Imaging hormone receptors in metastatic breast cancer patients

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Recommendations and technical aspects of $16\alpha$-[$^{18}\text{F}$]fluoro-$17\beta$-estradiol PET to image the estrogen receptor in vivo: the Groningen experience. Clin Nucl Med 2016; 41: 844-851

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Abstract

The estrogen derivative 16α-18F-fluoro-17β-estradiol (FES) is a PET tracer that has been used in a variety of preclinical and clinical studies to detect estrogen receptor (ER) expression, mainly in breast cancer, but also for other oncological indications. As a result of the success of these studies and the potential applications of the tracer, FES starts to be implemented in routine clinical practice. However, the number of centers using this tracer is still limited and many nuclear medicine physicians and medical oncologists are still unaware of the possibilities FES PET imaging offers. The aim of this article is therefore to give an overview of the main indications of FES-PET in oncology and to provide recommendations on correct use of this imaging technique. This includes precautions that have to be taken for patient preparation, procedures for the acquisition of the scans, the physiological distribution of the tracer, factors that might influence tracer uptake and guidance for image analysis, quantification of tracer uptake and reporting of the scans.

Keywords: estrogen receptor expression, imaging, breast cancer, ovarian cancer, FES-PET
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Cancer is a leading cause of death in the western world\(^1\), but remarkable progress has been made in unraveling the molecular pathways that drive tumorigenesis. This has led to the discovery of many novel molecular targets for anticancer treatment and, as a result, in the development of numerous targeted anticancer drugs. These targeted agents interfere with specific molecules that are critical for tumor progression. Personalized medicine aims to identify those patients who are most likely to benefit from a specific treatment. Molecular imaging enables non-invasively determination of the presence of relevant drug targets and other molecular properties in (metastatic) lesions throughout the whole body of an individual patient.\(^2\)

The estrogen receptor (ER) is over expressed in approximately 70% of patients suffering from primary breast cancer and is an important target for endocrine therapy. Current guidelines recommend the assessment of receptor status (ER, progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER-2)) and grade before starting a new line of therapy.\(^3\) ER expression of the tumor is the main indication to start antihormonal treatment, since success rate relies heavily on the ER status of the tumor, both in an adjuvant and in the metastatic setting. Thus assessment of functional ER status provides a rationale for endocrine therapy and is a predictive biomarker for treatment response. The golden standard for assessment of ER expression still remains immunohistochemistry on biopsy samples. Remarkably, this technique uses 1% ER positive cells as a cut-off point for ER positivity.\(^3\) Although the specificity of immunohistochemistry is high (almost 100%), it is not always feasible to obtain a suitable biopsy due to the location of the tumor, for safety reasons or because of sampling errors. Moreover, some studies have underlined the possibility of ER expression changing over time, resulting in heterogeneity of ER expression between primary tumor and its metastases, between lesions within a single patient, and even within a single lesion. Discordant ER expression between the primary breast tumor and the metastases was observed in 15-40% of the patients.\(^4,5\) In metastatic breast cancer, a single biopsy may therefore not be representative for the ER expression in other metastases throughout the body. Furthermore, performing biopsies from multiple lesions – if
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feasible – is far from patient friendly. PET imaging may overcome these issues and can possibly guide towards more personalized medicine.

The most frequently used tracer for detection of ER expression in breast cancer is $16\alpha$-$[^{18}\text{F}]$fluoro-$17\beta$-estradiol (FES). With its potential to serve as a prognostic as well as a predictive biomarker FES-PET has gained growing interest in research with over 20 studies registered at clinicaltrials.gov of which 11 are active at this moment (Table 1). However, guidelines on how to correctly acquire, analyze and interpret the scans are not available thus far. Uniformity is essential to compare upcoming studies and for warehousing of all the available data. Besides applications for research purposes, FES-PET is now more and more being introduced in routine clinical practice, especially for patients with a diagnostic dilemma.$^6$

At this moment, FES is produced only in a few institutions worldwide, but this number is growing and more centers in the vicinity of the production sites are able to perform the PET scan. The physical half-life of $^{18}\text{F}$ (110 min) should allow transportation of FES to other hospitals within a traveling distance of a few hours. As more and more nuclear medicine physicians and medical oncologists are getting access to the unique possibilities FES-PET imaging offers, the aim of this article is to give an overview of the main indications of FES-PET in oncology and to provide guidance for the most important aspects of this imaging technique, such as precautions that have to be taken for correct patient preparation, procedures for the acquisition of the scans, the physiological distribution of the tracer, factors that may influence tracer uptake and recommendations for image analysis, quantification of tracer uptake and reporting of the scans. These recommendations are based on extensive experience with FES-PET imaging acquired both in the research and in a clinical setting in Groningen and on available literature data.

