Radiolabeled Amino Acids: Basic Aspects and Clinical Applications in Oncology*

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As the applications of metabolic imaging are expanding, radiolabeled amino acids may gain increased clinical interest. This review first describes the basic aspects of amino acid metabolism, then continues with basic aspects of radiolabeled amino acids, and finally describes clinical applications, with an emphasis on diagnostic value. A special focus is on ^{11}C-methionine, ^{11}C-tyrosine, and ^{123}I-iodomethyltyrosine, because these have been most used clinically, although their common affinity for the L-transport systems may limit generalization to other classes of amino acids. The theoretic and preclinical background of amino acid imaging is sound and supports clinical applications. The fact that amino acid imaging is less influenced by inflammation may be advantageous in comparison with ^{18}F-FDG PET imaging, although tumor specificity is not absolute. In brain tumor imaging, the use of radiolabeled amino acids is established, the diagnostic accuracy of amino acid imaging seems adequate, and the diagnostic value seems advantageous. The general feasibility of amino acid imaging in other tumor types has sufficiently been shown, but more research is required in larger patient series and in well-defined clinical settings.

Key Words: radiolabeled amino acids; ^{11}C-methionine; ^{11}C-tyrosine; ^{123}I-iodo-α-methyltyrosine; tumor imaging

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Over the past few years, clinical interest in metabolic imaging of cancer has been growing. The most prominent example is the increasing application of ^{18}F-FDG and PET. FDG PET is now successfully used in many types of cancer to stage and restage disease and also to better differentiate between malignant and benign lesions. Increased anaerobic glycolysis, present in nearly all cancer cells, is the target for uptake of FDG.

Another interesting target for metabolic tumor imaging is the increased protein metabolism, to which radiolabeled amino acids can be applied, in cancer cells. With the increasing clinical application of FDG, clinical interest in imaging protein metabolism through radiolabeled amino acids is also expected to increase. These amino acid tracers may help in imaging areas in which FDG imaging is limited, such as the brain (because of high background FDG uptake), or in differentiating tumorous lesions from inflammatory lesions (because of high FDG uptake in macrophages, e.g., after radiotherapy).

In this article, we present a short overview of amino acid metabolism, describe the basic aspects of radiolabeled amino acids, and review published clinical applications in various types of cancer, with a special emphasis on diagnostic value. We have limited the description of clinical applications to those radiolabeled amino acids about which several articles exist. Thus, we focus especially on methionine (L-[methyl-^{11}C]-methionine [MET]), tyrosine (L-1-[^{11}C]-tyrosine [TYR]), and L-3-[^{123}I]iodo-α-methyltyrosine (IMT).

AMINO ACID AND PROTEIN METABOLISM

Proteins, which play crucial roles in virtually all biologic processes, are built from amino acids linked through peptide bonds. Of the basic set of 20 amino acids, 11 can be synthesized from metabolic pathways, whereas the other 9 must be obtained from dietary sources (essential amino acids). Protein synthesis starts with nuclear DNA transcription, which is followed by ribosomal translation of mRNA into protein, and is a coordinated interplay of more than 100 macromolecules (*1*). Amino acids either enter the cell from outside or are derived from intracellular protein recycling. Besides being the building blocks of proteins, amino acids are precursors for many other biomolecules, such as adenine, cytosine, histamine, thyroxine, adrenaline, melanin, and serotonin (*1*). In addition, amino acids can be crucial to metabolic cycles. For example, methionine is part of the activated methyl cycle, which is important as a methyl group donor in many biosynthetic steps (*1*).

Amino acid degradation and recycling is a constant and dynamic process. In degradation, the α-amino groups are removed and usually converted into urea. The resulting molecule is converted into metabolic intermediates that may

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be transformed in fatty acids, ketone bodies, or glucose (*1*). In the same way, surplus amino acids are used as metabolic fuel because they cannot be stored. Recycling represents reuse of amino acids by other tissues or cells and requires transport across cell membranes. With radiolabeled amino acids in mind, this transmembrane transport is an important factor in protein metabolism.

