N-Terminal Pro-Brain Natriuretic Peptide Levels and Risk of Death in Sickle Cell Disease

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Inherited Hemoglobin Disorders are the most common monogenic diseases worldwide. Pulmonary hypertension is an increasingly recognized complication of hemoglobinopathies such as sickle cell disease (SCD) and thalassemia and occurs in 10% to 30% of this population, suggesting that these diseases rank among the most common causes of pulmonary hypertension worldwide. In patients with SCD, pulmonary hypertension is associated with a high mortality rate and has been linked to anemia, high hemolytic rate, iron overload, and systemic vasculopathy. Unexpectedly, high pulmonary pressures do not appear to be associated with rates of vaso-occlusive pain crisis, the acute chest syndrome, or leukocytosis. This lack of association with selected vaso-occlusive events is consistent with a similar high prevalence of pulmonary hypertension in other hereditary hemolytic diseases, such as thalassemia intermedia, in which sickle hemoglobin, vaso-occlusive crisis, and acute chest syndrome are absent. A pathogenic link between pulmonary hypertension and N-terminal pro-brain natriuretic peptide (NT-proBNP) provide such information in patients with idiopathic pulmonary arterial hypertension.

Context Thirty percent of patients with sickle cell disease (SCD) develop pulmonary hypertension, a major risk factor for death in this population. A validated blood biomarker of pulmonary hypertension in SCD could provide important prognostic and diagnostic information and allow the exploration of the prevalence of pulmonary hypertension in participants in the 1996 Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH) Patients’ Follow-up Study. Levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) provide such information in patients with idiopathic pulmonary arterial hypertension.

Objective To determine the relationship between NT-proBNP levels and severity of pulmonary hypertension and prospective mortality in patients with SCD.

Design, Setting, and Participants NT-proBNP levels were measured in 230 participants in the National Institutes of Health (NIH) Sickle Cell Disease–Pulmonary Hypertension Screening Study (enrollment between February 2001 and March 2005) and in 121 samples from patients enrolled starting in 1996 in the MSH Patients’ Follow-up Study. A threshold level predictive of high pulmonary artery pressure and mortality was identified in the NIH Sickle Cell Disease–Pulmonary Hypertension Screening Study and used to define an a priori analytical plan to determine the prevalence and associated mortality of pulmonary hypertension in the MSH follow-up study.

Main Outcome Measures Severity of pulmonary hypertension and risk of all-cause mortality.

Results NT-proBNP levels were higher in patients with sickle cell pulmonary hypertension and correlated directly with tricuspid regurgitant jet velocity in the NIH cohort (R=0.50, P<.001). An NT-proBNP level of 160 pg/mL or greater had a 78% positive predictive value for the diagnosis of pulmonary hypertension and was an independent predictor of mortality (21 deaths at 31 months’ median follow-up; risk ratio, 5.1; 95% confidence interval, 2.1–12.5; P<.001; 19.5% absolute increase in risk of death).

Conclusions Pulmonary hypertension, as indicated by an NT-proBNP level of 160 pg/mL or greater, was very common in patients in the NIH study and in the MSH cohort. The MSH analysis suggests that rates of vaso-occlusive pain episodes in these patients were unrelated to risk of death; this risk was largely determined by occult hemolytic anaemia–associated pulmonary hypertension.
hemolytic anemia is also consistent with the role of intravascular hemolysis in the development of endothelial dysfunction and vasculopathy. However, the idea that pulmonary hypertension does not develop as a consequence of acute chest syndrome and repetitive cycles of pulmonary vascular vaso-occlusion, but rather as a consequence of hemolytic anemia, remains controversial. Additional uncertainty surrounds the contribution of left ventricular diastolic dysfunction to SCD-related pulmonary hypertension.

Brain natriuretic peptide (BNP) is a hormone released in response to cardiomyocyte stretch, and high levels reflect cardiac chamber volume and pressure overload. The prognostic importance of BNP has been demonstrated in several cardiovascular disorders. In patients with pulmonary arterial hypertension, BNP levels correlate with the severity of pulmonary artery pressure elevation and with right ventricular dysfunction. We therefore hypothesized that plasma levels of the N-terminal pro-BNP (NT-proBNP) would correlate with the severity of pulmonary hypertension and prospective risk of death in patients with SCD.

The validation of using NT-proBNP level for the diagnosis of pulmonary hypertension would allow for the evaluation of the prevalence and prognosis of pulmonary hypertension in other historical cohorts of patients, such as the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH). In that randomized, placebo-controlled study, the use of hydroxyurea diminished the incidence of painful episodes and acute chest syndrome. In addition, in a long-term observational follow-up study of these patients, those taking hydroxyurea experienced reduced mortality over 9 years of follow-up. Interestingly, pulmonary and cardiovascular disorders were major causes of death in this cohort, raising the question of the role of undiagnosed pulmonary hypertension as a contributor to mortality.