**Potential indications of FES-PET imaging**

Several studies have been performed to assess the potential applications of FES-PET (-CT) as an in vivo imaging tool in oncological diseases (Figure 1). Most frequent settings, and promising indications for clinical use, are summarized below:
Diagnosis and Staging

- **Breast cancer**: over 20 studies using FES-PET were performed in patients with breast cancer, particularly as a diagnostic tool. As such it has the potential to serve an important role patients with diagnostic dilemmas that cannot be solved by conventional imaging techniques and/or when a biopsy in a suspicious lesion is not feasible.\(^6\) Although current guidelines recommend to perform FDG-PET/CT when conventional work up is inconclusive FES-PET may be preferred in patients with ER-positive breast cancer, because FDG-PET cannot differentiate breast cancer metastases from metastases from other tumor types, or from benign, inflammatory or infectious lesions.\(^3\)

Particularly in a subgroup of patients with lobular breast cancer, which due to the loss of E-Cadherin grows in a less cohesive pattern, it is often difficult to detect the lobular tumor on physical examination and conventional imaging.\(^7\) Uptake on FDG PET in lobular breast cancer lesions are often lower compared to ductal breast cancer and hormone negative breast cancer.\(^8\)\(^9\)

An important advantage is that FES-PET is a whole body imaging technique, which is ideal for assessment of heterogeneous ER expression across metastases in the body, and for evaluating the expression of the ER both in the primary tumor and in metastatic disease.\(^10\) In 4 clinical trials, an overall specificity of 98% and a sensitivity of 84% of the FES-PET for ER in ER-positive patients was found. In ER-positive breast cancer patients that have high risk to develop metastases or have clinical signs, laboratory values or histology suggestive of the presence of metastases, FES-PET could be a valuable tool for tumor staging, and assessment of the change in ER expression over the time and the heterogeneity in ER status within the same patient.
Figure 1. Published articles with FES-PET, per indication (based on PubMed search and clinicaltrials.gov)

- **Ovarian cancer:** There are two known ER subtypes namely ERα and ERβ. In epithelial ovarian cancers both ERα and ERβ are expressed, in respectively 73% and 31% of tumors. Although the affinity of FES for ERα is 6.3 times higher than for ERβ\(^1\), FES-PET could be useful in the diagnosis of ovarian cancer in case of diagnostic dilemmas that remained despite conventional work up. In metastatic disease using FES-PET has a sensitivity of 79% and a specificity of 100% for ERα positive metastatic ovarian cancer lesions. Although uptake in metastases in the abdomen and pelvis could be obscured by physiological tracer uptake in the bowel this was mostly solved by the accompanying CT scan.\(^1\)

- **Uterine tumors:** FES-PET has been used for the detection of ERα in endometrial carcinoma\(^1\), and also in the benign setting of leiomyoma of the uterus, which can metastasize to distant locations (benign metastasizing leiomyoma).\(^1\) Furthermore, the use of this molecular imaging technique for the differential diagnosis between leiomyoma of the uterus and uterine sarcoma was published.\(^1\)

- **Other tumors:** although in this setting only a few studies have been performed, FES-PET could be helpful in all other oncological dilemmas in tumors with high probability of ER expression such as endometrial stromal sarcoma\(^1\), gastric carcinoma\(^1\), prostate cancer\(^1\) and meningeomas. In gastric carcinoma and prostate cancer, studies on imaging of the ER are still lacking, but might deserve further investigations.
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Treatment setting and follow-up