Amino Acid Transport Across Cell Membranes

Although all amino acids can diffuse into cells, the main movement of amino acids into cells occurs through carrier-mediated processes (*2,3*). Two groups of carriers can be designated. Carriers of the first group require sodium for maximal activity. The driving force that energizes this type of transport is provided by the sodium chemical gradient and the membrane electric potential and is maintained by Na^+ or K^+ adenosine triphosphatase. The second group of transport mechanisms is independent of sodium. In general, movement of an amino acid depends on its intra- and extracellular concentration, but transport is frequently associated with countertransport of a second amino acid, whose gradient has been established by one or more of the sodium-dependent carriers.

Few of the membrane transporter proteins have been identified, so investigators have relied on kinetic and competitive-inhibition analyses to define and characterize individual systems. Some 20 systems have been identified and are designated by letters. Among the main sodium-dependent systems, found in all tissues of nearly all species, are system A, system ASC, and system Gly (*2–5*). These systems usually transport amino acids with short, polar, or linear side chains such as alanine, serine, and glycine. The most important and ubiquitously found sodium-independent system is system L, but other systems such as system $B^{0,+}$ and system y^+ also exist. Sodium-independent systems are usually responsible for uptake of branched chain and aromatic amino acids, such as leucine, valine, tyrosine, and phenylalanine. In addition, some transport systems have been found only in specific tissues, such as system T, which specifically transports tyrosine, phenylalanine, and tryptophan into erythrocytes (*2*). The contribution of individual transport systems to the transport of a single amino acid may vary somewhat between different types of cells and species (*2–4*).

Regulation of amino acid transport is complex (*3,6*). Availability of nutrients is important. For example, in starvation, system A activity is increased by increasing the number of active A carriers, whereas system ASC appears unchanged (*6,7*). Apart from these adaptive responses to amino acid availability, hormones, cytokines, and cell volume changes are involved in transport regulation (*8,9*).

Amino Acid Metabolism in Malignancy

Amino acid transport is generally increased in malignant transformation (*10,11*). This increase in transport may be associated with specific cell surface changes in transformed cells. For example, amino acid transport system A is one of the few identified transport systems that is expressed strongly in transformed and malignant cells and appears to be a target of proto-oncogene and oncogene action (*12*). In general, however, the process of malignant transformation requires that cells acquire and use nutrients efficiently for energy, protein synthesis, and cell division. Therefore, increased transport of amino acids most likely is also an aspecific net result of increased demand for amino acids. Of the two major steps in protein metabolism, amino acid uptake and protein synthesis, the increased transport rate of amino acids may be more increased than protein synthesis. Several processes contribute to amino acid transport rather than to protein synthesis, including transamination and transmethylation, the specific role of methionine in initiation of protein synthesis (*1*), and the use of amino acids as glutamine for energy (*13*) or as precursors of nonproteins.

PRECLINICAL DATA FOR RADIOLABELED AMINO ACIDS

Nearly all amino acids have been radiolabeled to study potential imaging characteristics, usually for PET, because the replacement of a carbon atom by ^{11}C does not chemically change the molecule. These radiolabeled amino acids differ in ease of synthesis, biodistribution, and formation of radiolabeled metabolites in vivo. For these reasons, mainly [^{11}C-methyl]-methionine and tyrosine have been studied clinically. More recently, artificial amino acids such as IMT or L-3-[^{18}F]fluoro-α-methyltyrosine (FMT) (*14*), *O*-2-[^{18}F]fluoroethyl-L-tyrosine (FET) (*15*), [^{18}F]fluoro-L-phenylalanine (*16*), [^{18}F]-1-amino-3-fluorocyclobutane-1-carboxylic acid (*17*), [^{18}F]fluoro-L-proline (*18*), and [^{11}C-methyl]-α-aminoisobutyric acid (*19*) have been studied.

MET

The most frequently used radiolabeled amino acid is MET. The main reason is the convenient radiochemical production, which allows rapid synthesis with high radiochemical yield without the need for complex purification steps (*20*). However, this tracer has a considerable nonprotein metabolism and generates substantial amounts of nonprotein metabolites, making correct quantification of protein synthesis difficult (*21,22*).

