Serum Immunoreactive Erythropoietin in Children With Cyanotic and Acyanotic Congenital Heart Disease

By Per Hågæ, P. Mary Cotes, Janice A. Till, Barbara D. Minty, and Elliot A. Shinebourne

Serum immunoreactive erythropoietin (siEp) was measured in 27 cyanotic and 21 acyanotic children with congenital heart disease, age 4 months to 10 years. The geometric mean value was 9 mU/mL for each group with 95% range from 3 to 26 mU/mL and 4 to 22 mU/mL for the cyanotic and acyanotic subjects, respectively. The levels are similar to those found in normal adults using the same assay system. Three cyanotic subjects showed increased siEp values. One was anemic relative to his hypoxemia, and the other two showed signs of increasing hypoxia. There was a significant negative correlation between siEp and arterial oxygen content. However, siEp did not correlate significantly with hemoglobin, hematocrit, PaO₂, or SaO₂. Despite normal siEp levels, the cyanotic children showed compensatory erythropoiesis with significantly elevated hemoglobin and hematocrit levels, which did correlate inversely with PaO₂ and SaO₂. Arterial oxygen content was also significantly higher in the cyanotic subjects (p < 0.02). The cyanotic children seemed to display the same pattern as observed in man and animals exposed to prolonged hypobaric hypoxia, where after an initial rise in erythropoietin values the levels fall to normal, while increased erythropoiesis is sustained.

THE HYPOXEMIA of cyanotic congenital heart disease (CCHD) causes erythrocytosis, with a concomitant increase in blood volume.1,2 The erythrocytosis is classified as a secondary appropriate polycythemia because the increased red cell mass is a physiologic response to the inadequate tissue oxygenation.1

With increasing hematocrit the viscosity of blood rises, and this in turn may impair oxygen delivery to the tissues. The increased blood volume appears partly to counteract the increase in viscosity by lowering peripheral vascular resistance; there has been much debate however, on what is the optimal hemoglobin/hematocrit value for oxygen transport.3

In some patients with CCHD the erythrocytosis appears to be excessive, and they seem to benefit from reducing the hematocrit through partial exchange transfusion with plasma or 5% albumin.4,5

Traditionally the secondary polycythemias are considered to have increased erythropoietin (Ep) levels in serum or urine, in contrast to the primary polycythemias, which are epitomized by polycythemia rubra vera.1,3,4 In recent investigations employing radioimmunoassay, this distinction is generally upheld5; however, some studies have found that serum immunoreactive erythropoietin (siEp) levels in secondary hypoxia-induced erythrocytosis may be either normal or increased.6,8,11 Although the concept of secondary polycythemia may be advantageous in terms of a diagnostic work-up, the lumping together of widely different disease states as a single entity clearly is unsatisfactory.6

The subjects of the study were patients undergoing cardiac catheterization or about to have cardiac surgery. The catheterized patients were under general anesthesia with a FiO₂ of 33%, in accordance with hospital routine. Blood was sampled from the catheter at the start of the procedure. In the patients about to be operated on, blood was obtained from an arterial line after the patient was stabilized under general anesthesia.

Twenty-seven cyanotic children and twenty-one acyanotic children having congenital heart disease were studied. Their age ranged from 4 months to 10 years. Cyanosis or arterial desaturation was defined as a systemic arterial oxygen saturation (SaO₂) of less than 94%. Clinical findings are shown in Table 1. Growth was assessed on the standard height and weight charts of Tanner & Whitehouse (Castlemead Publications, UK). None of the patients showed signs of heart failure at the time of study. Four of the cyanotic and five of the acyanotic children were receiving antifailure treatment.

Only infants more than 4 months of age were studied so that measurements would not be influenced by hematological changes occurring at birth. Similarly, the nadir of the physiological anemia of infancy was avoided. Children who were preterm and/or small for gestational age were included only if they were older than one year of age at the time of the study. None of the subjects had evidence of renal disease, and all had normal serum levels of creatinine and urea.

