Erythropoietin Levels and the Medullary Hematopoietic Pool in Aplastic Anemia

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SUMMARY

The concentration of erythropoietin (EPO) was measured by radioimmunossy in 26 patients with aplastic anemia (AA). At the same time, the percentage of the area occupied by medullary hematopoietic pool was surveyed in cross sections of bone marrow biopsies from patients with AA. The mean serum EPO level was elevated in all patients with AA with significant differences between the severe and moderate groups and between the moderate and mild groups. The mean percentage of medullary hematopoietic pool in each group was not significantly different between the severe and moderate group and between the moderate and mild groups. A negative correlation was noted between the medullary hematopoietic pool and the serum EPO level in AA patients. Thus, the serum EPO level increased and medullary hematopoitic pool declined with increasing severity of the anemia. Comparison of the medullary hematopoietic pool between patients with AA of different severities showed that the pool increased in the order of severe < moderate < mild, and the differences between these groups of patients were all statistically significant.

INTRODUCTION

Erythropoietin (EPO) is a hormone which specifically stimulates red blood cell production and is the first hematopoietic factor to be purified and cloned for clinical application [9, 11]. Radioimmunoassay (RIA) of EPO has made the microquantitation of this hormone possible as a result of marked progress in the sensitivity and accuracy of measurement over recent years.

Clinical studies have shown that the blood level of EPO is negatively correlated with the hemoglobin and hematocrit levels in patients with various forms of anemia, except those with concurrent renal disease, rheumatoid arthritis, or cancer [2, 3, 5]. In patients with aplastic anemis (AA), the EPO level has been reported to be higher than that in other types of anemia [1, 8], probably due to a severe defect or deficiency of the hematopoietic stem cells and hematopoietic microenvironment. However, little is known about the relationship between the blood level of EPO and the medullary hematopoietic pool. In the present study, we measured the serum EPO level by RIA in patients with aplastic anemia (AA) and compared it with the measurements to the Hb and Ht values as well as the medullary hematopoietic pool estimated from marrow biopsy specimens.

SUBJECTS AND METHODS

A total of 26 Patients with previously untreated or treated AA examined at our hospital or affiliated institutions between July 1989 and

July 1993 were studied. They consisted of 10 males and 16 females, between 38 and 77 years old. Fortythree healthy volunteers consisting of 20 males and 23 females between 17 and 72 years, served as the controls.

Blood was taken from each subject in the early morning after fasting and a 1-ml aliquot of serum was stored frozen at −80℃ until use. The serum EPO concentration was determined using a commercial EPO RIA kit (Japan DPC Corporation). In brief, EPO was allowed to react with an anti-EPO antibody and then radioiodine-labeled r-hEPO was added. Thus, the assay was a 2-step dual antibody technique utilizing delayed competitive binding.

The medullary hematopoietic pool was determined as the percentage of the microscopic field occupied by nests (or islands) of hematopoietic cells on smears prepared from marrow biopsy specimens taken from the posterior illiac crest.

Using a tablet measure for micrometry (VM-30, Olympus Co.), the area of each nest (or island) of hematopoietic cells was measured on a television screen after microscopic images of a bone smear were projected. The medullary hematopoietic pool was calculated as the mean precent area of the nests (or islands) of hematopoietic cells in 3 microscopic fields with a mean area of 1.0×10^{-5} m^2.

Statistical analysis of the data was carried out using the t-test.

RESULTS

1) Serum EPO levels in the healthy controls

In the 43 healthy controls, the serum EPO level was 20.9 ± 6.5 mU / ml (mean ± SD), which was similar to previously reported values [10] and the Hb and Ht values were 1 4.0 ± 1.3 g / dl and 42 ± 4.3%, respectively.

2) Relationship of the serum EPO level to the medullary hematopoietic pool in AA patients

The relationship between the serum EPO level and the medullary hematopoietic pool was examined in 26 AA patients in whom the EPO level was determined concurrently with the bone marrow biopsy. These patients had a mean serum EPO level and medullary hematopoietic pool of 4578.4 ± 7337.5 mU / ml and 19.1 ± 15.4% respectively.

