USE OF INDIUM CHLORIDE SCINTIGRAPHY IN PATIENTS WITH MYELOFIBROSIS

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Various diagnostic modalities have been used to assess the degree and anatomic extent of erythroid elements in the marrow. Scintigrams of ^{111}In-chloride uptake have been suggested for this purpose, but data regarding the association of ^{111}In-chloride activity with erythroid elements are lacking. We compared the distribution of ^{111}In-chloride with results from bone marrow biopsies, ferrokinetic data, liver and spleen histology, and the clinical status in 21 patients (18 with myelofibrosis and 3 with other hematological disorders). In 15 patients the sacral uptake of ^{111}In-chloride correlated with marrow biopsy, ferrokinetic data, and clinical status. In five patients the scintigrams overestimated erythroid elements when compared with biopsy results but were consistent with ferrokinetic data and clinical status, uggesting that there is a limitation imposed o. marrow biopsies because of sampling. Activity in the liver and spleen did not correlate with the presence of erythropoietic elements assessed histologically. Indium-111-chloride scintigraphy appears to be a reliable means of assessing erythroid elements in bone marrow but not in the liver and spleen.

Various diagnostic modalities have been employed to assess the number and anatomic extent of erythroid elements. In particular, bone marrow biopsies and ferrokinetic studies have been used for the former purpose and bone marrow scans for the latter. Scans depicting the distribution of colloidal particles delineate the reticuloendothelial system while scans showing ^{111}In-chloride uptake are thought to delimit erythroid tissue.

We have investigated the distribution of ^{111}In-chloride by scintigraphy in patients with myelofibrosis and other disorders. The results are compared with bone marrow biopsies, clinical data, and ferrokinetic data from a majority of the patients and with liver and spleen histologic specimens from a few patients in order to assess the efficacy of scintigraphy for the detection of erythropoietic elements.

METHODS

Patient population and diagnostic modalities. Twenty-one patients were examined: 14 with myeloid metaplasia and myelofibrosis, two with lymphosarcoma and myelofibrosis, one with aplastic anemia, two with polycythemia vera and myelofibrosis, one with hypersplenism, and one with osteoporosis.

Twenty-five bone marrow scans were performed on these 21 patients. All patients had bone marrow biopsies; ten (50%) were within 1 week of their scan, one within 2 weeks, three within 1 month, three within 6 months, and two within 18 months. Sixteen patients had ferrokinetic studies; six (40%) were within 2 weeks of their scan, two within 1 month, two within 12 months, three within 18 months, and three within 24 months.

The liver was examined histologically in five patients; three by biopsy and two at postmortem examination. The time intervals ranged from 1 week to 6 months after scintigraphic examination.

The spleen was examined histologically in four patients, all within 3 months of their scintigraphic examination and bone marrow biopsy. Two specimens were obtained after splenectomy, one after splenic aspirate, and one at autopsy.

Scintigraphic technique. Patients were injected with carrier-free ^{111}In (2–3 mCi) at acid pH and anterior and posterior views were obtained with a dual-probe scanner* (with 5:1 minification) at 24 hr after in-

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* Ohio-Nuclear Scanner (Model 84) with 5-in. NaI crystals and 24L collimators.

jection. In some patients the scans were repeated at 48 hr after injection.

In most patients the radiopharmaceutical was injected intravenously without pretreatment. In four patients with saturated iron-binding capacities, ^{111}In-chloride was incubated with compatible plasma before injection in order to provide a carrier for the indium; one of these patients was also scanned after injection of the radiopharmaceutical without incubation in plasma. In a fifth patient with a saturated transferrin level, the radiopharmaceutical was injected immediately after transfusion with three units of frozen packed cells.

In all patients a liver–spleen scan was performed after intravenous injection of 2 mCi of ^{99m}Tc-sulfur colloid immediately after the bone marrow scan. The two studies were superimposed and the anatomic bounds of the liver and spleen were projected onto the bone marrow scan so that activity within and outside of the liver and spleen could be identified.

