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# Biologic Dosimetry in Thyroid Cancer Patients After Repeated Treatments with Iodine-131

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To estimate a cumulative dosimetric index that reflects the dose to the circulating lymphocytes after repeated treatments with $^{131}$I, biologic dosimetry was applied to 18 patients with differentiated thyroid carcinoma and neck relapse or lung metastases. **Methods:** Chromosomal aberrations were scored in peripheral blood samples that were obtained before and 4 days after each administration of 3.7 GBq $^{131}$I according to two methods, conventional cytogenetics and chromosome 4 painting. **Results:** The mean dosimetric index was equal to 0.5 Gy by both methods after the administration of 3.7 GBq $^{131}$I. Repeated administrations of $^{131}$I delivered the same dose each time, resulting in a cumulative dose from 1–3.5 Gy in the patients who had two to seven treatments. However, the estimated dose, based on the number of chromosomal aberrations on Day 4 and, above all, from the third treatment on, was considerably lower than the real dose absorbed by the lymphocytes. This may be linked to the phenomenon of apoptosis, which results in a loss of information during the course of repeated irradiation. **Conclusion:** Both chromosomal painting and conventional cytogenetics underestimate the cumulative dose after repeated $^{131}$I treatments. A complementary test measuring apoptosis may improve the dose estimates.

**Key Words:** biologic dosimetry; repeated iodine-131 treatments; thyroid cancer

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Biologic monitoring of the total-body dose in patients receiving radiation treatment and, in particular, in patients treated with $^{131}$I for differentiated thyroid carcinoma is important because its results can guide the subsequent treatment modalities (*1*). Dosimetry is also necessary to establish risk factors due to $^{131}$I exposure in these patients and in subjects exposed accidentally to $^{131}$I, such as those exposed during the nuclear power plant explosion at Chernobyl (*2*).

Until now, cumulative doses have been derived from numerical estimates based on an approximated geometric model (*3,4*). However, patients treated for differentiated thyroid carcinoma are hypothyroid, and iodine-concentrating metastases may considerably modify the dose to certain organs.

When patients are rendered hypothyroid before treatment, their renal iodine clearance is reduced, which increases the dose to the blood and bone marrow. Until now, no direct measurements have been performed in these patients, nor has there been a follow-up concerning the accumulated dose after repeated treatments with $^{131}$I.

Biologic dosimetry seems to be a valuable tool to address this question, even if it supplies only a dosimetric index that reflects the irradiation dose to peripheral lymphocytes (*5*).

The dicentric chromosome is the aberration of choice of biologic dosimetry because its production is almost specific for ionizing radiation and its natural occurrence is low. However, its unsuitability for measuring a dose received some years before the blood sampling is a major drawback (*6,7*). This drawback may now be overcome by scoring stable translocations by fluorescence in situ hybridization (FISH) with whole chromosome probe libraries (*8,9*). The persistence of these radiation-induced translocations may be used for retrospective biologic dosimetry (*10,11*).

In our previous reports (*12,13*), we estimated the dosimetric

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index, reflecting the dose to circulating lymphocytes in 50 thyroid cancer patients treated with $^{131}$I. On Day 4 after the first administration of 3.7 GBq $^{131}$I, the mean dosimetric index was 0.52 Gy [95% confidence interval (CI) = 0.48–0.60 Gy] by conventional cytogenetics and 0.47 Gy (95% CI = 0.42–0.51 Gy) by chromosome 4 painting, indicating that the results obtained by both methods were in close agreement with each other ($y = 1.03x$). We have also shown that, after a single treatment with $^{131}$I, these anomalies persisted for up to 2 yr with both methods (*13*). We have extended these studies to thyroid cancer patients who have had repeated treatments and followed them up for up to 3 yr during treatment. We wanted to know whether:

1. Biologic dosimetry based on scoring stable and unstable anomalies can estimate the cumulative radiation dose after repeated treatments; and
2. The cumulative dose to the circulating lymphocytes after repeated treatments with 3.7 GBq of $^{131}$I can serve to indicate the risk factors associated with these treatments.

## MATERIALS AND METHODS

### Patients

Eighteen patients (14 women, 4 men; age range 25–79 yr; mean age 48 yr) with differentiated thyroid carcinoma who had been treated with $^{131}$I (3.7 GBq) on two to seven occasions in the department of nuclear medicine at the Institut Gustave Roussy (Villejuif, France) since February 1993 were included in this study.