Because most clinical applications of MET have focused on brain tumors, studies of the uptake mechanisms have frequently used the same tissues and models. The accumulation rate of radiolabeled methionine, both in normal human brain tissue and in gliomas without disruption of the blood–brain barrier, decreased by 35% after infusion of branched chain amino acids. L-methionine accumulated 2.4 times as much as D-methionine (*23*). These findings by Bergstrom et al. (*23*) indicate specific carrier-mediated uptake as an important factor governing MET uptake. However, O'Tuama et al. (*24*) could not confirm these results using a phenylalanine overload in patients, although the total amount of unlabeled MET in the brain did decrease. The importance of blood flow in tumor MET uptake was

shown by Roelcke et al. (*25*), suggesting that at least part of MET uptake may result from passive diffusion, possibly in areas with a damaged blood–brain barrier. In cell uptake studies, MET transport is usually mediated through the L-transport system, with minor contributions from A and ASC (*2*,*5*).

Preclinical studies validating the possible use of MET in the evaluation of chemo- or radiotherapy generally show that MET uptake is reduced rapidly—more rapidly than FDG but less rapidly and less severely than DNA–RNA tracers such as [^{18}F]fluoro-deoxyuridine (*26*). Autoradiography confirmed MET uptake predominantly in viable tumor cells, with low uptake in macrophages and other cells. In agreement, Minn et al. (*27*) found that MET uptake correlated better than FDG with tumor proliferative activity in squamous cell head and neck cancer cell lines. In contrast, two other studies found increased MET uptake after irradiation and chemotherapy in ovarian carcinoma cells and colon carcinoma cells (*28*,*29*).

TYR

In the search for a radiolabeled amino acid to quantify protein synthesis, L-[1-^{11}C]-tyrosine was studied (*30*–*32*). Although radiosynthesis of this amino acid is difficult, a reliable automated synthesis system was developed and a metabolic model to quantify the protein synthesis rate was described and validated. TYR is largely incorporated in protein and generates only a small amount of labeled tissue metabolites on the time scale of ^{11}C PET studies (*32*). On the other hand, plasma metabolites (labeled proteins, labeled CO_2, and acid-soluble metabolites such as ^{11}C-L-dihydroxyphenylalanine) rise to 50% approximately 1 h after injection, requiring arterial sampling and metabolite correction for quantitative determinations of the protein synthesis rate.

Preclinical studies validating the application of TYR were published in the early 1990s. In a rat rhabdomyosarcoma model, Daemen et al. (*30*) found good agreement between tumor growth rate and TYR uptake—better than for FDG. Heat-induced inhibition of TYR uptake correlated well with tumor regression (*33*), but irradiation combined with hyperthermia did not reduce uptake (*34*).

IMT

The artificial amino acid IMT has generated much interest after the demonstration that uptake specifically reflects the increased amino acid transport in gliomas (*35*). In addition, the relative ease of preparation of IMT and its applicability for SPECT are of clinical interest. In fact, since the application of [^{75}Se]selenium-methionine in the 1970s and 1980s, IMT has been the first radiolabeled amino acid for tumor imaging to be used for SPECT.

IMT is rapidly taken up in brain tumors. Approximately 15–30 min after injection, uptake peaks. It is not incorporated into protein and slowly washes out of the tumors (~30% at 1 h after injection) (*36*,*37*). Tumor-to-background ratios in brain tumors are generally between 1.5 and 2.5. When patients received an infusion of a mixture of naturally occurring amino acids, absolute IMT uptake decreased by 53% in gliomas and by 45% in normal brain tissue (*35*). This basic study by Langen et al. (*35*) is now frequently cited as proof that IMT, despite an artificial nature that includes a large iodine atom and a methyl group, is still a substrate for the specific amino acid carriers in the blood–brain barrier. IMT is metabolically stable and is subject to only minor deiodination (*36*,*37*).

Although these observations were originally believed to be valid only for brain tumors, Jager et al. (*37*) found similar kinetics in a variety of extracranial tumors, such as breast cancer, lung cancer, soft-tissue sarcoma, and lymphoma. Apparently, IMT transport in tumors is similar to transport through the blood–brain barrier. These findings have widened the scope for clinical studies.

The main amino acid transport system involved in IMT uptake appears to be the L system, as found by three independent studies on IMT kinetics in tumor cell lines for glioma and lung cancer (*38*–*40*). IMT uptake seems to follow the same uptake route as does the native amino acid TYR (*40*). In contradiction to these findings, Deehan et al. (*41*) found IMT and TYR uptake to be governed mainly by blood flow and diffusion in a rat sarcoma model and suggested that tumor growth status is not related to amino acid uptake. Apart from being contradictory to cell line studies, this finding also contrasts with findings in human sarcoma patients, in whom IMT uptake significantly correlated with tumor proliferation indices (Ki-67, mitoses) and was not related to microvessel count (*42*).

