Accumulation of Indium-111-Labeled Granulocytes in Malignant Tumors

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In a retrospective study of 220 \(^{111}\text{In}\) granulocyte scintigrams from 208 patients, 25 patients had malignant neoplasms. Among these, tumor uptake of \(^{111}\text{In}\) activity was observed in ten patients (intense activity in two patients with non-Hodgkin’s lymphoma and colonic carcinoma, respectively; moderate uptake in a patient with non-Hodgkin’s lymphoma, and in a patient with an ovarian carcinoma; weak activity in three patients with cerebral neoplasms; and activity within otherwise “cold” metastatic lesions of the liver in three patients). Microscopic investigation following specific granulocyte staining revealed the greatest extent of granulocyte infiltration in the tumors which took up \(^{111}\text{In}\) activity, emphasizing the importance of tumor granulocyte infiltration as the single most important factor underlying tumor accumulation of \(^{111}\text{In}\) activity during \(^{111}\text{In}\) granulocyte scintigraphy.


Indium-111 granulocyte scintigraphy (\(^{111}\text{In}\) GS) has become a well-established method for the detection of occult infectious and inflammatory processes (1,2). Because infectious and neoplastic diseases may be indistinguishable on clinical grounds, and even after the application of ultrasound and CT scanning, \(^{111}\text{In}\) GS is considered a valuable diagnostic tool for the discrimination between tumors and localized infections. Sporadic cases of tumors mimicking abscesses on \(^{111}\text{In}\) GS have been reported, however, and the results of a recently published retrospective study (3), suggest that tumors may take up activity more frequently than previously thought. The present paper reviews the results of a retrospective study undertaken to throw some light on the frequency, pattern, and mechanism of \(^{111}\text{In}\) accumulation in malignant tumors during \(^{111}\text{In}\) GS.

MATERIAL AND METHODS

Patients

All \(^{111}\text{In}\) granulocyte scintigrams performed during a 4½-yr period, and the corresponding patient records were reviewed. Patients with histologically proven malignant neoplasms form the basis of the present study.

Two hundred twenty scintigrams from 208 patients were evaluated. Of these, 25 patients (1–88 yr, 17 females, 8 males) had localized malignant neoplasms (Hodgkin’s disease: three; non-Hodgkin’s lymphoma: three; cerebral metastases: four; cerebral or cerebellar glioma or glioblastoma: six; pulmonary sarcoma: one; nephroblastoma: one; colonic carcinoma: one; ovarian carcinoma: one; pelvic squamous cell carcinoma: one; hepatic metastases: four).

Granulocyte Isolation and Labeling

Autologous granulocytes were isolated from blood and labeled with \(^{111}\text{In}\)oxine as previously described (4). Briefly, three 25.7-ml samples of venous blood are anticoagulated with acid citrate dextrose (ACD). Platelet-poor plasma is prepared from one of the samples, the remaining two samples being subjected to dextran enhanced erythrocyte sedimentation and spontaneous granulocyte sedimentation during labeling in a plasma-buffer mixture.

The median dose injected was 11.3 MBq (range 4.7–18.4 MBq). The median fraction of cell-bound radioactivity in the injected samples (calculated in 13 cases) was 99.5% (range 98.1–99.7%). The corresponding erythrocyte-bound fraction was 0.5% (range: 0–18.2%). The granulocyte/mononuclear cell ratio in the injected sample was calculated in 15 cases. It ranged from 1.3 to 130.9 (median 14.0).

Considering the possibility of release of free \(^{111}\text{In}\) from circulating or disintegrating neutrophils, and the affinity of transferrin bound \(^{111}\text{In}\) for malignant tumors (5,6), we conducted a scintigraphic study following the injection of \(^{111}\text{In}\) chloride in the patient showing the fastest and most intense uptake of \(^{111}\text{In}\) after the injection of \(^{111}\text{In}\)-labeled granulocytes.
Image Acquisition

Following injection of the $^{111}$In-labeled granulocytes, gamma camera images of the thorax and upper abdomen, corresponding to the 173 keV and 247 keV photopeaks of $^{111}$In, were recorded on Polaroid film, with the patient placed in the supine position under the gamma camera parallel hole collimator (Maxi Camera 535, General Electric, Milwaukee, WI). Repeated static imaging of the thorax and abdomen (or the head when intracranial disease was suspected) was carried out for 30–45 min following the injection. Thereafter, 1-hr, 2-hr, 4-hr, and 18–24-hr images of the trunk and thighs (and/or head) were routinely obtained. Posterior and lateral views were included when considered necessary.

