Globin chain synthesis in myelodysplastic syndromes

G Chalevelakis, S Karaoulis, A G Yalouris, Th Economopoulos, N Tountas, S Raptis

Abstract
Globin chain synthesis was studied in the reticulocytes of 30 patients with various myelodysplastic syndromes (MDS) to determine the α/β globin chain synthetic ratio and its probable prognostic value. The mean (SD) value of the total α/β ratio was 0.82 (0.45) ranging from 0.05 to 1.73. The same ratio in 10 normal controls was 1.01 (0.04). This difference was significant. Furthermore, the α/β ratios were lower than normal in 14 patients (α-thalassaemia-like) (group I), almost within normal limits in 11 (group II), and higher than normal in 5 (β-thalassaemia-like) (group III). In each group almost all the FAB subtypes were represented. The addition of exogenous haem in several of the test samples resulted in a slight to pronounced increase in the α/β ratios, particularly in group I. In 92% of the high risk cases (refractory anaemia with excess blasts (RAEB), chronic myelomonocytic leukaemia (CMML)) or 87.5% of patients who finally developed acute non-lymphoid leukaemia (ANLL) low or normal α/β ratios were found. No significant correlation was noticed between α/β ratios and various haematological variables or survival.

It is concluded that in MDS the α/β ratio varied enormously across the entire population of patients, as well as within each FAB subtype, thereby restricting its prognostic value. Although haem deficiency may be implicated in some cases of MDS, why this should be remains unclear.

Myelodysplastic syndromes (MDS) are clonal disorders of the bone marrow, characterised by refractory peripheral cytopenias, despite an adequate bone marrow cellularity, and by a high incidence (20–60%) of progression to acute non-lymphoid leukaemia (ANLL).1-3 The morphological abnormalities of erythroid cells in both bone marrow (erythroid hyperplasia, megaloblasts, sideroblasts, multinuclearity) and peripheral blood (anisopikilocytosis, hypochromia),1 similar to those observed in thalassaemic syndromes, suggest disordered globin chain synthesis. Previous biosynthetic studies of selected patients, particularly those with primary sideroblastic anaemia, refractory anaemia with excess of blasts, or acute leukaemia, have produced conflicting results, ranging from a normal to a strongly imbalanced (lower or higher than unity) α/β ratio.6-11 Regarding sideroblastic anaemia, a recent report of normal α/β ratios which had not been affected by exogenous haem,12 contrasted with the widely accepted notion of an imbalance in α and β globin chain synthesis (diminished α/β ratio) and its correction by exogenous haem.6-7 We therefore decided (a) to determine the α/β ratio, relating it to various haematological variables, (b) to test whether it was affected by the addition of haem, and (c) to evaluate its possible prognostic value in a large series of unselected patients with MDS.

Methods
Thirty patients with MDS, 20 male and 10 female aged 61–84 (mean 73) years participated in the study. According to the FAB (French-American-British group) classification13 these patients were classified as follows: refractory anaemia two patients; refractory anaemia with ring sideroblasts (RAS) five; refractory anaemia with excess of blasts (RAEB) nine; RAEB in transformation (RAEB-T) six; and chronic myelomonocytic leukaemia (CMML) eight. All patients were transfusion dependent and the last transfusion had been given not less than three weeks previously. No patient had been treated with cytotoxic drugs. Ten haematologically and clinically normal subjects, aged 55–80 (mean 72) years, served as controls for peripheral blood studies and globin chain synthesis. Patients with HbA2 above 3.4%, or an abnormal osmotic fragility test, were not included so as to minimise the possibility of studying cases of heterozygous thalassaemia. The standard haematological determinations were performed as described by Dacie and Lewis.14