Other Amino Acids

The metabolic behavior of IMT and its fluorinated PET variants FMT and FET appears similar (*14*,*43*,*44*). Both FMT and FET accumulate rapidly (within 30 min) in normal brain tissue and in brain tumors and then wash out slowly. Minor FMT uptake was observed in the lipid, RNA–DNA, and protein fractions of mice bearing a human colorectal carcinoma. Uptake of FMT in mouse tumors was higher than uptake of FDG, with tumor-to-muscle ratios of approximately 3. Uptake significantly decreased after administration of large neutral amino acids (*45*). Similar kinetics were found for FET, which can be produced with high radiochemical yields.

The tryptophan metabolite ^{11}C-labeled 5-hydroxytryptophan has been used to study carcinoid tumors (*46*). Uptake in neuroendocrine tumors appears to be irreversible and specific.

Normal Distribution and Acquisition

A detailed description of normal and variant uptake of MET was recently published (*47*). In brief, low-grade uptake is found in the brain, and somewhat higher uptake is found in the salivary glands, lacrimal glands, bone marrow, and occasionally myocardium. Abdominal uptake in the liver and pancreas can frequently be seen, as well as intestinal uptake of varying degree. IMT, because renally ex-

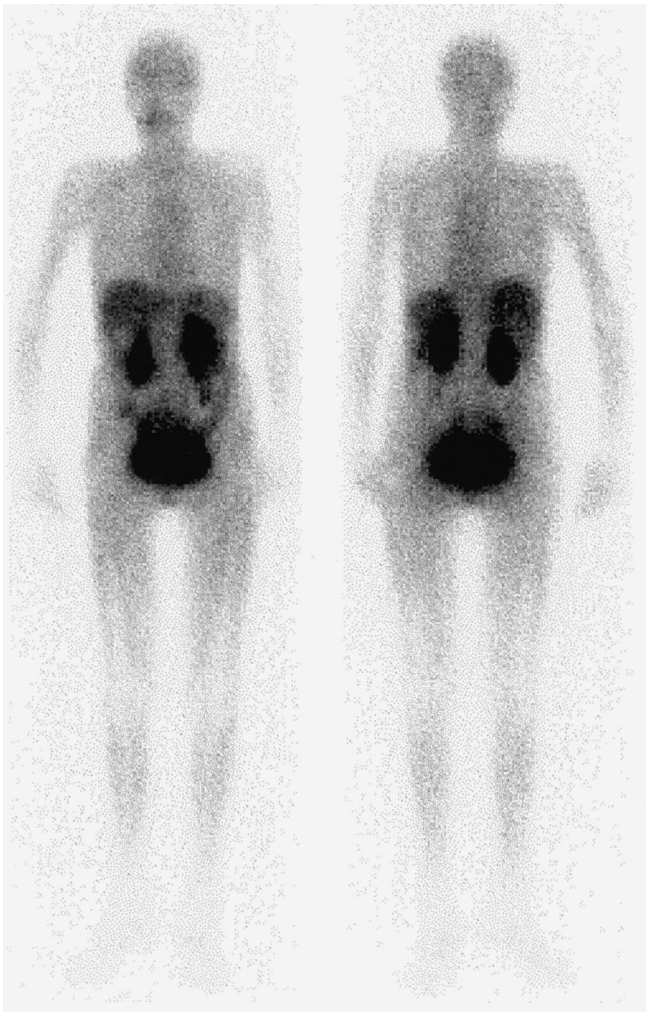

FIGURE 1. Anterior (left) and posterior (right) whole-body IMT scintigrams of healthy volunteer 30 min after injection show low-grade brain, liver, and spleen uptake and intense kidney and urinary system uptake.

creted, shows high uptake in the kidneys and bladder (*37*) (Figs. 1 and 2). Contrasting with IMT, uptake of MET and TYR in the pituitary gland and pancreas is high. MET and TYR show only moderate uptake in the renal cortex.