Hematological indices were determined using a Coulter Counter Model S 880 (Coulter Electronics). Serum ferritin was determined by radioimmunoassay (Amersham International, Chalfont, England); values lower than 16 μg/L signify iron deficiency in this assay. Blood gas measurements were performed on a Corning 178 pH/Blood Gas Analyzer (Corning Medical), and arterial oxygen
correlation was tested by linear regression. SiEp shows a log normal distribution and was therefore tested after logarithmic transformation; means were expressed as geometric means. Some of the acyanotic patients displayed hyperoxemia on being artificially ventilated at a FiO2 of 33% and PO2 values above 15 kPa were excluded from analysis. Two cyanotic patients (subjects II & III in Table 3) had siEp values 6.0 and 10.8 standard deviations, respectively, above the rest of the group. For this reason they were excluded from the statistical analysis. The study was approved by the Brompton Hospital Ethical Committee.

RESULTS

The estimates of siEp are shown in Fig 1 and Table 2. The geometric mean was 9 mIU/mL for each group, with a 95% range† from 3 to 26 mIU/mL for cyanotic and 4 to 22 mIU/mL for acyanotic subjects. The difference is not statistically significant. The levels are similar to those of 21 normal adults using the same assay system (geometric mean ± 95% range from 3 to 26 mIU/mL).

Three cyanotic patients (subjects I, II, III; Table 3) had siEp values 3.8, 7.6, and 13.5 standard deviations, respectively, higher than their counterparts (Fig 1). Subject I, with arterial oxygen saturation 80.9% and hemoglobin concentration 12.7 g/dL, may be considered anemic in relation to his hypoxemia. This relative anemia was most likely due to iron deficiency (serum ferritin 8 μg/L). This may explain the

*Values are means (range).

†Values are means (range), p < 0.05 for cyanotic vs acyanotic group (two-tailed unpaired t-test, degrees of freedom = 44). Centiles refer to those of normal children (Tanner & Whitehouse, Castlemead Publications 1983).

Abbreviations: ASD, atrial septum defect; PA, pulmonary atresia; PDA, patent ductus arteriosus; PS, pulmonary stenosis; TA, tricuspid atresia; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect; shunt, modified Blalock-Taussig aortopulmonary shunt; TAPVC, total anomalous pulmonary venous connections.

saturation was determined spectrophotometrically in a IL 282 CoOximeter (Instrumentation Laboratory). Standard PO2 (pH 7.40, 37°C) was measured as described by Holter et al.3 Blood from five normal children, age 19 months to 8 years, had levels of 3.3 ± 0.3 kPa* (mean ± SD), using this method.

SiEp was estimated by a modification of the radioimmunoassay method described and validated by the method of Egrie.12 1351 recombinant human erythropoietin (Amersham International, IM 178) was used as tracer antigen, and the assay diluent was 0.04 M phosphate, 0.01 M disodium EDTA (pH 7.5) containing 0.15 M NaCl, 0.01% sodium azide, and human plasmaprotein fraction (2 mL/100 mL) (Lister, Elstree; 4.5 g protein/100 mL). The WHO second international reference preparation of erythropoietin (2nd IRP) was used as standard.13 Serum samples from blood allowed to clot at room temperature were stored at −20°C until analysis. Each serum sample was tested in two assay tubes, usually at two-dose levels, but 12 sera were tested as two identical replicates. All samples were examined as a batch in a single assay to exclude interassay variability. Forty-nine samples tested in the assay at two or more dose levels gave consistent potency estimates at all dose levels, indicating parallelism of dose dilution curves of sera and the second IRP. The between assay coefficient of variation of estimates was 11%.

