Haematological determinants of cardiac involvement in adults with sickle cell disease†

Thibaud Damy1,2,3,4,5,6*, Diane Bodez1,2,3,4,5,6, Anoosha Habibi4,7, Aziz Guellich1,2,3,4,5,6, Stéphane Rappeneau1,2,3,4,5,6, Jocelyn Inamo8, Soulef Guendouz1,2,3,4,6, Justine Gellen-Dautremer7, Serge Pissard9, Sylvain Loric10, Orianne Wagner-Ballon11, Bertrand Godeau2,12, Serge Adnot13,14, Jean-Luc Dubois-Randé1,2,3,4, Luc Hittinger1,2,3,4, Frédéric Galactéros2,4,7,12,15, and Pablo Bartolucci2,4,7,12,15

Aims
Cardiac involvement is common in sickle cell disease (SCD). Studies are needed to establish haematological determinants of this involvement and prognostic markers. The aim of the study was to identify haematological factors associated with cardiac involvement in SCD and their impact on prognosis.

Methods and results
This longitudinal observational study was performed on 1780 SCD patients with SS or S-βthalassemia gene deletion referred to our centre. Six hundred fifty-six met our inclusion criteria (availability of a blood-workup and echocardiogram obtained <1 year apart, no heart valve surgery and no current pregnancy). Median age was 31 (interquartile range, 25–40) years, and median haemoglobin (Hb) was 87 (80–95) g/L. Left ventricular (LV) dilation, left atrial dilation, cardiac index (CI) >4 L/min/m², LV ejection fraction <55%, and tricuspid regurgitant velocity (TRV) ≥2.5 m/s were found in 35, 78, 23, 8.5, and 17% of patients, respectively. Compared with other patients, those in the fourth quartiles (Q4) of LV end-diastolic dimension index (LVEDDind) and left atrial dimension index (LAhDind) and those with high CI had significantly lower Hb, % foetal Hb (HbF), and red blood cell (RBC) counts; and significantly higher lactate dehydrogenase, bilirubin, and % dense RBCs. Independent haematologic determinants of Q4 LVEDDind and LADhDind were low RBC count and %HbF; high %dense RBCs were associated with LAhDind. Low %HbF and RBC count were associated with high CI. High %dense RBCs or no α-thalassemia gene deletion was associated with greater severity of anaemia and cardiac dilation and with higher CI. During the median follow-up of 48 (32–59) months, 50 (7.6%) patients died. Tricuspid regurgitant velocity ≥2.5 m/s was a predictor of mortality. The risk of death increased four-fold when left ventricular ejection fraction <55% was present also (P = 0.0001).

Conclusion
Cardiac dilation and CI elevation in patients with SCD are associated with haematologic variables reflecting haemolysis, RBC rigidity, and blood viscosity. Tricuspid regurgitant velocity ≥2.5 and LV dysfunction (even mild) predict mortality.

Keywords
Sickle cell disease • Anaemia • Cardiac remodelling • Haemolysis • Tricuspid regurgitant velocity • Prognosis

†This work has been performed at AP-HP Henri-Mondor Teaching Hospital, Créteil, France.

Received 22 December 2014; revised 21 August 2015; accepted 29 September 2015; online publish-ahead-of-print 29 October 2015

European Heart Journal (2016) 37, 1158–1167
Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2015. For permissions please email: journals.permissions@oup.com.
Introduction

Sickle cell disease (SCD) is one of the most common genetic disorders and has been recognized by the World Health Organization and United Nations as a global public health problem. The prevalence of SCD in industrialized countries is increasing steadily, in part due to therapeutic advances such as exchange transfusion and hydroxyurea therapy.\(^1\)\(^2\) The resulting increase in life expectancy is increasing the frequency of chronic damage to multiple organs, including the heart.\(^3\) Haematological abnormalities induced by the production of haemoglobin S (HbS) include haemolysis, red blood cell (RBC) rigidity, increased blood viscosity, and abnormal RBC adhesion. These abnormalities contribute to cause occlusion of small and large vessels with repeated episodes of ischaemia-reperfusion injury.

The pathogenesis of the organ dysfunctions seen increasingly in adults with SCD is poorly understood. Among organ dysfunctions, cardiac involvement has been associated with increased mortality.\(^3\) Chronic anaemia is associated with cardiac output (CO) elevation, cardiac chamber dilatation, and a compensatory increase in left ventricular (LV) mass; and pulmonary hypertension (PH) can lead to right ventricular (RV) dysfunction.\(^4\) However, the haematological risk factors for cardiac involvement remain unclear. Knowledge of these factors would help to adjust treatment strategies to the level of risk in each individual patient.

