Positron Tomographic Assessment of Estrogen Receptors in Breast Cancer: Comparison with FDG-PET and In Vitro Receptor Assays

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The purpose of this study was to assess the results of PET with 16α-[^{18}F]fluoro-17β-estradiol (FES) and [^{18}F]fluoro-2-deoxy-D-glucose (FDG) to validate the concordance between tumor estrogen-receptor (ER) status as determined by FES-PET and in vitro assays and to assess the relationship between tumor metabolic activity determined by FDG-PET and tumor ER status, both of which may provide information about tumor aggressiveness and prognosis. **Methods:** We studied 32 patients with primary breast masses and 21 patients with clinical or radiological evidence of recurrent/metastatic breast carcinoma. A diagnosis of breast carcinoma was subsequently proven in 43 patients (24 primary, 15 metastatic and 4 recurrent tumors). In vitro assessment of ER status was available for 40 malignant lesions (23 primary and 17 metastatic/recurrent). The patients underwent PET with both FES and FDG, and the uptake of each tracer within each lesion was evaluated qualitatively as well as semiquantitatively using the standardized-uptake-value (SUV) method. **Results:** We found good overall agreement (88%) between in vitro ER assays and FES-PET. This degree of agreement is similar to that observed between replicate in vitro assays (with discordances due to interlaboratory, interassay and specimen variability). We were, however, unable to demonstrate any significant relationship between tumor FDG uptake and ER status or between tumor FDG and tumor FES uptake in these patients. **Conclusion:** These results indicate that in vitro ER assays and/or FES-PET provide unique direct information about breast cancer ER status that cannot be obtained indirectly by FDG-PET.

Key Words: breast cancer; positron emission tomography; estrogen receptor; fluoroestradiol; fluorodeoxyglucose

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Breast cancer is the most common malignancy of women in North America and is the leading cause of death in women between the ages of 40 and 55. A number of factors have been identified that serve as predictors of prognosis or response to therapy in this disease, the estrogen receptor (ER) being one of the most important. Estrogen-receptor-positive cancers not only have a more favorable prognosis than do estrogen-receptor-negative cancers but, additionally, ER status determines the likelihood of response to hormonal therapy, with the response rate being roughly proportional to the concentration of ER in the tumor (*1*). Because of the importance of ER status in breast cancer, in vitro ER assays are routinely utilized to predict tumor response to therapy and patient prognosis. In clinical practice, however, these assays are imperfect tools for guiding therapy; only 55%–60% of patients with ER-positive tumors and 8%–10% of patients with ER-negative tumors respond to hormonal manipulation. Because of these limitations, we (*2*) and others (*3,4*) have sought to develop radionuclide imaging methods to help determine the functional status of the ER in vivo. If successful, such techniques could be utilized to select the preferred mode of therapy on an individual basis.

In previous work, we have shown that the ER status of both primary and metastatic breast cancers can be reliably evaluated in vivo by PET with the radiolabeled estrogen analog, 16α-[^{18}F]fluoro-17β-estradiol (FES). We have demonstrated that FES uptake in primary breast cancer is proportional to the ER concentration of the tumor measured by in vitro techniques (*5*). Furthermore, we have shown that FES accumulation within metastatic lesions of breast carcinoma is a receptor-mediated process that can be blocked by antiestrogen therapy (*6*).

PET has been used to study several other aspects of breast cancer pathophysiology. The most widely used radiopharmaceutical has been [^{18}F]fluoro-2-deoxy-D-glucose (FDG). This radiolabeled glucose analogue has been broadly applied in PET studies of cancer because of observations that nearly all malignant tumors exhibit increased uptake of FDG, presumably reflecting an increased rate of glycolysis in tumor tissue. Clinical studies in patients with known or suspected breast cancer have shown that FDG-

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PET is a reliable means for distinguishing benign from malignant breast masses and for evaluating the locoregional extent of tumor (*7–10*). FDG-PET also has been used to assess the response to therapy in patients with breast cancer; reduction in tumor FDG uptake during the course of chemotherapy or hormonotherapy indicates a good response of the tumor to treatment (*11*).

In a recent experimental study, Wahl et al. showed that administration of estrogen markedly increased FDG uptake in the ER-rich uterus of immature female rats. These investigators suggested that FDG uptake could serve, in some circumstances, as an index of the level of functional stimulation of tumor ERs (*12*). In addition, it has been shown that tumor FDG uptake measured by PET correlates well with the aggressiveness of several types of tumors, including primary brain tumors (*13,14*), malignant lymphomas (*15*) and breast cancer (*10*). In general, low-grade tumors have a better prognosis and lower FDG uptake than do high-grade tumors. Based on these observations, we speculated that there may be a relationship between the ER status of breast cancers and their FDG uptake; i.e., ER-positive tumors (with a more favorable prognosis) would be expected to have lower tumor FDG uptake than more aggressive ER-negative tumors.

We initiated the present study to determine whether there is a relationship between the metabolic activity of breast cancer as reflected by tumor FDG uptake and the ER status of the cancer as reflected by tumor FES uptake. In addition, we sought to reconfirm, in a larger number of patients than previously studied, the degree of agreement between tumor ER status as determined by FES-PET and by in vitro assays.