The scintigraphic studies were interpreted with reference to the distribution and relative amount of activity present. Quantification was arbitrarily assessed on a four-point scale: 0, no activity detectable; 1, activity just above body background; 2, increased activity allowing delineation of organ structures; 3, high activity allowing sharp delineation of organ structures (Fig. 1). These gradations were assessed by two of us without knowledge of the hematologic status of the patients or of biopsy or ferrokinetic data.

Bone marrow biopsies and histologic specimens. Bone marrow biopsies were obtained from the iliac crest. All specimens were reviewed by one observer as unknowns. Erythroid elements were graded on a four-point scale: 0, none; 1, decreased; 2, normal; 3, increased. The presence or absence of erythropoietic elements in histologic specimens of the liver and spleen was noted.

Technique for ferrokinetic studies. Ferrokinetic studies were performed by the Huff technique (*1*): 5 μCi of carrier-free ^{59}Fe as ferrous citrate were preincubated with plasma and injected intravenously. In 20 patients the plasma was the patient's own and in one patient with aplastic anemia compatible plasma was used to provide a carrier for iron.* External counting over the liver, spleen, precordium, and sacrum was done daily for 10 days with a single-probe collimated NaI detector; in two patients knee activity was recorded as well.

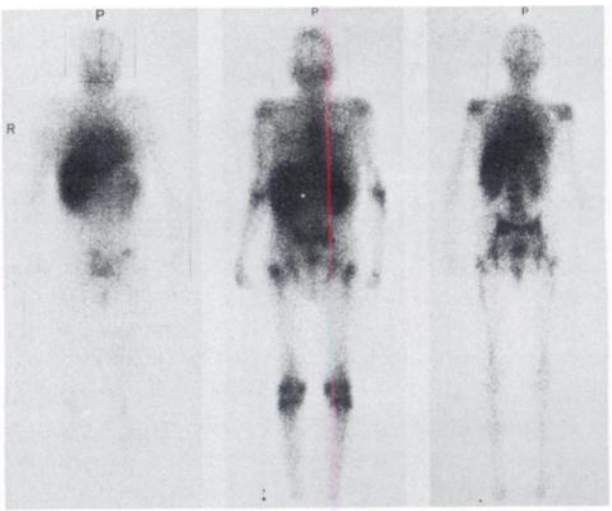

FIG. 1. Varying grades of ^{111}In-chloride uptake as visualized in bone marrow of these patients. All three show high liver activity and varying degrees of renal activity. Patient 15 (left photo) had aplastic anemia. There is no vertebral (Grade 0) and minimal sacral (Grade 1) activity; in addition, there may be bladder activity. Patient 11 (middle photo) had undergone splenectomy for myelofibrosis. High renal activity is probably related to patient's saturated iron-binding capacity. Distribution of indium activity in vertebrae and pelvis is good (Grade 2). There is peripheral extension to knees. Patient 21 (right photo) had undergone splenectomy for lymphosarcoma with myelofibrosis. Indium chloride activity in axial skeleton is high (Grade 3); vertebral structures and pelvic bones can be sharply defined.

Data were obtained in absolute units (cpm/μCi of administered ^{59}Fe). For purposes of comparison with scintigraphic and biopsy data, however, they were scaled as follows: 0, 0 cpm/μCi administered iron; 1, 1–150 cpm/μCi; 2, 151–300 cpm/μCi; 3, >300 cpm/μCi. The scale was developed on he basis of previous experience (*2*) in patients witl. myelofibrosis undergoing splenectomy. Sacral activity of less than 150 cpm/μCi at 24 hr in patients with myelofibrosis was associated with decreased erythroid activity and poor response to splenectomy. Patients with sacral activity of >150 cpm/μCi were able to maintain hematological stability after splenectomy. Thus, on our scale a value of 1 corresponds to decreased iron uptake whereas values of 2 and 3 suggest normal or increased uptake. The distinction between Grades 2 and 3 is arbitrary and may be unnecessary.

