Serum Erythropoietin (ESF) Titers in Polycythemia

By G. de Klerk, P. C. J. Rosengarten, R. J. W. M. Vet, and R. Goudsmit

Serum ESF titers were measured in 42 polycythemic patients using the fetal mouse liver cell bioassay. ESF titers in patients with secondary polycythemia differed significantly from those in patients with polycythemia vera (p < 0.0001). Among the 21 patients with secondary polycythemia, 1 patient had an ESF titer less than 10 mU/mL (the lower limit of sensitivity) and 20 had ESF titers that ranged between 11 and 112 mU/mL, with a mean titer of 56 mU/mL. Among the 21 patients with polycythemia vera, 13 patients had ESF titers less than 10 mU/mL and 8 had ESF titers ranging between 12 and 55 mU/mL, with a mean titer of 26 mU/mL. The mean hemoglobin concentration in the 8 patients with ESF titers greater than 10 mU/mL was significantly below that in the 13 polycythemia vera patients with ESF titers less than 10 mU/mL (p < 0.03). If ESF titers less than 10 mU/mL had been indicative of polycythemia vera and ESF titers greater than 10 mU/mL had been indicative of secondary polycythemia in patients with hemoglobin concentrations greater than 17.7 g/dL, but not indicative of either condition in patients with hemoglobin concentrations less than 17.7 g/dL, 71.5% of the polycythemic patients in this study would have been diagnosed correctly, 9.5% incorrectly, and in 19% the diagnosis would have remained uncertain. It was concluded that measurement of serum ESF titers using this in vitro bioassay can be of clinical importance in differentiating between polycythemia vera and secondary polycythemia.

PATIENTS with an increased red blood cell count, hemoglobin concentration, or hematocrit fall into two groups—those with absolute polycythemia where the total red cell mass is elevated and those with relative polycythemia where the red cell mass is normal but the plasma volume is reduced. Patients with absolute polycythemia can be classified as those with polycythemia vera and those with so-called secondary polycythemia.

Polycythemia vera is a myeloproliferative disorder characterized by excessive production of erythrocytes and usually of granulocytes and platelets also. On the contrary, the secondary polycythemias are all caused by increased erythropoietin (ESF) stimulation of red cell production by normal erythroid bone marrow. They can be divided into those in which increased ESF production is a physiologic response to tissue hypoxia leading to an appropriate compensatory increase of the circulating red cell mass and those in which ESF production is inappropriately increased. A decrease in oxygen supply sufficient to cause polycythemia may occur as the result of decreased arterial oxygen saturation as in chronic obstructive lung disease or cyanotic heart disease, increased blood oxygen affinity as with certain abnormal hemoglobins, and decreased renal blood flow as with renal artery stenosis. A nonphysiologic increase of ESF production is associated most commonly with tumors but also with benign disorders as renal cysts and hydronephrosis.

An accurate ESF measurement in patients with polycythemia could be of considerable help in the differential diagnosis between polycythemia vera and the many secondary polycythemias. Unfortunately, this has long been hampered by the insensitivity and crudeness of the current in vivo bioassay for ESF in the polycythemic mouse. We have measured serum ESF titers in patients with absolute polycythemia by the in vitro bioassay for ESF using fetal mouse liver cells. This method allows the quantitative detection of ESF in unconcentrated normal human sera. Its usefulness in differentiating between polycythemia vera and secondary polycythemia is reported herein.

MATERIALS AND METHODS

We used the fetal mouse liver cell bioassay for ESF. Details of the basic assay technique were described in the preceding paper. Serum ESF titers were determined in 21 patients with clinical and laboratory findings meeting the requirements of the Polycythemia Vera Study Group for a diagnosis of polycythemia vera. Among these patients, 13 were treated with regular phlebotomies.

RESULTS

Figure 1 shows the serum ESF titers found in 42 polycythemic patients. Patients with polycythemia vera patients with ESF titers less than 10 mU/mL (p < 0.03). If ESF titers less than 10 mU/mL had been indicative of polycythemia vera and ESF titers greater than 10 mU/mL had been indicative of secondary polycythemia in patients with hemoglobin concentrations greater than 17.7 g/dL, but not indicative of either condition in patients with hemoglobin concentrations less than 17.7 g/dL, 71.5% of the polycythemic patients in this study would have been diagnosed correctly, 9.5% incorrectly, and in 19% the diagnosis would have remained uncertain. It was concluded that measurement of serum ESF titers using this in vitro bioassay can be of clinical importance in differentiating between polycythemia vera and secondary polycythemia.