The medullary hematopoietic pool and the

serum EPO showed a significant negative correlation ($r = -0.514$ p<0.001). In AA, the serum EPO level was thus demonstrated to rise as the medullary hematopoietic pool decreased (**Fig. 1**).
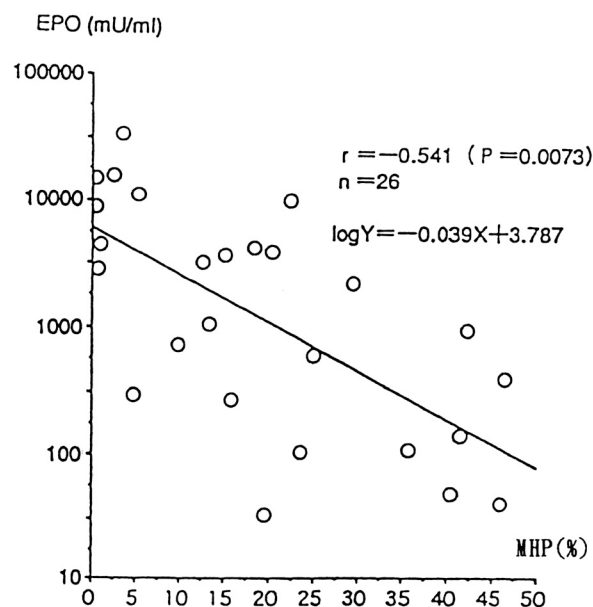

Fig. 1 Relationship between serum EPO level and medullary hematopoietic pool in patients with aplastic anemia (MHP : Medullary Hematopoietic Pool)

3) The serum EPO level and medullary hematopoietic pool in relation to the severity of AA

The 26 AA patients were divided into 3 groups according to the severity classification proposed by the Welfare Ministry's Specified Disease Idiopathic Dyshematopoiesis Research & Study Group and the relationship between the serum EPO level and the medullary hematopoietic pool was determined in each group. The 11 patients in the severe group had a mean serum EPO level of 9741.8 ± 9055.4 mU / ml and a mean hematopoitic pool of 8.0 ± 8.7%, and there was no correlation between these two variables. The 6 patients with moderate disease had a serum EPO level of 1664.2 ± 1377.2 mU / ml and a hematopoietic pool of 18.0 ± 7.7%, there being no correlation between the two variables. Furthermore, the 9 patients in the mild group had a mean serum EPO level and hematopoietic pool of 210.3 ± 257.6 mU / ml and 33.4 ± 14.3%, respectively, again with no correlation between the two parameters. On the other hand, a statistically significant
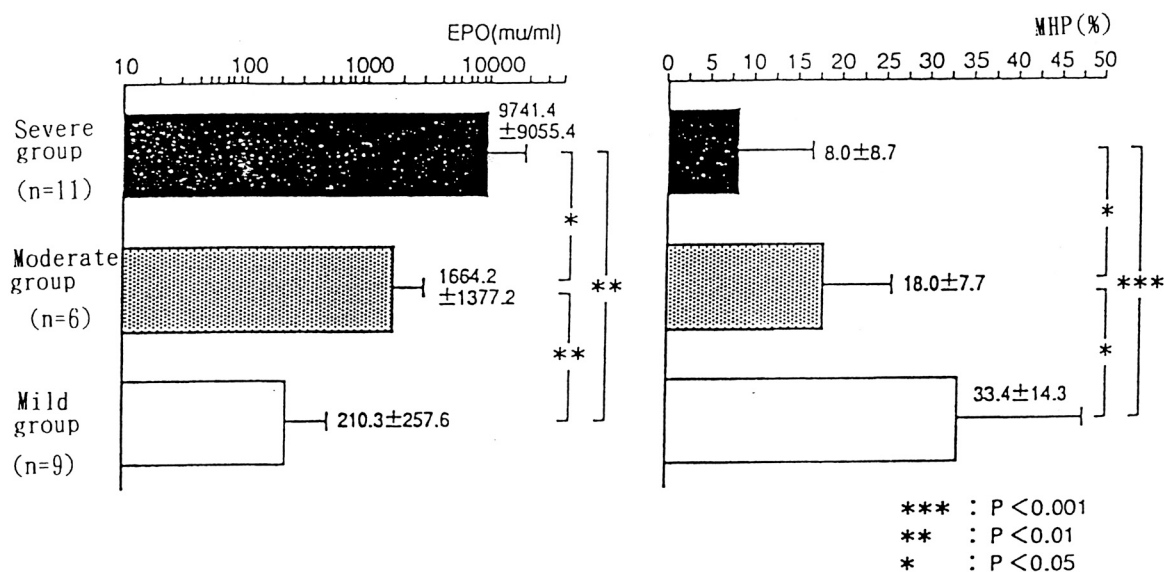
Fig. 2 Serum EPO level and medullary hematopoietic pool in every severity group of aplastic anemia (MHP : Medullary Hematopoietic Pool)

difference in serum EPO levels was observed between the severe and moderate groups ($p < 0.05$), and between the moderate and mild groups ($p < 0.01$). Intergroup comparison of the medullary hematopoietic pool also showed a significant difference between the severe and moderate groups ($p < 0.05$), and between the moderate and mild groups ($p < 0.05$) (**Fig. 2**).