RESULTS

Distribution of indium chloride. Since most of the patients in this series had myelofibrosis, the overall distribution of ^{111}In-chloride reflected primarily the patterns found in patients with this disease; the other patients had patterns representative of what might be expected in the absence of extramedullary hematopoietic elements. Several generalities were apparent: (A) at least some activity in the central

* Three other patients had saturated iron-binding capacities at the time of their ferrokinetic studies. Because this fact was not appreciated prior to the start of the ferrokinetic studies, no extrinsic carrier was provided.

skeleton (vertebrae and/or sacrum) was seen in all patients, (B) long bone activity was common (13 of 14 patients with primary myeloproliferative disorders), (C) activity in the liver was seen at 24 hr in all patients, (D) varying degrees of activity in the spleen were noted in 10 of 11 patients with myelofibrosis examined prior to splenectomy and in the three patients without clinical evidence for extramedullary hematopoiesis, (E) renal activity was seen in 9 of 14 patients with myelofibrosis and in two of two patients with lymphosarcoma and myelofibrosis. Minimal-to-moderate activity occurred in the presence of either saturated or unsaturated iron-binding capacities while high activity (Fig. 1, middle) occurred only in the presence of saturated levels.

There was no difference in either the distribution or relative uptake of ^{111}In-chloride detected at 24 or 48 hr.

Assessment of erythroid elements. There was little difficulty in grading consistently the degree of ^{111}In-chloride uptake by the bone marrow. There were no problems in distinguishing no activity (i.e., Grade 0) from little activity (i.e., Grade 1) or little activity from near-normal (i.e., Grade 2) activity. In some cases it was difficult to distinguish between Grade 2 and Grade 3 because this difference separated near-normal from normal activity; however, this difference may not be critical.

On the whole, the degree of ^{111}In-chloride uptake in the sacrum compared favorably with sacral uptake of ^{59}Fe, the presence of erythroid elements on marrow biopsy, and the transfusion requirement (Table 1). Thirteen patients had high (Grades 2–3) sacral indium activity and in eight of these patients (Nos. 1–3, 8, 16–18, 20), the other indices also supported the presence of normal erythroid elements. In 5 of these 13 patients (Nos. 4, 11, 13, 14, and 21), the sacral iron uptake and a stable clinical situation supported the presence of normal erythroid activity but the bone marrow biopsy suggested decreased erythroid elements; in two patients (Nos. 4 and 14) the biopsy preceded the isotopic studies by 18 months and this long time interval may have been the cause of the discordant results. In the other three patients the biopsy sample may not have been a representative one.

Eight patients (Nos. 5–7, 9, 10, 12, 15, 19) had decreased sacral indium activity; in seven of these the biopsy results suggested that the erythroid elements were decreased. In four of these patients, the sacral iron uptake values were low (0 or 1); in two they were normal and, of these, one patient (No. 12) had results strikingly different from his scintigraphic and biopsy data. His scintigraphic study showed high renal activity. If iron localized to the kidneys similarly, then it is possible the sacral probe viewed renal as well as sacral activity and gave a falsely elevated reading. In these patients, the clinical situation, when judged by the transfusion requirement, was variable and could not be predicted from the isotopic or biopsy data alone.

One patient (No. 19) with a normal marrow biopsy had poor uptake of ^{111}In-chloride by his marrow. His scintigraphic study was performed during a chemotherapeutic course of nitrogen mustard and busulfan for polycythemia vera whereas his biopsy had been performed before the institution of chemotherapy. These time differences are undoubtedly critical and may preclude accurate comparison of the examinations.

In two patients with myelofibrosis, ^{111}In-chloride activity was visualized around the knees and ^{59}Fe uptake in this region was significantly above precordial levels. No other patients with knee activity visualized scintigraphically had iron uptakes measured around the knees.

TABLE 1. COMPARISON OF SACRAL UPTAKE OF INDIUM CHLORIDE WITH THE TRANSFUSION REQUIREMENT, ERYTHROPOIETIC ELEMENTS ON BONE MARROW BIOPSY, AND THE SACRAL UPTAKE OF IRON