Here, our objective was to assess potential correlations between echocardiographic abnormalities and haematological features in adults with SCD. To this end, we conducted a cohort study at an SCD referral centre. We also assessed potential correlations linking echocardiographic and haematological findings to mortality during the median follow-up of 48 months.

Established cardiac risk factors for death in patients with SCD were tricuspid regurgitant velocity (TRV) ≥ 2.5 m/s most notably when left ventricular ejection fraction (LVEF) < 55%.

Methods

Since there is no intervention, IRB is not necessary for this type of studies in France. All participants including patients and controls gave their signed informed consent for study and genetic analyses in accordance with the Declaration of Helsinki.

Patients and controls

Inclusion criteria were SS and S-\(\beta^+\) thalassemia (S-\(\beta^+\) Thal) patients with age > 18 years old in stable condition, outpatient, with both echocardiography and blood tests within the same year as part of standard care, and hydroxyurea-naïve followed at the SCD referral centre of the Henri Mondor Teaching Hospital in Creteil, France, between 1999 and 2011. Exclusion criteria were other genotypes, pregnancy or history of heart valve surgery. Clinical data were considered when prospectively collected.

The control group used to assess the echocardiographic findings was composed of 25 age-matched healthy black volunteers.

Data collection

For each patient, we recorded age, sex, weight, height, and body mass index.

Haematological data

Blood samples were collected during routine outpatient visits. For each haematological variable, we used the mean of two or three measurements performed at steady state, defined as at least 1 month after the last acute event (vasoocclusive crisis, infection, acute chest syndrome, or other event requiring admission) and at least 3 months after the last blood transfusion. We collected the following variables: RBC and reticulocyte counts (LH 780 analyser, Beckman-Coulter, Brea, CA, USA); or manual count if needed), Hb concentration, haematocrit, mean corpuscular cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC), HbF percentage (%HbF, determined by cation-exchange high-performance liquid chromatography using the Variant Hb analyser (Bio-Rad, Hercules, CA, USA), and percentage dense red blood cell (%DRBC; assessed using the plathate density distribution technique\(^6\)\(^5\) with DRBCs defined as having density values > 1.11). Polymerase chain reaction tests on DNA from peripheral blood leukocytes were used to diagnose α-thalassemia (classified as α\(^3\)\(^7\), α\(^2\)\(^0\)\(^5\), or Mediterranean type) using specific primers. All laboratory analyses were performed at the clinical laboratories of the Henri Mondor Teaching Hospital, Creteil.

Echocardiography

All echocardiograms were performed by experienced cardiologists as recommended by the American Society of Echocardiography, using a Vivid Five or Seven machine (GE Healthcare, Chalfont St Giles, UK) at 3.4 MHz. Each recorded value was the mean of three consecutive cardiac cycles. Briefly, transthoracic two-dimensional, M-mode images and Doppler tracings were obtained from parasternal long-axis and apical four- and five-chamber views. Left ventricular end-diastolic dimension (LVEDD) and left atrial dimension (LAD) were measured in M-mode and the corresponding indices (LVEDD-index and LAD-index, respectively) were computed by normalization for body surface area (BSA). Left ventricular ejection fraction was measured using Simpson’s biplane method and considered to indicate systolic LV dysfunction when < 55%. Left ventricular outflow tract (LVOT) diameter was measured on two-dimensional long-axis parasternal views, and cross-sectional aortic area was calculated. Pulsed Doppler was used to estimate the LVOT velocity time integral (VTI) on the apical five-chamber view. Cardiac output was calculated as LVOT VTI cross-sectional aortic area heart rate (HR) and cardiac index (CI) as CO/BSA. Peak early diastolic mitral inflow velocity (E, cm/s) and peak mitral inflow velocity at atrial contraction (A, cm/s) were measured using pulsed wave Doppler, and the E/A ratio was calculated. Mean septal and lateral, early diastolic, mitral-anullos peak velocities (e’, cm/s) were estimated using colour tissue Doppler imaging. Mean septal and lateral E/e’ ratios and peak TRV were determined.

Statistical analysis

Continuous data are described as median (interquartile range) or percentages, as appropriate. We divided echocardiography values into quartiles, given the absence of published data from patients with SCD and no cardiac involvement. The first three LVEDD-index and LAD-index quartiles were combined and compared with the fourth quartiles (Q4). For CI, Q2 and Q3 combined were considered normal and compared with Q1 and Q4. Left ventricular ejection fraction was considered normal if ≥ 55%. Quantitative parameters were compared between groups using the Mann–Whitney non-parametric test. Simple linear regressions were performed for each haematological variable to assess potential associations with echocardiographic variables.

Pearson’s coefficients were computed to assess univariate correlations between haematological and echocardiographic variables. Associations of haematological and echocardiographic variables yielding P-values < 0.05 were entered into a multivariable regression model.
designed to identify haematological variables associated with echocardiographic variables.