MATERIALS AND METHODS

Patients

Fifty-three women (mean age 54 yr; range 26–76 yr) participated in this study. Two distinct groups of patients were included. Group 1 consisted of 32 patients with primary breast lesions who either had a suspicious breast mass and were scheduled to undergo biopsy (fine-needle aspiration, incisional biopsy or excisional biopsy) or had locally advanced breast carcinoma proven by biopsy or strongly suggested by clinical findings; 24 of these patients were ultimately proven to have malignant lesions. Group 2 consisted of 21 patients with clinical and/or radiographic evidence of metastatic or recurrent breast cancer (which was ultimately proven in 19). Histopathologic diagnosis was established in all Group 1 patients and in 17/21 Group 2 patients. At the time of the study, none of the 53 patients had undergone treatment for their primary or metastatic/recurrent tumors. This investigation was approved by the Human Studies Committee and the Radioactive Drug Research Committee of Washington University School of Medicine. Each patient gave informed consent prior to participating in the study.

Radiopharmaceutical Synthesis

FES was synthesized by a robotic adaptation of a previously described method (*16,17*). FES prepared by this method has high specific activity and high affinity for estrogen receptors (*17*). FDG was prepared by a robotic adaptation of standard methods as previously described (*18*).

PET

PET imaging was performed with either SuperPETT IIB (11 patients) or a Siemens (Des Plaines, IL) ECAT EXACT scanner (42 patients). The paired studies of any one subject were always performed on the same scanner. SuperPETT IIB is a whole-body, time-of-flight positron tomograph with intrinsic in-plane spatial resolution of 4.5 mm FWHM. The scanner was operated in the 14-slice mode, allowing for simultaneous collection of 14 overlapping sections at an interslice interval of 7 mm over an axial extent of 10.2 cm. Emission data were acquired in the low-resolution mode, and image reconstruction filters were selected to provide an inplane transaxial resolution of 11 mm FWHM. The Siemens ECAT EXACT is a whole-body device that acquires 47 simultaneous slices at a section interval of approximately 3.7 mm FWHM over an axial extent of 16.2 cm. This tomograph has a best-case reconstructed spatial resolution of 5.5 mm FWHM in both the axial and transaxial directions. Reconstructed spatial resolution under clinical imaging conditions is approximately 10 mm FWHM. With both scanners, a 10–15-min transmission scan was performed with a rotating $^{68}Ge/^{68}Ga$ rod source after each emission scan at each bed position. Transmission images were reconstructed, and backprojected attenuation files were generated for use in emission scan reconstruction. Images were acquired at 3 bed positions in 11 patients, 2 bed positions in 22 patients and 1 bed position in the remaining 20 patients. The contiguous two- or three-position image sets were then added to generate a volume image and reprojection images (by maximum-pixel-activity volume rendering).

FES-PET and FDG-PET studies were performed in random sequence on two separate days (34% had the FES study first and 66% had the FDG study first). In 81% of patients, the two studies were done within 3 days of each other (58% on consecutive days); the maximum interval between the two studies was 9 days. For the FES study, 6 mCi (222 MBq) FES was administered intravenously. Approximately 90 min later, the patient was positioned supine in the PET scanner so that the field of view included the lesion(s) of interest (as determined by physical examination or by reference to correlative imaging studies). A 30-min emission scan was performed for each bed position. Prior to the FDG-PET study, patients fasted for at least four hours. Ten millicuries (370 MBq) of FDG were administered intravenously and imaging began approximately 30 min later. Similar bed positions and imaging times were used for the FES and FDG studies.

Image Analysis

All PET images were evaluated qualitatively by at least two experienced nuclear medicine physicians. Based on the knowledge of normal biodistribution of the radiopharmaceuticals, foci of abnormal radiotracer accumulation were identified and recorded. Regions where abnormalities existed on clinical examination or radiographs were also specifically evaluated. On FDG-PET, all lesions were then graded as definitely or probably abnormal (categorized as representing tumor), equivocal, or normal (in the case of an abnormality identified on radiography or clinical examination for which no corresponding abnormality was present on PET). On FES-PET, images were reviewed for the presence (categorized as FES-positive) or absence (categorized as FES-negative) of focally increased uptake. In a given patient, FES and FDG images were reviewed independently. At least one of the observers was blinded to the clinical and correlative radiographic find-

ings. For final interpretation, the PET images were then correlated with the clinical, radiographic, and surgical findings and with the results of the clinical follow-up. There was 100% agreement between blinded and unblinded observers in PET image interpretation.