Images in amino acid studies are usually acquired within the first hour after tracer administration, because uptake and equilibration with tumor washout are usually rapid. For clinical purposes, the assumption of a steady state within the first 45 min is reasonable. Patients are usually studied while they are fasting, because extracellular amino acid concentrations clearly influence transport in vitro (*2,3*). In vivo evidence is limited to a small study by Lindholm et al. (*48*), who found decreased MET uptake after food ingestion in five patients. Because of different influences on various tissues, the impact of the nutrition state is complex, however. Lower uptake both in normal brain and in gliomas will result in unchanged tumor-to-background ratios but may diminish image quality. In other tumor types, unchanged tumor uptake and lower background uptake will cause higher ratios. For these reasons, it is preferable to study patients who are fasting.

Amino Acid Transport or Protein Synthesis Markers?

Radiolabeled amino acids that enter protein synthesis (e.g., TYR and, partly, MET) are believed to better reflect the malignant nature and increased proliferation rate of cancer cells than do amino acids that are only transported into the cell (e.g., IMT, FMT, FET, ^{11}C-aminoisobutyric acid, and, partly, MET). This belief is based on the idea that increased proliferation requires increased protein synthesis. However, as a consequence, amino acid transport is increased in malignancy as well, and this increase may be even more pronounced than the increase in protein synthesis. Although difficult to measure experimentally, the size of the intracellular amino acid pool is probably increased in malignancy. From this pool, amino acids may enter ribosomal protein synthesis, but not all amino acids are shuttled into protein synthesis (*1,11*). The fraction that enters protein synthesis and the fraction used for other purposes are different for different amino acids. The size of the fraction entering protein synthesis may indeed correlate with the proliferation rate, although protein synthesis for cellular maintenance will disturb this correlation. The fraction used for other purposes, however, may also (partly) correlate with proliferation. For example, the activity of the activated methyl cycle may be increased in malignant cells, cells may use amino acid intermediary metabolites for metabolic fuel, methionine is important in the initiation of translation, and some tumors produce secretory products from amino acids (*3,22*). All these nonprotein-synthesis processes contribute to increased transport rather than to increased protein synthesis. Furthermore, imaging shows the sum of both fractions, and the total amino acid signal is therefore also likely to correlate with proliferation.

The importance of transport is further supported by studies by Ishiwata et al. (*49*) and Daemen et al. (*50*), who

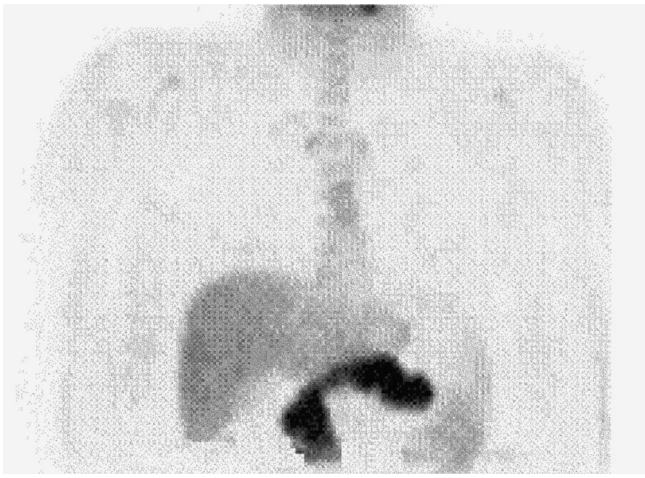

FIGURE 2. Maximum-intensity projection from TYR PET study shows normal distribution in chest and upper abdomen; low uptake in bone marrow, liver, and stomach; and intense uptake in pancreas.

reported significant MET uptake in murine tumors (in the nonprotein fraction) despite inhibition of protein synthesis. These studies support the idea that protein synthesis tracers may also reflect repair mechanisms (maintenance). As empiric proof of all these biochemical mechanisms, in vivo studies using IMT have shown significant correlations between tracer uptake (amino acid transport) and proliferation (*42*,*51*). For these reasons, amino acid transport tracers appear as valuable as protein synthesis tracers in clinical applications.

Specificity of Amino Acids

It is frequently suggested that amino acids are less troubled by interfering uptake in inflammatory tissues than is FDG, making amino acids more tumor specific. This suggestion is based on the fact that inflammatory cells have a low protein metabolism in comparison with glucose metabolism. Indeed, several (mainly in vitro) comparisons with FDG confirm lower amino acid uptake in inflammation (*52*–*54*). However, the list of nontumoral uptake of all radiolabeled amino acids is also long and includes ischemic brain areas, infarction, scar tissue, abscess, sarcoidosis, irradiated areas, hemangioma, and many other non-neoplastic processes (Fig. 3) (*37*,*54*–*57*). Active inflammatory cells also require amino acids, and the increased perfusion of infections may contribute even further to uptake of amino acids. Therefore, the tumor-specific nature of radiolabeled amino acids is probably better than that of FDG but is not absolute.

