Serum erythropoietin in the diagnosis of polycythaemia and after phlebotomy treatment

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Summary. Serum erythropoietin (S-Epo) was measured with a radiomunoassay method in 36 patients with polycythaemia vera, 17 patients with secondary polycythaemia and 14 patients with relative polycythaemia. The diagnoses were made without the aid of the S-Epo values. It was found that S-Epo was below the reference range in 34/36 patients with polycythaemia vera (mean 21±1:0 U/l), elevated in all cases of secondary polycythaemia (mean 121±7±242 U/l) and normal in all but one of the cases with relative polycythaemia (mean 7:0±2:5 U/l). Previous studies of S-Epo levels in the differential diagnosis of polycythaemia have shown significant differences between the means of the groups but a considerable overlap. After phlebotomy treatment to normal haematocrit levels, the S-Epo levels remained subnormal in most of the polycythaemia vera patients even after 18 months at a haematocrit around 45%. Two patients who had been kept at normal haematocrit for 6 and 7 years both had subnormal S-Epo.

We conclude that with an Epo assay method of high sensitivity and specificity it is possible to differentiate between different forms of polycythaemia with a high degree of certainty, even between patients with relative polycythaemia and polycythaemia vera patients.

The reason why S-Epo remains low in spite of a normal haematocrit in treated polycythaemia vera patients is not known.

In the differential diagnosis of polycythaemia it is essential to decide whether the polycythaemia is absolute or relative. In absolute polycythaemia there is a true increase in the red cell mass, which also leads to an increase in total blood volume. Relatively polycythaemia is defined as a reduction in plasma volume without any change in red cell mass. The measured Hb and haematocrit values, being concentration measurements, are elevated (Berlin, 1975). These two conditions can be differentiated by measuring red cell mass and total blood volume, from which also the plasma volume can be calculated. There are also methods for direct measurement of plasma volume (Bratteby, 1967).

An absolute polycythaemia may be primary or secondary. In primary polycythaemia (polycythaemia vera) there is an autonomous overproduction of red cells. In secondary polycythaemia the overproduction of red cells either is a response to perceived need, as in hypoxic pulmonary or cardiac disease, or an autonomous overproduction of erythropoietin (Epo), i.e. from a tumour or cyst. Determination of immunoreactive Epo in serum has been suggested as a method to differentiate between groups of patients with primary and secondary polycythaemia (Erslev et al, 1979; Cotes et al, 1986). However, the Epo levels in serum in the groups of patients have shown a considerable overlap which has reduced the clinical value of a single S-Epo estimation (Cotes et al, 1986; Egri et al, 1989; Najean et al, 1990). The explanation may be a non-specific interference of serum components at low S-Epo levels. In a preliminary study on seven patients with polycythaemia vera we found that they all had S-Epo levels below the reference range when measured with a sensitive and specific radioimmunoassay (Wide et al, 1989). In the present study we have investigated the possibility to use S-Epo levels, measured with this radioimmunoassay, to differentiate between relative, primary and secondary polycythaemia.

The present knowledge about the role of Epo in polycythaemia vera indicates that serum levels are depressed in response to an increased red cell mass and normalized after phlebotomy treatment (Erslev et al, 1979). S-Epo levels after phlebotomy treatment to normal haematocrit (PCV) in polycythaemia vera patients were also studied in the present investigation.

Patients

Polycythaemia categories. (i) Absolute or true polycythaemias. Polycythaemia vera and secondary polycythaemia are both
absolute polycythaemias with increased Hb and PCV, increased total blood volume, total Hb and red cell mass. (ii) Relative polycythaemia. Relative polycythaemia is due to reduced plasma volume. Hb and PCV are increased, but total Hb and red cell mass are normal.

67 Patients referred for investigation of polycythaemia to the Department of Haematology between 1987 and 1990 were included in the study. All patients were diagnosed without the aid of S-Epo measurements. All patients have been followed clinically for more than 1 year after diagnosis and in no case has the diagnosis been changed.

