African Journal of Medicine and Medical Sciences

Editor: O.A. Ladipo
Assistant Editors:
B.O. Osotimehin and A.O. Uwaifo

Volume 18
1989

BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Boston Melbourne

Afr. J. Med. med. Sci. (1989) **18**, 75–81.

Radioisotope investigations of haematological disorders (excluding sickle cell disease) in Sierra Leone

H. H. M. KNOX-MACAULAY

Radioisotope Unit, Departments of Medicine & Pathology, Connaught Hospital, Freetown, Sierra Leone

Summary

Using ^{51}Cr, ^{125}I, and ^{59}Fe, red cell survival, blood volumes and certain aspects of iron metabolism were investigated in Sierra Leoneans referred for further evaluation of various blood disorders. The data provided clarification of the nature of the anaemia in some patients and demonstrated the haemolytic and erythropoietic role of the spleen in others with splenomegaly. Blood volume values in healthy individuals were found to be similar to those of normal subjects in other populations. Increased plasma volumes commonly described in non-African patients with anaemia and splenomegaly were also recorded in this group of Sierra Leoneans. Information obtained from these studies indicates that facilities for radioisotope investigations would be an asset in the diagnostic evaluation of complex blood disorders and in haematological research in developing African countries.

Résumé

Utilisant le ^{51}Cr, le ^{125}I, et le ^{59}Fe, on a examiné la survivance des cellules rouges, des volumes de sang et quelques aspects du métabolisme de fer, chez des Sierra Léonais, atteints des diverses affections sanguines, nécessitants une évaluation additionnelle. Les données ont fourni une clarification de la nature de l'anémie de certaines malades et aussi ont montré le rôle hémolytique et erythropoiétique de la rate chez certaines d'autres, atteints de splénomégalie. Les valeurs du volume de sang chez les individus en bonne santé étaient pareils aux gens normaux dans les autres pays. L'augmentation des volumes du plasma qui s'est trouvée d'habitude chez des malades non africaines, atteints d'anémie et de splénomégalie était remarquée aussi parmi ce groupe de Sierra Léonais. Les renseignements obtenus de ces études indiquent que les moyens de recherche portant sur les radioisotopes seraient d'un grand avantage pour l'évaluation diagnostique d'affections sanguines complexes, et pour la recherche en hématologie dans les pays africains en voie de développement.

Present address: Professor H. Knox-Macaulay, Department of Pathology, College of Medicine & Medical Sciences, King Faisal University, P.O. Box 2114, Dammam-31451, Kingdom of Saudi Arabia.

Introduction

In Sierra Leone, the diagnosis of common blood disorders such as sickle cell disease and folic acid deficiency anaemia usually does not present any difficulties. However, some patients are referred to hospital for further diagnostic evaluation of complex haematological problems including ill-defined anaemias. Recently, radioisotopes have played a major role in the assessment of various blood diseases. The results of some of these studies in sickle cell disease have been reported previously [1]. This communication further demonstrates the usefulness of radioisotopes in helping to elucidate and analyse ill-defined forms of anaemia and functional abnormalities associated with splenomegaly in a developing country.

Subjects and methods

During an 18-month period, 12 patients (seven male and five female) were referred to the Radioisotope Unit for further evaluation of various haematological problems. Three subjects had obvious iron deficiency anaemia and their results are not included. Of the remaining

nine patients, five were anaemic with varying degrees of splenomegaly, two were anaemic without splenomegaly and two had an enlarged spleen but were not anaemic (Tables 1, 2 and 4). The following investigations were performed: (a) ^{59}Fe-ferrokinetics, i.e. plasma clearance, organ uptake and red cell incorporation; (b) survival and organ uptake of ^{51}Cr-labelled red cells; and (c) red cell and plasma volume measurements using ^{51}Cr, ^{125}I and ^{59}Fe.