Median follow-up was calculated for the whole population using Kaplan–Meier estimation with reverse meaning of the status indicator. We then built a univariate Cox proportional hazard model for a time-to-event analysis of all haematological and echocardiographic variables. The primary endpoint was all-cause mortality. Patients were censored at last follow-up. Variables yielding P-values <0.20 were used to build four multivariate Cox proportional hazard models that included clinical, haematological, echocardiographic, and all significant variables, respectively. Factors associated with mortality were identified by backward stepwise selection. P-values <0.05 were considered significant.

The best TRV cutoff for predicting death within 3 years was determined by receiver-operating characteristic (ROC) curve analysis followed by Youden’s test. Kaplan–Meier curves were plotted, and the log-rank test was performed to compare survival between groups stratified on TRV cutoffs used alone or in combination with other parameters. The probability of death at 36 months was assessed as a continuous function of TRV using a moving average estimator. The size of the averaging window was set to 10% of the available observations. The resultant curve was smoothed using a best-fit polynomial equation.

Statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Study population

Figure 1 is the patient flow chart. Of the 656 included patients, 634 had SS disease and 22 S-β-thal. Median age was 31 years (25–40) and 43% of patients were male. Median values of blood variables were as follows: Hb, 87 g/L (80–95); %HbF, 6% (3.3–10.0); %DRBC, 12% (7.0–19.0); lactate dehydrogenase, 6.01 μkat/L (4.92–7.85); and bilirubin, 50 μmol/L (36–74).

Cardiac involvement in patients with sickle cell disease and comparison with controls

Cardiac involvement diagnosed based on normal echocardiographic cutoff values in the general population was common among patients with SCD. Left ventricular and LA dilation were found in 35% (208/586) and 78% (419/537) of patients, respectively. Cardiac index was >4 L/min/m² in 23% (104/447) of patients and <2 L/min/m² in 24% (109/447). In contrast, LV systolic dysfunction (LVEF <55%) was present in only 8.5% (47/552) of patients, and only three patients had LVEF <35%. Since different TRV thresholds have been reported in the literature, we have determined the prevalence for each TRV intervals. Results are reported in Supplementary material online, Figure S1. Tricuspid regurgitant velocity was ≥2.5 m/s in 17% (114/656) of patients.

Supplementary material online, Table S1 reports the echocardiographic findings in the patients and age-matched controls. The patients had significantly higher LVEDDind, LADind, Cind, and LVEF values. Neither HR nor TRV differed between the two groups.

Associations among echocardiographic variables

Patients in Q4 for LVEDDind and LADind had higher CI values despite lower LVEF and almost similar HR values (Table 1). Patients in Q4 for LVEDDind, LADind, or CI and those with LVEF <55% had significantly higher TRV values LVEDDind and LADind correlated positively with each other (P <0.001, r =0.43). Left ventricular end-diastolic dimension index and LADind were weakly correlated with LVEF (P <0.001, r =0.31; and P = 0.01, r =0.14; respectively) and CI (P <0.001, r =0.23; and P <0.001, r =0.25; respectively). Left ventricular ejection fraction and CI were not correlated with each other (P = 0.6, r =0.03). By multiple regression analysis, TRV was correlated with LADind and LVEF.

Associations between echocardiographic and haematological variables

Patients in Q4 for LV and LA dilation exhibited several differences compared with other patients: lower Hb concentration and RBC count values, indicating greater anaemia severity; higher LDH, bilirubin, and reticulocyte count values, indicating greater haemolysis severity; lower %HbF; and higher %DRBC. Patients in Q4 for CI exhibited similar differences compared with Q2–Q3 patients (Table 1). Hb and RBC values and haemolysis markers increased from Q1 through Q4 of CI values. In contrast, systolic LV dysfunction (LVEF <55%) was not associated with markers for anaemia or haemolysis. Patients with LVEF <55% had higher platelet counts and lower MCV values than did the other patients.

Supplementary material online, Table S2 reports the correlations between echocardiographic and haematological variables. Greater anaemia severity correlated with greater LV and LA dilation, higher CI, and higher TRV. Anaemia severity did not correlate with LVEF, HR, or E/A. Higher reticulocyte counts indicating greater haemolysis severity correlated with greater LV and LA dilation and higher TRV; bilirubin, a marker not only for haemolysis, but also for liver dysfunction, correlated only with LVEDDind and CI. Higher %HbF had protective effects, whereas higher %DRBC was associated with worse dilation and higher TRV.

