Differential Polycythemia: A Comparative Study between Spurious Polycythemia and Pure Erythrocytosis

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FUKUSHIMA, Y., FUKUDA, M., TAKAHASHI, T., YOSHIDA, K. and MIURA, A.B. Differential Polycythemia: A Comparative Study between Spurious Polycythemia and Pure Erythrocytosis. Tohoku J. exp. Med., 1987, 153 (2), 103-110—The definite differential diagnosis between spurious polycythemia (SP) and pure erythrocytosis (PE) was tested. Serum erythropoietin (EPO) levels in 6 patients with PE were 12.8±3.7 mU/ml and were significantly lower than those of both 19 normal controls (28.5±15.0 mU/ml) and 9 patients with SP (21.3±10.2 mU/ml). Three of 11 patients with SP and 1 of 3 patients with PE had significantly higher marrow erythroid progenitor cells (CFU-Es) than those of the normal controls. Spontaneous CFU-E growth (CFU-E growth in the absence of added EPO) was found in 4 of 11 patients with SP, 1 of 3 patients with PE, and all patients with polycythemia vera. However, the number of spontaneous CFU-E was low in SP and PE. The measurements of serum EPO levels and CFU-E growth did not provide differentiation between PE and SP. Even if some patients, whose total red cell volumes are either higher than 12.5% above the mean predicted values or their CFU-E growth is great, are diagnosed as SP, consideration should be made that they might, in fact, have PE.

The development of technics for measurement of erythropoietin (EPO) and erythroid progenitor cell (CFU-E) growth has played an important role in the differential diagnosis of the polycythemias. The differences in CFU-E growth and blood EPO levels in both polycythemia vera (PV) and secondary erythrocytosis are useful diagnostic criteria. Spurious polycythemia (SP) has been a vague entity with no clear criteria, and pure erythrocytosis (PE) has been accepted as a distinct pathological entity only recently. We have many patients in whom it is difficult to differentiate between SP and PE. Differentiation between these patients is very important, however, because those patients with PE must be followed-up strictly. There are a few reports of blood EPO levels and CFU-E growth in PE patients (Greenberg and Golde 1977; Dainiak et al. 1979; Clement et al. 1982; Erslev and Caro 1984). Regarding SP patients, however, a
detailed investigation has not yet been made. In the present paper, we have examined whether the combined measurements of serum EPO levels and CFU-E growth can contribute to the differential diagnosis between PE and SP.

**Materials and Methods**

**Patients**

Eleven male patients with SP were included in this study. SP was diagnosed according to the following criteria: 1) PCV > 51%. 2) The measured total red cell volume (TRCV) was lower than 25% above each patient's mean predicted normal value (Pearson et al. 1978). 3) The measured plasma volume below the predicted value for height and weight, based on the data of Nadler et al. (1962). None of the patients had conditions conducive to either secondary erythrocytosis or a palpable spleen.

Six male PE patients were also examined. PE was diagnosed according to all the criteria of Najean et al. (1981), except for TRCV. All patients with PE had TRCVs that were greater than 25% above their mean predicted normal values (Pearson et al. 1978).

All patients with polycythemia vera (PV) (n = 7) met the criteria of the National Polycythemia Vera Study Group. They consisted of one male and six females. Three of the 7 patients received no therapy, and 4 patients were treated with $^{32}$P (n = 2), busulfan (n = 1), and pipobroman (n = 1).

Serum EPO levels in 19 normal controls, and marrow CFU-Es in 18 other normal controls, were measured.

**Measurement of red-cell and plasma volume**

TRCV and plasma volume were measured separately with $^{51}$Cr-labelled red cells and $^{131}$I-labelled albumin. The results were expressed as ml/kg.

**Fetal mouse liver cell (FMLC) bioassay for EPO**

Serum EPO titers were measured by FMLC bioassay in vitro modified by Dunn et al. (1975). Fetal liver cells were obtained from dd-Y mouse embryos after 14 days of gestation and were suspended in Eagle's Minimal Essential Medium contained 5% fetal calf serum (FCS). The culture tubes were preincubated for 24 hr at 37°C in humidified air with 5% CO$_2$. 0.5 $\mu$Ci $^{59}$Fe-ferric citrate was subsequently added to each culture tube. Following 4 hr of further incubation, heme was extracted. Radioactivity in the aliquots of the solvent layer was then determined by an autowell gamma counter. A minimum of three cultures was set up for each sample.