All patients had undergone total thyroidectomy before the first treatment with $^{131}$I, and none of them had been treated with external radiotherapy. A first treatment of 3.7 GBq $^{131}$I was administered 4–5 wk after total thyroidectomy for ablation of thyroid remnants while the patient was hypothyroid. Other treatments were administered for neck relapse or lung metastases at 4, 6 or 12 mo after the first treatment and then at a yearly interval.

A blood sample was obtained before each treatment, and another was obtained on day 4 after the administration of $^{131}$I.

A whole-body scan was performed 4 days after each administration of 3.7 GBq $^{131}$I using a homemade rectilinear digitized whole-body scanner. This scanner measures radioactive uptake in any focus, as well as whole-body retention of $^{131}$I.

This study was performed in accordance with local ethical rules, and all patients gave their informed consent.

Conventional cytogenetics methods were applied to score unstable aberrations (dicentrics and rings), and chromosome 4 painting (FISH) was performed to score stable aberrations (translocations and insertions), as described previously (*8,14*). Two hundred metaphases per blood sample were scored for each method.

### Lymphocyte Culture and Chromosome Preparation

Five milliliters of medium were added to 0.5 ml of blood sample and incubated at 37°C for 48 hr. The medium consisted of 5 ml of RPMI 1640 supplemented with 10% fetal calf serum, 0.1 ml of phytohemagglutinin M (Life Technologies, Inc., Grand Island, NY), 1% glutamine, 1 m*M* sodium pyruvate, 1% bromodeoxyuridine and antibiotics (penicillin and streptomycin). Colcemid (0.1 μg/ml) was added 2 hr before harvesting, and slides with chromosomes in metaphase were prepared after the standard methanol: acetic acid (3:1, v/v) procedure. The slides were stored at −20°C until use (*5*).

### Conventional Cytogenetics

The slides were stained by Fluorescent Plus Giemsa. Only complete metaphases (46 centromeres) were scored for dicentrics, rings and breaks under a light microscope (*5,15,16*).

### Fluorescence In Situ Hybridization

Whole chromosome 4 painting was performed with a specifically labeled fluorescein isothiocyanate probe (spectrum green, Life Technologies, Inc.) (*8,17*). The slides were analyzed under a fluorescence microscope by visual scoring of translocations, insertions, deletions and breaks.

### Statistical Analysis

The cumulative dosimetric index was obtained by plotting the number of chromosomal aberrations in peripheral lymphocytes on the dose–effect curve established by $^{131}$I in vitro exposure of normal lymphocytes (*12,13*).

The dosimetric index reflects irradiation of lymphocytes, which depends on the distribution of lymphocytes in organs where vascularization is highly heterogeneous. It may be compared to the bone marrow dose.

Bone marrow depression usually is maximal approximately 6 wk after therapeutic administration of $^{131}$I and recovers later on (*18,19*). This is accompanied by an apparent decrease in chromosomal anomalies at 3 mo when compared to Day 4. Because the population of lymphocytes is not the same in the course of treatment, we presented the results in the form of a histogram instead of a curve.

The cumulative dosimetric indices for the second, third and subsequent treatment were at first obtained by scoring the number of aberrations in the blood on Day 4 after each treatment without taking into account the number of anomalies already present before each treatment. Then, it was also estimated, correcting for chromosomal anomalies present, before each treatment and for the decrease in lymphocytes.

Because chromosome 4 in human lymphocytes represents 6.23% (*20*) of the total genome, we have compared the results obtained from painting chromosome 4 with those obtained from painting chromosomes 2, 3 and 5, which represent 20.4% of the total genome. We did not find any difference.

We used Wilcoxon's nonparametric test to compare the frequency of anomalies in women before and after menopause (eight and six, respectively).

## RESULTS

The results of the follow-up study are demonstrated in Figures 1 and 2, which show the frequencies of unstable anomalies (dicentrics and rings) detected by conventional cytogenetics and those of stable anomalies (translocations and insertions) detected by chromosome 4 painting after repeated treatments of 3.7 GBq $^{131}$I, respectively.