METHODS

Study Patients and Controls

In the National Institutes of Health (NIH) Pulmonary Hypertension Screening Study, 250 patients with SCD (all genotypes included) were enrolled between February 2001 and March 2005, of whom 230 had NT-proBNP levels measured at baseline. Among these 230 patients, 35 with moderate to severe pulmonary hypertension identified by echocardiography underwent right heart catheterization and functional assessment. Results from 195 of these patients were previously reported. Written informed consent was obtained from each patient for a protocol approved by the Intramural National Heart, Lung, and Blood Institute institutional review board. All laboratory assays were performed using the blood specimens that were collected prospectively at the time of initial enrollment. Forty-five healthy self-identified black individuals recruited from a list of volunteers maintained at the NIH Clinical Research Program volunteer office were evaluated as controls. Racial/ethnic background was assessed because of the known increased prevalence of SCD in certain ethnic populations and was classified by study volunteers.

The validation cohort included a subset of 121 patients enrolled in the MSH Patient’s Follow-up Study, which started in 1996. Laboratory assays were performed using the blood specimens that were collected prospectively at enrollment in the 1996 follow-up study. Three patients enrolled in the MSH cohort were also participants in the NIH Sickle Cell Disease–Pulmonary Hypertension Screening Study and were alive at the time of last follow-up.

Study Measurements

Transthoracic echocardiography was performed in all patients in the NIH cohort as previously described. Pulmonary hypertension was defined by a tricuspid regurgitant jet velocity of 2.5 m/s or greater. Thirty-seven right heart catheterization procedures were performed in 35 patients with tricuspid regurgitant jet velocity of 2.9 m/s or greater. A 6-minute walk test was performed in accordance with standard practice in 34 patients. Twenty-five patients underwent bicycle ergometry using standard incremental protocols as previously described.

Plasma NT-proBNP levels were measured by a sandwich immunoassay (Elecsys Analyzer; Roche Diagnostics, Manheim, Germany) in samples originally frozen and immediately stored at –80°C. The intra-assay and interassay coefficients of variation were 1.3% and 4.8%, respectively, and measurements have been previously performed in samples stored at –80°C for as long as 15 years. Plasma hemoglobin levels were measured on dilutions of patients’ plasma using an enzyme-linked immunosorbent assay (Bethyl Laboratories Inc, Montgomery, Tex).

Statistical Analysis

Results are presented as median and interquartile range (IQR), 95% confidence interval (CI), or percentage of participants with a given characteristic. Glomerular filtration rate was estimated by the abbreviated Modification of Diet in Renal Disease Study equation. Wilcoxon rank sum tests were used to compare the medians of continuous variables in 2 groups. Kruskal-Wallis nonparametric analysis of variance was used to compare NT-proBNP levels in healthy controls and in patients with and without pulmonary hypertension, with a Bonferroni adjustment for comparing differences between 2 groups. Receiver operating characteristic curves, sensitivity, specificity, likelihood ratios, and positive and negative predictive values of NT-proBNP level to diagnose pulmonary hypertension were determined. A test of the null hypothesis that the area under the receiver operating characteristic curve is 0.5 was performed using the Wilcoxon rank sum test. Bivariate correlations were assessed using the Spearman rank correlation coefficient.

In the NIH cohort, proportional hazards (Cox) regression was used to...
study relationships between covariates of interest and mortality in patients with SCD, in some cases log transformed to reduce the influence of extremely large values. Patients were censored at the point of their last contact with study staff if they did not have an event. The regression coefficients were tested for significant differences from zero by a likelihood ratio test. For patients in the MSH cohort, time to event or time to censoring was measured from the date of entry into the MSH Patients’ Follow-up Study. The risk ratio (RR) (hazard ratio) and 95% CI for each predictor were determined and Kaplan-Meier survival curves were calculated. The proportional hazards assumption was evaluated by assessing whether scaled Schoenfeld residuals showed a trend with time. Since this was a registry study, prospective power analysis was not performed.

The results of the analysis from the NIH cohort were used to define a priori the analytical plan for the cohort of patients enrolled in the MSH follow-up study. For the analysis of the relation of mortality to NT-proBNP level in the MSH cohort, a level of 160 pg/mL or greater (corresponding approximately to the 75th percentile for the NIH population) was prospectively defined as abnormal.

Logistic models were used to investigate associations of a variety of factors with NT-proBNP levels of 160 pg/mL or greater. Goodness of fit of the model was assessed using the Hosmer-Lemeshow statistic and a generalized coefficient of determination ($R^2$). Assessment of significance was by likelihood ratio test for the overall model and by ratio of coefficient to estimated standard error for individual predictors. All analyses were performed using SAS version 9.3 (SAS Institute Inc, Cary, NC), NCSS 2004 (Number Cruncher Statistical Systems, Kaysville, Utah), or GraphPad Prism version 4.0 (GraphPad Software, San Diego, Calif). $P<.05$ was considered statistically significant.