GLOBIN CHAIN SYNTHESIS
Peripheral blood (10 ml) was immediately transferred to tubes containing heparin. The leucocytes were removed using the method of Beuter et al.15 Then after washing three times in reticulocyte saline (NaCl 130 mmol/l, MgCl2,7·4 mmol/l, KCl 5 mmol/l) at 4°C, 0·6 ml of cells were removed from the top layer and resuspended in reticulocyte saline at 4°C. Two samples of 0·3 ml cells were obtained. These cells were suspended in three volumes of incubation mixture.16-18 The samples had been preincubated for 15 minutes as follows. Sample A was the control; sample B (used only in 16 cases) (table 1) contained...
haemin (Sigma Chemical Co), dissolved (by shaking it in boiling hot water for five minutes) in Na₂CO₃ (100 mmol/l) buffer (pH 8) at a final concentration of 0·4 mmol/l. Leucine (5·32 GBq/l) (148 × 10⁻⁹ Ci/l) (H) (Radiochemical Centre, Amersham) specific activity 2TBq mmol/l or 50 Ci/mmol was added in both samples. The incubation was stopped two hours later by adding a large volume of ice cold reticulocyte saline in which the cells were washed four times at 4°C.

DETERMINATION OF RADIOACTIVITY

Globin preparation, globin chain separation, and the determination of radioactivity incorporated in the globin chains were done according to previously described protocols.¹⁷¹⁸

Briefly, whole cell globin was prepared by the acid acetone method and the globin chains were separated using carboxymethyl cellulose (CM₅₉)-cellulose in 8M urea, 50 mmol/l mercaptoethanol with a linear Na₂HPO₄ (starting buffer 5 mmol/l), strong buffer 40 mmol/l gradient at pH 6·8. Aliquots of 0·2 ml for each fraction were mixed with 1 ml water and 10 ml of Bray’s scintillation fluid¹⁹ and counted for 10 minutes. The results were expressed as total counts incorporated into each chain per minute per ml (cpm/ml). For the determination of the specific activity the contents of the peak tube of each globin chain were dialysed against 12 hourly changes of 5 litres of 0·5% formic acid. The absorbency was then measured at 280 nm and 0·5 ml samples were mixed with 10 ml Bray’s solution for the determination of radioactivity. All samples were counted in duplicate to a minimum of 20 000 accumulated counts.²⁰

For statistical analysis Student’s paired t test and Pearson’s regression analysis were used when our data followed normal distribution; otherwise, we used the respective non-parametric tests (Wilcoxon’s rank sum test, Spearman’s correlation coefficient). Survival scores were estimated according to the Kaplan-Meier method.²¹

Results

The results are given in tables 1, 2, and 3.

GLOBIN CHAIN SYNTHESIS

Ten controls had α:β ratios close to unity (range 0·94 to 1·07, mean value 1·01 (0·04). These ratios were within the reported range for normal numbers of reticulocytes.¹³¹⁸²² The mean value of the α:β ratio in the patients with MDS was 0·82 (0·45). The difference was significant (p < 0·01). The specific activity ratio was measured in 15 cases and it was repeatedly close to the total count ratio. Fourteen (46%) patients (group I) had low ratios ranging from 0·05 to 0·75 (α-thalassaemia-like). Four were classified as CMML, four as RAEB-T, three as RAEB, two as RAS, and one as refractory anaemia. Eleven (37%) patients (group II) had α:β ratios almost within normal limits, ranging from 0·80 to 1·11 (RAEB five, CMML four, RAEB-T one and RAS one). The rest of the patients (17%) (group III) had slightly to noticeably raised α:β ratios, ranging from 1·26 to 1·73 (β-thalassaemia-like) refractory anaemia one, RAS two, RAEB one and RAEB-T one (table 2). There was no evidence of thalassaemia in family members, siblings, and children. Six patients with increased Hb F concentrations ranging from 2·8 to 10 (5·71 (2·75)) were equally distributed in groups I and II (RAS one, RAEB-T one, refractory anaemia one, RAEB two, CMML one). Hb A₂ was within normal limits, ranging from 2·9 to 3·4 (2·7 (0·48)), and none of the patients had inclusions of Hb H.

Within each of the five RAB subtypes a great variation in α:β ratios was observed; the differences in their mean values were not significant. A high proportion (66%) of patients with RAEB had normal ratios, whereas the patients with RAS and CMML had equally low and high, or low and within the normal range ratios, respectively (table 3). Eight (27%) patients finally progressed to ANLL (RAEB four, RAEB-T one, and CMML three). Four (RAEB one, RAEB-T one, CMML two) fell into group I (α:β ratio 0·29–0·60), three (RAEB two, CMML one) to group II (α:β ratio 0·80–1·08), and one (RAEB) into group III (α:β ratio 1·70). Ninety two percent (13/14) of patients with RAEB-T and CMML and 87·5% (seven of eight) of those who eventually developed ANLL had low or normal α:β ratios. The mean value of the α:β ratio in this subgroup of patients was 0·79 (0·48). The difference from the entire population of patients was not significant.