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polycythemia vera had lower ESF titers than patients with secondary polycythemia (p < 0.0001). In 13 of 21 patients with polycythemia vera, ESF titers were below 10 mU/ml. In the other 8 patients, ESF titers ranged between 12 and 55 mU/ml, with a mean value of 26 mU/ml. In 1 of 21 patients with secondary polycythemia, the serum ESF titer was below 10 mU/ml. In the other 20 patients ESF titers ranged between 11 and 112 mU/ml, with a mean value of 56 mU/ml.

The highest ESF level was found in the patient with cyanotic heart disease who had been phlebotomized several times in the preceding months.

Table 1 presents mean values of ESF titers, hemoglobin concentrations, and hematocrit levels in these 42 polycythemic patients and those obtained in 38 normal subjects in a previous study. ESF titers in patients with polycythemia vera were lower than those in normal subjects (p < 0.001). ESF titers in patients with secondary polycythemia did not differ significantly from those in normal subjects. Among the 21 patients with polycythemia vera, the 8 patients with ESF titers greater than 10 mU/ml had lower hemoglobin levels than the 13 patients with ESF titers less than 10 mU/ml (p < 0.03). No significant difference was found between hematocrit levels in both groups.

Figure 2 shows the serum ESF titers related to the hemoglobin concentration in 21 patients with polycythemia vera and 21 patients with secondary polycythemia. No significant correlation was found between hemoglobin concentration and ESF titer in the patients with secondary polycythemia (r = -0.17, Spearman rank correlation). Among the 8 polycythemia vera patients with ESF titers greater than 10 mU/ml, 5 had hemoglobin levels less than 17.7 g/dl, and among the 13 patients with ESF titers less than 10 mU/ml, 11 had hemoglobin levels greater than 17.7 g/dl (x^2 = 4.96, p < 0.03). If ESF titers less than 10 mU/ml were indicative of polycythemia vera and ESF titers greater than 10 mU/ml were indicative of secondary polycythemia in patients with hemoglobin concentrations greater than 17.7 g/dl but not indica-

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patients (n)</th>
<th>ESF (mU/ml)</th>
<th>Hemoglobin (g/dl)</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycythemia vera</td>
<td>21</td>
<td>&lt;10 - 555</td>
<td>18.6 ± 3.2*</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>ESF &lt;10 mU/ml</td>
<td>13</td>
<td>&lt;10</td>
<td>19.6 ± 2.8†</td>
<td>61 ± 8†</td>
</tr>
<tr>
<td>ESF &gt;10 mU/ml</td>
<td>8</td>
<td>26 ± 16</td>
<td>16.9 ± 1.6†</td>
<td>58 ± 6†</td>
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<tr>
<td>Secondary polycythemia</td>
<td>21</td>
<td>&lt;10 - 112</td>
<td>19.6 ± 2.2</td>
<td>60 ± 7</td>
</tr>
<tr>
<td>ESF &lt;10 mU/ml</td>
<td>1</td>
<td>&lt;10</td>
<td>19.8</td>
<td>60</td>
</tr>
<tr>
<td>ESF &gt;10 mU/ml</td>
<td>20</td>
<td>56 ± 28</td>
<td>19.6 ± 2.2</td>
<td>60 ± 7</td>
</tr>
<tr>
<td>Cyanotic heart disease</td>
<td>6</td>
<td>&lt;10 - 112</td>
<td>20.6 ± 3.9</td>
<td>63 ± 11</td>
</tr>
<tr>
<td>ESF &lt;10 mU/ml</td>
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<td>&lt;10</td>
<td>19.8</td>
<td>60</td>
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<tr>
<td>ESF &gt;10 mU/ml</td>
<td>5</td>
<td>80 ± 29</td>
<td>20.7 ± 3.9</td>
<td>64 ± 11</td>
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<tr>
<td>Obstructive lung disease</td>
<td>8</td>
<td>47 ± 19</td>
<td>19.6 ± 1.7</td>
<td>59 ± 5</td>
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<tr>
<td>Renal artery stenosis</td>
<td>1</td>
<td>76</td>
<td>19.6</td>
<td>59</td>
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<tr>
<td>Renal cysts</td>
<td>2</td>
<td>19 ± 6</td>
<td>18.6 ± 1.3†</td>
<td>56 ± 0</td>
</tr>
<tr>
<td>Tumors</td>
<td>4</td>
<td>59 ± 25</td>
<td>18.7 ± 0.6</td>
<td>57 ± 3</td>
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<tr>
<td>Normal subjects</td>
<td>38</td>
<td>39 ± 24</td>
<td>14.7 ± 1.3</td>
<td>44 ± 3</td>
</tr>
</tbody>
</table>

*Mean ± SD.
†Different at p < 0.03.
‡Not significantly different.
§Different at p < 0.0001.
¶Different at p < 0.001.
‖Not significantly different.