### RESULTS

#### NT-proBNP Level Changes

The characteristics of patients evaluated in the NIH cohort and in healthy black controls are shown in Table 1. The median NT-proBNP level was 72 (IQR, 30-160) pg/mL in individuals with SCD and 29 (IQR, 16-49) pg/mL in healthy volunteers ($P<.001$). In patients with pulmonary hypertension, NT-proBNP levels were higher (median, 206 [IQR, 81-701] pg/mL) than those of patients without pulmonary hypertension (median, 47 [IQR, 26-104] pg/mL) and healthy volunteers (median, 29 [IQR, 16-49] pg/mL) ($P<.001$) (Figure 1). Using a tricuspid regurgitant jet velocity of 2.5 m/s or greater as the standard, a logistic value that predicts prospective mortality in the SCD population, the area under the receiver operating characteristic curve using NT-proBNP level to diagnose pulmonary hypertension was 0.81 (95% CI, 0.74-0.87; $P<.001$) (Figure 2). An NT-proBNP level of 160 pg/mL or greater representing the 75th

### Table 1. Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NIH Cohort</th>
<th>MSH Cohort</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Median (IQR)*</td>
<td>No.</td>
</tr>
<tr>
<td>Age, y</td>
<td>230</td>
<td>33 (27-44)</td>
<td>121</td>
</tr>
<tr>
<td>Women, No. (%)</td>
<td>230</td>
<td>138 (60)</td>
<td>121</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>228</td>
<td>9.5 (8.2-10.6)</td>
<td>119</td>
</tr>
<tr>
<td>White blood cell count, ×10^3/µL</td>
<td>228</td>
<td>9.8 (7.6-11.8)</td>
<td>120</td>
</tr>
<tr>
<td>Platelet count, ×10^3/µL</td>
<td>228</td>
<td>346 (272-437)</td>
<td>119</td>
</tr>
<tr>
<td>Reticulocyte count, ×10^3/µL</td>
<td>216</td>
<td>212 (143-340)</td>
<td>110</td>
</tr>
<tr>
<td>Fetal hemoglobin, %</td>
<td>229</td>
<td>5.8 (2.0-11.5)</td>
<td>119</td>
</tr>
<tr>
<td>HbSC disease, No. (%)</td>
<td>229</td>
<td>41 (18)</td>
<td>NA</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>227</td>
<td>0.7 (0.5-0.8)</td>
<td>120</td>
</tr>
<tr>
<td>Blood urea nitrogen, mg/dL</td>
<td>227</td>
<td>8 (6-11)</td>
<td>104</td>
</tr>
<tr>
<td>Lactate dehydrogenase, U/L</td>
<td>206</td>
<td>313 (229-413)</td>
<td>NA</td>
</tr>
<tr>
<td>Alanine aminotransferase, U/L</td>
<td>227</td>
<td>21 (17-33)</td>
<td>112</td>
</tr>
<tr>
<td>Aspartate aminotransferase, U/L</td>
<td>225</td>
<td>35 (24-51)</td>
<td>114</td>
</tr>
<tr>
<td>Alkaline phosphate, U/L</td>
<td>226</td>
<td>96 (69-125)</td>
<td>118</td>
</tr>
<tr>
<td>Total bilirubin, mg/dL</td>
<td>225</td>
<td>2.1 (1.3-3.5)</td>
<td>118</td>
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<tr>
<td>Albumin, g/dL</td>
<td>226</td>
<td>4.1 (3.9-4.3)</td>
<td>119</td>
</tr>
<tr>
<td>Ferritin, mg/L</td>
<td>221</td>
<td>257 (98-937)</td>
<td>NA</td>
</tr>
<tr>
<td>Uric acid, mg/dL</td>
<td>226</td>
<td>5.9 (4.5-7.1)</td>
<td>119</td>
</tr>
<tr>
<td>Tricuspid regurgitant jet velocity, m/s</td>
<td>226</td>
<td>2.3 (2.0-2.6)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: HbSC, hemoglobin SC; IQR, interquartile range; MSH, Multicenter Study of Hydroxyurea in Sickle Cell Anemia; NA, not available; NIH, National Institutes of Health.

†Statistical comparison (Wilcoxon test of location or Z test) made between sickle cell patient populations; values from healthy controls are presented as normal reference and not used for statistical comparison.

SI conversion factors: To convert creatinine values to µmol/L, multiply values by 88.4; blood urea nitrogen values to mmol/L, multiply by 0.357; and bilirubin values to µmol/L, multiply by 17.1.
percentile for the population provided a sensitivity of 57%, a specificity of 91%, a likelihood ratio of 6.33, and a positive predictive value of 78%.