Six dicentrics were found in 3540 control metaphases by conventional cytogenetics, and seven translocations for chromosome 4 were found in 3600 metaphases before $^{131}$I administration (equivalent to 19 translocations per 1000 cells in the total genome, according to Lucas' formula). A total of 19,280 cells were scored by conventional cytogenetics, and 17,474 cells were scored by chromosome 4 painting 4 days after repeated treatment of 3.7 GBq $^{131}$I.

When the influence of age and sex on the frequency of chromosomal aberrations after the treatment of $^{131}$I was evaluated, the results of the two methods indicated that there was no significant difference ($p = 0.71$ and 0.69, respectively, for conventional cytogenetics; and $p = 0.10$ and 0.45, respectively, for chromosome 4 painting). This finding agrees with several published reports (*21–23*), whereas others did not observe this tendency (*24–27*).

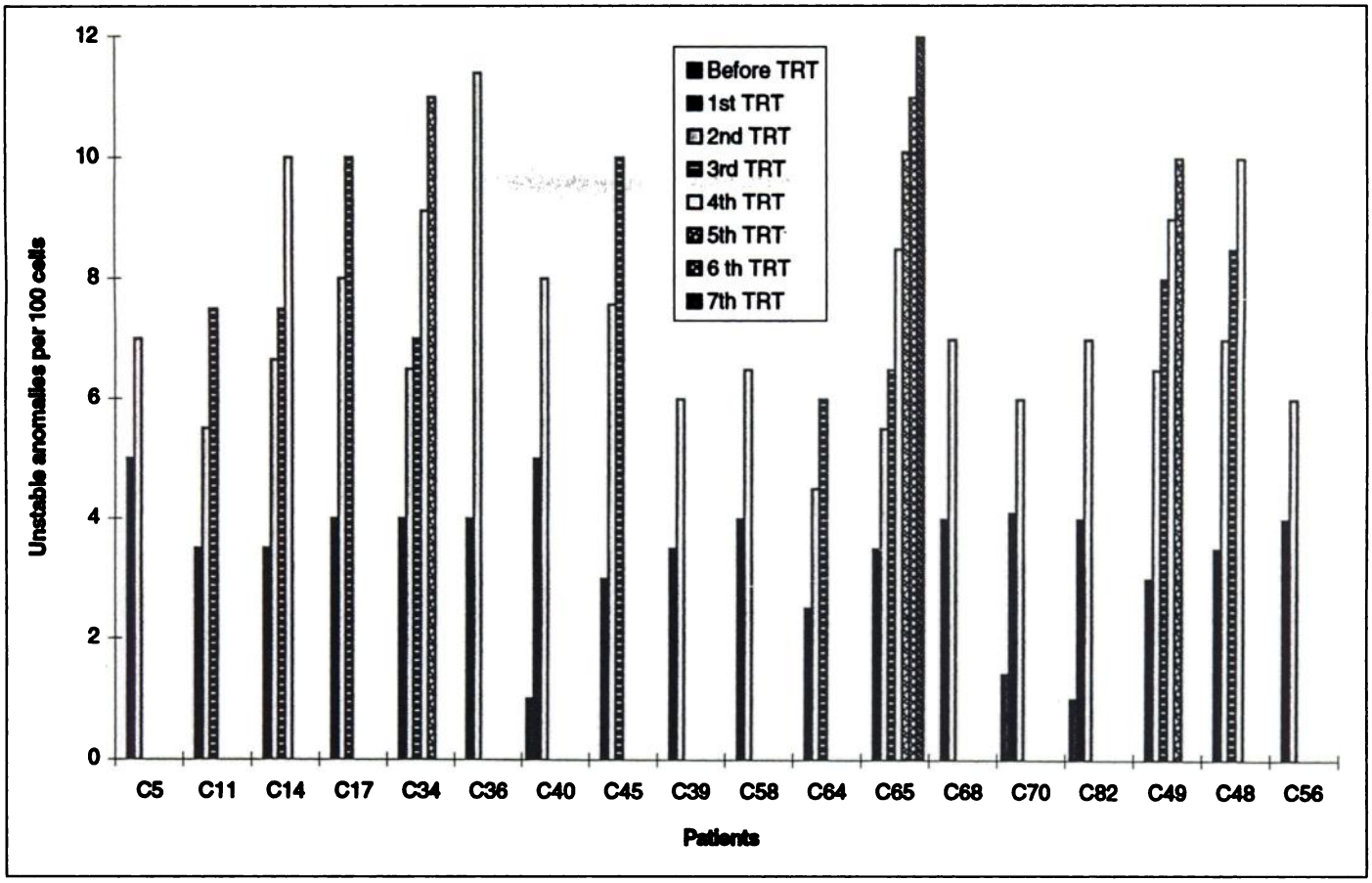


**FIGURE 1.** Frequency of unstable aberrations measured on Day 4 after repeated administrations 3.7 GBq $^{131}$I by conventional cytogenetics.