DISCUSSION

RIA has recently achieved widespread use as a reliable and sensitive method for the quantitation of serum EPO levels [7, 13].

Erslev et al. [4, 5] reported an inverse correlation between the serum EPO level and the Ht, except in patients with concurrent renal disease, rheumatoid arthritis, or cancer. There are also reports stating that anemia in premature infants and AIDS patients is associated with paradoxically low serum EPO levels [12, 14]. However the serum EPO level generally rises in anemia.

AA is a hematologic condition characterized by a quantitative decrease of hematopoietic stem cells resulting from a severe defect or suppression of bone marrow function. The pat hogenetic factors, proven or hypothetical, may be classified as intrinsic and extrinsic. Possible intrinsic pathogenetic

factors include quantitative and qualitative defects of the hematopoietic stem cells themselves, while immunologic abnormalities of the hematopoietic environment and the process of hematopoiesis have been suggested as extrinsic factors [11]. The quantitative decrease of hematopoietic cells is an outstanding pathophysiologic feature of AA and the colony-forming capacity of late erythroid precursor cells (colony-forming unit-erythroid : CFU-E) is generally strongly depressed. On the other hand, the sensitivity to EPO begins with BFU-E and peaks with CFU-E [6]. This probably explains why a reactive elevation of the serum EPO concentration occurs in AA patients. In fact, AA is frequently associated with severe anemia and exceedingly high serum EPO levels, but nevertheless an inverse correlation is still noted between the serum EPO concentration and both the Hb and Ht values.

Our study revealted that AA patients had extremely high EPO levels compared to healthy individuals and that the serum EPO level showed a negative correlation with both Hb and Ht. Since the target of circulating EPO may include a wide variety of cell types in the erythroid lineage, encompassing early erythroid precursor cells (BFU-E), late erythroid precursor cells (CFU-E) and basophilic erythroblasts (BEb), it seems important

to examine the relationship between the serum EPO level and the medullary hematopoietic pool (normally functioning bone marrow). Therefore, we determined the serum EPO concentrations concurrently with bone marrow biopsy in 26 AA patients. Our findings showed that there was a negative correlation between the serum EPO level and the medullary hematopoietic pool ($r = -0.514$), indicating that the EPO concentration increased as the medullary hematopoietic pool diminished. After classifying the AA patients into 3 groups (i, e., severe, moderate and mild accrding to the Welfare Ministry classification) the serum EPO level and hematopoietic pool were compared in the individual groups. As a result, a significant difference in serum EPO was observed between the severe and moderate groups as well as between the moderate and mild groups ($p < 0.001$).

The medullary hematopoietic pool was also significantly different between the severe and moderate groups as well as between the moderate and mild groups ($p < 0.01$ and $p < 0.001$, respectively). These findings clearly indicate that as AA becomes more severe, the serum EPO level rises and the medullary hematopoietic pool diminishes. The Welfare Ministry's classification is a comprehensive system based on the granulocyte, platelet, and reticulocyte counts, but not on the severity of anemia. However, the present study demonstrated that both the serum EPO level and the hematopoietic pool vary noticeably with the severity of AA, Therefore, the serum EPO level may not only be useful in evaluating the severity of this disease but may also provide an index for the estimation of bone marrow function.

CONCLUSION

1. In AA patients, serum EPO levels decreased in the order of severe > moderate > mild, being significantly different between any two of these disease severity groups.
2. A negative correlation was noted between the medullary hematopoietic pool and the serum EPO level in AA patients. Thus, the serum EPO level increased and the medullary hematopoietic pool declined with increasing severity of the anemia.
3. Comparison of the medullary hematopoietic

pool between patients with AA of different severities showed that the pool increased in the order of severe < moderate < mild, and the differences between these groups of patients were all statistically significant.

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