**NT-proBNP Levels and Functional Capacity, Pulmonary Hypertension, and Left Ventricular Diastolic Dysfunction**

NT-proBNP levels negatively correlated with peak oxygen consumption and 6-minute walk distance. Echocardiographic markers of pulmonary hypertension and right ventricular function, including tricuspid regurgitant jet velocity, right atrial area, right ventricular area, and diameter of the vena cava, were associated with high NT-proBNP levels. NT-proBNP levels also correlated with left atrial size and left ventricular mass index and with echocardiographic markers of diastolic dysfunction such as early-late ventricular filling ratio, deceleration time, and early-late mitral annular velocity ratio obtained by tissue Doppler but did not correlate with measures of left ventricular area or ejection fraction (Table 2).

In patients undergoing right heart catheterization, NT-proBNP levels directly correlated with pulmonary artery systolic, diastolic, and mean pressures; pulmonary vascular resistance; and the transpulmonary pressure gradient. NT-proBNP levels also correlated negatively with cardiac output. The association between NT-proBNP level and pulmonary capillary wedge pressure in this relatively small sample of patients was weaker and did not achieve statistical significance (Table 2).

These data suggest that in patients with SCD and pulmonary hypertension, elevations in NT-proBNP levels are primarily determined by right ventricular dilation secondary to intrinsic pulmonary vascular disease, rather than by left ventricular systolic or diastolic dysfunction. However, left ventricular diastolic dysfunction did contribute to pulmonary hypertension in a subgroup of patients with elevated NT-proBNP levels. The correlation between NT-proBNP level and invasive measures of pulmonary hypertension is likely to be stronger in these catheterized patients with more severe pulmonary hypertension than in those individuals with milder elevations in pulmonary arterial pressures.

**NT-proBNP Levels and Mortality (NIH Cohort)**

Two hundred nine patients were alive at the time of last follow-up, with a median follow-up time of 31 (range, 1.3-49) months. There were 21 deaths during the study; in these patients, the median follow-up time was 19 (range, 1.6-42) months. At 40 months the cumulative survival was 94% in patients with NT-proBNP levels of 30 pg/mL or less (25th percentile) and 93% in those with NT-proBNP levels greater than 30 pg/mL and less than 160 pg/mL, whereas it was 74% in patients with NT-proBNP levels of 160 pg/mL or greater (75th percentile). There was no apparent difference in mortality between patients with NT-proBNP levels at or below the 25th percentile (30 pg/mL) and those with levels between the 25th and 75th percentiles ($P=.85$, log-rank test).

The unadjusted risk ratio for death of patients with log-transformed NT-proBNP levels in the 75th percentile compared with those having levels in the 25th percentile was 2.1 (95% CI, 1.4-2.9; $P<.001$) and 1.8 (95% CI, 1.1-2.9; $P=.03$), respectively, after adjustment for age, glomerular filtration rate, and white blood cell count (Table 3). Risk of death at 30 months after enrollment was 2.8% for patients with NT-proBNP levels in the 25th percentile and 22.3% for those with levels in the 75th percentile (difference, 19.5%; 95% CI, 6.2%-32.7%). Ferritin level was also a univariate predictor of death in this cohort (RR, 4.6; 95% CI, 2.1-10.1; $P<.001$), and its addition to the multivariate model decreased the strength of association between NT-proBNP level and mortality (RR, 1.5; 95% CI, 0.9-2.5; $P=.14$), consistent with the previously described association of iron overload with pulmonary hypertension and ventricular dysfunction.$^2$ Adjustment for the presence of systemic
NT-proBNP levels and mortality in sickle cell disease

Table 2. Spearman Correlation Analysis of N-Terminal Pro-Brain Natriuretic Peptide With Functional and Hemodynamic Parameters—NIH Cohort

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No.</th>
<th>R</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional capacity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak oxygen consumption, L/min</td>
<td>25</td>
<td>-0.55</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>6-minute walk distance, m</td>
<td>34</td>
<td>-0.54</td>
<td>.01</td>
</tr>
<tr>
<td>Echocardiography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricuspid regurgitant jet velocity, m/s</td>
<td>226</td>
<td>0.50</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>128</td>
<td>-0.05</td>
<td>.53</td>
</tr>
<tr>
<td>Diameter of vena cava, mm</td>
<td>211</td>
<td>0.22</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Left atrial size, mm</td>
<td>211</td>
<td>0.22</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Right atrial area, cm²</td>
<td>161</td>
<td>0.09</td>
<td>.24</td>
</tr>
<tr>
<td>Right ventricular area, cm²</td>
<td>160</td>
<td>0.28</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Left ventricular mass index</td>
<td>158</td>
<td>0.18</td>
<td>.02</td>
</tr>
<tr>
<td>Right heart catheterization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary artery pressure, mm Hg</td>
<td></td>
<td></td>
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<tr>
<td>Systolic</td>
<td>37</td>
<td>0.59</td>
<td>.002</td>
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<tr>
<td>Diastolic</td>
<td>37</td>
<td>0.37</td>
<td>.02</td>
</tr>
<tr>
<td>Mean</td>
<td>37</td>
<td>0.43</td>
<td>.006</td>
</tr>
<tr>
<td>Pulmonary vascular resistance</td>
<td>37</td>
<td>0.51</td>
<td>.001</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure, mm Hg</td>
<td>37</td>
<td>0.30</td>
<td>.07</td>
</tr>
<tr>
<td>Cardiac output, L/min</td>
<td>37</td>
<td>-0.43</td>
<td>.006</td>
</tr>
<tr>
<td>Transpulmonary pressure gradient</td>
<td>37</td>
<td>0.40</td>
<td>.03</td>
</tr>
</tbody>
</table>

