Sickle cell disease
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Chapter 7

N-terminal pro-B-type natriuretic peptide, tricuspid jet flow velocity and death in adults with sickle cell disease

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ABSTRACT

Both elevated N-terminal prohormone brain natriuretic peptide (NT-proBNP) levels (>160 pg/mL) and tricuspid regurgitant flow velocity (TRV ≥ 2.5 m/s) have been related to the presence of pulmonary hypertension and risk of early death in patients with sickle cell disease (SCD). Eighty-five consecutive ambulatory SCD patients were followed for 6 years during which 12 deaths occurred. Baseline NT-proBNP levels, TRV and mortality were analyzed. Hazard ratio (HR) for mortality with baseline NT-proBNP ≥ 160 pg/ml was 10.0 [CI 2.9-34.4], \( P < 0.001 \), adjusted for age 6.4 [CI 1.6-25.2], \( P = 0.008 \). HR for mortality with TRV ≥ 2.5 m/s was 1.6 [CI 0.5-5.2], \( P = 0.4 \). After adjustment for elevated TRV the HR for mortality with baseline NT-proBNP ≥ 160 pg/ml was 11.0 [CI 3.1-38.4], \( P < 0.001 \). Elevated NT-proBNP levels were associated with death in SCD, independent of an elevated TRV.
INTRODUCTION

In countries with comprehensive care programs for managing sickle cell disease (SCD) most patients survive into adulthood, even though the median life expectancy of especially sickle cell anemia patients is still under 45 years. [1, 2] Complications such as pulmonary hypertension (PH) have been shown to contribute to mortality in adult sickle cell patients. [3] Several groups have reported echocardiographically defined PH (increased tricuspid regurgitant jet flow velocity (TRV) ≥ 2.5 m/s) to occur in up to one third of adult sickle cell anemia patients and to constitute an important risk factor for early death, with mortality rates as high as 40% in 6 years after diagnosis. [4-8] The actual prevalence of PH in SCD is lower, however, when employing right heart catheterization (RHC). [9, 10] Plasma levels of NT-proBNP have been investigated in SCD in addition to TRV attempting to increase the probability of identifying patients with PH, with recent guidelines advising to use NT-proBNP levels as a surrogate for PH screening if transthoracic Doppler screening is not available. [11-14] NT-proBNP is the biologically inert product of the prohormone proBNP and is released upon atrial stretch and failing of the ventricular myocardium. NT-proBNP levels are increased in patients with heart failure and are strongly associated with the risk of cardiovascular diseases in the general population. [15] Plasma NT-proBNP levels ≥160 pg/ml (the 75th percentile in the NIH sickle cell cohort) [11] have been reported to be predictive of mortality in sickle cell patients. [11, 16] Interestingly, even though both NT-proBNP and TRV may indicate the presence of PH in SCD, in a recent published study 64 patients with sickle cell did have an elevated TRV and 140 patients an elevated NT-proBNP, while only 6.2% of patients had both elevated TRV and NT-proBNP. [8] In order to further elucidate the relation of NT-proBNP, TRV and mortality in SCD we analyzed these parameters in relation to mortality in a well-defined prospective Dutch cohort of patients with SCD.

METHODS

Patients

This study was designed as a prospective cohort study and its design is described elsewhere. [17] In short, consecutive adult outpatients with SCD were included with the following criteria: patients ≥ 18 years with sickle cell anemia (HbSS) or compound heterozygous states HbSβ⁰-thalassemia (HbSβ⁰-thal), HbSβ⁺-thalassaemia (HbSβ⁺-
thal and sickle-hemoglobin C (HbSC). Exclusion criteria were: history of chronic obstructive pulmonary disease, congestive heart failure or poorly controlled asthma, painful crisis and/or acute chest syndrome in the preceding 4 weeks and blood transfusion within 3 months prior to performing the tests (trans-thoracic echocardiograms and laboratory tests). The protocol was approved by the Medical Ethical Committees of participating centers and conducted in agreement with the 2008 revised Helsinki declaration.