CLINICAL APPLICATIONS

In analyses of the clinical value of diagnostic methods in general, the levels of diagnostic performance as described by Fryback and Thornbury (*58*) are helpful (Table 1). Analyzing radiolabeled amino acid studies in this way makes clear that most of the studies mentioned above can be assigned to level 1 because they provide preclinical evidence on feasible applications in human cancer. In the following description of clinical applications, we will see that studies on the use of radiolabeled amino acids usually address level 2.

TABLE 1
Levels of Diagnostic Tests

Level	Diagnostic test	Seeks answers to whether the application . . .
1	Feasibility	is feasible
2	Accuracy	is sufficiently sensitive and specific
3	Diagnostic value	performs well in relation to other tests
4	Therapeutic value	results in better treatment
5	Patient and societal value	results in better survival and quality of life, at acceptable cost

Table is slightly adapted from (*58*).

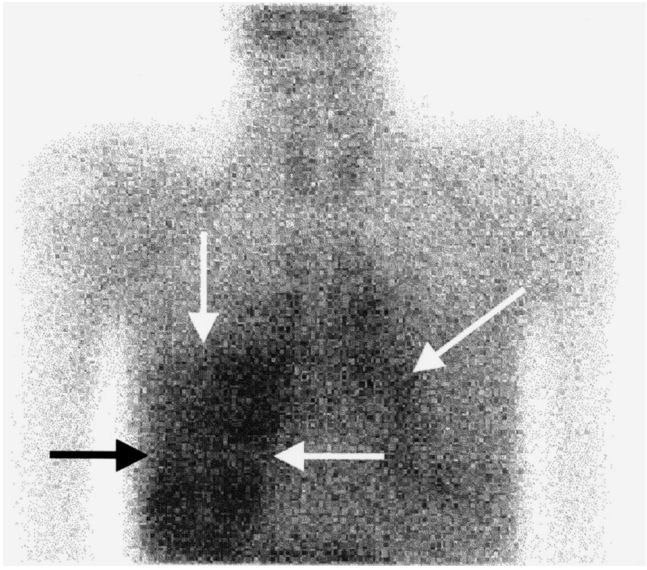

FIGURE 3. Planar IMT image obtained 1 wk after 60 Gy radiotherapy in patient with non–small cell lung carcinoma in right middle lobe shows nonspecific increased uptake in irradiated field (arrows).

Brain Tumors

Most amino acid studies have been performed on brain tumors and have used MET PET. In contrast to FDG, background uptake of amino acids in normal brain tissue is low, providing good contrast with tumor uptake. However, the number of studies using the SPECT tracer IMT has increased rapidly since the first application in 1989 (*59*). ^{11}C-labeled tyrosine and 2-[^{18}F]fluorotyrosine have been applied to a lesser extent (*60*). In brain tumor management, nuclear medicine techniques may supplement such excellent anatomic imaging modalities as CT and MRI. For example, information on tumor grade, optimal biopsy locations, the degree of intracerebral infiltration, and recurrence provided by PET or SPECT is likely to be clinically helpful (*61*).

Detection. Generally, high sensitivities are reported. For example, Ogawa et al. (*62*) found an excellent 97% sensitivity for MET PET in 32 patients with high-grade tumors but only 61% in low-grade tumors. Mosskin et al. (*63*) found a patient-based sensitivity of 84% ($n = 38$) in a study using stereotactic biopsies from tumor and normal areas. In addition, biopsies showed MET uptake in nontumor tissue in 5 patients, indicating that tumor specificity of MET is not perfect.