In addition to the above subjective analysis, regions of interest (ROIs) were drawn around areas of increased tracer accumulation to determine the local tissue accumulation of radiopharmaceutical. A standardized uptake value (SUV) was then calculated for these areas (*19*). The SUV is a decay-corrected measurement of activity per unit volume of tissue (nanocuries per milliliter) adjusted for administered activity per unit of body weight (nanocuries per kilogram). The SUVs for both FDG and FES were multiplied by appropriate recovery coefficients for lesions smaller than 2.5 cm. Lesion size (for both malignant and benign lesions) was determined by physical examination or correlative radiographic studies. In patients with multiple lesions, only the index lesion of primary clinical interest and/or that with histopathologic verification was chosen for semiquantitative analysis. On FES-PET, tumors with an SUV ≥ 1.0 were categorized as FES-positive and those with a tumor SUV < 1.0 were categorized as FES-negative.

In Vitro ER Assays

Quantitative measurement of ER concentrations was performed by the conventional ligand-binding (radioreceptor assay) method on fresh or frozen tumor. A tumor with an ER level > 3 fmole/mg protein was defined as ER-positive; a tumor with ER level ≤ 3 fmole/mg protein was defined as ER-negative. Immunohistologic assessment of ER status was performed using the avidin-biotin-peroxidase complex technique (*20*) on paraffin sections.

The results of assays were known for 23 of the 24 Group 1 patients with pathologically proven primary breast cancers (by immunohistochemical staining in 13 and by quantitative assays in 10). An ER assay was not performed on the tumor in the remaining patient. Information concerning the ER status of at least one of the lesions was available in 10 of the 19 Group 2 patients who had metastatic/recurrent breast cancer and of only the original primary tumor in 7 additional Group 2 patients. The ER status of the metastatic/recurrent lesions was assumed to be the same as that of the primary tumors in these seven patients. In the remaining two Group 2 patients, information about tumor ER status was not available. The ER status of biopsied material was determined by immunohistochemical staining in 11 patients, by quantitative assays in 8 and by both in 2.

Statistical Analysis

An unpaired Student's t-test was used to determine whether there was a significant difference in FDG uptake between ER-positive and ER-negative tumors. The relationship between tumor FDG uptake and tumor FES uptake was assessed by linear regression.

RESULTS

Group 1 (Primary Breast Masses)

Of the 32 women studied in this group, 24 were found to have primary breast carcinoma and 8 had benign breast lesions. The size of the breast masses ranged from 1.0 to 10.0 cm in maximum diameter. In two patients, the entire breast was involved. A summary of pertinent data for this group of patients is shown in Table 1.

On FES-PET, lesions of primary breast cancer were judged to be FES-positive in 6 patients and FES-negative in 18. The results of FES-PET and in vitro ER assays of tumor were in agreement in 19 primary breast cancers (13 ER-negative/FES-negative; 6 ER-positive/FES-positive). There was disagreement in four patients (ER-positive/FES-negative), yielding an agreement rate of 82%. The mean SUV ($\pm$ s.d.) for ER-positive tumors (including the four patients in whom the FES-PET and in vitro assay results were in disagreement) was 1.9 ± 1.6 (range 0.5–5.2). For ER-negative tumors, the mean SUV was 0.5 ± 0.2 (range 0.2–0.9) (Fig. 1). The tumor with unknown ER status was FES-negative. No abnormal FES uptake was noted within the benign breast lesions and the mean SUV for these lesions was 0.6 ± 0.2 (range 0.5–0.7).

On FDG-PET by qualitative analysis, uptake in the primary tumor was judged to be definitely abnormal in 14 and probably abnormal in 8 of the 24 patients with primary breast cancer. FDG uptake was graded equivocal in the remaining two patients with breast cancer (patients 19 and 24) and in one patient with a benign breast mass (Patient 30). Lesion uptake was judged to be normal in the remaining seven benign breast masses (Table 1). The mean SUV was 1.05 ± 0.41 (range 0.6–1.8) for benign breast lesions and 4.5 ± 2.8 (range 1.2–11.6) for breast cancers (Fig. 1). By quantitative analysis, with a cutoff SUV value of 2.0 (determined retrospectively), FDG-PET correctly identified 21 of 24 patients with breast cancer and 8 of 8 patients with benign breast lesions (sensitivity 88% and specificity 100%).

Group 2 (Metastatic or Recurrent Disease)

Of the 21 women studied in this group, 15 were found to have metastatic disease (confirmed by biopsy in 13 and by radiographic and clinical assessment in 2); 4 had locally recurrent breast cancer (confirmed by biopsy in 3 and by radiographic and clinical assessment in one); and 2 had benign lesions. Pertinent data in this group of patients are summarized in Table 2.

On FES-PET, lesions of metastatic/recurrent breast cancer were judged to be FES-positive in 11 patients and FES-negative in 8. There was agreement between the results of the FES-PET and in vitro ER assays in 16 of 17 lesions (7 ER-negative/FES-negative, 9 ER-positive/FES-positive). There was one disagreement (ER-positive/FES-negative); hence, the rate of agreement was 94% in this group of patients. The mean SUV in ER-positive metastatic/recurrent tumors (including the one instance in which FES-PET and in vitro results were in disagreement) was 2.3 ± 1.7 (range 0.5–6.6). For ER-negative tumors, the mean SUV was 0.5 ± 0.2 (range 0.2–0.8). The tumors with unknown ER status were both FES-positive (SUVs of 2.0 and 1.2, respectively). No abnormal FES uptake was noted in the two benign lesions (SUVs of 0.2 and 0.4, respectively) (Fig. 1).