Plasma NT-proBNP levels and Trans-thoracic echocardiography

Trans-thoracic echocardiography was performed according to local clinical practice every 2 years in steady state. TRV ≥ 2.5-2.9 m/sec was considered elevated, while a TRV > 2.9 m/sec was considered severely elevated. [4] TRV was considered normal in patients with trace or no tricuspid regurgitation (undetectable values were assigned a value of 1.3 m/s). [4] Plasma NT-proBNP levels were quantitated in EDTA anticoagulated plasma employing an electrochemiluminescence immunoassay (Roche).

Statistical analysis

IBM SPSS Statistics 19, SPSS Inc, Hong Kong, PRC was used for data analysis. HbSS and HbSβ₀-thal patients were grouped together as were the relatively milder HbSC and HbSβ⁺-thal genotypes. Non-parametric data are expressed as median with interquartile range (IQR). To assess differences between groups the Mann-Whitney rank-sum test was used. Kaplan-Meier estimator and Cox proportional-hazards regression were used for survival data analysis. Univariate survival analysis was performed on all variables of interest to obtain hazard ratios (HR) with confidence intervals (CI). Without enough events to do a proper multivariate analysis, only 2 variables were entered at the same time in the Cox proportional-hazards regression. A p value of < 0.05 was considered statistically significant.

RESULTS

Clinical characteristics

Eighty-five patients were followed for a median of 82 months (IQR 75-85) and the mean age at baseline was 34 years (median age 30; IQR 23-47). From 81 patients (56 HbSS/HbSβ₀-thal and 25 HbSC/HbSβ⁺-thal) baseline trans-thoracic echocardiographic results were available. In 25 (31%) a TRV ≥ 2.5 m/s was measured at baseline
(22 (39%) HbSS/HbSβ⁰-thal patients and 3 (12%) HbSC/HbSβ⁺-thal patients). A TRV > 2.9 m/s was measured in two HbSS patients. Twenty of 56 patients who had a TRV < 2.5 m/s at baseline developed an increased TRV (≥2.5 m/s) during follow-up (5.3% per year). From 77 patients (44 HbSS/HbSβ⁰-thal and 23 HbSC/HbSβ⁺-thal) baseline NT-proBNP levels were available. In 14 patients a NT-proBNP value ≥ 160 pg/ml was measured at baseline (all HbSS/HbSβ⁰-thal). Three patients were lost during follow-up, including 1 patient with a TRV ≥ 2.5 m/s (2.65) at baseline with a NT-proBNP level of 362 pg/ml.

**Survival**

During the follow-up period, twelve (14%) patients (11 HbSS and 1 HbSβ⁰-thal) died. Median age at death was 53 years (IQR 37-60) (Table 1). Median TRV at baseline for the patients who died (all-cause mortality) was 2.4 (1.5-2.6) m/s versus 2.1 (1.3-2.5) m/s in survivors (P = 0.28). The death rate for patients with a TRV ≥ 2.5 m/s was 20% versus 12.5% for patients with a TRV < 2.5 m/s (HR of 1.6 [CI 0.5-5.2], P = 0.4) (Figure 1A). Since age at baseline showed a statistically significant correlation with baseline TRV (Sᵣ = 0.265, P = 0.045) (but not to NT-proBNP) we introduced age as a possible confounder in a multi-variate cox-regression analysis, resulting in a HR for mortality in patients with TRV ≥ 2.5 m/s of 1.1 [CI 0.3-3.7], P = 0.9. Age in an univariate analysis resulted in a HR for mortality for patients older than the median age of the cohort (>30 years)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sex</th>
<th>Age at time of death</th>
<th>Cause of death</th>
<th>Place of death</th>
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<tr>
<td>Sβ⁰</td>
<td>M</td>
<td>54</td>
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<td>hospital</td>
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<tr>
<td>SS</td>
<td>M</td>
<td>23</td>
<td>Consequences of tongue carcinoma</td>
<td>home</td>
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<td>F</td>
<td>58</td>
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<td>hospital</td>
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<tr>
<td>SS</td>
<td>F</td>
<td>67</td>
<td>Painful crisis, lactate acidosis, sepsis /reanimation</td>
<td>hospital</td>
</tr>
<tr>
<td>SS</td>
<td>M</td>
<td>57</td>
<td>Kidney failure / Iron overload</td>
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<tr>
<td>SS</td>
<td>M</td>
<td>36</td>
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<tr>
<td>SS</td>
<td>F</td>
<td>74</td>
<td>Unknown</td>
<td>home</td>
</tr>
<tr>
<td>SS</td>
<td>F</td>
<td>61</td>
<td>Chest syndrome, reanimation</td>
<td>hospital</td>
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<td>F</td>
<td>38</td>
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<td>F</td>
<td>49</td>
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<td>hospital</td>
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<tr>
<td>SS</td>
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<tr>
<td>SS</td>
<td>F</td>
<td>51</td>
<td>Myocardial infarction</td>
<td>hospital</td>
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</tbody>
</table>