**CFU-E assay**

The plasma clot method modified by Terasawa et al. (1979) was employed to assay CFU-E formation (Flow, McLean, VA, USA). The cultures were set up in duplicate, consisting of 20% FCS, 30% $\alpha$-medium (Flow), 10% EPO (1.0 U/culture), 10% beef embryo extract (GIBCO, Grand Island, NY, USA), 10% deionized bovine serum albumin (Sigma, St. Louis, MO, USA), 10% buffy coat cells, and 10% citrated AB type serum.

Sheep plasma EPO (Connaught, Step III) was used in both assays.

Both methods employed have been described in detail elsewhere (Fukushima et al. 1984).

**Statistical analysis**

The student's t-test and $\chi^2$ test were used for statistical comparisons.

All results are given as means ± s.d.
RESULTS

The values obtained for hematological analysis at the time of EPO and CFU-E assay are shown in Table 1. The values of age, PCV, leukocyte, body hematocrit, and plasma volume were not significantly different among three groups. The TRCVs of patients with either PE or PV were significantly greater than the TRCVs of patients with SP \((p<0.05)\). These values were also significantly greater than each predicted value \((p<0.01)\). Plasma volumes of patients with SP were significantly lower than the predicted values \((p<0.05)\), but those of patients with PE were not. The hematocrit ratios of patients with PV were greater than those of patients with SP \((p<0.01)\). Platelets of patients with PV were significantly greater than those of patients with SP \((p<0.05)\) and PE \((p<0.05)\), respectively.

Five of the 11 patients with SP (45\%) and 4 of the 6 patients with PE (67\%) were obese men. The degree of obesity \([\text{weight}/(\text{height}-100) \times 0.9) \times 100 > 110\%\] ranged from 114 to 133\% (128±8\%) in SP, and from 112 to 127\% (119±6\%) in PE. Seven patients with SP (64\%) and 4 patients with PE (67\%) were smokers. Among them, 5 patients with SP and 3 patients with PE were heavy smokers (>20 cigarettes/day). Five patients with SP (45\%) and 3 patients with PE (50\%) were found to have hypertension. The incidence of obesity, smoking

| Table 1. Hematological data in patients with SP, PE and PV |
|------------------------------|----------------|----------------|----------------|----------------|----------------|
|                              | Mean           | Range          | Mean           | Range          | Mean           | Range          |
| Age (years)                  | 50.3±15.3      | 49.4±14.6      | 58.1±9.1       | 30–74          | 27–65          | 46–72          |
| Sex (subject number)         | Male 11        | Male 6         | Male 1         | Female 6       | Male 1         | Female 6       |
| PCV (%)                      | 55.0±4.0       | 55.8±4.0       | 52.7±2.9       | 51.2–61.7      | 51.7–61.7      | 50.0–59.0      |
| Red cell volume (ml/kg)      | 28.4±5.7       | 38.6±3.6       | 38.6±2.9       | 20.5–37.1      | 36.0–44.9      | 35.9–41.3      |
| Predicted red cell volume    | 27.9±1.8       | 28.8±2.5       | 25.6±4.9       | 25.8–31.2      | 26.6–33.1      | 24.4–34.1      |
| (ml/kg)                      | 32.8±4.2       | 34.6±5.7       | 39.0±4.6       | 25.8–41.3      | 28.2–40.0      | 32.5–42.9      |
| Plasma volume (ml/kg)        | 37.4±2.6       | 38.6±3.3       | 38.4±4.3       | 34.9–42.6      | 35.6–44.3      | 32.6–45.6      |
| Predicted plasma volume (ml/kg) | 46.2±4.1     | 52.9±3.1       | 51.5±2.9       | 40.8–53.3      | 48.5–56.3      | 49.3–55.4      |
| Body hematocrit (%)          | 0.88±0.04      | 0.92±0.04      | 0.99±0.06      | 0.82–0.95      | 0.89–0.98      | 0.95–1.06      |
| Hematocrit ratio             | 7.2±1.6        | 5.5±0.8        | 12.8±6.0       | 5.3–11.0       | 4.6–6.5        | 5.5–21.8       |
| Leukocyte \((\times 10^9)/liter\) | 218±85        | 225±24         | 615±276        | 102–336        | 206–258        | 187–1039       |
| Platelet \((\times 10^9)/liter\) | 55±10         | 60±10          | 65±10          | 50–70          | 45–80          | 40–90          |
and hypertension was not significantly greater in the PE group than in the SP group ($\chi^2$ test).