- **Breast cancer:** Next to diagnosing and staging of patients, FES-PET can be used for the selection and early response prediction of antihormonal therapy (this latter still in a research setting). In progressive patients, despite several lines of antihormonal therapy, FES-PET can be used to assess whether metastases still express ER and thus to provide a rationale for another line of antihormonal therapy, or – in case of a negative FES-PET (no ER expression in the metastases) – to switch to another treatment option. Low or absent FES uptake can be considered as a good predictor of endocrine resistance.\(^\text{21,22}\) For example, in a recent study the value of FES-PET to identify patients with acquired hormone resistant disease (low or absent tracer uptake in metastases) who are unlikely to respond to estradiol therapy was demonstrated.\(^\text{23}\) Another study found that the degree of ER blockade after institution of tamoxifen treatment predicted responsiveness to anti-estrogen therapy.\(^\text{24}\) Furthermore molecular imaging could provide an important imaging tool in therapy decision making by measuring the probable responsiveness to intended endocrine drugs: patients with a maximum standardized uptake value \((\text{SUV}_{\text{max}})\) in tumor metastases below 1.5 are unlikely to respond.\(^\text{22,25,26}\)

FES-PET/CT could guide dose finding strategies as it is able to visualize the pharmacodynamics of ER modulators/degraders. In small patient groups \((n=16-47)\), the decrease in FES uptake after initiating treatment with an ER antagonist predicted response to tamoxifen and fulvestrant. The quantification of ER binding (receptor saturation) during antihormonal therapy can be used to adjust the drug dose in individual patients.\(^\text{26}\)

In addition Yang et al. showed the value of FES-PET imaging to predict response to neoadjuvant chemotherapy, especially when compared to FDG-PET: a low ratio between FES and FDG uptake was correlated with highly proliferative disease that might benefit from chemotherapy, while an high ratio between these two imaging techniques would identify those patients that would benefit from endocrine therapy.\(^\text{27}\)

- **Ovarian cancer:** Currently, most ovarian cancer patients are treated with chemotherapy; whether the ER status is correlated with response
to chemotherapy is still unknown. FES PET might provide a rationale to initiate endocrine treatment in patients ERα-positive lesions. However, clinical studies are required to support this indication.

- **Uterine tumors:** FES-PET can be used for monitoring treatment response and is able to provide a rationale for ER targeted therapy in endometrial stromal sarcoma patients; however, this finding is, at this moment, only demonstrated by a case report.\(^{17}\)

**Pharmacokinetics of FES**

FES is a fluorinated analogue of estradiol and therefore its biodistribution depends on the presence of functional ER in normal tissue, primary tumor and metastases. The metabolism of FES is similar to that of other estrogens. Several studies have shown that the physiological biodistribution of FES in humans and small animals is similar to that of estradiol: the tracer first accumulates in the liver where it is metabolized into polar conjugates (sulfate and glucuronide), with subsequent excretion into bile. Then its metabolites are excreted by the bile ducts, and pass through the small intestine. Because of the enterohepatic circulation only a small percentage of tracer is distributed in the large intestine, where little uptake can be seen. The main elimination route for estrogens is via urine, while only 7% of the administered tracer is excreted by feces.

FES has chemical properties similar to estradiol, the main and most potent agonist of ER. In the uterus estrogens stimulate the endometrial growth, while in the ovaries the receptor is important to maintain the feedback loop with the pituitary for the synthesis of hormones. In breast tissue, estrogens serve as growth factors for ductal proliferation. As a consequence, specific physiological uptake of FES can be seen in the pituitary gland, uterus, ovaries and in breast tissue of premenopausal women. Uterine FES uptake is roughly SUV 2.5 in the myometrium and SUV 4.0-6.0 in the endometrium.\(^{28,29}\)
Table 1. Active FES studies on clinicaltrials.gov, June 2016