*Table 1. Causes of death of patients during follow-up.*

MOF=multi-organ failure
of 4.9 (CI 1.1-22.2), \( P = 0.041 \). Since the only deaths that were recorded occurred in the HbSS/HbS\( \beta \)\(^0\)-thal genotype group, the survival analysis was repeated for this genotype group separately. The HR for mortality in HbSS/HbS\( \beta \)\(^0\)-thal patients with a TRV ≥ 2.5 m/s versus patients with a TRV < 2.5 m/s was 1.1 (CI 0.4-3.4), \( P = 0.9 \).

There was a significant difference in plasma levels of hemoglobin, LDH, ferritin, BNP and NT-proBNP between patients who were alive at follow-up compared to levels in the deceased patients. When the analysis was limited to the HbSS/HbS\( \beta \)\(^0\)-thal patients only differences in plasma levels of ferritin, BNP and NT-proBNP between survivors and non-survivors remained statistically significant (Table 2). Using the previously defined cut-off value for NT-proBNP of 160 pg/ml as a risk factor for early death, [11, 16] patients with NT-proBNP levels ≥ 160 pg/ml had a HR of death of 10.0 (CI 2.9-34.4, \( P < 0.001 \)), compared to patients with NT-proBNP levels < 160 pg/ml (Figure 1B). HR for mortality in the HbSS/HbS\( \beta \)\(^0\)-thal genotype group alone for patients with NT-proBNP levels ≥ 160 pg/ml was 6.3 (CI 1.8-21.6, \( P = 0.003 \)) vs patients with NT-proBNP levels < 160 pg/ml.

Figure 1. Kaplan Meier survival curves by tricuspid regurgitant jet flow velocity and N-terminal pro-B type natriuretic peptide.

Grey dotted line: all-cause mortality; grey line: without the patient who died as the consequences of a tongue carcinoma.

1A Kaplan Meier survival analysis of risk of mortality for sickle cell patients with TRV ≥ 2.5 m/s versus patients with TRV < 2.5 m/s. The death rate for patients with a TRV ≥ 2.5 m/s was 20% versus 12.5% for patients with a TRV < 2.5 m/s (Hazard ratio of 1.6 (CI 0.5-5.2, \( P = 0.4 \)).

1B Kaplan Meier survival analysis of risk of mortality for sickle cell patients with NT-proBNP levels ≥ 160 pg/ml versus patients with NT-proBNP levels < 160 pg/ml. The hazard rate for death rate for patients with NT-proBNP levels ≥ 160 pg/ml was 10.0 (CI 2.9-34.4, \( P < 0.001 \)), compared to patients with NT-proBNP levels < 160 pg/ml.
N-terminal pro-B-type natriuretic peptide, tricuspid jet flow velocity and death in adults with sickle cell disease

< 160 pg/ml. Excluding the patient who died of head and neck cancer did not affect the results with regard to both TRV and NT-proBNP. The increased baseline levels of NT-proBNP that were seen in patients who deceased compared to baseline levels in survivors was seen in both patients with TRV < 2.5 m/s (169 (70-565) pg/ml vs. 78 (40-129) pg/ml respectively) as well as in patients with TRV ≥ 2.5 m/s (865 (265-5553) pg/ml vs. 70 (42-114) pg/ml respectively) (Figure 2).

Table 2.