^{59}Fe-plasma clearance, organ uptake and red cell incorporation

Serum transferrin from the subjects studied was labelled with ^{59}Fe by a modification of Cavill's method [2]. The procedure involved adding 0.5 ml of [^{59}Fe]ferric citrate (Radiochemical Centre, Amersham) to 5 ml of patient's and normal subject's serum and incubating the serum at 37°C for 1 h before passage through an anion exchange resin column to remove unbound [^{59}Fe]ferric citrate. After sterilizing the eluate containing bound [^{59}Fe]transferrin by passing it through a Millipore filter, ^{59}Fe-plasma clearance (Fig. 1) was then determined as described previously in this population [1]. Organ uptake of ^{59}Fe was evaluated during the first 6 h (Fig. 2) and periodically over the ensuing 20–30 days (Fig. 3). The degree of incorporation of ^{59}Fe into red cells was also determined (Fig. 4).

^{51}Cr red cell survival

This was measured by a modification [1] of the technique recommended by the International Committee for Standardization in Hematology (ICSH) Panel [3]. Organ uptake of ^{51}Cr-labelled red cells was assessed by the method of the ICSH Panel [4].

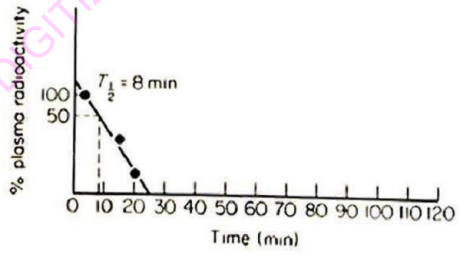

Fig. 1. ^{59}Fe plasma clearance: rapid clearance of ^{59}Fe from plasma in subject C with refractory anaemia, hyperplastic bone marrow and hepatosplenomegaly (plotted on semi-log paper).

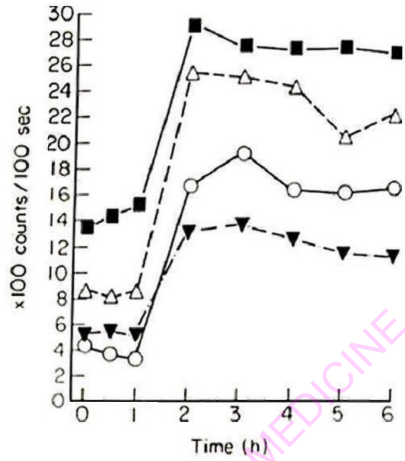

Fig. 2. ^{59}Fe organ-surface counts: curves showing significant uptake of ^{59}Fe by the liver and spleen but minimal uptake by the bone marrow during the first 6 h after the intravenous administration of ^{59}Fe-labelled serum transferrin into subject C. Subject H with idiopathic myelofibrosis also showed similar ^{59}Fe uptake curves. (■) Liver, (△) spleen, (○) heart, (▼) marrow.

Red cell volume

Red cell volume was estimated by the use of ^{51}Cr-labelled red cells following the procedure recommended by the ICSH panel [5].

Plasma volume estimation

Using radio-iodinated human serum albumin ([^{125}I]HSA), plasma volume was estimated according to the method recommended by the ICSH panel [5]. Plasma volume values were also calculated from the ^{59}Fe plasma clearance data.

Whole blood volume estimation

Whole blood volume was calculated from the various red cell volume, plasma volume and venous haematocrit (H_V) values (Tables 3 and

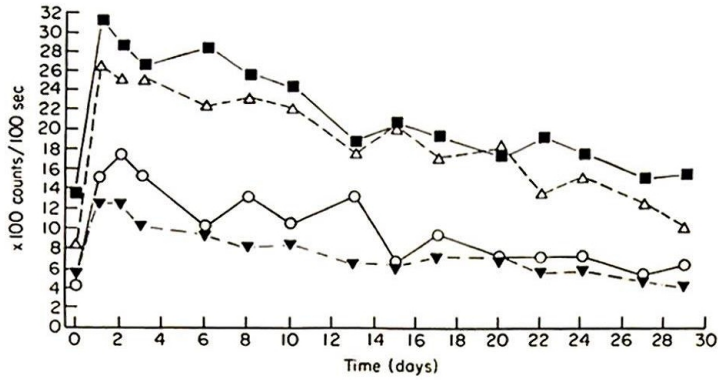

Fig. 3. ^{59}Fe organ-surface counts: curves showing persistence of low uptake of ^{59}Fe by the bone marrow and relatively high uptake by liver and spleen over a period of 29 days in subject C. Subject H with idiopathic myelofibrosis also showed a similar pattern. (■) Liver, (△) spleen, (○) heart, (▼) marrow.