Patient No.	Diagnosis	$InCl_3$ activity (0–3) (scintigraphy)	Erythroid elements (0–3) (biopsy)	^{59}Fe uptake at 24 hr (0–3)
1	Myelofibrosis	2	2	3
2	Myelofibrosis	2	1–2	3
3	Myelofibrosis	2	2	–
4	Myelofibrosis	2	1†	2
5	Myelofibrosis	1	1	–
6	Myelofibrosis	0–1	1	1†
7*	Myelofibrosis	1–2	1	2
8	Myelofibrosis	3	2	3†
9*	Myelofibrosis	1	0–1	1†
10	Myelofibrosis	1	1	1†
11	Myelofibrosis	2	1	3†
12	Myelofibrosis	1	1	3
13	Myelofibrosis	2	0‡	2
14	Myelofibrosis	2	1†	2†
15*	Aplastic anemia	1	1	0
16	P. vera	2–3	2	–
17	Hypersplenism	3	2–3	3
18	Osteoporosis	3	2	–
19	P. vera	1	2‖	–
20	Lymphosarcoma with myelofibrosis	2	2	3
21	Lymphosarcoma with myelofibrosis	3	1	2

* Transfusion required at the time of scintigraphic study.

† ≧18 months before indium chloride study.

‡ Inadequate amount of marrow from biopsy for proper evaluation.

‖ Performed during a course of chemotherapy; biopsy performed before institution of chemotherapy.

TABLE 2. COMPARISON OF INDIUM CHLORIDE ACTIVITY AND ERYTHROPOIETIC ELEMENTS IN LIVER

Patient No.*	Diagnosis	$InCl_3$ activity (0–3) (scintigraphy)	Erythroid elements (biopsy)	Time interval between studies
3	Myelofibrosis	3	scattered	3 months
4	Myelofibrosis	3	scattered	6 months
21	Lymphosarcoma with myelofibrosis	3	none	6 months
15	Aplastic anemia	3	none	3 months
17	Hypersplenism	2	none	1 week

* With reference to Table 1.

TABLE 3. COMPARISON OF INDIUM CHLORIDE ACTIVITY AND ERYTHROPOIETIC ELEMENTS IN SPLEEN

Patient No.*	Diagnosis	$InCl_3$ activity (0–3) (scintigraphy)	Erythroid elements (specimen source)	Time interval between studies
3	Myelofibrosis	3	scattered (autopsy)	3 months
5	Myelofibrosis	3	scattered (splenectomy)	1 week
12	Myelofibrosis	1	scattered (aspirate)	1 month
17	Hypersplenism	3	none (splenectomy)	1 week

* Corresponds with numbers in Tables 1 and 2.

Assessment of extramedullary hematopoietic elements. Five of the 21 patients had liver biopsies (Table 2) and four had histological evaluation of the spleen (Table 3). There was no relationship between the amount of ^{111}In-chloride in the liver or spleen assessed scintigraphically and the presence of erythropoietic elements assessed histologically.

One patient (No. 12) with myelofibrosis and splenic erythropoiesis had poor splenic visualization on two scintigraphic studies, one performed with and one without extrinsic transferrin as carrier for the indium. Indium-111-chloride activity was visualized in the central and peripheral skeleton on both studies.

Effects of therapy on the distribution of indium chloride. The effects of splenic irradiation and splenectomy on the distribution of ^{111}In-chloride was examined in two patients with myelofibrosis. One patient was examined only 3 days and 4½ months after splenectomy; the distribution of ^{111}In-chloride was identical both times. Scintigraphic patterns unchanged except for spleen size were found in the second patient examined immediately prior to and 4 months after 600 rads to the spleen.

Scintigraphic technique. The necessity for extrinsic carrier in patients with saturated transferrin levels could not be established from this study. The one patient (No. 19) with myelofibrosis examined with and without an extrinsic carrier had identical scintigraphic patterns on both studies. Two other patients who were studied with indium chloride and an extrinsic carrier as well as with ^{59}Fe without an extrinsic carrier showed central skeletal isotopic activity on both studies. The patient with aplastic anemia in whom both the scintigraphic and ferrokinetic studies were performed with an extrinsic carrier showed minimal sacral activity on both studies.

DISCUSSION

The need for a noninvasive method for detecting the anatomic extent of erythropoietic elements is obvious, both for the determination of suitable therapeutic modalities as well as for the assessment of therapy. Iron radionuclides are ideal physiologically but unsuitable technically for this purpose: ^{59}Fe emits high-energy gamma radiation and ^{52}Fe is a positron emitter. Indium-111-chloride has been proposed as an alternative agent for scintigraphic examination of the marrow because of its transportation in the plasma by transferrin (*3*) and its suitable energy characteristics.