**Serum EPO levels**

Fig. 1 shows serum EPO levels, both in normal controls, and in patients with SP, PE, and PV. Serum EPO levels in normal controls were $28.1 \pm 15.0$ mU/ml ($n = 19$). Serum EPO levels in patients with SP, PE and PV were $21.3 \pm 10.2$ mU/ml ($n = 9$), $12.8 \pm 3.7$ mU/ml ($n = 6$) and $17.1 \pm 9.4$ mU/ml ($n = 7$), respectively. Serum EPO levels in patients with PE were significantly lower than those in both normal controls ($p < 0.01$) and patients with SP ($p < 0.05$). Serum EPO levels of SP and PV patients were not significantly lower than those of the normal controls.

**Bone marrow CFU-E formation**

Fig. 2 shows the number of CFU-Es per $1 \times 10^6$ bone marrow cells at 1.0 U/ml concentration of EPO, as well as in the absence of added EPO (spontaneous CFU-E) in both normal controls and in patients with SP, PE, and PV. The number of marrow CFU-Es in the normal controls was $235 \pm 81/1 \times 10^6$ cells ($n = 18$); that in PE was $158, 228$ and $610/1 \times 10^6$ cells ($332 \pm 243/1 \times 10^6$ cells, $n = 3$);
that in SP ranged from 123 to 661/1 x 10^5 cells (314±185/1 x 10^5 cells, n = 11), and that in PV ranged from 355 to 499/1 x 10^5 cells (419±52/1 x 10^5 cells, n = 7). Three of 11 patients with SP, and 1 of 3 patients with PE had high marrow CFU-Es which were higher than 2 s.D. of those of the normal controls. The remainder in both groups had marrow CFU-Es within normal limits. The number of marrow CFU-Es of the PV patients was significantly higher than that of the normal controls (p <0.01), but was not significant in comparison with that of the SP patients.

Spontaneous CFU-E growth was found in 4 of 11 patients with SP, 1 of 3 patients with PE, and in all of the patients with PV. It ranged from 0 to 30/1 x 10^5 cells (6.3±10.4/1 x 10^6 cells) in SP, was 0, 0 and 10/1 x 10^5 cells (3.3±5.8/1 x 10^5 cells) in PE, and ranged from 162 to 381/1 x 10^5 cells (250±70/1 x 10^5 cells) in PV. The spontaneous colony number in patients with PV was significantly higher than that of patients with SP (p <0.01) and PE (p <0.01).

Follow up study

Eight of the 11 patients with SP and 4 of the 6 patients with PE were followed-up from 6 months to 8 years following their original examination. None
of the patients developed PV or acute leukemia. Their PCVs were maintained over 51.1%. In 5 other cases of SP and PE, who were followed-up within 6 months, the laboratory findings were about the same as they were at the first examination.

**DISCUSSION**

Although SP has been a vague entity, most patients with SP have such characteristic clinical features as mild obesity, moderate hypertension and compulsive smoking. Although patients with PE have not been reported to have such clearly defined clinical features, we have found that the incidence of obesity, smoking, and hypertension among them is similar. Therefore, we were unable to differentiate them by the clinical features alone. Differentiation between them must be based on laboratory data.