Abnormal foci of $^{111}$In activity were graded as weak, moderate, or intense on anterior view 18–24-hr postinjection images, using the bone marrow activity of the lumbar spine 18–24-hr postinjection as reference, i.e., the intensity of a lesion with moderate $^{111}$In uptake corresponds to that of the spine, weakly positive lesions are definitely seen but with lesser activity than the spine, and intense $^{111}$In uptake exceeds the activity level of the lumbar spine.

Microscopic Method

Using a scoring system (0-1-2-3), four specimens from each tumor were evaluated blindly for neutrophils, necrosis, hemorrhage, and vascularization after staining with hematoxylin and eosin, and the neutrophil azurophil granule stain a-naphthyl AS-D chloroacetate esterase.

The number of neutrophils was assessed semiquantitatively (0 = no neutrophils, 3 = numerous neutrophils, the scores 1 and 2 representing intermediate numbers of cells) in four random fields (25 X objective) in each specimen, and the average score of the total of 16 fields calculated. The necrosis, hemorrhage, and vascularization scores were obtained from two representative fields in each specimen, using a 2.5-X objective (screen 10 mm) for searching, and a 10-X objective (screen 2.3 mm) for scoring.

RESULTS

In two patients (non-Hodgkin's lymphoma and colonic carcinoma) the neoplastic tissue showed an intense accumulation of $^{111}$In activity (Figs. 1 and 2), two patients (non-Hodgkin's lymphoma and ovarian carcinoma) showed a moderate uptake (Fig. 3), and three patients (cerebral metastasis: one; cerebral glioma: two) showed weak uptake corresponding to the tumors (Fig. 4). Photopenic lesions were seen in four patients with hepatic metastases. In three of these, however, the picture was not that of a purely cold lesion, as the 18–24-hr images (and the 2–4-hr scans in addition in one case) showed some degree of tumor $^{111}$In uptake (Figs. 5 and 6). Technetium-99m sulfur colloid scintigraphy confirmed the presence of cold lesions as shown on the initial $^{111}$In granulocyte scans in all four patients, and computerized $^{111}$In-$^{99m}$Tc subtraction (7) performed in two of the three patients with $^{111}$In accumulation in metastases confirmed their content of excess $^{111}$In activity.

In the four patients with intense or moderate tumor

**FIGURE 1**

A: 42-yr-old woman with a centrocytic-centroblastic lymphoma in partial remission for 4 yr following whole-body irradiation and chemotherapy. $^{111}$In granulocyte scintigraphy was performed after 10 wks' unexplained fever. Sequential scintigraphy showed a tracer accumulation of increasing intensity (starting 5 min after the injection) in the left side of the lesser pelvis and—to a lesser extent—along the aorta and close to the right common iliac vessels. At explorative laparotomy the tracer accumulations were shown to represent lymphomas (microscopy: lymphomas heavily infiltrated with neutrophils; score: 3). B: Scintigrams obtained 3 wk after those of Fig. 1A (prior to institution of chemotherapy), following the injection of 12.7 MBq $^{111}$In chloride. No tumor uptake is seen.
uptake of $^{111}$In, the activity started to accumulate at 5 min, 30 min, 2-hr, and 2-hr after the injection, respectively. The three weakly accumulating cerebral tumors became visible at 2-hr, 2-hr, and 20-hr postinjection, respectively.

Twelve patients were febrile (>38.0°C) (all six patients with lymphomas, all four patients with hepatic metastases, the patient with an ovarian carcinoma, and the patient with a pelvic squamous cell carcinoma), i.e., six of the 12 patients with tumor associated fever showed $^{111}$In uptake in their tumors, as did four of the 13 afebrile patients.

Neutrophil leukocytosis ($>8.0 \times 10^9/l$) was seen in 7/11 patients with a $^{111}$In score of zero, and in 6/9 patients with scores 1-2-3. Neutrophil counts were not available in five patients with cerebral tumors.