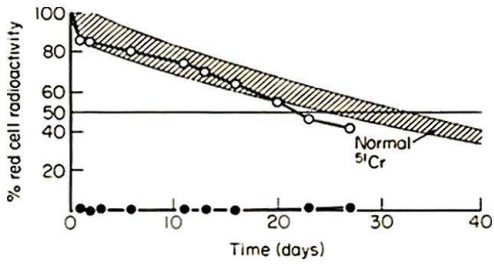

Fig. 4. Radio-iron incorporation (●) and red cell survival (○) curves of subject C: The ^{59}Fe graph shows clearly the negligible incorporation of radio-iron into the red cells; ^{51}Cr red cell survival curve shows a slightly reduced $T_{1/2}$ of 22 days; and normal ^{51}Cr red cell survival curves predrawn on imported graph paper from data based on 'Caucasian' population (▨).

4). The body haematocrit (H_B) was derived from the quotient of the ^{51}Cr-red cell volume and the total blood volume (Tables 3 and 4).

Normal reference values for the blood volume and ferrokinetic studies were established in six healthy male volunteers. However, values for which there were no normal volunteers had to be obtained from the literature [6,7]. Examination of a bone marrow aspirate was carried out on the referred subjects.

Results

Ferrokinetic and radiochromate red cell survival data are summarized in Tables 1 and 2. The

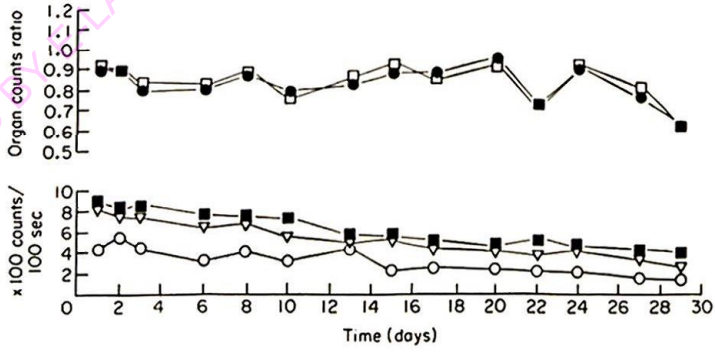

Fig. 5. ^{51}Cr organ-surface counts: low spleen/liver counts ratio following injection of ^{51}Cr-labelled red cells into subject C indicates that possibly increased splenic sequestration is not a significant factor in reduced mean red cell life-span of 52 days (Table 2). (●--●) Spleen/heart ratio, (□--□) spleen/liver ratio, (■) liver, (△) spleen, (○) heart, (▼) marrow.

Table 1. ^{59}Fe-ferrokinetic data

Subject	Referral diagnosis	Hb (g/dl)	Plasma $T_{1/2}$ ^{59}Fe (min)	T_{max} ^{59}Fe rbc inc. (days)	^{59}Fe rbc inc. max. (%)
A	Sickle cell trait with unexplained anaemia	11.2	70	14	98.8
B	Unexplained anaemia	7.5	44	12	60.0
C	Refractory anaemia with splenomegaly	7.3	8	6	1.1
D	Refractory anaemia with splenomegaly	7.5	30	10	62.3
E	Tropical splenomegaly syndrome	10.0	52	11	91.5
F	Tropical splenomegaly syndrome	10.0	50	3	92.0
G	Idiopathic splenomegaly	7.8	27	14	69.0
H	Idiopathic myelofibrosis	15.3	40	24	71.7
J	Cirrhosis with splenomegaly	12.5	33	11	50.0

Hb = haemoglobin, $T_{1/2}$ ^{59}Fe = plasma half clearance time of ^{59}Fe, T_{max} ^{59}Fe rbc inc = time taken for maximal incorporation of ^{59}Fe into red cells, ^{59}Fe rbc inc. max = maximum percentage of ^{59}Fe incorporated into red cells ($T_{1/2}$ ^{59}Fe in six normal male subjects were 55, 60, 75, 60, 115 and 116 min respectively).