Table 2 lists the haematological variables associated with the echocardiographic variables. Multivariable regression models identifying the haematologic determinants of the cardiac variables are shown in Table 2. Briefly, LVEDDind was determined by RBC and %HbF;
<table>
<thead>
<tr>
<th>LVEDDind (mm/m²)</th>
<th>P-value</th>
<th>LADind (mm/m²)</th>
<th>P-value</th>
<th>CI (L/min/m²)</th>
<th>P-value</th>
<th>LVEF (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1–Q3 ≤ 33</td>
<td>0.68</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q4 &gt; 33</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>491</td>
<td>156</td>
<td>466</td>
<td>150</td>
<td>129</td>
<td>259</td>
<td>129</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31 (25;40)</td>
<td>31 (25;38)</td>
<td>34 (28;41)</td>
<td>31 (24;41)</td>
<td>31 (24;41)</td>
<td>31 (26;38)</td>
<td>31 (25;40)</td>
</tr>
<tr>
<td>Males (%)</td>
<td>218 (44)</td>
<td>62 (38)</td>
<td>194 (44)</td>
<td>53 (35)</td>
<td>56 (43)</td>
<td>66 (43)</td>
<td>35 (70)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22 (20;24)</td>
<td>20 (19;22)</td>
<td>22 (20;24)</td>
<td>20 (19;22)</td>
<td>22 (20;24)</td>
<td>21 (19;22)</td>
<td>20 (19;24)</td>
</tr>
<tr>
<td>RBC (10^12/L)</td>
<td>250 (176,312)</td>
<td>252 (179,327)</td>
<td>242 (169,304)</td>
<td>265 (200,331)</td>
<td>216 (130,300)</td>
<td>250 (190,310)</td>
<td>216 (130,300)</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>87 (81;93)</td>
<td>89 (81;94)</td>
<td>88 (80;93)</td>
<td>88 (82;95)</td>
<td>85 (76;93)</td>
<td>88 (81;93)</td>
<td>88 (83;95)</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>8 (4.8;7.6)</td>
<td>6.9 (5.4;9.2)</td>
<td>6.0 (4.8;8.7)</td>
<td>6.6 (5.5;8.7)</td>
<td>5.2 (4.3;7.0)</td>
<td>6.1 (5.1;7.9)</td>
<td>6.6 (5.0;8.4)</td>
</tr>
<tr>
<td>%DRBC</td>
<td>12 (6.17)</td>
<td>16 (8.23)</td>
<td>11 (6.17)</td>
<td>15 (10.22)</td>
<td>12 (6.17)</td>
<td>12 (7.17)</td>
<td>15 (8.22)</td>
</tr>
<tr>
<td>LDH (µkat/L)</td>
<td>204 (310;370)</td>
<td>242 (169,304)</td>
<td>242 (169,304)</td>
<td>265 (200,331)</td>
<td>216 (130,300)</td>
<td>250 (190,310)</td>
<td>216 (130,300)</td>
</tr>
<tr>
<td>Platelets (10^9/L)</td>
<td>304 (300,441)</td>
<td>360 (300,441)</td>
<td>360 (300,441)</td>
<td>360 (300,441)</td>
<td>360 (300,441)</td>
<td>360 (300,441)</td>
<td>360 (300,441)</td>
</tr>
<tr>
<td>Tricuspid valve inflow</td>
<td>30 (25;38)</td>
<td>34 (28;41)</td>
<td>31 (25;38)</td>
<td>34 (28;41)</td>
<td>31 (25;38)</td>
<td>34 (28;41)</td>
<td>31 (25;38)</td>
</tr>
<tr>
<td>LA/IVS</td>
<td>22 (20;24)</td>
<td>20 (19;22)</td>
<td>22 (20;24)</td>
<td>20 (19;22)</td>
<td>22 (20;24)</td>
<td>21 (19;24)</td>
<td>21 (19;22)</td>
</tr>
<tr>
<td>CI (L/min/m²)</td>
<td>2.9 (2.6;3.5)</td>
<td>2.6 (2.3;3.3)</td>
<td>2.9 (2.6;3.5)</td>
<td>2.7 (2.3;3.3)</td>
<td>3.1 (2.6;3.8)</td>
<td>2.9 (2.6;3.4)</td>
<td>2.7 (2.4;3.1)</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>87 (80;95)</td>
<td>50 (45;60)</td>
<td>60 (50;70)</td>
<td>60 (50;70)</td>
<td>60 (50;70)</td>
<td>60 (50;70)</td>
<td>60 (50;70)</td>
</tr>
<tr>
<td>E (cm/s)</td>
<td>50 (46.78)</td>
<td>50 (46.78)</td>
<td>50 (46.78)</td>
<td>50 (46.78)</td>
<td>50 (46.78)</td>
<td>50 (46.78)</td>
<td>50 (46.78)</td>
</tr>
<tr>
<td>A (cm/s)</td>
<td>41 (35;51)</td>
<td>41 (35;51)</td>
<td>41 (35;51)</td>
<td>41 (35;51)</td>
<td>41 (35;51)</td>
<td>41 (35;51)</td>
<td>41 (35;51)</td>
</tr>
<tr>
<td>E/A</td>
<td>1.4 (1.2;1.6)</td>
<td>1.6 (1.2;1.6)</td>
<td>1.6 (1.2;1.6)</td>
<td>1.6 (1.2;1.6)</td>
<td>1.6 (1.2;1.6)</td>
<td>1.6 (1.2;1.6)</td>
<td>1.6 (1.2;1.6)</td>
</tr>
<tr>
<td>TRV (m/s)</td>
<td>2.2 (1.9;2.4)</td>
<td>2.3 (1.9;2.5)</td>
<td>2.2 (1.9;2.4)</td>
<td>2.3 (1.9;2.5)</td>
<td>2.2 (1.9;2.4)</td>
<td>2.3 (1.9;2.5)</td>
<td>2.2 (1.9;2.4)</td>
</tr>
</tbody>
</table>