TABLE 1
Summary of Clinical and Imaging Data for Group 1 Patients

Patient no.	Age (yr)	Tumor type and maximum diameter (cm)	FDG		FES		ER status
			Visual	SUV	Visual	SUV	
1	60	Mucinous adeno ca (5.0)	++	3.2	(−)	0.7	Negative
2	44	Inflammatory ductal ca (Entire breast)	+	2.2	(−)	0.2	Negative
3	49	Inflammatory ductal ca (Entire breast)	+	2.7	(−)	0.2	Negative
4	65	Poorly differentited adeno ca (6.0)	++	10.6	(−)	0.6	Negative
5	58	Inflammatory ductal ca (Entire breast)	++	5.4	(−)	0.4	Negative
6	71	Inflammatory ductal ca (Entire breast)	++	3.2	(−)	0.3	Negative
7	62	Malignant cell (2.5)	+	2.9	(−)	0.5	NE
8	39	Moderately differentiated adeno ca (3.8)	++	5.0	(−)	0.8	Negative
9	65	Inflammatory ductal ca (Entire breast)	++	8.3	(+)	5.2	Positive
10	68	Inflammatory ductal ca (Entire breast)	++	4.0	(+)	1.9	Positive
11	45	Inflammatory ductal ca (Entire breast)	+	2.7	(−)	0.5	Positive
12	76	Inflammatory ductal ca (Entire breast)	++	4.8	(−)	0.5	Negative
13	67	Lobular ca (5.0)	+	1.7	(+)	1.5	Positive
14	33	Moderately differentiated adeno ca (4.0)	++	11.6	(−)	0.7	Negative
15	71	Inflammatory ductal ca (Entire breast)	++	6.0	(+)	4.0	Positive
16	36	Ductal ca (4.0)	+	2.4	(−)	0.7	Positive
17	56	Adeno ca (3.0)	++	3.8	(+)	1.8	Positive
18	57	Invasive lobular ca (7.0)	+	2.6	(+)	2.5	Positive
19	56	Inflammatory ductal ca (Entire breast)	±	1.2	(−)	0.5	Negative
20	45	Inflammatory ductal ca (Entire breast)	+	2.0	(−)	0.2	Negative
21	40	Adeno ca (1.5)	++	4.3	(−)	0.5	Positive
22	34	Ductal ca (5.0)	++	6.0	(−)	0.5	Positive
23	58	Inflammatory ductal ca (Entire breast)	++	9.7	(−)	0.9	Negative
24	67	Moderately differentiated ca (1.5)	±	1.5	(−)	0.5	Negative
25	43	Fibroadenoma (1.2)	−	1.0	(−)	0.3	NA
26	53	Fibroadenoma (1.5)	−	1.0	(−)	0.2	NA
27	68	Fibroadenoma (1.0)	−	0.6	(−)	0.9	NA
28	26	No malignancy (2.0)	−	0.6	(−)	0.5	NA
29	48	Fibroadenoma (3.0)	−	0.9	(−)	0.8	NA
30	45	Intraductal papiloma (2.5)	±	1.8	(−)	0.8	NA
31	35	Fibrocystic changes (1.0)	−	1.0	(−)	0.5	NA
32	75	Fibrocystic changes and chronic inflammation (2.0)	−	1.5	(−)	0.7	NA

++ = definitely abnormal; (−) = FES-negative; + = probably abnormal; NE = not evaluated; (+) = FES-positive; ± = equivocal; − = normal; NA = not applicable.

In 13 of the 19 patients with metastatic/recurrent disease, multiple sites were evaluated on FES-PET. A total of 45 foci were evaluated in these 13 patients. Although 42 of these different sites demonstrated concordant results of FES-PET (i.e., all FES-positive or all FES-negative), one site in one patient (Patient 34) and two sites in another patient (Patient 40) were discordant with the other lesions in those patients. Thus, within-patient discordance of FES uptake was demonstrated in 2 of the 13 women (15%).

On FDG-PET by qualitative analysis, tumor uptake was judged to be definitely abnormal in 12 and probably abnormal in 7, which is consistent with breast cancer. The studies of two patients (Patients 45 and 51), categorized as probably abnormal, had lesions with SUVs in the benign