<table>
<thead>
<tr>
<th>Study No.</th>
<th>No. Patients</th>
<th>Tumor Type</th>
<th>Aim</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02374931</td>
<td>10</td>
<td>Desmoid</td>
<td>To establish the avidity of desmoid tumors on $^{18}$F-FES PET/CT imaging</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02409316</td>
<td>75</td>
<td>Breast</td>
<td>To evaluate the $^{18}$F-FES PET/CT uptake as a predictor of progression-free survival in endocrine refractory recurrent or metastatic breast cancer patients starting a new therapy regimen</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02398773</td>
<td>99</td>
<td>Breast</td>
<td>To evaluate the predictive value of FES PET/CT to endocrine therapy in patients with newly diagnosed metastatic breast cancer</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01986569</td>
<td>94</td>
<td>Breast</td>
<td>To determine a positive and negative percent agreement between immunohistochemistry and FES</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02149173</td>
<td>50</td>
<td>Breast</td>
<td>To measure the effect of endocrine targeted therapy on the estrogen receptor expression and estradiol binding to the receptor using serial FES PET and FDG PET</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT00816582</td>
<td>100</td>
<td>Breast</td>
<td>To determine whether FES can predict clinical benefit to fulvestrant in postmenopausal women with recurrent or metastatic ER+ breast cancer who are candidates for further hormonal therapy</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01957332</td>
<td>200</td>
<td>Breast</td>
<td>To evaluate the clinical utility of experimental PET scans in the setting of MBC at first presentation</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02559544</td>
<td>50</td>
<td>Breast</td>
<td>To evaluate FES PET/CT uptake as a predictor of progression free survival in endocrine refractory recurrent or metastatic breast cancer patients starting a new therapy regimen including endocrine targeted therapy</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02233621</td>
<td>100</td>
<td>Endometriosis</td>
<td>To assess sensitivity of FES PET for diagnosing endometriosis compared to the gold standard in women care for suspected endometriosis</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01720602</td>
<td>15</td>
<td>Breast</td>
<td>To assess the change in estrogen receptor expression measured as the change in SUV using not FES PET after 2 and 8 weeks of vorinostat and recruiting aromatase inhibitor therapy</td>
<td>Active, not recruiting</td>
</tr>
<tr>
<td>NCT01153672</td>
<td>8</td>
<td>Breast</td>
<td>To assess the change in estrogen receptor expression, measured as the change in SUV using not FES PET after 2 and 8 weeks of vorinostat and recruiting aromatase inhibitor therapy</td>
<td>Active, not recruiting</td>
</tr>
</tbody>
</table>
Technical procedures of FES-PET imaging

Patient preparation
No specific patient preparations have to be made, except when the FES-PET scan is accompanied by a diagnostic CT scan. Then, the same procedural guidelines as for FDG-PET/CT imaging can be followed. Some medical aspects have to be taken into account before planning the FES-PET, such as:

- A review of the medical history, with specific attention to: current and previous treatment received, the ER status of the primary tumor, the ER status of metastases (if available), the results of other imaging techniques and the reason for performing the scan (for example, diagnostic dilemma or therapy rationale).
- In case of diagnosis and staging, treatment with ER antagonists (e.g. tamoxifen or fulvestrant) should be stopped for ≥5 weeks before performing the scan. Especially for tamoxifen clearance of the drugs may take up to 8 weeks. Aromatase inhibitors as well as luteinizing hormone-releasing hormone (LHRH) agonists can be continued.
- Liver metabolism: despite the rapid hepatic metabolism of FES, slightly decreased liver function is unlikely to affect quantitative measurements of ER expressing tumors outside the liver. No studies have been performed with patients with severe hepatic impairment.
- If the FES-PET is performed together with a diagnostic CT scan, renal function should be evaluated and kidney failure excluded.

Patient instructions
Other than discomfort at the injection site, adverse events have never been reported. As FES is injected as a bolus, after reaching physiological concentrations, it returns to sub physiological levels within an hour. After injection the patients should be scanned within 120 minutes, but not before 20 minutes as FES concentrations in the blood reaches a peak after 10-20 minutes and remains fairly constant between 20 and 120 minutes. In most studies image acquisition is performed 60 +/- 10 minutes after tracer administration for logistical reasons. No results have been published on scanning 20 minutes post injection.
During the waiting period between injection and scanning, similar instructions have to be followed as for FDG-PET. However, patients are allowed to talk and move and theoretically there is no need to fast before the scan. To reduce the radiation burden to the patient and to avoid artifacts due to high radioactivity levels in the urine, drinking 1 liter of water before the procedure and 0.5 liter after tracer injection is suggested. Fasting has been suggested by several studies to reduce bowel accumulation due to bile excretion.

**Table 2.** Scanning time per bed position, based on administered activity and body weight (mCT Biograph, Siemens)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>100-150 MBq</td>
<td>&gt; 150 MBq</td>
</tr>
<tr>
<td>0-60 kg</td>
<td>2 min</td>
</tr>
<tr>
<td>60-90 kg</td>
<td>4 min</td>
</tr>
<tr>
<td>&gt; 90 kg</td>
<td>6 min</td>
</tr>
</tbody>
</table>

**Tracer production**

The production of FES is generally based upon the two-step one-pot radiolabeling procedure starting from the precursor 3-O-(Methoxymethyl)-16,17-O-sulfuryl-16-epiestriol (32). A critical step in the labeling procedure is the hydrolysis step, as incomplete hydrolysis of the intermediate gives rise to the formation of radioactive side-products and low yields. A recent study, however, suggested that this issue can be avoided using another acid for the hydrolysis reaction.