Data are median (interquartile range) unless otherwise indicated.

LVEDDind, left ventricular end-diastolic dimension indexed to body surface area; LADind, left atrial dimension indexed to body surface area; CI, cardiac index; LVEF, left ventricular ejection fraction; BMI, body mass index (kg/m²); RBC, red blood cells; MCV, mean corpuscular volume; Hb, haemoglobin; HbF, foetal Hb; %DRBC, percentage of dense RBCs; LDH, lactate dehydrogenase; E, early transmitral flow velocity; A, late transmitral flow velocity; E’, early diastolic mitral annular velocity; TRV, peak tricuspid regurgitant velocity.
Table 2  Five multivariable regression models to identify the relationship between haematological variables and each echocardiographic variable separately

<table>
<thead>
<tr>
<th>Variables</th>
<th>Multiple R</th>
<th>P-value</th>
<th>Standardized coefficient</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDDind (mm/m²)</td>
<td>0.33</td>
<td>&lt;0.001</td>
<td>-0.25</td>
<td>-1.84; -0.73</td>
</tr>
<tr>
<td>RBC (10¹²/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbF (%)</td>
<td></td>
<td></td>
<td>-0.22</td>
<td>-0.22; -0.07</td>
</tr>
<tr>
<td>LAind (mm/m²)</td>
<td>0.35</td>
<td>&lt;0.001</td>
<td>-0.19</td>
<td>-1.72; -0.35</td>
</tr>
<tr>
<td>RBC (10¹²/L)</td>
<td></td>
<td></td>
<td>-0.15</td>
<td>-0.19; -0.01</td>
</tr>
<tr>
<td>HbF (%)</td>
<td></td>
<td></td>
<td>0.22</td>
<td>0.02; 0.13</td>
</tr>
<tr>
<td>DRBC (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI (L/min)</td>
<td>0.24</td>
<td>0.001</td>
<td>-0.21</td>
<td>-0.47; -0.14</td>
</tr>
<tr>
<td>RBC (10¹²/L)</td>
<td></td>
<td></td>
<td>0.14</td>
<td>-0.04; -0.01</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td></td>
<td></td>
<td>-0.17</td>
<td>-0.21; -0.01</td>
</tr>
<tr>
<td>Platelets (10⁹/L)</td>
<td>0.26</td>
<td>0.024</td>
<td>-0.19</td>
<td>-0.002; -0.014</td>
</tr>
<tr>
<td>TRV (mmHg)</td>
<td>0.18</td>
<td>&lt;0.001</td>
<td>0.19</td>
<td>0.35; 0.73</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td></td>
<td></td>
<td>-0.16</td>
<td>-0.086; -0.006</td>
</tr>
<tr>
<td>HbF (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LVEDDind, left ventricular end-diastolic dimension indexed to body surface area; LAind, left atrial dimension indexed to body surface area; CI, cardiac index; LVEF, left ventricular ejection fraction; TRV, tricuspid regurgitant velocity; RBC, red blood cells; %HbF, percentage of foetal haemoglobin; %DRBC, percentage of dense RBCs; MCV, mean corpuscular volume.

Risk factors for mortality

Median follow-up was 48 (32, 59) months in the overall population. Of the 656 patients, 50 (7.6%) died and 38 (5.8%) were lost to follow-up and censored at their last visit. Factors significantly associated with mortality by univariate analysis (P < 0.20) in this patient subset were older age, lower LVEF, higher %DRBC, and higher TRV.