Abbreviations: E/A ratio, early-late ventricular filling ratio; Em/Am ratio, early-late mitral annular velocity ratio; NIH, National Institutes of Health.

Table 3. Cox Proportional Hazards Regression Analysis of Mortality—NIH Cohort

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>RR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted NT-proBNP levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log₁₀(NT-proBNP)</td>
<td>2.1 (1.4-2.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>&gt; 160 pg/mL</td>
<td>5.1 (2.1-12.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Adjusted NT-proBNP levels†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log₁₀(NT-proBNP)</td>
<td>1.8 (1.1-2.9)</td>
<td>.03</td>
</tr>
<tr>
<td>&gt; 160 pg/mL</td>
<td>3.1 (1.2-8.1)</td>
<td>.02</td>
</tr>
<tr>
<td>Age, y</td>
<td>2.6 (1.3-4.9)</td>
<td>.006</td>
</tr>
<tr>
<td>GFR†</td>
<td>1.9 (1.1-3.3)</td>
<td>.02</td>
</tr>
<tr>
<td>White blood cell count</td>
<td>1.6 (1.1-2.4)</td>
<td>.02</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; GFR, glomerular filtration rate; NIH, National Institutes of Health; NT-proBNP, N-terminal pro-brain natriuretic peptide; RR, risk ratio.

*From likelihood ratio test.
†Adjusted for age, GFR, and white blood cell count. For log NT-proBNP, RR for age and white blood cell count is given for 25th relative to 25th percentile, calculated as geometric coefficient × (25th percentile – 25th percentile); RR for GFR is given for 25th relative to 75th percentile, calculated as geometric coefficient × (75th percentile – 75th percentile).
‡Negative association with mortality, i.e., higher values are associated with a lower risk of mortality.

NT-proBNP levels and pulmonary hypertension and mortality (MSH Cohort)

We measured NT-proBNP levels in stored samples from 121 participants in the 1996 MSH Patients' Follow-up Study to explore the prevalence and associated risk of pulmonary hypertension in that well-characterized patient cohort (Table 1). The patients enrolled in the MSH cohort were more anemic, had lower reticulocyte counts, lower fetal hemoglobin levels, and higher alanine aminotransferase levels. These findings likely reflect the greater severity of vaso-occlusive disease in the MSH patients, a criterion for study entry.

During a median follow-up of 47 months (range, 1-61), 24 deaths occurred in the MSH cohort. An abnormal NT-proBNP level (≥160 pg/mL) was selected a priori to identify patients in the MSH cohort with pulmonary hypertension. An NT-proBNP level hypertension (RR, 1.9; 95% CI, 1.2-2.9; P = .008), fetal hemoglobin levels (RR, 2.1; 95% CI, 1.4-3.0; P < .001), hydroxyurea use (RR, 2.1; 95% CI, 1.5-3.1; P < .001), and the number of emergency department visits per year (RR, 2.2; 95% CI, 1.6-3.1; P < .001) did not change the relationship between NT-proBNP levels and mortality. Adjustment for tricuspid regurgitant jet velocity decreased the strength of the association between NT-proBNP levels and the presence of pulmonary hypertension.

Kaplan-Meier estimates of survival demonstrated a mortality break point in patients with NT-proBNP levels at or above the 75th percentile (corresponding to an absolute value of 160 pg/mL) when compared with those individuals having levels below the 75th percentile (Figure 3). Using this cut-off value, the unadjusted RR for death of patients with NT-proBNP levels of 160 pg/mL or greater when compared with those having levels less than 160 pg/mL was 5.1 (95% CI, 2.1-12.6; P < .001) and 3.1 (95% CI, 1.2-8.1; P = .02), respectively, after adjustment for age, glomerular filtration rate, and white blood cell count. Addition of tertritin level to the multivariate model decreased the strength of association between NT-proBNP levels of 160 pg/mL or greater and mortality (RR, 2.5; 95% CI, 0.9-6.7; P = .07). Adjustment for presence of systemic hypertension (RR, 3.3; 95% CI, 1.1-9.3; P = .03), fetal hemoglobin level (RR, 5.1; 95% CI, 2.1-12.5; P < .001), hydroxyurea use (RR, 5.7; 95% CI, 2.2-14.7; P < .001), and the number of emergency department visits per year (RR, 6.2; 95% CI, 2.4-15.8; P < .001) did not change the relationship between NT-proBNP levels of 160 pg/mL or greater and mortality. Adjustment for tricuspid regurgitant jet velocity decreased the strength of the association between NT-proBNP levels of 160 pg/mL or greater and mortality (RR, 2.8; 95% CI, 1.0-7.8; P = .04).
of 160 pg/mL or greater was present in 30% of patients in the MSH cohort in 1996, suggesting a prevalence of pulmonary hypertension that is comparable to that in recent reports.\(^2\)\(^3\)