The same was true when results for women before and after menopause were compared ($p = 0.77$ for conventional cytogenetics; and $p = 0.48$ for chromosome 4 painting).

The mean dosimetric index of the 18 patients on Day 4 after the first administration of 3.7 GBq of $^{131}$I was equal to 0.54 Gy (95% CI = 0.49–0.58 Gy) by conventional cytogenetics and 0.52 Gy (95% CI = 0.42–0.58 Gy) by chromosome 4 painting. This is in accordance with our previous data (*12,13*).

Table 1 shows the mean dosimetric index after each treatment corrected for chromosomal anomalies present before treatment and for the decrease in lymphocytes on Day 4. The estimated dose after each treatment is comparable to that after the first administration of $^{131}$I. Thus, the cumulative dose equal to the sum of all the single doses, was 1–3.5 Gy after two to seven treatments, respectively.

Table 2 shows the mean cumulative dosimetric index by conventional cytogenetics and by chromosome 4 painting after repeated treatment with 3.7 GBq of $^{131}$I, based only on scoring chromosomal anomalies on Day 4 after each treatment. The estimated doses vary considerably and range from 0.8 to 1.23 Gy after two to seven treatments, respectively.

## DISCUSSION

The frequency of chromosomal aberrations before iodine treatment is somewhat higher than the values reported in the literature (*28,29*) because the number of patients studied is low. However, it is clear that there is a higher frequency of translocations than dicentrics in the control blood samples.

The dose delivered by each treatment of 3.7 GBq of $^{131}$I (Table 1) is about 0.5 Gy, as estimated by both methods. By taking into account the aberrations present before and the decrease in the lymphocytes after treatment, we demonstrated that repeated administrations of $^{131}$I have the same cytogenetic impact.

This dosimetric index is 2–4 times higher for the dose to the blood than the results based on the International Commission on Radiological Protection calculations (0.13 Gy) (*30*), which are derived from individuals with normal thyroid function and normal metabolic activity. Thyroid cancer patients are hypothyroid at the time of $^{131}$I administration. This hypothyroid status decreases renal clearance of radioiodine and thus increases whole-body exposure. Four days after the administration of $^{131}$I, the dosimetric index correlated with whole-body $^{131}$I retention (*12*).

Repeated administrations of $^{131}$I deliver the same dose each time, resulting in a cumulative dose from 1 to 3.5 Gy in the patients who had two to seven treatments (Table 1). However, the estimated dose based on the number of chromosomal aberrations on Day 4 was only 0.5–1.23 Gy (Table 2) by both methods.

Our study indicates that both methods are suitable for biologic dosimetry in thyroid cancer patients after the first and second treatment with $^{131}$I. However, neither conventional cytogenetics nor chromosomal painting is able to establish a reliable retrospective biologic dosimetry based only on scoring chromosomal aberrations after the third treatment with 3.7 GBq of $^{131}$I. This finding suggests the disappearance of chromosomal anomalies after iterative administrations of $^{131}$I and may be related to the death of lymphocytes with multiple chromosomal anomalies. A test designed to indicate cellular apoptosis