HEMATOLOGICAL DATA AND THEIR CORRELATION WITH THE α:β RATIO

The observed differences in the mean values of the haematological findings among the three principal groups were not significant, except

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**Table 1** Effect of haemin (0·4 mmol/l) on α:β globin synthetic ratio in 16 patients with various subtypes of MDS

<table>
<thead>
<tr>
<th>Case No</th>
<th>1</th>
<th>2</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>15</th>
<th>16</th>
<th>18</th>
<th>19</th>
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<th>26</th>
<th>27</th>
<th>28</th>
<th>30</th>
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<tr>
<td>FAB subtype</td>
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<tr>
<td>CMML</td>
<td>RAEB</td>
<td>RAS</td>
<td>RAEB</td>
<td>CMML</td>
<td>RAEB-T</td>
<td>RAEB-T</td>
<td>RAEB</td>
<td>CMML</td>
<td>RAEB</td>
<td>RAS</td>
<td>RAEB-T</td>
<td>RAEB</td>
<td>CMML</td>
<td>RAEB</td>
<td>RAS</td>
<td>RAEB-T</td>
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<tr>
<td>Mean (SD) value</td>
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<tr>
<td>Without haemin (A)</td>
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<td>0·58</td>
<td>0·38</td>
<td>0·05</td>
<td>0·06</td>
<td>0·75</td>
<td>0·62</td>
<td>1·00</td>
<td>0·92</td>
<td>1·08</td>
<td>1·11</td>
<td>1·00</td>
<td>1·05</td>
<td>1·34</td>
<td>1·60</td>
<td>1·70</td>
<td>1·26</td>
<td>0·91 (0·48)</td>
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<tr>
<td>With haemin (B)</td>
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<tr>
<td>0·79</td>
<td>0·80</td>
<td>0·09</td>
<td>0·87</td>
<td>0·82</td>
<td>0·72</td>
<td>1·05</td>
<td>0·93</td>
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<td>1·23</td>
<td>1·28</td>
<td>1·85</td>
<td>1·43</td>
<td>1·62</td>
<td>1·70</td>
<td>1·43</td>
<td>1·11 (0·49)</td>
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<tr>
<td>Percentage change</td>
<td>+36</td>
<td>+105</td>
<td>+80</td>
<td>+1350</td>
<td>+9</td>
<td>+16</td>
<td>+5</td>
<td>+1</td>
<td>+5</td>
<td>+11</td>
<td>+28</td>
<td>+76</td>
<td>+7</td>
<td>+1</td>
<td>0</td>
<td>+13</td>
</tr>
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</table>
**Table 2** Haematological variables and α:β globin chain synthetic ratio (mean (SD) and range) in patients, their subgroups (I, II, III), and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage of total</th>
<th>Sex ratio M:F</th>
<th>PVC%</th>
<th>Reticulocytes %</th>
<th>White cells (10^12/l)</th>
<th>Platelets (10^12/l)</th>
<th>S-Fe (μmol/l)</th>
<th>HbA1c %</th>
<th>Bone marrow blasts %</th>
<th>α:β ratio (cpm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients (n = 30)</td>
<td>100</td>
<td>73 (9)</td>
<td>18:12</td>
<td>29 (4)</td>
<td>1:2:7 (154)</td>
<td>12 (11:5)</td>
<td>180 (104)</td>
<td>21 (12)</td>
<td>2:7 (0:48)</td>
<td>10:2 (9:4)</td>
</tr>
<tr>
<td>Group I (n = 14)</td>
<td>46</td>
<td>73 (9)</td>
<td>11:3</td>
<td>29 (3:3)</td>
<td>0:04 (0:61)</td>
<td>1:5 (3:12:5)</td>
<td>183 (96)</td>
<td>22 (11)</td>
<td>2:7 (0:5)</td>
<td>10:8 (9:5)</td>
</tr>
<tr>
<td>Group II (n = 11)</td>
<td>37</td>
<td>71 (7)</td>
<td>6:5</td>
<td>30 (4:8)</td>
<td>2:2 (2:1)</td>
<td>11 (10)</td>
<td>160 (120)</td>
<td>18 (11)</td>
<td>2:76 (0:43)</td>
<td>9:5 (9:6)</td>
</tr>
<tr>
<td>Group III (n = 5)</td>
<td>17</td>
<td>75 (11)</td>
<td>3:2</td>
<td>28 (5)</td>
<td>0:54 (0:15)</td>
<td>4:4 (3:7)</td>
<td>146 (113)</td>
<td>28 (15)</td>
<td>2:86 (0:43)</td>
<td>10:4 (11:3)</td>
</tr>
<tr>
<td>Controls (n = 10)</td>
<td>100</td>
<td>72 (28)</td>
<td>6:4</td>
<td>45 (3)</td>
<td>0:7 (0:3)</td>
<td>6:4 (1:3)</td>
<td>220 (53)</td>
<td>25 (7:5)</td>
<td>2:5 (0:6)</td>
<td>1:01 (0:04)</td>
</tr>
</tbody>
</table>