Experience with TYR is more limited. Pruim et al. (*56*) used TYR PET for both primary and recurrent brain tumors and found 20 of 22 tumors positive for uptake (sensitivity, 91%) (Fig. 4). Also, metastases and cerebral lymphomas were visualized. Wienhard et al. (*60*) found increased uptake and transport rates of 2-[^{18}F]-fluorotyrosine in brain tumors ($n = 15$). Uptake appeared related more to amino acid transport than to protein synthesis. In addition, the

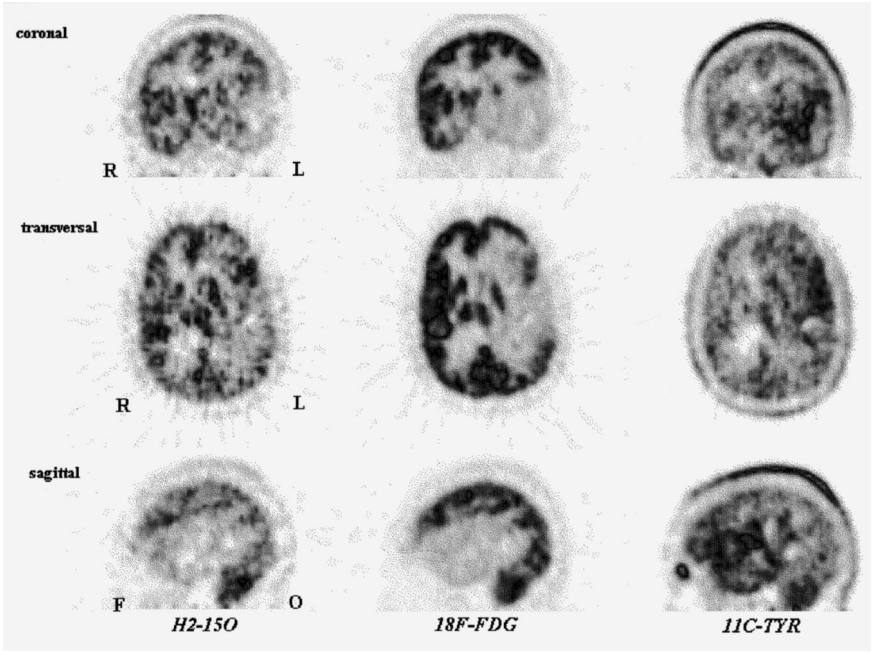

FIGURE 4. Coronal, transverse, and sagittal images obtained using $H_2{}^{15}O$ (perfusion, left column), FDG (middle column), and TYR (right column) in patient with large, low-grade astrocytoma in left temporoparietal region show that tumor is not intensely perfused and glucose metabolism is low. However, large area of irregularly increased amino acid uptake is clearly seen, and amino acid uptake is noted in lacrimal gland.

SPECT tracer IMT is taken up in nearly all brain tumors, both astrocytomas and oligodendrocytomas, but also in lymphomas and metastases, as evidenced by many studies. Reported sensitivities in the detection of malignancy range from 85% to 100% (Table 2).

Tumor Grading. Nearly all studies on tumor detection also addressed the feasibility of tumor characterization and grading, comparing uptake both between benign and malignant processes and between various grades of malignancy. This clinically useful aspect is supported by in vitro studies, in which MET uptake and IMT uptake were shown to correlate with proliferation markers (*51,64*). Somewhat surprising is that this relationship was not confirmed for TYR uptake ($n = 20$) (*65*).

Different MET accumulation in vivo was shown between low-grade astrocytomas and oligodendrogliomas, with uptake in astrocytoma being near background uptake but all oligodendrogliomas showing high uptake (*66*). The authors, Derlon et al. (*66*), suggested that this difference could be clinically useful. Good and possibly clinically useful differentiation (without overlap) between skull base meningiomas and benign neuromas was suggested by Nyberg et al. (*67*) (total $n = 18$). The largest study was performed by Herholz et al. (*68*), who found 79% accuracy in distinguishing glioma from non-neoplastic lesions in 196 patients with a suspected brain tumor.