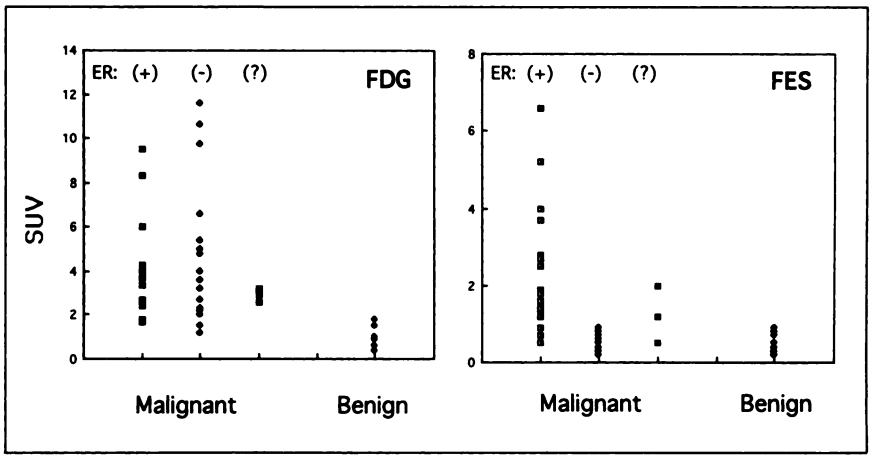

FIGURE 1. Semiquantitative results (expressed as SUV) for lesional FDG (left) and FES uptake (right). Scatter plot on the left shows the lack of relationship between tumor FDG uptake and ER status, designated as positive (+), negative (−) or indeterminate (?). Scatter plot on the right shows good separation between ER-positive and ER-negative cancers by FES-PET.

range. No abnormally increased FDG uptake was seen within the two benign breast lesions (SUVs of 0.4 and 0.6, respectively). The mean SUV was 3.7 ± 1.8 (range 1.7–9.5) for metastatic/recurrent breast cancer lesions. The sensitivity and specificity of FDG-PET for differentiating lesions of metastatic/recurrent breast carcinoma from benign lesions (considering only the 21 lesions subjected to quantitative analysis that were histopathologically confirmed or were of primary clinical interest), with a cutoff SUV value of 2.0, were 89% and 100%, respectively.

Relationship between Tumor ER Status and FDG Uptake

Comparison of the SUVs for FDG in malignant lesions showed no significant relationship with tumor ER status (Fig. 1). The mean SUV for FDG in ER-positive tumors was 4.0 ± 2.1 and for ER-negative tumors was 4.5 ± 3.0 ($p < 0.65$). These results indicate that the ER status of breast cancer cannot be predicted by assessing tumor FDG uptake. Additionally, we found no significant correlation between tumor FDG uptake and tumor FES uptake in 43 malignant lesions subjected to quantitative analysis ($r = 0.15$; p = ns) (Fig. 2).

Representative paired FES-PET and FDG-PET of patients with ER-positive and ER-negative primary and metastatic breast cancer are shown in Figures 3–5.

DISCUSSION

In breast cancer, the hormone-receptor status of the tumor defines not only the likelihood of response to hormonal therapy, but prognosis as well. Hormone-sensitive breast cancer is a less-aggressive disease than hormone-resistant cancer; it occurs more commonly in postmenopausal women and is characterized by longer disease-free intervals and survival. Overall, the median survival in patients with ER-positive tumors is several times longer than for patients with ER-negative breast cancer.

Currently, the ER status of breast cancer is assessed by in vitro assays (quantitative or qualitative). These assays, however, have limitations: Only 55%–60% of ER-positive tumors identified by these assays respond to hormonal therapy and, conversely, approximately 8%–10% of ER-negative cancers show a favorable response to hormonal therapy. The conventional ligand-binding (quantitative) method requires a sample of fresh or fresh-frozen tissue of adequate size and adequate tumor cell density. This is very important, as breast cancer has a wide degree of epithelial cellularity, and this results in heterogeneous receptor expression within the tumor. In addition, hormone receptors are not reliably determined by this assay in biopsies of osseous metastatic lesions or in samples of bone marrow, ascitic fluid or pleural fluid (*21,22*). The assay results may be false-negative due to high blood levels of estrogen hormones (in premenopausal women or those on estrogen replacement therapy) and the presence of hemorrhage or necrosis in the sample. The immunohistochemical assay is less dependent on sample size and is able to determine ER status of tumor cells in bone biopsy specimens and malignant effusions. As typically performed, however, this assay is only qualitative and has limited value in patients on therapy, because the receptor may be identified irrespective of whether the lesion is still hormone responsive (*23*). Both types of assays also suffer from interlaboratory variability due to differences in methodology and the lack of uniformly accepted cutoff values for discriminating ER-positive from ER-negative tumors.

The accuracy of ER status determination by the quantitative (ligand-binding) or qualitative (immunohistochemical) methods is comparable. Studies comparing immunohistochemical methods with ligand-binding techniques have found concordant results in 80%–95% of specimens (*24*). Neither of the receptor assays, however, completely predicts the response to hormonal manipulation in breast cancers.