There are several differences between the physiology of injected indium and iron, however, and thus far data regarding the concordance between the location of indium chloride and the presence of active erythropoiesis are sparse. The clearance of 50% of radioactive iron from the circulating plasma takes approximately 90 min (*4*) whereas that for indium takes 6–10 hr (*5–7*). In the normal person 80% of the labeled iron appears in the circulating red blood cells by 8–10 days (*4*) whereas only 4% of the indium is present in the cells at this time. (*8*). On the other hand, in vitro studies of transport of ^{59}Fe-labeled transferrin and ^{111}In-labeled transferrin by mature erythrocytes and reticulocytes show similar handling of the two agents with greater accumulation in the reticulocytes than in mature erythrocytes (*9,10*). Studies of the bone marrow uptake of ^{59}Fe-transferrin, ^{111}In-transferrin, and ^{99m}Tc-sulfur colloid in rabbits before and after irradiation indicate that irradiation leads to a decrease in the uptake of ^{59}Fe but not of ^{111}In or sulfur colloid (*11*). One study in man comparing ^{111}In and sulfur colloid has not confirmed this observation (*10*). Thus, on the basis of these data alone, it appears hazardous to

equate indium chloride activity with active erythropoiesis.

Our study has attempted to resolve these differences by comparing the results of indium chloride scintigraphy with bone marrow biopsies, ferrokinetic studies, and, in some cases, liver and spleen histology. We have selected as our study group patients with myeloproliferative disease because of the opportunity they afford in assessing extramedullary hematopoietic elements. A few other patients were included for comparative purposes. No normal patients could be studied because of the radiation dose to the bone marrow (about 4 rads/mCi) from this procedure.

In 15 patients the sacral indium chloride uptake, the marrow biopsy, and the clinical status were in agreement. In five patients the scan and clinical status suggested normal activity and the bone marrow biopsy decreased activity; in these patients it is possible that a sampling problem existed in the marrow biopsy. In the one patient with discordant results among the clinical status, marrow biopsy, and ^{111}In-chloride examination, it is possible that high-dose marrow-suppressive chemotherapy altered the erythroid elements at the time of the scintigram thus precluding accurate comparison with a marrow biopsy performed before the chemotherapeutic trials.

Thus, practically, it appears that scintigraphy of the bone marrow with ^{111}In-chloride provides a useful measure of erythroid elements in the marrow. In the absence of marrow-depressing chemotherapeutic agents, the appearance of ^{111}In-chloride activity suggests erythroid elements and its absence suggests the absence of erythroid elements. The sampling problem inherent in bone marrow biopsies, particularly in patients with fibrotic marrows, is not present in this method.

The lack of association of liver and splenic uptake of ^{111}In-chloride with erythropoietic elements is disturbing and indicates that evaluation of the isotopic distribution in these organs at 24 and 48 hr alone cannot be used for diagnostic purposes. Early studies with ^{52}Fe (*12,13*) showed liver uptake in several patients with hypoplastic marrows. In the majority of the patients, however, liver activity appears to have been absent. These patterns are not surprising since the reticuloendothelial cells of the liver can store iron if red blood cell production is decreased. A similar storage mechanism may be operating with indium. Alternatively, it is possible that ^{111}In-chloride may be phagocytized by the reticuloendothelial cells of the liver. These mechanisms cannot be differentiated from this study.

Whether indium accumulates in the reticuloendothelial cells of the bone marrow cannot be determined from this study; autoradiography with a more suitable indium isotope (e.g., ^{114}In) or differential centrifugal separation of the erythroid and reticuloendothelial cells of the marrow is required to answer this question.

Our scintigraphic technique involving direct intravenous injection of the ^{111}In-chloride or injection after preincubation with compatible plasma in the presence of saturated iron-binding capacities does not confirm the necessity or utility of the latter procedure. Both ^{59}Fe and ^{111}In were obtained carrier-free and in the doses used about 1 pg of tracer was added. Because the iron-binding protein is normally able to bind 300 μg of iron/100 ml of plasma, it is possible that most physiological or pathophysiological states can accommodate 1 pg of tracer added to the entire plasma pool.

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