The table above shows the haematological variables and α:β globin chain synthetic ratio (mean (SD) and range) in patients, their subgroups (I, II, III), and controls. The values are presented as percentages of total, sex ratios, mean (SD), range, and some other parameters such as white cells, platelets, S-Fe, and HbA1c.

**Discussion**

Several studies on α:β globin chain synthesis ratios in MDS have shown conflicting results. In primary sideroblastic anaemia (FAB-RAS) and various subtypes of MDS the reported α:β ratios ranged from much less than normal to higher compared with those of group I (p < 0.05) and group III (p = 0.01). Furthermore, among the total number of patients and in the subgroups I, II, and III there was no significant correlation between α:β ratios and various haematological variables, including reticulocytes, white cells, platelets, and myeloblasts in bone marrow.

**Effect of Haemoglobin on the α:β Ratio**

The addition of exogenous haem (0.4 mmol/l) to the incubation mixture in reticulocytes in 16 cases with MDS (table 1) resulted in a significant increase in the mean value of the α:β ratio from 0.91 (0.48) (sample A) to 1.11 (0.44) (sample B). The observed positive haem effect was more evident in group I compared with groups II and III (p = 0.01) and was not associated with serum iron concentration or with bone marrow iron storage. In case 10 (group I) with sideroblastic anaemia the addition of exogenous haem was unable to correct substantially the initially extremely low α:β ratio (sample A: 0.05, sample B: 0.09). In cases 13, 15, and 28 with subnormal serum iron (3.5–6.4 mmol/l) haem had a minimal or a negligible effect. In contrast, in case 25 (RAEB) (group II) the initially normal (1.05) α:β ratio was increased to β-thalassaemic level (1.85) after the addition of exogenous haem. The serum iron concentration in this last case was normal (21.4 μmol/l).

**Correlation between α:β Ratio and Survival**

Twenty-two patients had died to date (group I n = 10, group II n = 8, group III n = 4). Four patients were lost to follow up and four are still alive. There was no significant correlation between α:β ratios and survival. There was also no significant difference in survival among groups I, II, and III.

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significant correlation was found, however, between \( \alpha: \beta \) ratios and survival or various haematological variables either in the total population of patients or in each FAB subtype.

In sideroblastic anaemia the activity of \( \delta \)-aminolaevulinic acid synthetase, which is the first and rate limiting enzyme in the haem synthetic pathway, is often depressed, resulting in a depletion of intracellular haem. The addition of exogenous haem in the incubation mixture stimulates considerably the synthesis of both globin chains and particularly that of the \( \alpha \) chain by preventing the synthesis of a transamination inhibitor that is accumulated in haem deficiency states. \(^{29,30} \) Similar iron deficiency and sideroblastic anaemia. This leads to a rise in the initially diminished \( \alpha: \beta \) ratio. \(^{6} \) Similar results were reported in human reticulocytes after specific inhibition of haem synthesis with Isoniazid. \(^{31,32} \) By contrast, Peters et al observed no important stimulatory effect of exogenous haem on globin chain synthesis in sideroblastic anaemia. \(^{12} \)