<table>
<thead>
<tr>
<th></th>
<th>HbSS/Sβ⁰-thal</th>
<th>HbSC/Sβ⁺-thal</th>
</tr>
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<tr>
<td>Lost to follow-up</td>
<td>59</td>
<td>26</td>
</tr>
<tr>
<td>Deceased / Alive at follow-up</td>
<td>Deceased 45</td>
<td>Alive 25</td>
</tr>
<tr>
<td>Age at baseline (y)</td>
<td>49 (32-55)</td>
<td>27 (21-42)</td>
</tr>
<tr>
<td>Female/male</td>
<td>8:4</td>
<td>34:11</td>
</tr>
<tr>
<td>On hydroxycarbamide (%)</td>
<td>50 %</td>
<td>38 %</td>
</tr>
<tr>
<td>TRV m/s</td>
<td>2.4 (1.5-2.6)</td>
<td>2.2 (1.3-2.6)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>7.9 (7.1-8.9)</td>
<td>8.9 (7.7-9.7)</td>
</tr>
<tr>
<td>HbF (%)</td>
<td>10.0 (3.1-18.4)</td>
<td>8.3 (5.1-14.9)</td>
</tr>
<tr>
<td>Leukocytes (10e9/l)</td>
<td>9.4 (7.4-11.0)</td>
<td>8.5 (6.9-10.5)</td>
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<tr>
<td>Reticulocytes (%)</td>
<td>6.7 (5.1-9.7)</td>
<td>7.6 (5.9-10.6)</td>
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<td>Bilirubin Total (μmol/l)</td>
<td>30 (22-49)</td>
<td>49 (30-78)</td>
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<td>LDH (U/l)</td>
<td>327 (365-716)</td>
<td>415 (319-629)</td>
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<tr>
<td>Creatinine (μmol/l)</td>
<td>94 (44-125)</td>
<td>51 (42-61)</td>
</tr>
<tr>
<td>Ferritin (μg/l)</td>
<td>968 (387-1700)</td>
<td>205 (77-490)</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>179 (40-540)</td>
<td>45 (25-88)</td>
</tr>
<tr>
<td>NT-proBNP (pg/ml)</td>
<td>177 (77-871)</td>
<td>82 (45-129)</td>
</tr>
</tbody>
</table>

Table 2. Characteristics at baseline of survivors versus non-survivors.
For these analyses all patients are included, also those without TRV measurement at baseline.

In our previous analysis, multivariate regression analysis showed hemoglobin and glomerular filtration rate at baseline to correlate significantly with log transformed NT-proBNP levels in SCD. [17] Given the low number of deaths during follow-up, a multivariate survival analysis was performed for only two variables at time, always including NT-proBNP levels. Including age in the multi-variate cox-regression analysis changed the HR for mortality in patients with NT-proBNP levels ≥ 160 pg/ml in 6.4 (CI 1.1-22.5, \( P = 0.008 \)). None of analyzed variables that could affect NT-proBNP plasma levels (such as age, renal function, TRV or hemoglobin levels) could solely explain the
increased HR for death for patients with NT-proBNP plasma levels of ≥ 160 pg/ml (see supplemental table).

Figure 2. Distribution of levels of NT-proBNP in deceased patients and patients who survived according to baseline values of tricuspid regurgitant jet flow velocity.

Levels between survivors were not significantly different ($P = 0.394$), as were levels between non-survivors ($P = 0.109$).

**DISCUSSION**

In the present study baseline TRV values ≥ 2.5 m/s were not related to the risk of death in this well-defined cohort of 85 adult sickle patients. However, our data confirm that in adult patients NT-proBNP measured at a routine outpatient visit has predictive value for mortality in patients with SCD. [8, 11, 16, 18] NT-proBNP levels have initially been related to the presence of PH in patients with SCD,[11, 12, 16] but as we did not perform RHC in our cohort we cannot determine whether elevated NT-proBNP levels in our patients are indeed reflective of PH in most patients. [9] In our previous report baseline TRV and NT-proBNP values correlated significantly but weakly to each other, [17] whereas others did not find any significant relation between the two. [18] Therefore, elevated NT-proBNP levels in patients with SCD are likely to be determined by many factors such as diastolic dysfunction, [19] inflammation and recurring tissue hypoxia, [18] age, hemolytic anemia, [17] renal function [17] and iron overload. [11, 16, 18] Plasma NT-proBNP as a single test taken in the clinically asymptomatic state may therefore very well be reflective of the severity of the generalized vasculopathy and organ dysfunction characteristic of SCD. Objective tools to accurately assess and predict SCD severity have been sought for decades. [20, 21] Plasma NT-proBNP may
well be a much needed widely available general marker of SCD severity in adult patients for daily clinical practice.