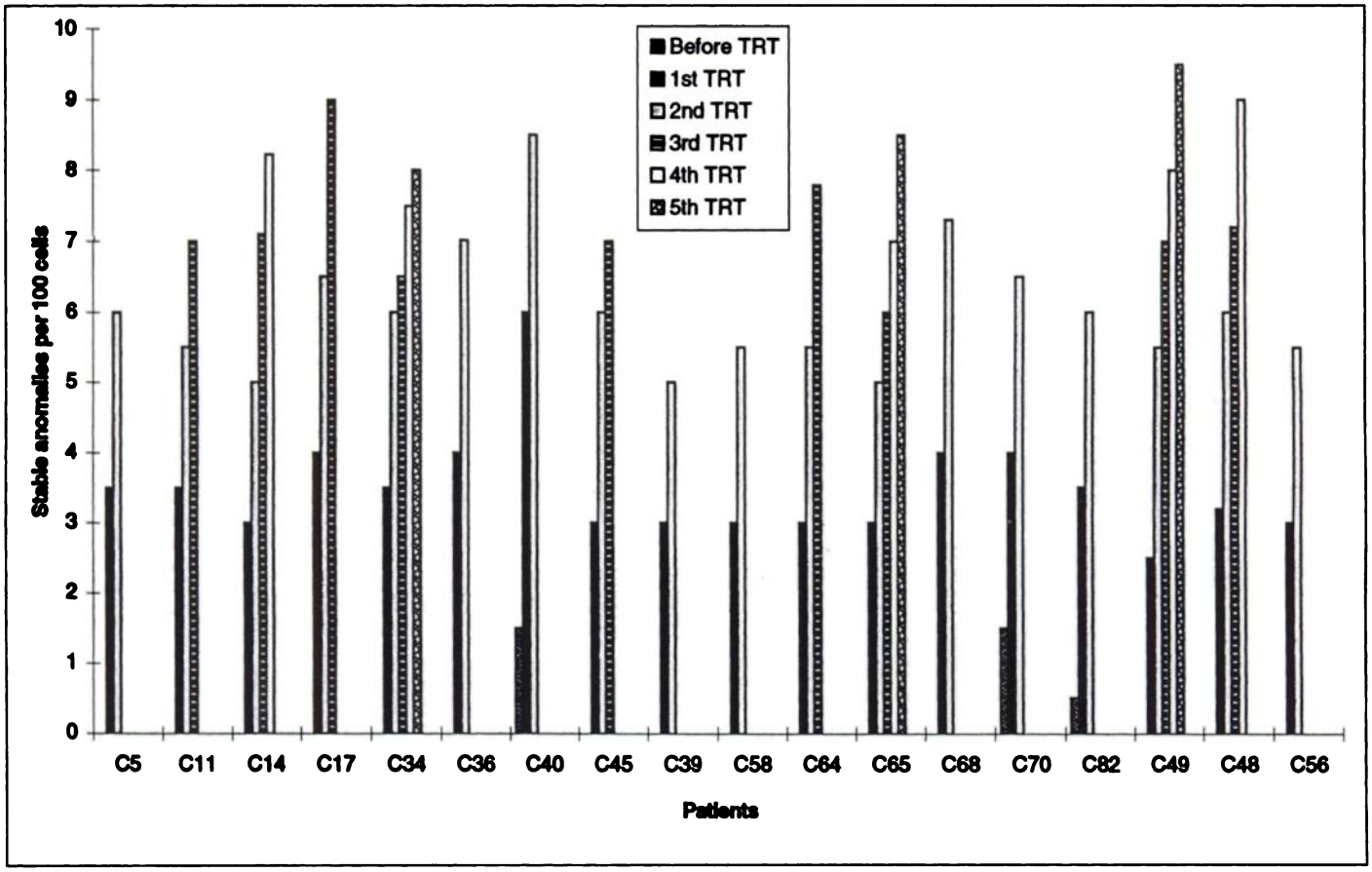


**FIGURE 2.** Frequency of stable aberrations measured on Day 4 after repeated administrations of 3.7 GBq $^{131}$I by chromosome 4 painting.

qualitatively as well as quantitatively could be used to improve the dose estimates after repeated treatments (*31*).

## CONCLUSION

The direct estimation of the dose will provide a more accurate quantification of the risk incurred by exposure to $^{131}$I, which, until now, has been based exclusively on approximate statistical calculations of cumulative $^{131}$I doses (*32–34*). Conventional cytogenetics and chromosome painting are useful techniques for biologic dosimetry in $^{131}$I treated thyroid cancer patients. They take into account all physiopathologic parameters and permit the correction of certain mathematical estimations. However, their limit lies in the underestimation of cumulated doses after repeated treatments with $^{131}$I. This study will be continued to improve radiobiologic and radiopathologic data.


