA and degree of heart failure in patients as described by CMR and the lower was cardiopulmonary exercise capacity.

Discussion

To the best of our knowledge, this is the first prospective study that relates BNP and haematological parameters to functional heart failure data from CMR and cardiopulmonary exercise testing in patients with EA. The main findings of our study are the following: (i) plasma BNP levels and haematological parameters, serving as a measure of heart failure, volume overload, and pulmonary perfusion, were increased above normal levels in patients with EA and (ii) these parameters correlated with functional data from CMR and exercise testing, and may be useful as prognostic markers in patients with EA. Our results may help to assess the severity of EA and stratify patients in a simple way, using cost-effective laboratory parameters (BNP, Hct, Hb, and red blood cell count).

BNP is a well-established marker and prognosticator for heart failure in the general population11 and in patients with CHD.12 An increased BNP level is the result of increased cardiac chamber wall stress, resulting from increased blood volume typical of heart failure.13 BNP levels are increased in most types of CHD with RV volume and pressure overload,13,14 and are generally lower in patients with RV dysfunction compared with those with LV heart failure.23 The R/L-Volume-Index, as an expression of right heart volume overload and heart failure in patients with EA, correlates well with BNP levels.

Most EA patients live with persisting RV failure of different degrees, often increasing over time. In some patients, LV failure develops at some stage of their life.5,6 Why, when, and which patient is in danger to contract LV dysfunction is not entirely clear. It is possible that with increasing right heart volumes and altered transversal (i.e. right to left) mechano-kinetics LV function is also increasingly impaired, and that the observed increase in BNP reflects this transition from still balanced to deteriorating an RV–LV interaction with decreased LVEF. Golecki et al.6 found altered shape and function of the LV in EA and a correlation with RV function measured by TAPSE, but not with RV volume. Other groups have reported on interaction between LV and RV in different congenital malformations.24,25 In our cohort, some patients with high R/L-Volume-Index also have low LVEF, which may be a hint that in EA right-to-left interaction of all right- and left-sided cardiac chambers is of importance.
and that an increase in BNP levels reflects a disturbance in this balance. We therefore hypothesize that, in severe EA, increasing right-sided heart volumes also impair LV function through altered right-to-left mehano-kinetics which result in an increase of BNP.

It remains difficult to grasp these changes and put them into reliable measurements. Recently, CMR feature tracking has been introduced as a method to quantify ventricular function by use of strain and strain rate—similar to echocardiographic speckle tracking. Possibly, this newer technique may be an approach to unveil the changes in fRV and LV function and their interdependence in EA.

Decreased pulmonary perfusion with physical exercise, or situations of stress that necessitate a high cardiac index over many years, could represent subclinical right heart failure in EA patients. Our results suggest that patients with more severe forms of EA based on CMR methods have decreased exercise capacity, which may entail recurrent or continuous subclinical right heart volume overload and pulmonary hypoperfusion. This is supported by the correlation of BNP and Hct with right heart functional parameters (such as fRV EF, right-sided cardiac volumes, and TR).

It is well known that pulmonary hypoperfusion represents one mechanism of cyanosis in CHD. Possibly, recurrent hypoperfusion may play a role in the observed increase in Hb and Hct in severe EA. However, none of our patients had overt cyanosis and unfortunately, we did not measure oxygen saturation during exercise. Thus, the underlying mechanism of increased Hct and Hb in severe EA remains elusive. Nevertheless, we suggest that increases in Hct and Hb in EA patients should be regarded as markers of caution that these patients may develop clinical heart failure more than those with normal laboratory values.

We suggest that the R/L-Volume-Index in conjunction with BNP should serve as global prognostic parameters in EA and should be evaluated further in larger studies. Concomitant increases in Hb and Hct may add further information and a note of caution to classify an EA patient’s severity of disease and functional impairment.

Study limitations
We examined EA patients in a prospective and observational design without a healthy reference group. However, normal values for laboratory parameters, and CMR functional parameters have been defined previously. We deemed it appropriate to focus on pathological values in a patient cohort and compare these findings within the group to define disease severity and explore correlations. Our sample size was small, which is due to the rarity of the disease and single-centre recruitment. However, our patient cohort is comparable to that of other published studies. Changes in PA flow reserve during exercise were not evaluated, neither was transcatheter oxygen saturation since this was not part of the study protocol. We cannot present a conclusive mechanism for the observed Hb and Hct increase in severe EA. Finally, our data depict a single time-point in EA, which is known to be a progressive disorder. A prospective multicentre study would be needed to confirm our findings and for better risk stratification of this disease.

Conclusions
BNP and Hct were increased in patients with severe EA and correlated well with parameters from functional CMR and cardiopulmonary exercise testing. We suggest that BNP in conjunction with the R/L-Volume-Index from CMR should be employed among other markers of heart failure in the clinical follow-up of EA patients to monitor for disease severity. Additionally, an increase in Hb and Hct in EA patients should be regarded as a hint towards a higher clinical and functional severity of EA. Further large prospective studies are necessary to confirm these findings.

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