Statistical difference was tested by unpaired student's t-test; correlation was tested by linear regression. SiEp shows a log normal distribution and was therefore tested after logarithmic transformation; means were expressed as geometric means. Some of the acyanotic patients displayed hyperoxemia on being artificially ventilated at a FiO2 of 33% and PO2 values above 15 kPa were excluded from analysis. Two cyanotic patients (subjects II & III in Table 3) had siEp values 6.0 and 10.8 standard deviations, respectively, above the rest of the group. For this reason they were excluded from the statistical analysis. The study was approved by the Brompton Hospital Ethical Committee.

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Three cyanotic patients (subjects I, II, III; Table 3) had siEp values 3.8, 7.6, and 13.5 standard deviations, respectively, higher than their counterparts (Fig 1). Subject I, with arterial oxygen saturation 80.9% and hemoglobin concentration 12.7 g/dL, may be considered anemic in relation to his hypoxemia. This relative anemia was most likely due to iron deficiency (serum ferritin 8 μg/L). This may explain the
raised siEp concentration. Subjects II and III had both shown symptoms and signs of increasing cyanosis and hypoxia during the months leading up to hospital admission. In addition, subject III suffered from iron deficiency anemia, as judged from red cell indices and serum ferritin (Table 3).

Hemoglobin concentration and hematocrit were significantly higher in the cyanotic than the acyanotic subjects (p < 0.001, n = 46). Arterial oxygen content (1.36 x Hb x SaO2) was also significantly higher in the cyanotic group (p < 0.02, n = 44) (Table 2).

The siEp concentrations did not show any significant correlation with hemoglobin concentration or hematocrit, or with systemic arterial oxygen tension (PaO2) or SaO2. This was so both for the cyanotic and acyanotic patients as well as for the two groups combined. However, there was a significant negative correlation between siEp and arterial oxygen content in the cyanotic group (r = -0.56, P < 0.01, n = 25) (Fig 2). The inverse correlation was also significant for all patients combined, although to a lesser extent (r = -0.43, P < 0.01, n = 44).

Hematocrit was inversely correlated with arterial PO2 and oxygen saturation for both the cyanotic group and all subjects combined (p < 0.05 in all instances). Hemoglobin concentration showed the same pattern, but the relation did not attain significance in the cyanotic group. The best correlation was obtained between hematocrit and SaO2 in the total sample (r = -0.74, P < 0.001, n = 44).

The low siEp concentrations found in the cyanotic group were unexpected. Our findings are not in agreement with either the general notion of raised Ep levels in secondary

table 2. Hematologic and Physiologic Data of the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Cyanotics</th>
<th>Acyanotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythropoietin (mIU/mL)</td>
<td>25 9 (3-26)</td>
<td>21 9 (4-22)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>25 15.1 ± 1.8*</td>
<td>21 11.7 ± 1.4</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>25 0.46 ± 0.05*</td>
<td>21 0.35 ± 0.04</td>
</tr>
<tr>
<td>PO2 (kPa)</td>
<td>25 7.2 ± 1.2</td>
<td>17 &gt;9.9</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td>25 84.0 ± 7.8</td>
<td>19 97.5 ± 1.2</td>
</tr>
<tr>
<td>Arterial O2 content (mL/dL)</td>
<td>25 17.1 ± 2.2</td>
<td>19 15.5 ± 1.8</td>
</tr>
<tr>
<td>P50 (kPa)</td>
<td>9 3.9 ± 0.4</td>
<td>4 3.5 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SD, except for erythropoietin values, which are geometric means (95% range).

*P < 0.001 when compared with the values of acyanotics (two-tailed unpaired t-test, degrees of freedom = 44).

†1 kPa = 7.5 mm Hg; 1 mm Hg = 0.133 kPa.

‡P < 0.02 when compared with the values of acyanotics (two-tailed unpaired t-test, degrees of freedom = 42).

Fig 2. Serum immunoreactive erythropoietin levels vs oxygen content (1.36 \times Hb \times SaO2) of the cyanotic subjects. The correlation is statistically significant (r = -0.56, P < 0.01, n = 28).