To allow FES production for clinical studies, the labeling procedure has been adapted to enable fully automated tracer production with either a fixed-tubing or cassette-based synthesis module. Nowadays not only the cyclic-sulfate precursor is commercially available, but also dedicated disposable labeling kits containing all required chemicals and disposables can be readily purchased. This highly facilitates the production of FES in accordance with Good Manufacturing Practice guidelines. Thus, FES can typically be produced in 20-30% radiochemical yield, which is sufficient for distribution to centers in the vicinity of the production site.
Figure 2. FES-PET scan of a patient with metastasized breast cancer. (A) low intensity MIP image to analyze the uptake in the liver: in this case homogenous (no liver metastases), (B) high intensity MIP image to analyze all other tissues: multiple bone lesions with high ER expression can be seen.

**Image acquisition**

FES is a lipophilic radiopharmaceutical with a volume of ≤ 20 mL containing < 10% ethanol, administrated by intravenous injection. The mass injected should be ≤ 5 μg and the administered activity dose is usually a fixed dose of 200 MBq (the drug substance is obtained by dilution of radioactivity with 0,9% NaCl). Higher doses might be safe but have not been tested. The specific activity of FES is typically higher than 25.000 GBq/mmol. Consequently a 200 MBq injection of FES results in less than 3 μg of FES injected. Radiation dosimetry studies show that organ doses due to FES-PET are comparable with those associated with other commonly performed nuclear medicine studies and potential radiation risks are well within acceptable limits. The organs that receive the highest dose are the liver (0.13 mGy/MBq), gall bladder (0.10 mGy/MBq) and urine bladder (0.05 mGy/MBq). The effective dose equivalent is 0.022 mSv/MBq, which corresponds to a radiation burden of 4.4 mSv for an injection of 200 MBq of FES; this is comparable to the radiation burden of FDG. The recommended minimum dose is 100 MBq, although this may depend on the performance
of the PET camera used (e.g. sensitivity, time-of-flight) and the body weight of the patient.

For scan acquisition and processing, the same protocol can be followed as for FDG-PET. During the scan, the arms of the patient should ideally be placed above the head, in order to avoid artifacts. If this is not possible, the arms can be positioned alongside the body. Since FES-PET usually contains insufficient anatomical detail to localize lesions, especially in the abdomen, multimodality imaging (PET/CT) is required to provide anatomical information. For the vast majority of applications, axial anatomical scan coverage from the skull base to mid-thigh is recommended. The scanning time per bed position depends on the weight of the patient and administered dose (Table 2, based on mCT Biograph Siemens camera).

**Interpretation, quantification and reporting:**
Some issues should be taken into account when analyzing and reporting a FES-PET scan: the reason why the scan was performed, areas with physiological uptake, metabolism and excretion. Lesions on conventional imaging techniques should be evaluated with FES-PET. Depending on the research question both metastases that show increased ER expression and metastases that do not, have to be examined. Also hitherto unknown lesions, not visible on conventional imaging techniques should be described. Furthermore, if the purpose of the diagnostic imaging was to confirm the ER status for therapy decision making, it is of importance to quantify the overall uptake and (heterogeneity in) ER status of metastases.

For the interpretation of the results, the following aspects should be taken into account:
- Physiological uptake of the tracer in the gastrointestinal tract and urinary tract has to be evaluated (see above).
- Best visual analysis method is to first look at low intensity levels at the liver uptake: homogeneous uptake suggests the absence of lesions (although ER-positive lesions with similar uptake as the liver cannot be excluded), “cold spots” could indicate benign cysts, ER negative lesions, but also lesions with low ER expression or even high expression, “hot spots” (uptake > physiological liver uptake) indicate
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ER positive lesions. Care should be taken that the gall bladder has in general higher physiological uptake than the liver. For the evaluation of lesions in all other tissues the intensity level should be put higher (Figure 2).