Surgical specimens, biopsies, and/or smears obtained within 2 wk of the scintigraphic study were investigated in 20 cases. In five cases, autopsy material obtained from 10 to 30 days after the scintigraphic investigation was studied. Granulocyte infiltration was seen in 19 of the 25 tumors (Table 1). The score averaged 0.73 in the 15 patients without tumor accumulation of $^{111}$In, and 1.80 in the ten patients with tumor $^{111}$In uptake (p < 0.01; Mann-Whitney's rank sum test). In the four patients with liver metastases the granulocyte infiltration scores were 2, 2, 2, and 1. The corresponding $^{111}$In granulocyte scores were 3, 2, 1, and 0, respectively.

The presence of tumor necrosis could be evaluated microscopically in 20 specimens, tumor hemorrhage in 20 specimens, and degree of vascularization in 17 samples. We thereby found signs of necrosis in 13 tumors, hemorrhage in 11, and signs of vascularization in all 17 evaluable tumors. Neither phenomenon was correlated with the result of $^{111}$In granulocyte scintigraphy.

**DISCUSSION**

Based on the results of $^{111}$In granulocyte scintigraphy in previously published large series of patients it can be concluded that malignant lesions rarely produce positive images with labeled leukocytes (2,8-12). Sporadic cases of $^{111}$In leukocytes accumulating in skeletal (2, 11,13,14) and nonosseous (7,8,15-19) neoplasms have been reported. In most of these, tumor uptake has been modest, in contrast to the more intense activity characteristic of abscesses, allowing the two to be differen-
84-yr-old female patient with 1 mo's unexplained fever. Following the injection of $^{111}$In labeled granulocytes, a large cold area involving the major part of the left liver lobe was seen initially. The photopenic lesion was partially filled out during subsequent imaging from 2 hr to 21 hr postinjection. The extent of the lesion is evident on the $^{99m}$Tc sulfur colloid scintigram. Following a rapid deterioration in the patient's condition, she expired. At autopsy, the hepatic lesion was identified as a metastasis from a bile duct carcinoma. Microscopic granulocyte score: 2.

Apart from these cases, only McDougall et al. (8) have reported $^{[111]}$In leukocyte uptake in lymphomas. By carrying out sequential imaging we found an early and rather intense accumulation of activity in three cases of breast carcinoma metastasizing to the lungs, and a case of Hodgkin's disease in peripheral lymph nodes. Apart from these cases, only McDougall et al. (8) have reported $^{[111]}$In leukocyte uptake in lymphomas.

FIGURE 6
57-yr-old woman with 6 mos' intermittent fever and no focal symptoms. Following the injection of $^{111}$In labeled granulocytes, a large photopenic lesion in the left liver lobe was seen initially, being partially filled out 20 hr p.i., however, The "absence" of the left liver lobe and the adjacent part of the right lobe is evident on the $^{99m}$Tc sulfur colloid scintigram. At surgery, a large metastatic process (adenocarcinoma) involving the left liver lobe was found. Microscopic granulocyte score: 2.
patients, a pattern usually considered characteristic of frank abscess formation (3,20,21). In two of the three cases of cerebral malignancies, and in the patients with hepatic metastases, the early scans drew attention to possible abnormalities, thereby facilitating proper imaging 18-24-hr postinjection (which in all cases showed the most intense tumor uptake).

The mechanisms underlying the uptake of $^{111}$In by tumors following the injection of $^{111}$In-labeled granulocytes have been badly elucidated. Our results emphasize the importance of tumor granulocyte infiltration as the single most important factor. The cause of this infiltration is not known, nor is its frequency. Even if necrotic tumor tissue may elicit an inflammatory response, this phenomenon was of minor importance in the present study. This is in accordance with a case of $^{111}$In uptake in a non-necrotic tumor containing a large number of inflammatory cells described by Fortner et al. (3). We have previously demonstrated an accelerated accumulation of $^{111}$In-labeled granulocytes at inflammatory sites in febrile patients (22). Our present findings give a hint of a connection between the body temperature and the intensity of $^{111}$In activity as well, both probably reflecting the release of locally derived chemoattractants. As karyorrhexis in the cell nuclei of necrotic tumors can be mistaken for neutrophils, the importance of using a staining method specific for granulocytes prior to microscopy should be stressed.