Standard P50 was measured in arterial blood from 13 patients. In cyanotic subjects (n = 9), the mean P50 was 3.9 kPa (range 3.3-4.4 kPa), which was significantly higher than in normal children (P < 0.02, n = 12). No correlation with siEp values was noted. There was a positive correlation between P50 and hemoglobin and hematocrit levels that did not attain significance. However, when both cyanotic (n = 9) and acyanotic (n = 4) patients were combined, the relations became significant (r = 0.71, P < 0.01 vs hemoglobin, and r = 0.72, P < 0.01, vs hematocrit).

From three cyanotic patients who had aortopulmonary shunt operations, serial samples were collected for siEp assay in conjunction with blood gas sampling. For each patient, PaO2 did not improve during the study period, and siEp levels remained unchanged (Fig 3). In another patient, age 3 years, blood was sampled on two occasions, 5 months apart. During this period, her condition remained stable, and estimates of siEp were 7 mU/mL and 11 mU/mL on the two occasions, with PaO2 of 8.3 and 8.6 kPa, respectively.

DISCUSSION

The low siEp concentrations found in the cyanotic group were unexpected. Our findings are not in agreement with either the general notion of raised Ep levels in secondary

Table 3. Data on the Three Subjects Showing Increased siEp Levels

<table>
<thead>
<tr>
<th>Subjects</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Cardiac diagnosis</td>
<td>TGA + VSD</td>
<td>TGA + PA + VSD + shunt</td>
<td></td>
</tr>
<tr>
<td>Erythropoietin (mIU/mL)</td>
<td>12</td>
<td>211</td>
<td>2695</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.7</td>
<td>15.0</td>
<td>16.2</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.41</td>
<td>0.47</td>
<td>0.54</td>
</tr>
<tr>
<td>MCV (fl)*</td>
<td>79</td>
<td>86</td>
<td>64</td>
</tr>
<tr>
<td>MCH (pg)*</td>
<td>24.5</td>
<td>27.6</td>
<td>19.1</td>
</tr>
<tr>
<td>Ferritin (g/L)</td>
<td>8</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>PO2 (kPa)</td>
<td>7.5</td>
<td>5.0</td>
<td>5.9</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td>80.9</td>
<td>63.9</td>
<td>64.0</td>
</tr>
</tbody>
</table>

polycythemia nor with the raised levels reported in adults with CCHD in other radioimmunoassay studies. In fact, Koefler and Goldwasser found the patients with cyanotic heart disease to have the highest average concentration among the secondary polycythemia subjects. Our findings cannot be explained by insufficient severity of hypoxemia, since the PaO2 ranged from 5.0 to 8.9 kPa, with an even spread of values.

A FiO2 of 33% is used in general anesthesia to counteract the negative effects of artificial ventilation on oxygenation. Some acyanotic subjects displayed hyperoxemia when ventilated in this way, and there is a possibility that the SaO2 of some of the cyanotic patients with a high pulmonary blood flow was also increased by artificial ventilation.