- In case of ambiguous lesions upon visual analysis of the scan, tracer uptake in the lesion should be quantified as $\text{SUV}_{\text{max}}$. Based on earlier preclinical and clinical studies that correlated FES PET with immunohistochemistry, a lesion with a $\text{SUV}_{\text{max}} > 1.5$ should be considered ER positive.

- FES is lipophilic and therefore patients with increased fat mass may have a lower tumor FES uptake than leaner patients, because of an increased pharmacological distribution volume.

- In the majority of patients, uptake at the injection site and in blood vessels can be seen despite extensively flushing or longer administration times. The cause of this accumulation is still unknown, but is probably due to sticking of the tracer to the vessel wall/endothelial cells. As the amount of tracer in these blood vessels is negligible ($< 1\%$ of the administered dose) no influence is to be expected for uptake in the tumours (Figure 3).

**Figure 3.** Physiological distribution of FES-PET with accumulation in the liver (1), excretion by gastrointestinal tract (2), kidneys (3) and bladder (4). In most patients also high uptake is seen in the injected vessel (5).
In the final scan report, at least the following items have to be mentioned:

- Description of the areas with physiological uptake, metabolism and excretion
- Description of areas with increased (SUV$_{\text{max}} > 1.5$) ER expression
- Evaluation of ER expression (positive or negative) in lesions observed by other available imaging techniques (CT, MRI, FDG-PET)
- If the scan was performed to solve a diagnostic dilemma (Figure 4): describe if there is FES uptake in the equivocal lesion, if there is uptake quantify it and report if the lesion is ER positive or ER negative
- If the scan was performed to look for heterogeneity within all known metastases: describe which metastases show increased ER expression and which metastases do not
- If the scan was performed for therapy rationale, describe the overall ER status of the metastases
- Describe aspecific findings (see below, factors influencing uptake)

Figure 4 Patient with breast cancer, presenting with coughing and shortness of breath. FDG-PET (left image) was performed to search for metastases. Slightly elevated FDG uptake is visible in mediastinal and hilar lymph nodes, which could be due to metastases but also to inflammation/reactive lymph nodes. FES-PET (right image) shows high ER expression in multiple lymph nodes in mediastinum and hili and also in the neck (left side), in accordance with lymph node metastases with high ER expression (proven by immunohistochemistry).
Factors influencing FES uptake

The accumulation of FES can be affected by both external and intrinsic factors (Table 3).

**Intrinsic factors**

Based on the results of 312 FES-PET scans various factors might influence FES uptake.37

- *Estradiol levels and menopausal status*: It is expected that circulating estrogens in premenopausal patients (>30 pg/ml) affect FES uptake, leading to lower accumulation in tumours, due to competition of the tracer with the physiological hormone for the ER binding site. Peterson *et al.*, however did not show evidence that premenopausal estradiol levels impacted FES uptake in 82 patients with estradiol levels >30pg/mL.

- *SHBG*: in analogy with estradiol, circulating FES is mostly bound to one of two proteins in plasma (SHBG and albumin). After injection approximately 45% of the tracer is bound with high affinity but low capacity to SHBG and this percentage depends on the plasma concentration of SHBG. SHBG level is significantly inversely correlated with FES uptake in the tumor.38

- *BMI*: a significant correlation was also described between BMI and uptake of the tracer. In contrast to what could be expected based on the lipophilic nature of FES, patients with a higher BMI showed a higher tumor uptake of FES. However, this effect did not persist when FES uptake values were adjusted for lean body mass rather than body weight in kilograms.37

- *Tumor location*: due to high physiological uptake of FES in the liver, FES-PET is not the optimal imaging technique to detect liver metastases. A correlation between the photopenic appearance and the metastatic lesions with tracer accumulation inferior to the physiological distribution in the surrounding healthy liver tissue has been described.6 However, in these cases both ER positive and ER negative hepatic lesions could appear with lower uptake than the uptake in surrounding normal liver parenchyma. Therefore, FES-PET is not recommended when a patient is expected to have only liver metastasis, although occasionally uptake in the liver metastases can be higher than in normal liver tissue. In a recent
lesion based analysis, performed on 91 patients, it was also demonstrated that uptake of FES in metastases differs per location: an overall lower uptake of FES was found in lung and brain metastases compared to bone lesions or lymph node metastases.\textsuperscript{39} Furthermore, lesions in the gastro-intestinal tract are often difficult to assess due to excretion of the tracer. Bone marrow infiltration can sometimes be seen as a diffuse uptake.