By multivariate analysis, TRV was a predictor of mortality (Table 3). The best TRV cutoff for predicting mortality was 2.5 m/s (area under the ROC curve, 0.70; 95% confidence interval, 0.57–0.83; P = 0.0001). The Kaplan–Meier analyses are shown in Figures 2 and 3. The subgroup of 36 patients with both TRV ≥ 2.5 m/s and LVEF < 55% had the shortest survival time, with a median of mortality of 4 years. Additional differences between this subgroup and the other patients were significantly higher LDH values and trends toward higher %DRBC and reticulocyte count values. Of the nine patients in this subgroup who underwent genetic testing for α-thalassemia, seven had no deletions and two had one deletion.

The probability of death at 36 months was assessed as a continuous function of TRV using a moving average estimator Figure 4. The probability of death was low in patients with a TRV < 2.5 m/s. Beyond this value, the risk evolves linearly with 50% probability of death at TRV ~ 3.2 m/s.

Discussion

Cardiovascular events were a major cause of death among adults with SCD in numerous studies. The pathophysiology of cardiac involvement in SCD remains unclear. In our study, LV and LA dilation, cardiac output elevation, and increased TRV were common. Cardiac involvement was significantly associated not only with

Haematologic and echocardiographic variables according to α-thalassemia status and percentage dense red blood cells (Supplementary material online, Table S5)

Genetic testing for α-thalassemia was performed in 358 patients. Patients with one or no α⁻³.⁷ deletions had greater severity of anaemia and haemolysis compared with those with two α⁻³.⁷ deletions. No significant differences were noted between patients with one vs. no α⁻³.⁷ deletion. Compared with patients with two α⁻³.⁷ deletions, those with one α⁻³.⁷ deletion had greater LV and LA dilation and lower CI with similar LVEF values.

Patients in Q4 for %DRBC had significantly greater LV and LA dilation than did the other patients.
markers for haemolysis, but also with %HbF, %DRBC, and α-thalassemia status. Tricuspid regurgitant velocity $\geq 2.5$ m/s was associated with 3-year mortality. The risk of death was greatest among patients who had both TRV $\geq 2.5$ m/s and LVEF $\geq 55\%$ (Figure 3).

**Cardiac involvement in sickle cell disease**

The pathophysiology of left-sided heart disease is not well studied in SCD. The disease process is probably multifactorial with multiple overlapping mechanisms that lead to both cardiac structural and functional modifications. The major admitted mechanism is the compensatory high output state and volume overload due to SCD anaemia and chronic tissue hypoxia. In our study, over 25% of patients with SS disease had LV and LA dilation and $\approx 25\%$ had high, and 25% low, CO values, in keeping with earlier clinical and autopsy studies. In contrast, systolic LV function as assessed based on LVEF was normal in most patients in our study and in previous work involving screening echocardiography. However, the absence of correlation between LVEF and CI, together with the low CI values in about one-fourth of patients, suggests underestimation of the prevalence of systolic LV dysfunction. A study of the end-systolic stress–volume index at rest demonstrated depressed LV contractile performance with compensation by an increase in stroke volume via a preload increase and afterload decrease, with the result that LVEF remained normal.

**Correlations between haematological and echocardiographic variables**

Several previous studies in SCD showed normal LV function in patients with severe chronic anaemia, whereas others documented varying degrees of LV dysfunction with significantly decreased fractional shortening or normal systolic time intervals. The chronic anaemia of SCD results in a hyperdynamic state with enhanced or near-normal LV function and decreased peripheral vascular resistance. Despite the increase in CO, HR is only slightly elevated. The abnormal loading conditions associated with anaemia lead to LV dilation with an increase in LV stroke volume. Other factors including iron overload, immunogenic factors, microcirculation damage due to vaso-occlusive crisis, associated valvular disease, may all participate in cardiac remodelling and dysfunction.