Sixty-month survival was 94% in patients with NT-proBNP levels of 30 pg/mL or less and 83% in those with NT-proBNP levels greater than 30 pg/mL, whereas it was 51% in patients with NT-proBNP levels of 160 pg/mL or greater. There was no apparent difference in mortality between patients with NT-proBNP levels of 30 pg/mL or less and those with levels greater than 30 pg/mL and less than 160 pg/mL (P = .16, log-rank test).

An NT-proBNP level of 160 pg/mL or greater was a univariate predictor of mortality (RR, 2.8; 95% CI, 1.2-6.6; P = .02) (Figure 3), with risks of death at 47 months after enrollment of 14.1% for patients with levels less than 160 pg/mL and 26% for those with levels of 160 pg/mL or greater (difference, 11.9%; 95% CI, 5.9%-29.8%). This relationship remained significant for log-transformed levels (RR, 2.6; 95% CI, 1.2-4.0; P = .002) and after adjustment for other covariates such as age (RR, 2.7; 95% CI, 1.1-6.7; P = .03), systemic hypertension (RR, 2.8; 95% CI, 1.3-6.6; P = .02), white blood cell count (RR, 2.6; 95% CI, 1.1-6.3; P = .02), fetal hemoglobin level (RR, 2.8; 95% CI, 1.2-6.5; P = .01), use of hydroxyurea (RR, 2.8; 95% CI, 1.2-6.5; P = .02), and rate of vaso-occlusive crisis (RR, 3.1; 95% CI, 1.3-7.4; P = .01). Adjustment for glomerular filtration rate decreased the strength of association between NT-proBNP levels of 160 pg/mL or greater and mortality (RR, 1.9; 95% CI, 0.7-4.9; P = .19). Similarly, in a multivariate model including all these covariates, the risk ratio for death of patients with NT-proBNP levels of 160 pg/mL or greater when compared with those having levels less than 160 pg/mL decreased to 1.9 (95% CI, 0.7-5.4; P = .20).

Mechanisms for Elevated NT-proBNP Level

In the NIH cohort, univariate predictors of a high NT-proBNP level included a low hemoglobin level (R = −.41, P < .001) and high levels of lactate dehydrogenase (R = 0.31, P < .001), aspartate aminotransferase (R = 0.23, P < .001), and total bilirubin (R = 0.29, P < .001), suggesting an association between NT-proBNP and hemolytic anemia. High NT-proBNP levels were also associated with markers of renal dysfunction (high levels of serum creatinine [R = 0.22, P < .001] and blood urea nitrogen [R = 0.26, P < .001]), liver dysfunction (high serum alkaline phosphatase level [R = 0.22, P < .001] and low serum albumin level [R = −0.30, P < .001]), iron overload (high serum ferritin level [R = 0.21, P < .001]), and high serum uric acid level (R = 0.21, P = .001). Advanced age was also correlated with high NT-proBNP levels (R = 0.37, P < .001).

In the MSH cohort, univariate predictors of a high NT-proBNP level included advanced age (R = 0.35, P < .001), low hemoglobin level (R = −0.54, P < .001), and high levels of creatinine (R = 0.24, P < .001), blood urea nitrogen (R = 0.28, P = .004), total bilirubin (R = 0.21, P = .02), and cell-free plasma hemoglobin (R = 0.27, P = .01). There was no correlation between NT-proBNP levels and prospective rates of painful episodes or the acute chest syndrome, hydroxyurea use or fetal hemoglobin levels, and leukocyte and platelet counts. Overall, the strongest association was observed for hemoglobin (Figure 4).

Table 4 illustrates the results of a logistic regression analysis of independent clinical and laboratory factors associated with a high NT-proBNP level. In the NIH cohort these factors included advanced age, low hemoglobin levels, and high levels of lactate dehydrogenase and ferritin, while in the MSH cohort these factors included low hemoglobin levels and high blood urea nitrogen levels.