In 1988, we reported a noninvasive in vivo technique with potential utility for assessing ER status in patients with breast cancer. We found an excellent quantitative correlation ($r = 0.97$) between the ER concentration measured in vitro and FES uptake determined in vivo by PET (*5*). Subsequently, we demonstrated that FES-PET has a high sensitivity (93%) for detecting lesions of ER-positive

TABLE 2
Summary of Clinical and Imaging Data for Group 2 Patients

Patient no.	Age (yr)	Index lesion (Additional lesions)	Lesion type	FDG		FES		ER status of M or R/primary
				Visual	SUV	Visual	SUV	
33	58	Breast	R	++	3.6	(−)	0.2	Negative
34	41	Chest wall (soft tissue, axillary and supraclavicular LNs)	R	++	3.4	*(+)	1.2	NE/Positive
35	37	Axilla (mediastinal and supraclavicular LNs)	M	++	6.6	(−)	0.3	Negative
36	58	Chest wall (humerus, soft-tissue mass, axillary and mediastinal LNs)	R	++	4.0	(−)	0.8	Negative
37	45	Supraclavicular node (contralateral supraclavicular LNs)	M	+	1.7	(+)	1.4	Positive
38	59	Pelvis (breast)	M	+	2.3	(−)	0.5	Negative
39	45	Pleura	M	++	4.1	(+)	1.6	Positive
40	46	Pleura (ribs, mediastinal LNs, lung, spine)	M	++	3.7	*(+)	1.3	NE/Positive
41	66	Lung (breast)	M	++	3.7	(−)	0.9	Positive
42	59	Lung (hilum, spine)	M	+	2.7	(+)	2.8	NE/Positive
43	50	Lung (hilum)	M	+	2.7	(−)	0.4	NE/Negative
44	53	Pleura (spine, lung)	M	++	6.0	(+)	3.7	NE/Positive
45	69	Pelvis	M	++	9.5	(+)	2.7	Positive
46	47	Spine (chest wall, mediastinal LNs)	M	++	3.2	(−)	0.7	NE/Negative
47	42	Chest wall (pleura, spine, mediastinal LNs)	M	+	2.6	(+)	2.0	NE
48	61	Chest wall	R	+	2.7	(+)	6.6	Positive
49	49	Breast (axilla)	M	+	3.2	(+)	1.2	NE
50	57	Breast	M	+	1.8	(+)	1.2	Positive
51	46	Breast (axilla, intramammary LNs)	M	++	3.2	(−)	0.8	NE/Negative
52	50	Lung	Granulomatous disease	−	0.4	(−)	0.2	NA
53	44	Spine	No malignancy	−	0.6	(−)	0.4	NA

*Discordant lesion.
R = recurrent disease; ++ = definitely abnormal; (−) = FES-negative; (+) = FES-positive; NE = not evaluated; M = metastatic disease; + = probably abnormal; − = normal; NA = not applicable.

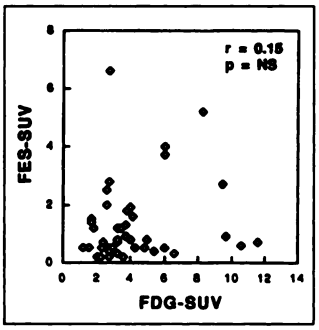

FIGURE 2. Scatter diagram demonstrates lack of correlation between SUVs for FDG and FES in 43 malignant lesions subjected to quantitative analysis.

metastatic breast cancer. In addition, we showed that FES uptake in metastatic breast cancer is likely to be a receptor-mediated process because it is blocked by antiestrogen therapy (*6*).

In the current study, we have confirmed in a larger number of patients that FES-PET is a reliable in vivo technique for evaluating the ER status of breast cancer (primary, recurrent or metastatic). The results of FES-PET correlated well with those of conventional in vitro ER assays (Fig. 1). The overall rate of agreement between the results of in vitro ER assays and the results of FES-PET was 88%, which is similar to that observed with in vitro assays (with disagreements explained by such factors as

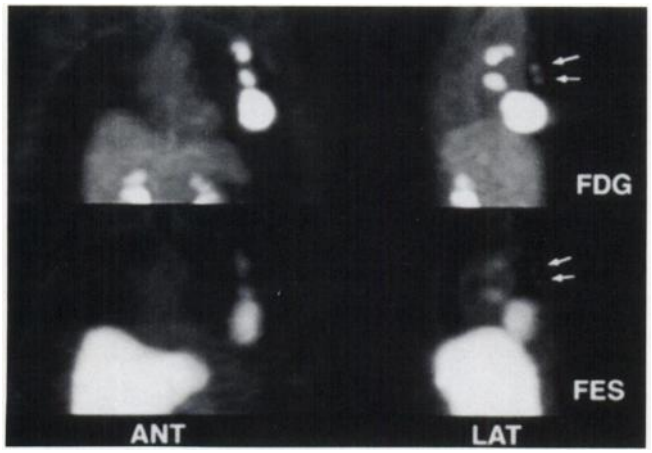

FIGURE 3. Anterior and left lateral volume-rendered maximum-activity-reprojection FDG and FES images from a patient with locally advanced breast cancer demonstrate concordant localization of both tracers in this ER-positive tumor. There is uptake in the primary left breast mass (4.5 cm diameter), in left axillary nodal metastases and in internal mammary nodal metastases (arrows).