Polycythaemia vera was diagnosed in 36 patients. They all had PCV > 53%. Total blood volume (mean 84 ml/kg body weight), total Hb (mean 13-6 g/kg) and red cell mass (43-6 ml/kg) were all increased. One or more of the following secondary criteria were present: leucocytosis, thrombocytosis, splenomegaly, bone marrow changes in the form of fibrosis, megakaryocyte increase, increased cellularity. The leucocyte alkaline phosphatase (LAP) score was elevated or normal. None of these patients had pulmonary or hypoxic cardiac disease. An ultrasound or CT scan of the abdomen was performed in most cases to exclude the presence of a tumour.

Secondary polycythaemia was diagnosed in 17 patients. PCV was > 53%, total blood volume (mean 79 ml/kg), total Hb (mean 13·6 g/kg) and red cell mass (mean 43·5 ml/kg) were all increased. A positive diagnosis of the underlying cause of the polycythaemia was found in all cases: hypoxia due to pulmonary disease in six patients, cardiomyopathy in three, cardiac shunting, thrombosis of the vena cava and sleep apnoea in one each, overproduction of Epo due to renal carcinoma in three and cystic kidneys in two patients.

Relative polycythaemia was diagnosed in 14 patients. Total Hb (mean 57-4 g/kg) and red cell mass (mean 29·6 ml/kg) was normal, whereas plasma volume was decreased. In most cases the plasma volume was estimated from total blood volume and red cell mass. In a few patients direct measurement of the plasma volume was performed (see below). The reduction in plasma volume was caused by: diuretics, two; acute nephritis with polyuria, one; alcohol abuse, two; mental stress, nine.

The PCV was kept at around 45% by phlebotomy treatment. Some of the patients were also treated with hydroxyurea. S-Epo was measured at various times during phlebotomy treatment. Two patients diagnosed before 1987 were included in this part of the study in order to investigate serum Epo levels after a long period with a normalized PCV.

METHODS

S-Epo was measured with a radioimmunoassay as previously published (Wide et al., 1989). The assay uses labelled human recombinant Epo (Boehringer Mannheim) and a rabbit antibody to purified human urinary Epo. The 2nd International Reference Preparation was used as a standard.

The detection limit was 0·5 U/l, the within-run coefficient of variation 6-7% and the between-run variation 11-19%. The reference range for a population of 57 healthy individuals was 3·3-13·5 U/l, mean 6·7 U/l. There was no significant difference between men and women. Samples were collected between 8 and 12 a.m. and stored at -20°C until assayed.

Total Hb and red cell mass were measured using the patient's erythrocytes labelled with 51Cr. Plasma volume was in a few cases measured by the use of labelled albumin, in the other cases estimated from total blood volume and red cell mass (Bratteby, 1967). Hb and PVC were measured with a Coulter Counter.

RESULTS

The mean S-Epo level was subnormal in 34/36 patients with polycythaemia vera, mean 2·1 ± 1·0 U/l. The patients with relative polycythaemia (reduced plasma volume) had S-Epo levels within the reference except in one case (Fig 1), mean 7·0 ± 2·5 U/l. The patients with secondary polycythaemia all had elevated S-Epo levels within a wide range, mean 121·7 ± 242 U/l. The difference was statistically significant (P < 0·001) between all three groups (see Fig 1 and Table I). There was no significant correlation between S-Epo and Hb in any of the groups.