### Table 3 Variables associated with all-cause mortality in the overall population

<table>
<thead>
<tr>
<th>Univariate</th>
<th>Wald</th>
<th>P-value</th>
<th>HR (95% confidence interval)</th>
<th>Multivariate yielding P &lt; 0.20</th>
<th>Wald</th>
<th>P-value</th>
<th>HR (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>3.51</td>
<td>0.06</td>
<td>1.03 (0.99–1.06)</td>
<td></td>
<td>Age (years)</td>
<td>4.45</td>
<td>0.035</td>
</tr>
<tr>
<td>Sex, male vs. female</td>
<td>2.60</td>
<td>0.11</td>
<td>1.60 (0.91–2.75)</td>
<td></td>
<td>Sex, male vs. female</td>
<td>2.55</td>
<td>0.11</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.54</td>
<td>0.46</td>
<td>0.46 (0.95–1.13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRBC (%)</td>
<td>2.87</td>
<td>0.09</td>
<td>1.04 (0.99–1.09)</td>
<td></td>
<td>DRBC (%)</td>
<td>2.87</td>
<td>0.09</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>0.49</td>
<td>0.48</td>
<td>1.44 (0.52–3.97)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbF (%)</td>
<td>0.23</td>
<td>0.64</td>
<td>0.97 (0.86–1.10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>0.22</td>
<td>0.64</td>
<td>0.97 (0.86–1.10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC (10¹²/L)</td>
<td>0.11</td>
<td>0.74</td>
<td>0.65 (0.47–0.86)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRV (m/s)</td>
<td>15.21</td>
<td>0.0001</td>
<td>3.92 (1.97–7.78)</td>
<td>TRV (m/s)</td>
<td>15.75</td>
<td>&lt;0.001</td>
<td>6.81 (2.64–17.56)</td>
</tr>
<tr>
<td>LVEDDind (mm/m²)</td>
<td>2.72</td>
<td>0.11</td>
<td>0.88 (0.75–1.03)</td>
<td>LVEDDind (mm/m²)</td>
<td>2.58</td>
<td>0.11</td>
<td>0.90 (0.80–1.02)</td>
</tr>
<tr>
<td>LAVind (mm/m²)</td>
<td>0.43</td>
<td>0.84</td>
<td>0.99 (0.90–1.10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>0.21</td>
<td>0.65</td>
<td>1.01 (0.98–1.04)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI (L/min/m²)</td>
<td>0.59</td>
<td>0.44</td>
<td>0.84 (0.54–1.31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>0.016</td>
<td>0.90</td>
<td>1.00 (0.93–1.07)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/A</td>
<td>0.96</td>
<td>0.33</td>
<td>0.39 (0.06–2.53)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/e'</td>
<td>0.59</td>
<td>0.44</td>
<td>1.15 (0.81–1.62)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRV (m/s)</td>
<td>9.40</td>
<td>0.002</td>
<td>5.41 (1.84–15.9)</td>
<td>TRV (m/s)</td>
<td>11.9</td>
<td>0.001</td>
<td>4.72 (1.95–11.43)</td>
</tr>
<tr>
<td>LVEDDind (mm/m²)</td>
<td>0.63</td>
<td>0.43</td>
<td>0.95 (0.83–1.08)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRBC (%)</td>
<td>0.38</td>
<td>0.54</td>
<td>0.98 (0.93–1.04)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.18</td>
<td>0.89</td>
<td>1.00 (0.95–1.06)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex, male vs. female</td>
<td>0.08</td>
<td>0.77</td>
<td>0.87 (0.33–2.28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations as in Tables 1 and 2. HR, hazard ratio.
By multivariate analysis, greater anaemia severity as reflected by lower RBC counts and Hb levels, as well as qualitative RBC abnormalities were associated with LV enlargement and CI elevation in our study. Our finding that %HbF was associated with LVEDDind, LADind, CI, and TRV is consistent with the ability of HbF to limit HbS polymerization and thus sickling and to improve RBC properties. This protective effect contrasts with the hypothesis that increased level of HbF leads to a lower tissue oxygen delivery due to its high affinity for oxygen. Thus, it is supposed that high HbF percentage may enhance the ‘anaemic’ stimulus and leads to increased cardiac output and cardiac dilatation. This was not supported by our data. We hypothesize that HbF, by decreasing the

**Figure 2** Kaplan–Meier estimates of overall survival according to the best tricuspid regurgitant velocity cutoff determined by receiver-operating characteristic curve analysis and Youden’s test. Patients with unmeasurable tricuspid regurgitant velocity were included in the group with tricuspid regurgitant velocity <2.5 m/s.

**Figure 3** Kaplan–Meier estimates of overall survival according to tricuspid regurgitant velocity cutoff and left ventricular ejection fraction.
HbS polymerization rate, leads to a better oxygen transport. We have recently demonstrated that SS RBCs containing higher levels of HbS polymers have an impaired ability to carry oxygen to tissues and thus, could not be as efficient as RBCs containing higher %HbF, in terms of oxygen delivery despite their lower oxygen affinity. Moreover, haemorrhheological properties are impaired in SS RBCs containing lower HbS polymers. Other adaptive mechanisms including corpuscular ones may overcome this "anaemic stimulus". Metabolic changes have been proposed including of calcium, zinc, and calmodulin that might play an important role in the intrinsic adaptation of red cell.

Our results suggest that RBC counts and qualitative properties may be more closely associated with cardiac abnormalities than the Hb level. We also found that LA dilation was associated with %DRBC, a subpopulation of RBCs characterized by a low HbF content, high MCHC and high density (>1.11). Higher %DRBC was associated with increases in haemolysis, RBC rigidity, and blood viscosity. Dense red blood cells have increased the MCHC related, in part, to dehydration due to potassium loss. Dense red blood cell having a very shortened lifespan, their percentage is associated to the haemolysis level. The increased rigidity and tendency to aggregate exhibited by DRBCs result in an increase in blood viscosity. Finally, DRBCs may have a limited O2 transport capacity that increases the severity of tissue oxygen deprivation over that expected from the total Hb level. Our group has reported a higher prevalence of chronic organ damage with high %DRBC values.