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**TABLE 1**
Mean Dosimetric Index Corrected for Chromosomal Anomalies Present Before Treatment and for the Decrease in Lymphocytes After Each Treatment with 3.7 GBq of Iodine-131

| | | Conventional cytogenetics | | Chromosome 4 painting | |
|---|---|---|---|---|---|
| Treatment no. | Patient no. | Dose (Gy) | 95% CI (Gy) | Dose (Gy) | 95% CI (Gy) |
| 1 | 18 | 0.54 | 0.49–0.58 | 0.52 | 0.42–0.58 |
| 2 | 18 | 0.55 | 0.44–0.60 | 0.51 | 0.42–0.61 |
| 3 | 9 | 0.51 | 0.39–0.59 | 0.53 | 0.42–0.62 |
| 4 | 5 | 0.50 | 0.35–0.61 | 0.53 | 0.39–0.62 |
| 5 | 3 | 0.52 | 0.35–0.62 | 0.52 | 0.38–0.63 |
| 6 | 1 | 0.50 | 0.36–0.63 | nd | nd |
| 7 | 1 | 0.49 | 0.38–0.62 | nd | nd |

nd = not determined.

**TABLE 2**
Mean Dosimetric Index After Repeated Administrations of 3.7 GBq of Iodine-131, as Measured on Day 4 Without Any Correction

| | | Conventional cytogenetics | | Chromosome 4 painting | |
|---|---|---|---|---|---|
| Treatment no. | Patient no. | Dose (Gy) | 95% CI (Gy) | Dose (Gy) | 95% CI (Gy) |
| 1 | 18 | 0.54 | 0.49–0.58 | 0.52 | 0.42–0.58 |
| 2 | 18 | 0.81 | 0.76–0.96 | 0.84 | 0.63–0.98 |
| 3 | 9 | 0.91 | 0.82–0.98 | 1.02 | 0.81–1.15 |
| 4 | 5 | 1.01 | 0.72–1.02 | 1.13 | 0.9–1.5 |
| 5 | 3 | 1.1 | 0.94–1.3 | 1.18 | 0.95–1.6 |
| 6 | 1 | 1.15 | 0.85–1.46 | nd | nd |
| 7 | 1 | 1.23 | 0.92–1.55 | nd | nd |

nd = not determined.

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# Evaluation of the In Vivo Biodistribution of Yttrium-Labeled Isomers of CHX-DTPA-Conjugated Monoclonal Antibodies


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We evaluated the in vivo stability and biodistribution of four isomers (CHX-A', CHA-A'', CHX-B' and CHX-B'') of 2-(*p*-isothiocyanatobenzyl)-cyclohexyl-diethylenetriaminepentaacetic acid (CHX-DTPA), a recently developed backbone-substituted derivative of DTPA. **Methods:** The ligands were conjugated to monoclonal antibody B3, a murine IgG1 kappa, and labeled with $^{88}$Y at 55.5–66.6 MBq/mg (1.5–1.8 mCi/mg). Nontumor-bearing nude mice were injected intravenously with 55.5–66.6 kBq (1.5–1.8 μCi) of $^{88}$Y-labeled B3 conjugates and with $^{125}$I-labeled B3 as an internal control. The mice were then killed at 6, 24, 48, 96 and 168 hr postinjection. **Results:** At 168 hr, the concentration of $^{88}$Y in processed bone of either CHX-A' [4.6% injected dose (ID)/g] or CHX-A'' (4.0%ID/g) was less than that of either the CHX-B' (21.9%ID/g) or B'' (12.1%ID/g) ligands. The two ligands CHX-B'' and CHX-B' were not acceptable for yttrium labeling of antibody because of their high and progressive bone accumulation. The accumulation of $^{88}$Y in bone of CHX-B' was five times greater than that of CHX-A' at 168 hr. The CHX-A'' cleared from the circulation slightly faster than CHX-A' without releasing the yttrium and showed the lowest uptake by bone of any of the four isomers. The accumulation in the other normal organs was similar for all four isomers of $^{88}$Y-CHX-B3 conjugates. **Conclusion:** Although the CHX-B'' and CHX-B' were not acceptable for labeling with yttrium, the CHX-A' and CHX-A'' were suitable, indicating that differences in stereochemistry can greatly influence stability of radionuclide in the chelate.



**Key Words:** DTPA; chelates; stereoisomers; enantiomers; antibody

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