Table 2. ^{51}Cr red cell survival data

Subject	Referral diagnosis	Hb (g/dl)	Retics. (%)	^{51}Cr $T_{1/2}$ (days)	MCLS (days)	S/L counts ratio at $T_{1/2}$ ^{51}Cr
A	Sickle cell trait with unexplained anaemia	11.2	0.6	35	108	1.2
B	Unexplained anaemia	7.5	0.5	ND	ND	ND
C	Refractory anaemia with splenomegaly	7.3	0.1	22	52	0.7
D	Refractory anaemia with splenomegaly	7.5	1.0	25	68	1.2
E	Tropical splenomegaly syndrome	10.0	0.3	22	47	0.7
F	Tropical splenomegaly syndrome	10.0	2.2	32	85	1.2
G	Idiopathic splenomegaly	7.8	3.2	17	30	2.0
H	Idiopathic myelofibrosis	15.3	0.5	29	95	0.8
J	Cirrhosis with splenomegaly	12.5	1.8	25	73	1.0

Hb = haemoglobin, Retics. = reticulocyte count, ^{51}Cr $T_{1/2}$ = half-life of ^{51}Cr-labelled red cells, MCLS = mean red cell life span, ND = not determined, S/L = spleen/liver.

results of blood volume studies and H_B/H_V ratios in the six normal volunteers and nine subjects are presented in Tables 3 and 4 respectively. ^{59}Fe and ^{51}Cr curves of one of the subjects (subject C) are shown in Figs 1–5. Plasma volume was increased in all the anaemic subjects except two (subjects A and F; Table 4) as well as in the non-anaemic individuals with splenomegaly. Whole blood volume was also increased in six of the nine subjects studied. Interestingly, plasma volume values obtained with the use of ^{59}Fe were essentially similar to those derived from using ^{125}I.

Discussion

The referred subjects (Tables 1, 2 and 4) fell into three groups: (a) unexplained anaemia only (subjects A and B), (b) anaemia with splenomegaly (subjects C–G), and (c) splenomegaly without anaemia (H and J). In the first group, ^{59}Fe-ferrokinetics excluded abnormalities of iron utilization in both subjects, whilst normal ^{51}Cr studies indicated that haemolysis was not a factor in the anaemia of B (Tables 1 and 2). Mild reticulocytosis suggestive of haemolysis was seen in only one of the five subjects in the second group. ^{51}Cr data, however, revealed a significant reduction in the mean red cell life span of all (C–G) of them (Table 2); this indicates that haemolysis was a significant feature in the pathogenesis of their anaemia. Radio-isotope studies are therefore extremely useful in evaluating anaemic patients with splenomegaly — a common clinical problem in tropical countries — when the conventional criteria of haemolysis are lacking. The increased spleen/liver (S/L) counts ratio of 2.0 provides supporting evidence for significant sequestration of red cells by the spleen in subject G (Table 2). Thus it is very likely that splenectomy would be helpful in correcting the anaemia of this subject. Normal S/L counts ratio in the other four subjects of the 'anaemia cum splenomegaly' group does not necessarily exclude a pathological sequestering role for the spleen [8].

The non-anaemic subjects with marked splenomegaly (subjects H and J) were referred specifically for a preoperative evaluation of the possible therapeutic effect of splenectomy. In subject J with a reduced mean red cell life span of 73 days (Table 2) and marrow erythroid hyperplasia (this is not reflected in his reticulocyte count of 1.8%), splenectomy would probably have been a useful procedure; in subject H, with idiopathic myelofibrosis, a high splenic uptake of ^{59}Fe (very similar to that shown for subject C in Figs 2 and 3) combined with a maximum ^{59}Fe red cell incorporation of about 70% after 24 days (Table 1), strongly suggests a significant role for the spleen as an organ of erythropoiesis. Therefore, splenectomy in the

Table 3. Blood volumes (including red cell and plasma volumes) and body/venous haematocrit ratios in six normal male subjects