Log NT-proBNP levels and the proportion of patients with levels of 160 pg/mL or greater were not different in patients originally assigned to receive hydroxyurea when compared with those who received placebo in the MSH trial (P = .93 and P = .59, respectively). In a general linear model with log NT-proBNP level as the outcome and hydroxyurea use and fetal hemoglobin level as the predictors, neither hydroxyurea use (P = .97) nor fetal hemoglobin level (P = .92) were significant, suggesting that hydroxyurea use or fetal

**Figure 3. Kaplan-Meier Survival Curves by N-Terminal Pro-Brain Natriuretic Peptide (NT-proBNP) Level—NIH and MSH Cohorts**

<table>
<thead>
<tr>
<th>No. at Risk</th>
<th>NIH Cohort</th>
<th>MSH Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>160 pg/mL</td>
<td>NT-proBNP &lt;160 pg/mL</td>
<td>NT-proBNP ≥160 pg/mL</td>
</tr>
<tr>
<td>160 pg/mL</td>
<td>&lt;160</td>
<td>172 159 125 95 27</td>
</tr>
<tr>
<td>≥160 pg/mL</td>
<td>160</td>
<td>58 47 37 22 8</td>
</tr>
</tbody>
</table>

*P values were derived from proportional hazards model. Median follow-up, 30 (range, 1.3-49) months in the National Institutes of Health (NIH) cohort and 47 (range, 1-61) months in the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH) cohort.*

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hemoglobin level did not affect baseline level of NT-proBNP.

COMMENT

An analysis of 2 different groups of patients with SCD reveals that NT-proBNP level provides diagnostic and mechanistic information about the development of pulmonary hypertension and identifies patients at the highest risk of death. This is the first readily available laboratory biomarker to provide prognostic information in this population since white blood cell counts and fetal hemoglobin levels were demonstrated to be independent predictors of death in the Cooperative Study of Sickle Cell Disease (CSSCD). Our results suggest that in patients with SCD, an elevated NT-proBNP level largely reflects the severity of right ventricular dysfunction associated with pulmonary arterial hypertension rather than left ventricular filling abnormalities and is strongly associated with advanced age, renal insufficiency, iron overload, and hemolytic anemia. In the CSSCD population, clinical events such as frequent vaso-occlusive crises and acute chest syndrome predicted early mortality. Our data suggest that the number of episodes of vaso-occlusive crisis and the acute chest syndrome are not associated with pulmonary hypertension and that pulmonary hypertension represents the single largest determinant of prospective risk of death in this population.

Brain natriuretic peptide levels have been shown to correlate with functional capacity, severity of pulmonary hypertension, and prognosis in patients with pulmonary arterial hypertension. In catheterized patients in our study, NT-proBNP levels correlated with markers of pulmonary arterial hypertension and right ventricular dilatation to a greater extent than indirect markers of left ventricular dysfunction such as pulmonary capillary wedge pressure. In the entire cohort, NT-proBNP levels correlated strongly with tricuspid regurgitant jet velocity and to a lesser extent with echocardiographic markers of diastolic dysfunction and left atrial size, a finding that has been previously reported in patients with left ventricular diastolic dysfunction. These data suggest that although the etiology of pulmonary hypertension is likely to be multifactorial, pulmonary vascular disease is the dominant pathophysiological process responsible for the pulmonary hypertension observed in patients with SCD and that left ventricular diastolic dysfunction is a significant but less important contributor.

Despite the association between NT-proBNP level and tricuspid regurgitant jet velocity, its sensitivity and specificity for the diagnosis of a tricuspid regurgitant jet velocity of 2.5 m/s or greater is not optimal. However, both assays provide prognostic information, and neither assay represents a gold standard for the diagnosis of pulmonary arterial hypertension. For example, the sensitivity of echocardiography compared with right heart catheterization in patients with other forms of pul-

Table 4. Logistic Regression Model of N-Terminal Pro-Bra

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>NIH Cohort</th>
<th>MSH Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Age, y</td>
<td>3.0 (2.0-6.2)</td>
<td>2.2 (1.3-3.7)</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>0.5 (0.3-0.9)</td>
<td>0.2 (0.1-0.6)</td>
</tr>
<tr>
<td>Lactate dehydrogenase, U/L</td>
<td>2.3 (1.4-4.0)</td>
<td>2.2 (1.3-3.7)</td>
</tr>
<tr>
<td>Ferritin, mg/L</td>
<td>1.5 (1.1-1.9)</td>
<td>1.5 (1.1-1.9)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; MSH, Multicenter Study of Hydroxyurea in Sickle Cell Anemia; NIH, National Institutes of Health; OR, odds ratio.

*Odds ratio is given for 75th relative to 25th percentile, calculated as estimated coefficient/estimated standard error. From Wald χ² test on (estimated coefficient/estimated standard error).2

The negative correlation of NT-proBNP and hemoglobin levels was minimally influenced by hydroxyurea use. MSH indicates Multicenter Study of Hydroxyurea in Sickle Cell Anemia; NIH, National Institutes of Health.

