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Reduced Erythropoitein Levels as a Cause of Anaemia in Patients with Lung Cancer

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Abstract—*Measurements of erythropoietin (Ep) levels in patients with the anaemia of chronic disorders due to malignant disease have given variable results. This variation may be due to the wide range of malignancies studied and the assay method (whole animal) used. In this study Ep levels were measured, using the foetal mouse liver assay, in* 39 *patients with lung cancer and* 19 *controls. Twelve patients had reduced haemoglobin levels (Hb less than* 11.5 g/dl *for males and* 11 g/dl *for females) and the features of the anaemia of chronic disorders. Their mean Ep level was* 0.21 iu/ml. *This was significantly lower than for the normal controls, whose mean value was* 0.31 iu/ml ($P < 0.02$). *This data supports the concept that lack of an appropriate Ep response to anaemia is one factor in the genesis of anaemia in malignancy.*

INTRODUCTION

MANY patients with malignant disease have a mild non-progressive anaemia characterised by normochromic normocytic erythrocytes, a low serum iron and iron binding capacity but normal or increased marrow iron stores associated with a reduction in sideroblast formation. Cartwright termed this the Anaemia of Chronic Disorders: a condition in which red cell life span is reduced and it is the failure of the marrow to fully compensate for this reduction that renders the patient anaemic [1].

Erythropoietin (Ep) plays a major role in the control of erythrocyte production [2] and previous workers have reported variable levels in the anaemia of chronic disorders associated with malignancy [3–5].

The foetal mouse technique for measuring Ep is particularly suitable for measuring low levels of Ep [6] and has not been previously used in patients with malignant disease. It was therefore decided to measure Ep levels in patients with neoplastic disease using this method. Only patients with lung cancer were studied since such patients rarely suffer significant blood loss from tumour ulceration and by using patients with only one basic pathology it was hoped to reduce the variables present in the study.

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PATIENTS AND METHODS

Control subjects for Ep estimations were 12 males and seven females attending the outpatient laboratory in whom no significant pathology was subsequently found. All had normal full blood counts and haematological indices and normal serum irons and total iron binding capacities (TIBC) (Table 1). For the sake of comparison the Ep levels in seven patients with uncomplicated iron deficiency anaemia were also measured (Table 1). Thirty-nine patients were studied: 34 had small cell lung cancer, the remainder squamous cell tumours. The full blood count, serum iron, TIBC, B12 and folate were determined using standard techniques. A bone marrow aspirate and trephine were obtained in all but two patients and the serum ferritin measured in most cases. In addition to standard cytological examination, the marrow was stained with Prussian blue and scored for iron on a scale of 0 (no stainable iron) to 4 (markedly increased). Serum ferritin levels were measured using an immuno-radiometric method [7]. Ep was measured with the Foetal Mouse Liver Cell assay as described by Dunn *et al.* [6]. In all cases the results satisfied the criteria for statistically valid parallel line bio-assays [8]. In particular the relationship between the logs of the standard and test dose and the response (that is counts per minute from the 59 iron incorporated into haemoglobin) always showed significant linear regression and increasing response with increasing dose; furthermore they showed neither significant difference from linearity nor significant difference from para-

Table 1. *Details of Hb and iron status in groups of patients: values are means with standard deviation in brackets*

Group	Hb	Fe	TIBC	Marrow Iron	Ferritin
I	14.3 (1.3)	15.4 (5.7)	57.8 (9.1)	1.9 (1.0)	2.6 (0.6)
II	10.4 (0.9)	7.8 (6.7)	47.0 (6.4)	2.5 (0.8)	2.8 (0.4)
Controls	14.9 (0.9)	21.5 (3.8)	59.0 (6.9)	Not done	Not done
Iron deficient	8.7 (1.1)	6.0 (2.2)	69.6 (21.3)	Not done	Not done

Units: Hb, g/dl; Fe, μmol/l; TIBC, μmol/l.
Marrow iron 0 — no stainable iron to 4 — markedly increased.
Ferritin expressed as $\log_{10}$ of value in μg/l (ferritin distribution is approximately log normal).

llelision to one another.

The standard Ep preparation used was International B (0.5 iu/ml), kindly supplied by Dr. I. Bangham, World Health Authority for Biological Standards, London. At least four and usually five quadruplicate dilutions of test and standard were made for each assay. In the latter case the range of concentrations of test (or standard) material in the tissue culture medium was from 0.012 ml per ml to 0.606 ml per ml. The minimal detectable dose was 0.006 iu ml. The coefficient of variation for between assays in this laboratory was 0.039 and within assays 0.038. Results of the assay are expressed in iu/ml. Serum to be tested was stored at −20°C and prior to assay all serum samples were incubated at 60°C for 30 min to inactivate complement.

RESULTS

Fifteen patients (11 males, four females) had no significant haematological abnormality (Hb ≥ 13.5 g/dl for males, ≥ 13 g/dl for females) with normal indices. All had normal or increased stainable iron in the bone marrow and there was no evidence of iron or any dietary deficiency (Table 1). The mean Hb for this group was 14.3 g/dl. These patients formed group I.