Subject	^{51}Cr RCV (ml/kg)	^{125}I PV (ml/kg)	^{59}Fe PV (ml/kg)	BV (^{51}Cr RCV+ ^{125}I PV) (ml/kg)	BV (^{125}I PV/1−H_V) (ml/kg)	H_B	H_V	H_B/H_V
K	29.0	47.0	48.5	76.0	85.7	0.38	0.43	0.88
L	ND	44.4	48.2	ND	78.5	ND	0.48	ND
M	31.0	39.3	44.3	70.3	67.9	0.44	0.46	0.96
N	27.9	41.7	40.3	75.6	81.0	0.37	0.45	0.82
P	ND	36.0	41.5	ND	60.2	ND	0.44	ND
Q	28.5	46.2	44.2	74.7	78.3	0.38	0.43	0.88
Mean ± s.d.	29.1 ± 1.2	42.4 ± 3.9	44.6 ± 3.1	74.2 ± 2.3	75.3 ± 8.6			

RCV = red cell volume, PV = plasma volume, BV = whole blood volume, H_B = body haematocrit, H_V = venous haematocrit, ND = not determined.

Table 4. Blood volumes (including red cell and plasma volumes) and body/venous haematocrit ratios in referred patients

Subject	Referral diagnosis	^{51}Cr RCV (ml/kg)	^{125}I PV (ml/kg)	^{59}Fe PV (ml/kg)	BV (^{51}Cr RCV+ ^{125}I PV) (ml/kg)	BV (^{125}I PV/1−H_V) (ml/kg)	H_B	H_V	H_B/H_V
A	Sickle cell trait with unexplained anaemia	25.4	51.7	51.5	77.1	78.2	0.33	0.34	0.97
B	Unexplained anaemia	ND	79.4	58.6	ND	104.5	ND	0.24	ND
C	Refractory anaemia with splenomegaly	22.4	72.0	76.0	94.4	93.5	0.24	0.23	1.04
D	Refractory anaemia with splenomegaly	13.9	60.6	65.0	74.5	79.6	0.19	0.24	0.79
E	Tropical splenomegaly syndrome	29.0	66.5	67.5	95.5	95.0	0.303	0.30	1.01
F	Tropical splenomegaly syndrome	13.0	38.3	40.4	51.3	57.2	0.25	0.33	0.76
G	Idiopathic splenomegaly	13.8	72.2	76.4	86.0	96.3	0.16	0.25	0.64
H	Idiopathic myelofibrosis	41.2	69.4	71.7	110.6	121.8	0.37	0.43	0.86
J	Cirrhosis with splenomegaly	35.6	65.4	68.2	101.0	103.8	0.35	0.37	0.95

H_B = body haematocrit, H_V = venous haematocrit, RCV = red cell volume, PV = plasma volume, BV = whole blood volume, ND = not determined.

absence of a cellular and functioning bone marrow is likely to lead to the development and inexorable progression of anaemia.

The results of blood volume studies in the normal male subjects (Table 3) are similar to those of normal male individuals in other population groups [6] while blood volume data for the referred subjects (Table 3) are similar also to those of non-African subjects with anaemia and splenomegaly [6]. There were, however, some additional interesting observations. First, almost identical plasma volume values were obtained using ^{59}Fe and ^{125}I. ^{59}Fe can therefore be used for simultaneous ferrokinetic and blood volume studies thereby reducing costs — an important consideration for developing countries. Secondly, the slightly low haemoglobin concentration of the female subject (A) with sickle cell trait was most likely the result of a moderate increase in plasma volume rather than an absolute decrease in red cell volume. Thirdly, the H_B/H_V ratios in the healthy Sierra Leonean subjects covered a wide range (Table 3), and are in agreement with findings in other nationalities [9,10]. The increase in H_B and H_B/H_V ratios in the subjects with splenomegaly is possibly due to intrasplenic pooling of red cells.

Our experience at the Radioisotope Unit in Freetown indicates that using simple equipment, the introduction of radionuclide techniques into teaching and large general hospitals in developing countries will increase the range of diagnostic facilities, improve the standard of clinical care and enhance the level of haematological research in these institutions. Moreover, the same relatively inexpensive counting equipment can also be used for non-haematological investigations [11], thus decreasing the cost/benefit ratio of the service.

Acknowledgments

My thanks are due to Mr O. A. Smith and Mr A. Scott for their technical assistance. I would also like to thank Mr Gulraiz Butt for the typescript.

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(*Accepted* 22 *June* 1988)