Figure 4. Negative Correlation Between Levels of N-Terminal Pro-Bra

Brain natriuretic peptide levels have been shown to correlate with functional capacity, severity of pulmonary hypertension, and prognosis in patients with pulmonary arterial hypertension. In catheterized patients in our study, NT-proBNP levels correlated with markers of pulmonary arterial hypertension and right ventricular dilatation to a greater extent than indirect markers of left ventricular dysfunction such as pulmonary capillary wedge pressure. In the entire cohort, NT-proBNP levels correlated strongly with tricuspid regurgitant jet velocity and to a lesser extent with echocardiographic markers of diastolic dysfunction and left atrial size, a finding that has been previously reported in patients with left ventricular diastolic dysfunction. These data suggest that although the etiology of pulmonary hypertension is likely to be multifactorial, pulmonary vascular disease is the dominant pathophysiological process responsible for the pulmonary hypertension observed in patients with SCD and that left ventricular diastolic dysfunction is a significant but less important contributor.

Despite the association between NT-proBNP level and tricuspid regurgitant jet velocity, its sensitivity and specificity for the diagnosis of a tricuspid regurgitant jet velocity of 2.5 m/s or greater is not optimal. However, both assays provide prognostic information, and neither assay represents a gold standard for the diagnosis of pulmonary arterial hypertension. For example, the sensitivity of echocardiography compared with right heart catheterization in patients with other forms of pul-

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monary hypertension ranges from 79% to 100%, and the specificity ranges from 60% to 98%. Echocardiography provides additional clinical information, such as the assessment of ventricular and valvular function. Overall, these studies support the use of both screening measurements in this population for the identification of patients with hemodynamically significant pulmonary hypertension who are at high risk for death.

Recent studies reveal a 30% prevalence of pulmonary hypertension in adults with SCD. Using NT-proBNP level as a surrogate for the presence of pulmonary hypertension, we found the same prevalence in the MSH cohort, a finding that indicates that pulmonary hypertension was remarkably common over the last decade in patients with SCD. NT-proBNP level was also an independent predictor of death in both an unselected population of patients with SCD of different severities screened at the NIH and in a selected group of MSH participants with homozygous sickle hemoglobin disease and frequent episodes of vaso-occlusive crises.

In the MSH cohort, the use of hydroxyurea did not appear to protect against the development of pulmonary hypertension or death related to pulmonary hypertension. While this finding is consistent with observations in the NIH cohort, it must be interpreted with caution given the relatively small number of patients included in the MSH analysis, the relatively short duration of hydroxyurea therapy, and the modest associated increases in fetal hemoglobin levels. It is also conceivable that although hydroxyurea decreases hemolytic rate and increases red blood cell survival, the magnitude of this response may not be sufficient to prevent the development of hemolysis-associated endothelial dysfunction. The lack of association between pulmonary hypertension and fetal hemoglobin levels or hydroxyurea use could also have been the result of a survivor effect among patients in the MSH follow-up study.

An apparent central process in the development of pulmonary hypertension in patients with SCD is hemolytic anemia. As a result of hemolysis, hemoglobin is released into plasma, where it reacts with and destroys nitric oxide, resulting in a state of intrinsic resistance to nitric oxide–dependent vasodilation. In addition, hemolysis also releases erythrocyte arginase into plasma, which depletes l-arginine, the substrate for nitric oxide synthesis, by its conversion to ornithine. The association between markers of hemolytic anemia and NT-proBNP level provide further epidemiologic evidence for a mechanistic link between hemolysis and pulmonary hypertension. Hemolysis and impaired nitric oxide bioavailability likely also increase the risk of intravascular thrombosis. Hemolysis also leads to the accumulation of plasma and tissue redox-active heme and iron, which contributes to the generation of reactive oxygen species that can exacerbate ischemia-reperfusion injury, thrombosis, and endothelial and smooth muscle proliferative responses.

A number of clinical complications of SCD are associated with low hemoglobin levels, including pulmonary hypertension, priapism, and leg ulcers, suggesting that there may exist a clinical subphenotype of SCD caused by chronic hemolytic anemia.

In conclusion, these studies suggest that a high NT-proBNP level is associated with pulmonary hypertension and is a major risk factor for death in patients with SCD. Our findings also provide further support for a mechanistic link between hemolytic anemia and pulmonary hypertension. The MSH analysis suggests that rates of pulse episodes in this small sample of seriously ill patients were unrelated to risk of death; we speculate that this risk was largely determined by hemolytic anemia–associated occult pulmonary hypertension.

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**Author Contributions:** Dr Machado had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Analysis and interpretation of data:** Machado, Steinberg, Bonds, Kato, Taveira-DaSilva, Ballas, Blackwelder, Xu, Hunter, Waclawiw, Castro, Gladwin.

**Drafting of the manuscript:** Machado, Anthi, Steinberg, Kato, Blackwelder, Barton.

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**Statistical analysis:** Machado, Blackwelder, Xu, Barton, Waclawiw.

**Obtained funding:** Barton, Gladwin.

**Administrative, technical, or material support:** Anthi, Bonds, Sachdev, Taveira-DaSilva, Ballas, Hunter, Castro, Gladwin.

**Study supervision:** Machado, Bonds, Barton, Gladwin.

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