After phlebotomy treatment to normal PCV (45–47%) PCV was checked monthly and phlebotomy performed at levels higher than 45%, S-Epo remained subnormal in most of the patients with polycythaemia vera, even after several years (Fig 2). In one patient after 6 and 7 years of phlebotomy and with a normal PCV, S-Epo was 0·86 and 1·7 U/l, respectively, and in another it was 1·2 U/l after 6 years. In only a few patients did the S-Epo reach the reference range during phlebotomy. In one patient, however, there was a temporary increase to 13 U/l after 16 months of treatment (Fig 3). Treatment with hydroxyurea did not influence S-Epo levels after phlebotomy; there was no difference between patients with such treatment and those without. In a patient with secondary polycythaemia due to cardiac disease S-Epo increased from 18 to 87 U/l after phlebotomy (Fig 4).
Serum Epo in Polycythaemia and after Treatment

Table I. Mean ± SD for Hb, haematocrit and S-Epo at diagnosis in the three groups.

<table>
<thead>
<tr>
<th></th>
<th>Hb (g/dl)</th>
<th>Haematocrit (%)</th>
<th>S-Epo (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycythaemia vera</td>
<td>184±16</td>
<td>57.1±4.3</td>
<td>2.1±1.0</td>
</tr>
<tr>
<td>Relative polycythaemia</td>
<td>172±11</td>
<td>51.1±2.6</td>
<td>7.0±2.5</td>
</tr>
<tr>
<td>Secondary polycythaemia</td>
<td>182±0.9</td>
<td>54.7±2.3</td>
<td>121.7±242</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Low serum Epo values have been reported in polycythaemia vera before, but not so consistently as in this study. In several studies significant differences in mean S-Epo levels have been found between the groups of polycythaemia, but overlap between the groups has reduced the predictive value of a single value (Cotes et al. 1986; Egri et al. 1989; Najean et al. 1990). In a previous study one of the authors showed that when S-Epo was determined after heat treatment of the samples, the polycythaemia vera patients had lower levels than healthy controls (Birgegard et al. 1982). This indicated that the radioimmunoassay methods used at that time were influenced by some non-specific serum effect in the low range, obscuring a true difference between subnormal and normal S-Epo levels. In the present investigation, a subnormal S-Epo was found in 34/36 polycythaemia vera patients and only in one patient with relative polycythaemia. This indicates that with this method, without heat treatment of the samples, it is possible to identify polycythaemia vera patients with a high degree of certainty. The improved differentiation is probably due to both an increased sensitivity and specificity of the S-Epo assay method.

Relative polycythaemia, due to a reduced plasma volume, theoretically should not interfere with the erythropoietic homeostasis, and therefore the finding of normal Epo levels in this group is to be expected. Our results indicate that the combination of an increased PCV and Hb and a normal S-Epo level speaks in favour of a relative polycythaemia.

The definition of secondary polycythaemia includes an augmented erythropoiesis due to increased Epo secretion, either from a tumour or due to hypoxia. We found elevated levels in all these patients. However, some patients only had slightly elevated S-Epo, and there was no correlation between the degree of Epo increase and the severity of polycythaemia. It seems that even a small Epo increase can cause a severe polycythaemia. This has previously been noted in patients with slightly elevated S-Epo levels after renal transplantation. Our results indicate that an increased S-Epo in polycythaemia indicates secondary polycythaemia.

With regard to the diagnostic use of S-Epo estimations we conclude that a subnormal S-Epo level in a patient with polycythaemia makes it unnecessary to search for tumours...
and other causes of secondary polycythaemia. An elevated S-Epo level in an untreated patient, on the other hand, seems to exclude the diagnosis of polycythaemia vera and makes such a search necessary.

Why was S-Epo not normalized in all polycythaemia vera patients after phlebotomy treatment? Most of the patients still had subnormal values in spite of a long period at normal PCV. In a previous study (Wide et al. 1989) we found a mean S-Epo value within the reference range in eight patients treated with phlebotomy, which is in contrast to the present findings. The patients were randomly selected in both cases. We have no other explanation for the discrepancy, except that in the present study numbers are greater. It is possible that the Epo-producing cells have been rendered inactive by lack of stimulation over a long period of time. This would be an analogy with the atrophy of inactivity seen in other situations in other hormone-producing cells, for example, in the adrenal cortex after long-term cortisone treatment.

ACKNOWLEDGMENTS

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