Both the lack of an association of TRV to mortality and the relatively low mortality in our cohort are in contrast with earlier studies in the United States (US). [4-8] Our relatively small sample size may contribute to these discrepancies even though TRV was significantly related to mortality in SCD in a US study of comparable size. [5] The low number of patients with severe PH in our cohort is unlikely to explain these differences as mortality was also high in the NIH PHT Screening Study in patients with a TRV between 2.5 and 2.9 m/s. [22] Furthermore, pertaining to the echocardiographic findings our patient cohort does seem representative as both the prevalence of a TRV>2.5m/s as well as the incidence of TRV elevation during follow-up were comparable to previous data. [5, 7] Exclusion of patients on a chronic transfusion program as well as the relatively younger age of our cohort may, at least in part, explain these discrepancies. The relatively low mortality in our cohort in itself may also explain the lack of a significant association between TRV and mortality. It is unclear at this time what belies the difference in mortality between our cohort and the US cohorts. Mortality was also relatively low in another recently described European SCD cohort. [23] Factors such as genetic differences, differences in study inclusion criteria, health care organization and accessibility, may all contribute.

In conclusion, in this prospective cohort plasma levels of NT-proBNP were associated to mortality in patients with SCD. This association was completely independent of an elevated TRV. We suggest that NT-proBNP might not only be used as a screening tool to identify patients with PH in SCD but also to identify patients at risk of early death independent of the presence of PH. Whether initiating treatment with hydroxycarbamide[14] or intensifying treatments of sickle cell patients with increased levels of NT-proBNP results in clinical benefit should now be subject of prospective clinical trials.
REFERENCES


### Supplemental table.

<table>
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<tr>
<th>Total cohort</th>
<th>Hazard ratio</th>
<th>CI*</th>
<th>P value</th>
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<tr>
<td>NT-proBNP ≥160 pg/ml vs &lt; 160 pg/ml</td>
<td>10.0</td>
<td>2.9-34.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Hazard rate for mortality for NT-proBNP, adjusted for:

- Age
  - Per year: 6.4, 1.6-25.2, 0.008
- TRV
  - Per m/s: 11.0, 3.1-38.4, <0.001
- Genotype
  - HbSS/β° vs HbSC/ HbSβ°+: 6.3, 1.8-21.6, 0.003
- Hydroxycarbamide
  - Yes vs No: 7.8, 2.1-28.7, 0.002
- GFR (CG)
  - < 100 vs ≥ 100 ml/min: 9.2, 2.2-38.9, 0.003
- Hemoglobin
  - Per mmol/l: 8.0, 1.8-34.7, 0.005
- Ferritin
  - Per μg/l: 8.4, 2.2-29.6, 0.002

### HbSS/HbSβ°-thal genogroup

<table>
<thead>
<tr>
<th>Hazard ratio</th>
<th>CI</th>
<th>P value</th>
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<tr>
<td>NT-proBNP ≥160 pg/ml vs &lt; 160 pg/ml</td>
<td>6.3</td>
<td>1.8-21.6</td>
</tr>
</tbody>
</table>

Hazard rate for mortality for NT-proBNP, adjusted for:

- Age
  - Per year: 4.2, 1.1-16.1, 0.036
- TRV
  - Per m/s: 6.9, 2.0-24.0, 0.002
- Hydroxycarbamide
  - Yes vs No: 5.4, 1.5-19.1, 0.009
- GFR (CG)
  - < 100 vs ≥ 100 ml/min: 5.05, 1.1-22.8, 0.035
- Hemoglobin
  - Per mmol/l: 6.64, 1.8-25.1, 0.005
- Ferritin
  - Per μg/l: 5.14, 1.4-18.6, 0.013

### Supplementary Table I.

Multivariate (cox regression) analysis of NT-proBNP.

*95% Confidence Interval.