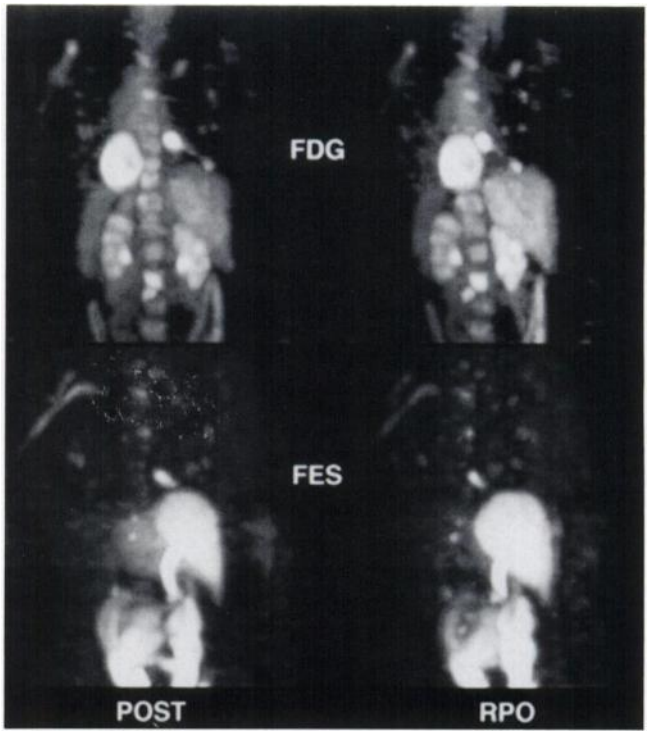

FIGURE 5. Anterior and right posterior oblique volume-rendered maximum-activity-reprojection FDG and FES images from a patient with breast cancer metastatic to bone demonstrate concordant localization of both tracers in this ER-positive tumor in innumerable skeletal metastatic foci, particularly in the vertebrae and ribs. Moderately intense FDG uptake in myocardium was visualized in this patient despite her fasting state. Renal excretion of FDG and hepatobiliary excretion of FES are demonstrated. The FES images also visualize activity along the course of proximal left arm veins consequent to tracer injection on this side.

interlaboratory variability, interassay variability, and specimen variability). The ER status of all five patients with apparent false-negative results of FES-PET was determined by the qualitative immunohistochemical method. Only one of these patients was treated with hormonal therapy and she did not respond (based on clinical follow-up for 13 mo after initiation of hormonal therapy). In addition, when multiple tumor sites were assessed in a given patient, concordance was present in 85% of the lesions. This is comparable to the level of concordance identified by in vitro ER determinations, when multiple sites in a single patient have been biopsied for quantitative receptor analysis (*24*). This confirms our earlier observations that FES-PET is a reliable method for assessing the ER status of breast cancers.

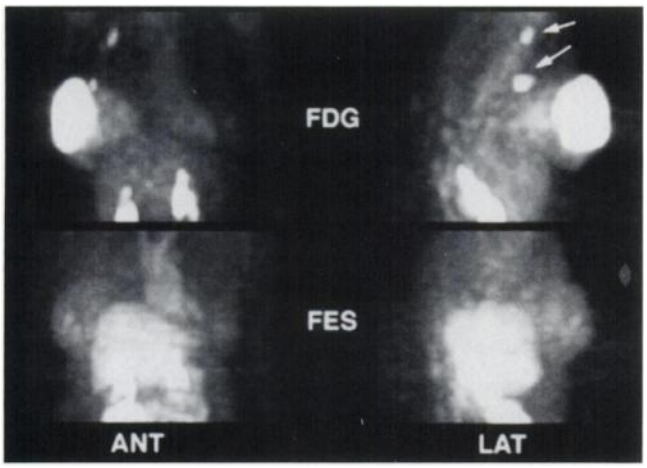

FIGURE 4. Anterior and right lateral volume-rendered maximum-activity-reprojection FDG and FES images from a patient with locally advanced breast cancer demonstrate discordant localization of both tracers in this ER-negative tumor. There is FDG uptake in the primary fungating right breast mass (8.0 cm diameter) and in right axillary nodal metastases (arrows) but no FES uptake in either site.

An in vivo technique, such as FES-PET, has several potential advantages compared with in vitro assays. Not only can the ER status of the primary cancer be assessed, but that of regional or distant metastatic lesions (some of which may be relatively inaccessible) can be determined with this technique obviating biopsy of each lesion. This is information of potential clinical importance, because discordance between the primary and metastatic lesions in individual patients has been reported, reflecting the heterogeneous nature of breast cancer. FES-PET, unlike in vitro assays, assesses the entire tumor volume rather than a single piece of the tumor. In addition, this in vivo technique has the ability to address, at least in part, the heterogeneity of receptor expression within individual lesions and to detect regions with low ligand binding because of hemorrhage or necrosis. Given the fact that there may be significant intratumoral ER heterogeneity, undersampling can be a significant problem in determining true ER status by in vitro assays. These advantages suggest that FES-PET should reveal the actual biological availability of tumor ERs for interaction with antiestrogen agents. Accord-

ingly, FES-PET may be a useful adjunct to guide systemic therapy in patients with breast cancer.