Absence of α-thalassemia deletions was associated with greater LV and LA dilation and with lower CI values. These deletions are known to decrease intracellular HbS concentrations and therefore %DRBC values and to improve SS RBC deformability. These effects decrease the risk of damage to organs, most notably the kidneys.

**Prognostic value of echocardiographic and haematological variables**

In a recent epidemiological study, most of deaths were attributed to cardiovascular causes. Tricuspid regurgitant velocity has been extensively studied in SCD as a marker for PH, and TRV elevation is among established risk factors for death. It is hypothesized that high output state leads to LV dilation and irreversibly damages the pulmonary vasculature and contributes to the pathogenesis of PH. In our study, TRV ≥ 2.5 m/s was a predictor of 3-year mortality. The adverse prognostic impact of PH has been reported previously and ascribed to pulmonary artery vasculopathy. Right-heart catheterization studies done by our group and others in adults with SCD have established that PH cases are equally divided between a pre-capillary mechanism, i.e. pulmonary vascular remodelling, and a post-capillary mechanism, i.e. LV dysfunction. In keeping with these findings, TRV in the present study was associated with lower LVEF and higher LADind, two changes usually associated with high LA filling pressures and, therefore, with a risk of post-capillary PH. The risk of death was highest in patients with both TRV ≥ 2.5 m/s and LVEF ≤ 55%.

In a cohort of 263 adults with SCD, TRV correlated strongly with markers for haemolysis including bilirubin and LDH. We also found correlations between echocardiographic abnormalities and markers for haemolysis such as low Hb, high bilirubin, and high %DRBC. Although the mechanisms by which haemolytic anaemia leads to pulmonary vasculopathy are incompletely understood,
nitric oxide (NO) may play a central role in the pathophysiology of PH complicating SCD.\textsuperscript{2} Haemoglobin and haem released by RBCs during haemolysis increase the oxidative stress and scavenge NO, decreasing its bioavailability and inducing damage to endothelial cells.\textsuperscript{48,49} Furthermore, arginase released by RBCs degrades the NO precursor arginine, thereby limiting the production of NO.\textsuperscript{46,47}

In clinical practice, a small decrease in LVEF is not considered as alarming sign. Moreover, right catheterization is not systematically performed in front of a TRV $>2.5$ m/s because with this cutoff, only 25% shows PH.\textsuperscript{41} The present study shows that patients with even a small increase in TRV and a small decrease in LVEF ($<55\%$), they had a significant higher risk of mortality. This should alert the practitioner to adapt therapeutically strategies.

**Clinical implications**

This study indicates that echocardiography, a low-cost and easily accessible exam, provides valuable information for risk stratification in SCD patients. It should be systematically performed in the follow-up these patients.

Special attention should be given in presence of any TRV beyond $2.5$ m/s, especially when LVEF is $<55\%$ as they indicate poor prognosis. Thus any change in these parameters should alert the practitioner to adapt therapeutic strategies and adopt effective measures to prevent cardiovascular complications in order to improve the overall survival of those patients.

**Limitations**

The single-centre design may limit the general applicability of our findings. Furthermore, the full range of haematological variables was not available for all patients. We did not separate pre-capillary PH from post-capillary PH, as right heart catheterization was not performed routinely. Our study has focused mainly on echocardiographic variables and their haematologic determinant. Therefore, other parameters such as coronary artery disease, arterial hypertension, and vascular disorders may also participate in the global effect on cardiovascular observed in SCD.\textsuperscript{48,49}

**Conclusion**

Left cardiac chamber dilation was common in our adult patients with SCD. This abnormality was associated with greater anaemia and haemolysis severity, higher %DRBC, lower %HbF, and absence of concomitant a-thalassemia deletions. TRV $>2.5$ m/s predicted death within 3 years, and the risk of death in patients with TRV $>2.5$ m/s was increased near four-fold when LVEF was $<55\%$. These findings support routine echocardiographic monitoring of adults with SCD to detect risk factors for mortality. The potential effects of treatments on these risk factors and mortality remain to be investigated.

**Supplementary material**

Supplementary material is available at European Heart Journal online.

**Authors’ contributions**


**Acknowledgement**

We thank A Wolfe MD for editing the English-language.

**Funding**

This work was supported by AREMCAR (Association pour la Recherche et l’Etude des Maladies Cardiovasculaires), a non-profit organization.

**Conflict of interest:** none declared.

**References**