**Extrinsic factors**

- *Previous antihormonal treatment:* Since ER antagonists block the binding site of the ER, tracer uptake will be affected. Treatment with ER antagonists (such as fulvestrant and tamoxifen) should therefore be stopped for $\geq 5$ weeks before the FES-PET scan is acquired. Whether this time is sufficient to completely eliminate competitive binding is unknown, particularly for fulvestrant, which has a half-life of 40 days and both blocks and degrades estrogen receptors. A longer drug-free period, therefore, might be needed, but often is not feasible in clinical practice. As aromatase inhibitors do not affect the ER, but inhibit the conversion of testosterone into estrogens by aromatase, its use does not influence the tracer uptake and may therefore be continued.\textsuperscript{22,39} In some cases it is expected that these type of drugs could even increase uptake, as a result of lower plasma estrogen levels.

- *Other therapies:* in some patients, aspecific uptake can be seen in regions of the body that underwent radiotherapy in the past.\textsuperscript{40} It remains unclear whether the increased tracer accumulation is due to enhanced extravasation owing to epithelial damage, or to tracer binding to infiltrating immune cells that express ER$\beta$ (Figure 5).\textsuperscript{41}

- *Resolution of the camera:* as in all other nuclear medicine imaging techniques, the detection of lesions depends partially on the size of the tumor in relation to the spatial resolution of the PET camera. Recent developments in hybrid camera imaging led to a better spatial resolution of 2-4 mm and if the ER expression level is high enough even small lesions of a few millimeters can be visualized by FES-PET.
Table 3. Factors influencing FES uptake

<table>
<thead>
<tr>
<th>Intrinsic factors</th>
<th>Extrinsic factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of the lesion</td>
<td>Previous antihormonal treatment</td>
</tr>
<tr>
<td>Menopausal status</td>
<td>Previous radiotherapy</td>
</tr>
<tr>
<td>SHBG level</td>
<td>Spatial resolution of the camera system</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
</tr>
<tr>
<td>ER status of the primary tumor</td>
<td></td>
</tr>
<tr>
<td>ER status of metastases</td>
<td></td>
</tr>
</tbody>
</table>

Figure 5 FES-PET of a patient with metastasized breast cancer and previous radiotherapy. Note the uptake in axillary lymph nodes (1) and in bone metastases (2). Also diffuse heterogeneous uptake in the lungs (3) is visible as result of previous radiotherapy.

Conclusions and future perspectives

Although FES-PET is not yet performed on a large scale, its use is increasing due to the need for better characterization of breast cancer to enable more personalized treatment. Personalized medicine is booming in the oncological world and in this setting, FES-PET seems a promising biomarker to be used for this purpose. If available, FES-PET could thus be considered as a non-invasive method for the detection of lesions expressing ER, mainly in metastatic breast cancer patients. The number of research studies with this tracer is increasing and several multicenter studies are ongoing to reveal the added value of FES-PET for several indications. Most studies are performed in the oncologic setting; however some ongoing studies are
investigating the feasibility of FES-PET in benign disease. For example, the Vanderbilt-Ingram Cancer Center is trying to establish the avidity of desmoid tumors on FES-PET/CT imaging and correlates FES uptake with degree of ER expression by immunohistochemistry. The purpose of another ongoing study is to assess the sensitivity of FES for diagnosing endometriosis compared to the gold standard, which is histological confirmation at biopsy or excision of lesions performed during laparoscopy, in women suspected of endometriosis and for whom laparoscopy is already scheduled. Not only in detection of disease, but also for the purpose of determining pharmacodynamics of new ER targeted therapies, FES-PET could be of invaluable importance. In a phase I trial in healthy volunteers, FES PET was used to determine ER occupancy in the uterus and brain during targeted treatment with RAD1901.\textsuperscript{42} It may be expected that the interest for this imaging technique will grow in the forthcoming years and that this technique will be implemented in daily clinical practice and even will be included in future diagnostic guidelines. Despite several publications, no specific recommendations were available for nuclear medicine physicians that described how to correctly acquire, analyze and interpret the FES-PET scan. Here, we described the technical aspects of the FES-PET scan that can be used as a first recommendation paper for everyone who wants to implement this imaging technique in his/her imaging facility. Thus, we hope that FES-PET may become available for more patients and can contribute to better diagnosis and treatment selection.
References


Chapter 2