Levels of significance were determined using the two-sided Wilcoxon rank sum test.
In this study we have demonstrated that serum ESF titers in patients with polycythemia vera are significantly lower than those found in normal subjects. In the majority of these patients, ESF levels were below the lower limit of sensitivity of the fetal mouse liver cell bioassay (10 mU/ml). However, in 8 of 21 patients with polycythemia vera, serum ESF titers were greater than 10 mU/ml and in the lower part of the normal range. These patients with ESF titers greater than 10 mU/ml had lower hemoglobin levels than those with ESF titers less than 10 mU/ml. This suggests that ESF production in patients with polycythemia vera is still under the influence of physiologic control mechanisms. Such a functional ESF response would be compatible with potential humoral regulation of erythropoiesis in polycythemia vera.

The secondary polycythemias are all caused by increased ESF production. This is often an appropriate response to conditions associated with tissue hypoxia.

In the present study we have found that ESF titers in patients with secondary polycythemia are in or slightly above the normal range. That the ESF levels are only moderately increased in these patients may be due to adequate compensation of the impaired tissue oxygen supply by the elevated red cell mass. Furthermore, the increased erythropoietic activity of the bone marrow in polycythemic patients may lead to relatively low serum ESF titers. Adequate tissue oxygen supply at polycythemic hemoglobin levels may explain the lack of a positive correlation between hemoglobin levels and ESF titers in patients with secondary polycythemia. Determination of serum ESF titers may be of clinical importance in the differential diagnosis of polycythemia.

Using the polycythemic mouse assay Erslev et al. measured ESF levels in highly concentrated plasma extracts from normal subjects and polycythemic patients. All patients with polycythemia vera and 24% of the patients with secondary polycythemia had undetectable ESF levels (less than 5 mU/ml). In patients with secondary polycythemia, ESF levels ranged from <5 to 3000 mU/ml. Normal subjects had ESF levels ranging from <5 to 18 mU/ml. Koeffler et al., using a radioimmunoassay for ESF, found that among 26 patients with polycythemia vera, 24 had ESF titers less than 30 mU/ml, and among 33 patients with secondary polycythemia, 31 had ESF titers greater than 30 mU/ml. The mean ESF titer was 17.5 ± 8.4 mU/ml (±SD) in patients with polycythemia vera, 94.3 ± 101.2 mU/ml in patients with secondary polycythemia, and 14.9 ± 4.2 mU/ml in normal subjects.

Comparison of these studies with our present study...

**DISCUSSION**

Polycythemia vera is generally considered a myeloproliferative disorder. The pathogenesis seems to be related to the evolution of an abnormal pluripotent stem cell with subsequent expansion of committed stem cell pools occurring most prominently in the erythroid line. Although the increased oxygen-carrying capacity of the blood in patients with polycythemia vera has been shown to lead to suppression of ESF production, studies both in vivo and in vitro suggest that erythropoiesis in polycythemia vera is not completely independent on normal humoral regulators such as ESF.
reveals a marked interassay variation. This was also shown by Lange et al. in a recent review of published reports on ESF levels in normal subjects. Such variation may be due to differences in immunoreactive versus bioactive ESF, loss of ESF associated with concentration methods, and the sensitivity of bioassays to the effects of inhibitors or nonspecific stimulators of erythropoiesis that may be present in crude sera. In the study of Erslev et al. as well as in our present study, ESF titers in patients with polycythemia vera were significantly below those found in normal subjects. On the contrary, Koeffler et al. found the ESF titers to be similar in patients with polycythemia vera and in normal persons. Thus far, this discrepancy is unexplained.

The studies of Erslev et al., Koeffler et al., as well as our present study all demonstrate that ESF titers in patients with polycythemia vera are significantly lower than those in patients with secondary polycythemia. However, in all three studies it was found that ESF titers in both groups of polycythemic patients partly overlap. In the present study this phenomenon may be considered to be due to the relatively high ESF levels found in polycythemia vera patients with only moderately elevated hemoglobin concentrations. The polycythemia vera patients with ESF titers greater than 10 mU/ml did not differ from those with titers less than 10 mU/ml with respect to age, therapy, and cardiopulmonary and renal function as assessed by routine clinical methods.

If ESF titers less than 10 mU/ml had been indicative of polycythemia vera and ESF titers greater than 10 mU/ml had been indicative of secondary polycythemia in patients with hemoglobin concentrations greater than 17.7 g/dl, 71.5% of the polycythemic patients included in this study would have been diagnosed correctly, 9.5% incorrectly, and in 19% of these patients the diagnosis would have remained uncertain. Measurement of serum ESF titers in polycythemic patients by this bioassay in vitro may be useful in differentiating between polycythemia vera and secondary polycythemia.

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