Our study clearly shows that, as in other FAB subtypes of MDS, even in sideroblastic anaemia, the \( \alpha: \beta \) ratio varied widely (0-34–1.73) with no apparent or definite haem effect (table 1). The presence of HbH inclusions in those cases with very low \( \alpha: \beta \) ratios is to be expected. The absence, therefore, is probably related to an enhanced proteolytic degradation of free \( \beta \) chain in erythroid cells in MDS. The finding that a strongly positive haem effect was noticed, not only in group I with low ratios but even in cases 24 and 25 with normal initial values in group II (table 1), indicates that depletion of intracellular haem may contribute to or obscure the imbalance of \( \alpha \) and \( \beta \) globin chain synthesis in MDS. It was also claimed by Peters et al that the pattern of globin chain synthesis in MDS may be affected by the presence of non-haem proteins with a charge similar to that of one of the globin chains, in spite of adequate removal of white cells, or even by changes in proteolytic degradation of globin chain. We agree with the authors' view that the very low reticulocyte counts in these patients do not permit studies of this kind. It is essential, however (a) to remove the white cells, which, in disorders like MDS, may be sufficiently increased to create problems even in reticulocyte studies, and (b) to rely only on samples with good chromatographic separation and high incorporation of labelled amino acids.

Apart from these technical or metabolic reasons and the aforementioned effect of intracellular haem (not associated with iron deficiency) the great variation of the \( \alpha: \beta \) ratios, which we found, must be mainly attributed to the heterogeneity (even within each FAB subtype) derived from the genetic and clonal instability of these disorders. \(^{4,5,13,34} \)

These features of MDS may explain the major controversy over globin synthesis and its relation with haem in primary sideroblastic anaemia. The basic molecular defect(s) in MDS remain(s) unknown, but it is probably associated with the leukaemic process and it influences the various mechanisms of molecular regulation occurring at the level of transcription or processing of globin mRNA, as has been suggested in acquired haemoglobin H disease. \(^{35–38} \)

In conclusion, although the wide variation of \( \alpha: \beta \) globin chain synthetic ratio reduced its value as a prognostic factor in MDS, including sideroblastic anaemia, further studies at globin gene level using erythroid cells from all FAB subtypes of MDS may give important information on the molecular regulation of globin gene expression or even the leukaemic process in these poorly defined disorders.

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26 Markham RE, Butler F, Goh Kong-OO, et al. Erythro-
leukaemia manifesting d-thalassaemia. Hemoglobin 1983;
7:71-8.
27 Sheffer R, Cividadi G Zabaran Y, Tamars H, Okon E.
Disturbed pattern of globin chain synthesis in childhood
monosites 7 myeloproliferative syndrome. Br J
features of chronic, myelomonocytic leukaemia: a
modified Bournemouth score gives the best prediction
29 Neuwirt J, Ponka P. Regulation of haemoglobin synthesis.
30 Maxwell CR, Rabinovitz M. Evidence of an inhibitor in the
control of globin synthesis by hemin in a reticulocyte
31 Legon S, Jackson RT, Hunt T. Control of protein synthesis
32 Franco RS, Hogg JW, Martelo 0. The effect of INH-
 inhibited heme synthesis on globin synthesis. J Lab Clin
Med 1979;93:679-86.
33 Chalevelakis G, Yalouris AG, Lyberatos G, Economopoulos
Th, Hatziioanou J, Raptis S. Effect of isoniazid, a haem
inhibitor, on globin chain synthesis in reticulocytes from
non-thalassaemic and 8-thalassaemic subjects. J Clin
34 Raskind W, Tirmali N, Jacobsen R, et al. Evidence for a
multistep pathogenesis of a myelodysplastic syndrome.
35 Weatherall DJ, Old J, Langley J, et al. Acquired
haemoglobin H disease in leukaemia pathophysiology and
36 Veer A, Koscioiek BA, Bauman AW, Rowley RT. Acquired
haemoglobin H disease in idiopathic myelofibrosis. Am J
37 Yoo D, Schechter GP, Amigable AN, Nienhuis AW.
Myeloproliferative syndrome with sideroblastic anaemia
and acquired hemoglobin H disease. Cancer 1980;45:
78-83.
38 Anagnou N, Chehbo B, Colbert D, et al. Acquired hemo-
globin H disease and leukaemia. Blood 1981;58
(Supplement 1):52a.