The important key about differential diagnosis of polycythemia is the volume of the circulating red blood cell. Measurements of TRCV are useful in distinguishing absolute from relative erythrocytosis. Absolute erythrocytosis has been interpreted that TRCV is higher than 36 ml/kg for men, and 32 ml/kg for women. However, Pearson et al. (1978) proposed that males with measured TRCVs greater than 25% and females with measured TRCVs greater than 30% above their mean normal TRCVs may be regarded as having absolute polycythemia. In our study, we adopted this interpretation. Since the mean normal TRCVs were variable, the formula for predicting a normal mean TRCV based on Nadler et al. (1962) was regarded as more precise than ml/kg expression. Using this method, two male patients, whose TRCVs were higher than 36 ml/kg, were diagnosed as SP because they were 119% and 121% of their predicted values. All patients with PE and PV had TRCVs higher than 36 ml/kg for men, and 32 ml/kg for women.

There are no clear definitions of SP. We decided the criteria of SP as defined above (see Materials and Methods) by referring to Humphrey et al. (1980) and Watts and Lewis (1983). Since Humphrey et al. (1980) have defined SP as being when the measured TRCVs are less than 12.5% above the mean predicted values, three of our eleven patients with SP might not be diagnosed as SP (119, 121 and 121%). Blood EPO levels in patients with SP have been reported to be within the normal range (Erslev 1983). In this study, serum EPO levels in patients with SP tended to be lower than normal values, but were not significant. Although PCVs were high in SP, TRCVs and body hematocrits were not high except for a few cases. Three patients with SP had significantly higher CFU-E numbers than those in the normal controls. It may be that these patients have PE. In fact, two of three patients had TRCVs higher than 12.5% above the predicted values, but one of them had a normal TRCV (23.5 ml/kg). Therefore, some SP patients, whose TRCVs are either high or their CFU-E growth is great (defined as borderline cases), must be followed-up carefully.

PE is a condition of absolute erythrocytosis, and is suspected of being a
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A subgroup of PV with only a pure red cell lineage anomaly (Clement et al. 1982). If this is true, serum EPO levels in patients with PE should be lower than the normal values. However, Erslev and Caro (1984) reported that one third of the patients with PE have increased titers, while two thirds have low titers, suggesting that there are at least two PE groups. In the present study, serum EPO levels in the PE patients were significantly lower than those in both the normal controls \((p<0.01)\) and the SP patients \((p<0.05)\). Significantly lower EPO titers for PE were reasonable since the TRCVs of patients with PE were significantly higher than both those of patients with SP \((p<0.05)\) and the predicted values \((p<0.01)\). Unfortunately, a CFU-E assay was done for only three out of six patients. One of these had a significantly higher CFU-E growth. Patients with SP and PE, who had higher CFU-E growth in 1.0 U/ml concentration of EPO, had a few CFU-E colonies in the absence of EPO.

In this study, spontaneous CFU-E growth in normal controls was not examined; however, we did assay it at another time by the use of different lot number of FCS. In an earlier study, we found that in 15 out of 19 normal controls, spontaneous CFU-E growth was not found. In the remainder, it ranged from 5 to 15/10^6 cells. Therefore, the small number of CFU-Es in our patients with SP and PE was not regarded as significant in distinguishing them. In respect to CFU-E growth in PE, it is reported that there are either spontaneous CFU-E growth (Greenberg and Golde 1977; Dainiak et al. 1979; Clement et al. 1982), or no growth (Clement et al. 1982). In our study on the CFU-E assay, PE could not be distinguished from SP.

Patients with PV had significantly higher CFU-E numbers both in the presence and in the absence of EPO, although some patients had already been treated. Therefore, the diagnosis of PV was easy.

We clarified that serum EPO levels in patients with SP tended to decrease, and that those with PE were significantly lower than both those of the normal controls and SP group. However, the ranges of serum EPO levels in PE patients \((7.8-18.9 \text{ mU/ml})\) were contained in those in SP patients \((7.3-37.0 \text{ mU/ml})\). From the view point of CFU-E growth, it is suggested that some of the patients with PE might be among those patients diagnosed as SP. Significant spontaneous CFU-E growth was not found in the patients with PE. Although only a few patients with PE were examined, these results have supported Gilbert’s suggestion that EPO and CFU-E assays are valuable, but may not be as specific as had been assumed (Gilbert 1977).

In conclusion, it is difficult to differentiate PE from SP, especially in borderline cases. We also found that patients with SP, where either the TRCVs are higher than 12.5% above the mean predicted values, or where the CFU-E growth is great, might be diagnosed as PE.
References