Although we found a difference in microscopic granulocyte infiltration between tumors with and without $^{111}$In accumulation, the correlation between the results of scintigraphic and microscopic scoring was not perfect. Specifically, nine patients with slight granulocyte infiltration, and one patient with moderate infiltration did not show $^{111}$In uptake on imaging. In addition, we observed weak $^{111}$In uptake in a glioma without microscopic signs of granulocyte infiltration. The surgically obtained specimen showed some degree of vascularization which might have contributed to the $^{111}$In uptake. The absence of $^{111}$In uptake in patients with microscopic signs of slight granulocyte infiltration does not seem surprising in view of the semiquantitative techniques, the problems of obtaining representative tissue samples (applying in particular to the hepatic metastases), and the time lag from scanning to tissue sampling.

Indium-111 released in vivo from leukocytes binds to transferrin, and the $[^{111}$In$]_{transferrin}$ complex may accumulate in tumors (5,6). Because the level of free $^{111}$In in plasma is low following the injection of $^{111}$In-labeled granulocytes (23), and because considerably less activity is injected in $[^{111}$In$]_{granulocyte}$ studies than in the quoted $[^{111}$In$]_{chloride}$ studies (5,6), this mechanism seems of minor importance. This conclusion is supported by the absence of tumor visualization following $[^{111}$In$]_{chloride}$ injection in our patient showing the most intense and rapid tumor $^{111}$In uptake after the injection of $^{111}$In-labeled granulocytes.

The previously reported low incidence of $[^{111}$In$]_{granulocyte}$ accumulation in malignant tumors definitely contrasts with our findings and some degree with those of Fortner et al. who recorded a 12% incidence of tumor leukocyte uptake (3). They did not describe their tumor patients in detail. The incidence of neoplasia in our patient material is lower than that of Fortner et al. (11% versus 20%) which may suggest a difference in the composition of the patient population. However, as we do not know at present which tumors take up $[^{111}$In$]_{granulocytes}$ most avidly, the significance of a possible difference of course is uncertain.

Another factor of potential importance is the functional state of the injected $[^{111}$In$]_{granulocytes}$. There is much to suggest that the presence of plasma during isolation and labeling protects the granulocytes against in vitro activation (24). We keep the granulocytes in a plasmatic environment throughout the isolation and labeling procedure, and subject the cells to gentle centrifugation steps. Judged from the intensity of initial pulmonary $[^{111}$In$]_{granulocyte}$ uptake (4), and the speed of $[^{111}$In$]_{granulocyte}$ accumulation at inflammatory sites (25), our granulocyte isolation and labeling method is gentle. Accordingly, we recorded a sensitivity of $[^{111}$In$]_{granulocyte}$ scintigraphy of 97% in a study of 159 patients with suspected nonosseous infection (25).

It has been suggested that mononuclear cells in the mixed leukocyte preparations used by most investigators may be responsible for tumor visualization during $[^{111}$In$]_{granulocyte}$ scintigraphy (3,19). As the polymorphonuclear/mononuclear cell ratio exceeded ten in the majority of our patients, this explanation does not seem plausible.

The sequential scintigraphic approach seems of particular value in the detection of $[^{111}$In$]_{granulocyte}$ accumulation in hepatic tumors, as knowledge of the true extension of the neoplastic process, as shown on the early scans, facilitates the assessment of granulocyte uptake on the delayed scans.

In conclusion, our results support the suggestion that

### TABLE 1

Corresponding Microscopic and Scintigraphic Scores

<table>
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<th>Intensity of $^{111}$In activity</th>
<th>0</th>
<th>1</th>
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<tr>
<td>Granulocyte score</td>
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<td>5</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
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<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
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1 Microscopically assessed degree of tumor granulocyte infiltration.

2 Scintigraphically assessed degree of tumor accumulation of $^{111}$In (1 = weak, 2 = moderate, 3 = intense).
the accumulation of $^{111}$In-labeled granulocytes in malignant tumors is more frequent than previously supposed. This is of great practical importance, as $^{111}$In granulocyte scintigraphy has been considered a valuable tool for overcoming the difficulty of computed tomography and ultrasound in differentiating between tumors and abscesses. Even if recording of the intensity and temporal pattern of focal $^{111}$In accumulation may facilitate image interpretation, tumor uptake of $^{111}$In granulocytes definitely should be regarded as a possible cause of positive $^{111}$In granulocyte scintigraphy.

REFERENCES