There were 12 patients in group II (nine male, three female) with Hb ≤ 11.5 g/dl for males ≤ 11 g/dl for females, mean Hb for the group was 10.4 g/dl. These patients tended to have low serum irons and TIBCs in the low or normal range and all but one had normal or increased amounts of stainable iron in the marrow (Table 1); this patient did not have a bone marrow performed but had a serum iron of 27.3 μmol/l, a TIBC of 53 μmol/l and a serum ferritin of 1059 μg/l, thus excluding iron deficiency anaemia. This group of 12 patients represent patients with the anaemia of chronic disorders.

Five patients had Hb between 11.6 and 12.8 g/dl falling between groups I and II. Five other patients had marrow infiltrated by tumour, another had frank iron deficiency anaemia and one patient had a megaloblastic marrow. These 12 patients will not be considered further.

Individual results for patients in groups I and II and the control group are shown in Fig. 1. Mean Ep for the control group was 0.31 iu/ml and that for the anaemic iron deficient patients 1.7 iu/ml. Group I patients had a mean Ep of 0.29 iu/ml and group II a mean Ep of 0.21 iu/ml. Analysis of results using the Wilcoxon test for unpaired data shows the difference in distribution of the results between the control group and group II to be significant at the 2% level.

DISCUSSION

Previous reports of Ep levels in the anaemia of chronic disorders have been conflicting. Zucker *et al.* [3] studied serum Ep in 25 patients with advanced malignancy and a wide variety of tumours including myeloma, leukaemia and gastro-intestinal neoplasms. The patients had a mean Hb of 10.3 g/dl and all had the features of the anaemia of chronic disorders. Twenty two of the patients had elevated Ep levels and there was a significant positive correlation between Ep and Hb.

Other authors have found reduced Ep levels. Douglas and Adamson examined six patients with a variety of solid tumours and a mean Hb of 10.3 g/dl (4); they found Ep levels (actually 'erythropoietin stimulating factor' excretion rates) to be reduced below that expected for the level of anaemia, but to be within or near the 95% confidence limits for control patients.

Firat and Banzon measured Ep in 12 patients with a mean Hb of 7.5 g/dl and differing solid tumours [5]. They found a significant reduction compared with non-anaemic controls.

In a more recent study Dainiak *et al.* were unable to detect erythropoietic activity in the serum of patients with haematological and non haematological malignancies and a range of Hb levels [9]. Other workers have measured Ep levels in patients with cancer [10, 11] but either the numbers have

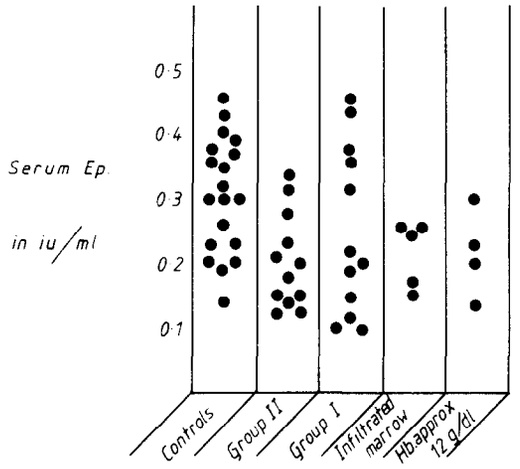

Fig. 1. Ep levels in various groups of patients with malignant disease. For details see text.

been small or it has been difficult to dissect out data relating to patients with cancer from patients with inflammatory disease.

The studies mentioned above have two features in common. They include patients with a variety of neoplasms and all used an *in vivo* whole animal type Ep assay. By contrast the present study was restricted to patients with lung cancer and the assays have all been of the *in vitro* foetal mouse cell culture type. It is suggested that this gives the present study advantages over those quoted. Haematological malignancies have a high incidence of bone marrow involvement which may affect erythropoiesis [12]. Patients with infiltrated marrows were not included in the final analysis of this study. Gastro intestinal tumours are frequently ulcerated leading to blood loss and the possibility of this being an independent cause of the anaemia. These problems did not occur with the pulmonary tumours considered here. It was also intended by using patients with only one underlying pathology, to study a more homogenous group and thus reduce some of the variation that might have been introduced into earlier studies by the large variety of malignancies involved.

The whole animal Ep assay is limited in its ability to measure low concentrations of Ep [2]. The choice of the foetal mouse method is thus preferable if low levels are to be measured.

The results from the control patients in this study are higher than those reported by others but of the same order as found by Napier *et al.* using the same assay system [13]. The Ep levels found in the group with iron deficiency anaemia indicate that high levels are readily detected. Possible causes of the anaemia of chronic disorders in patients with cancer include reduced Ep levels, reduced marrow sensitivity to Ep [12] and reduced iron reutilisation [14]. With these in mind this study has demonstrated a significant reduction in Ep levels amongst patients with lung cancer and mild anaemia compared with controls. Non-anaemic patients had a reduction in Ep levels which was not statistically significant. Ep has been shown to be the chief hormone regulating the erythron [2]. There seems little doubt that the failure of an appropriate Ep response is one of the factors in the genesis of the anaemia in malignant disease.

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