Oxygen delivery to the tissues depends on many factors, in particular on the total number of circulating erythrocytes, PaO2, SaO2, the position of the oxygen-hemoglobin dissociation curve, cardiac output, and regional blood flow. A possible reason for these low siEp values in children with CCHD is that their hypoxemia was totally compensated, and consequently no tissue hypoxia was experienced. Compensatory mechanisms to counteract tissue hypoxia clearly operated in the patients of our study group. We did not measure red cell mass, but hemoglobin levels and hematocrit were well above normal in our cyanotic patients and, moreover, were inversely correlated with PO2 and SaO2, indicating increasing production according to increasing needs. A rightward shift of the oxygen-hemoglobin dissociation curve is considered advantageous for oxygen delivery when lowered PaO2 is caused by right to left shunting of blood, which is in line with experimental findings. We found an increase in standard P50 in the cyanotic patients tested in our study (Table 2). Furthermore, for all subjects tested, P50 was correlated significantly with hemoglobin concentrations and hematocrit. Most importantly, the arterial oxygen content was found to be significantly higher in the cyanotic than in the acyanotic subjects (Table 2). However, although we have shown compensatory adjustments at work, whether total compensation of hypoxemia occurs is not answered by this investigation. Total compensation for any deficiency state in a child would entail "normal" growth and development. Our patients showed reduced growth, both in height and weight, as is a common finding in CCHD. However, the growth impairment was not significantly more severe in the cyanotic than the acyanotic group. Hypoxia seems to be only one of several factors, albeit an important one, responsible for impaired growth in congenital heart disease. A recent study found an inverse association between cognitive function and time of corrective surgery (6 months to 6 years) in children with simple transposition, suggesting that the length of hypoxemic exposure has an adverse effect and that the hypoxia is not totally compensated. On balance, we believe that total correction of tissue hypoxia most likely is not achieved and that the low Ep values cannot be fully explained through such a mechanism. One may argue that through reduction in growth, oxygen consumption, and physical exercise, oxygen needs are reduced to a lower level whereby equilibrium is reestablished through lower demands. However, such a concept seems unlikely in infants and children because of the major emphasis during this period on growth and development.

When exposed to hypobaric hypoxia, both experimental animals and humans show an initial rise in erythropoietin concentration that falls gradually to normal levels in the ensuing days or weeks. Even though the Ep levels return to normal, increased erythropoiesis is sustained, with a continuous rise in hemoglobin concentrations and hematocrit levels. Looking at other subjects chronically exposed to hypoxia, we find little data on erythropoietin secretion in persons living permanently at high altitude. However, in one study, urinary Ep was increased in only two out of six subjects. In patients with severe hypoxic lung disease with an associated polycythemia, siEp was above normal in only 5 out of 10 patients. Thus, from both experimental work in animals and humans as well as clinical studies, the axiom of a direct relationship between the degree of hypoxemia and serum or urinary erythropoietin concentrations is not borne out under long-term hypoxic conditions. We believe the cyanotic patients in the present study fit the pattern outlined above and that their low siEp levels may be explained in the context of chronic hypobaric hypoxia.

The raised siEp concentrations of the three patients in Table 3 must be explained differently. Subject 1 was relative to his cyanotic heart disease anemic (SaO2, 80.9%) at the time of study, most likely due to iron deficiency, and this may be the reason for his elevated siEp value. The other two subjects had both shown severe signs and symptoms of increasing cyanosis and hypoxia during the preceding months. They were thus not in a "stable chronic state," and both in humans and animals exposed to prolonged hypoxic conditions, it has been shown that increasing the hypoxic stimulus causes a renewed rise in Ep levels.

Our finding of a significant negative correlation between siEp levels and arterial oxygen content in CCHD (Fig 2) indicates that oxygen supply is a factor in the regulation of erythropoietin production even when the Ep concentrations are in the "normal" range.

Single determinations of concentrations of erythropoietin both as a measure of the production rate of the hormone and as a reflection of the serum levels over time may be seen as wanting. Diurnal variations in siEp values in humans, as well as fluctuating levels in a polycythemic adult, have been observed. Fig 3 shows that multiple samples from the same individual showed fairly stable levels of siEp. This indicates that the single measurements of the patients in our study can reasonably be expected to give good estimates of the steady state siEp levels.

ACKNOWLEDGMENT

We wish to acknowledge the willing cooperation of the nursing and medical staff of the Paediatric Department and of the technical staff of the Haematology and Cardiac Departments of the Brompton Hospital. We wish to thank WHO for provision of the International Reference Preparation of erythropoietin and Dr J.C. Egie (Amgen, CA) for antisera used in the erythropoietin radioimmunoassay.

REFERENCES

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