Warburg was the first to suggest that many malignant tumors have a higher rate of anaerobic glycolysis compared with normal tissues (*25*). FDG is a glucose analog that is now widely used to evaluate regional glucose metabolism in a variety of cancers. With specific reference to breast cancer, FDG-PET appears to have considerable utility for differentiating benign from malignant breast lesions, for evaluating the locoregional and distant extent of breast cancer, and for assessing the efficacy of therapy in patients with breast cancer (*7–10*). Although not a primary focus of the current study, our results confirm that FDG-PET is able to differentiate benign and malignant breast masses with a high degree of accuracy (sensitivity 88%, specificity 100%). These results are similar to those reported by Adler et al., Nieweg et al. and Tse et al. (*8–10*). We also observed axillary and internal mammary nodal uptake of FDG in many of our patients with primary breast cancer (Figs. 2 and 3), but this study was not designed to assess the reliability of FDG-PET in the detection of nodal metastases.

Wahl et al. (*12*) demonstrated increased uterine FDG accumulation in association with an increase in uterine size following estrogen administration to immature female rats, suggesting that increased cell proliferation and augmented glucose metabolism were mediated through an ER-response pathway in this ER-rich organ. They concluded that FDG may be a suitable tracer for detecting the metabolic effects of ER stimulation or repression and, thus, for assessing receptor function in tumors, such as breast cancers, where receptor function influences the behavior of the lesion. A relationship between tumor FDG uptake assessed by PET and tumor grade and aggressiveness has been demonstrated for several different types of tumors (*10,13–15*). Specifically, in the case of breast cancer, Adler et al. have shown that FDG accumulation is correlated with the pathologic grade of the tumor (*10*). These observations, in conjunction with the well-established relationship between breast cancer aggressiveness and tumor ER status, led us to hypothesize that there may be a relationship between glucose metabolism and ER status in breast cancers: patients with ER-negative breast cancer would be expected to have higher tumor FDG uptake than patients with ER-positive breast cancer. If this were true, tumor FDG uptake could be used as a surrogate marker of ER status or of the functional state of ER stimulation in breast cancer. To test this hypothesis, we compared tumor FDG uptake with tumor ER status (assessed both by in vitro assay and FES-PET) in patients with advanced breast cancer prior to initiation of systemic therapy. We were unable to demonstrate any significant difference in tumor FDG uptake between ER-positive and ER-negative tumors in these patients (Fig. 1). Moreover, there was no significant correlation between tumor FDG uptake and tumor FES uptake (Fig. 2). These results suggest that in vitro ER assays and/or FES-PET provide unique direct information about breast cancer ER status that cannot be obtained indirectly by FDG-PET.

CONCLUSION

In this larger patient series, we have confirmed our earlier observation that FES-PET reliably assesses the ER status of breast carcinoma lesions. We found good agreement between the results of in vitro assays and those of this in vivo technique. We have further demonstrated that there is no discernible relationship between tumor FDG uptake and ER status in this group of untreated patients. As suggested by the experimental animal study of Wahl et al. (*11*), it is possible that there will be changes in FDG uptake following hormonal therapy in ER-positive tumor that can be used to assess tumor response to therapy. This hypothesis needs investigation, and we have initiated studies at our institution to evaluate this problem in patients with breast cancer.

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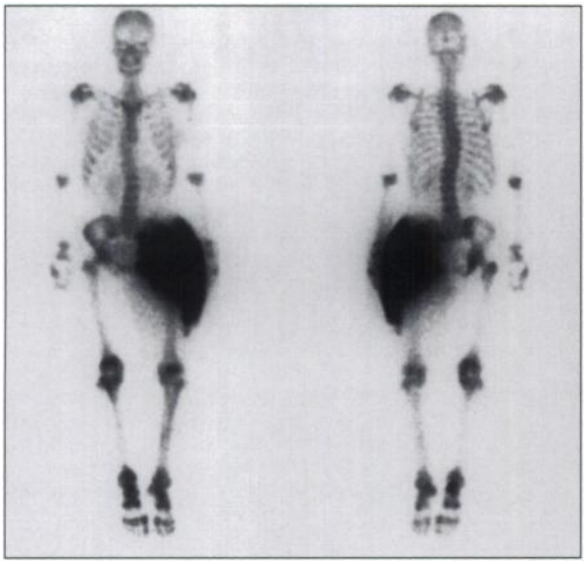
(continued from page 9A)

FIRST IMPRESSIONS:
OSTEOGENIC SARCOMA INVOLVING THE HIP REGION



PURPOSE
A 19-yr-old woman presented with a 1-yr history of increasing pain and swelling in the left hip. A biopsy from the head of the left femur, which was taken at presentation, indicated a benign tumor (i.e., chondroblastoma). Planar whole-body images (Fig. 1) show markedly extensive tracer uptake in the left hemipelvis and hip region. The first impression was presence of an artifact: radioactive contamination. Histologic examination confirmed osteogenic sarcoma involving the bone and soft tissue.

TRACER
Technetium-99m-methylene diphosphonate, 24 mCi

ROUTE OF ADMINISTRATION
Intravenous

TIME AFTER INJECTION
Four hours

INSTRUMENTATION
Elscint Helix (dual-head) SPECT camera

CONTRIBUTORS
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