The role of IMT for tumor grading is controversial. Kuwert et al. (*55*) could differentiate high-grade tumors

TABLE 2
Clinical Studies Using IMT in Brain Tumors

Study	Year	No. of patients	Purpose	Sensitivity (%)	Remarks and findings
Biersack (*59*)	1989	10	Detection	100	First study
Langen (*36*)	1990	32	Detection	88	
Kuwert (*55*)	1995	53	Detection	50–82	Differentiation between high grade, low grade, and benign; specificity 83%–100%
Weber (*75*)	1997	19	Detection	97	IMT uptake ratios superior to those of FDG PET
Langen (*115*)	1997	14	Detection	100	Similar to MET PET
Woesler (*116*)	1997	23	Detection	83	Differentiation between high and low grades; IMT similar to FDG PET
Grosu (*76*)	2000	30	Detection	100	Significant impact on radiotherapy planning
Guth (*84*)	1995	17	Evaluation	82	Recurrence detection
Molenkamp (*117*)	1998	11	Evaluation	100	Detection of progression in low-grade childhood tumors
Kuwert (*118*)	1998	27	Evaluation	78	Recurrence detection; specificity 100%
Bader (*69*)	1999	30	Evaluation	75–100	Detection of recurrence, grades 2–4; superior to FDG PET

from benign lesions with 82% sensitivity and 100% specificity in the largest study reported ($n = 53$). Separating high-grade from low-grade tumors resulted in 71% sensitivity and 87% specificity, whereas differentiation of low-grade tumors from non-neoplastic lesions was much more difficult, with a sensitivity of 50% and a specificity of 100%. Others, however, could not identify such differences and found IMT SPECT not to be suitable for noninvasive tumor grading (*64*,*69*). Minor uptake of IMT was also described for some non-neoplastic lesions such as infarctions and inflammation (*55*). More recently, much higher uptake was described for a desmoplastic ganglioneuroma, another benign process (*70*). Nearly all reports comparing IMT SPECT with FDG PET or with MET PET suggested that IMT SPECT is equally useful for routine clinical purposes (Table 2; Fig. 5) (*64–66*,*71*). However, PET resolutions frequently are converted to SPECT resolutions in such studies, and tumor-to-brain ratios are still higher for MET PET than for IMT SPECT (*35*)

Tumor Delineation. Many studies have shown that the margins of tumors, as assessed by MET or IMT uptake, are frequently wider than the anatomic boundaries, as assessed by MRI or CT (*63*,*67*,*71–74*). This fact is explained by the lack of contrast enhancement in CT and MRI studies in areas within the tumor with an intact blood–brain barrier. This phenomenon may be even more pronounced in low-grade tumors and in diffuse gliomatosis (*73*). Also, in comparison with FDG PET, this better tumor delineation is reported both for MET and for IMT (*74*,*75*). In a recent study using MRI and IMT SPECT fusion images, IMT SPECT led to a significant change in planning of irradiation volumes (*76*). Derived from these good delineation properties, an interesting new application has recently arisen. MET or FDG scanning is combined with activation studies using radiolabeled water ($H_2{}^{15}O$) to depict tumor extension in relation to functional brain areas (*77*). This application may contribute to the planning of surgical margins.

Biopsy Localization. Stereotactic biopsies of localizations based on either MET or FDG PET were shown to be more successful at finding tumor tissue than were biopsy trajectories based on CT only (*78*). Especially strong uptake reduction of MET in necrotic parts or especially high uptake in anaplastic parts may influence the planning and results of brain biopsies. Planning of biopsy trajectories was suggested to improve with use of TYR, particularly in low-grade glioma (*79*).

Evaluation of Therapy. Detection of recurrent or residual viable tumor can be troublesome in brain tumors treated by surgery or irradiation. In vitro evidence is somewhat conflicting, but clear demonstrations that MET PET is suitable for following up the effects of such treatment have been published (*80–82*). For example, Wurker et al. (*80*) showed a dose-dependent reduction in uptake in low-grade gliomas ($n = 10$) up to 1 y after brachytherapy, whereas FDG uptake was unchanged. Sonoda et al. (*83*) found no MET uptake in six of seven cases of radionecrosis that were difficult to assess using MRI or CT. Using IMT, several studies revealed good sensitivity and specificity for the detection of viable tumor tissue in previously treated patients (Table 2). IMT SPECT has been suggested to complement MRI and CT in patients in whom detection of recurrent disease was difficult (*84*). Remarkably, the protein synthesis rate determined using TYR PET was unchanged in 8 of 10 patients after radiotherapy (*85*).

Conclusion. The diagnostic accuracy (level 2, Table 1) of radiolabeled amino acid studies in brain tumors has sufficiently been shown. For detection, radiolabeled amino acids show adequate sensitivity and specificity. For grading, however, the role of these studies is still controversial, because conflicting reports exist. In addition, thresholds frequently are defined retrospectively—a methodologically suboptimal choice—and the true clinical impact is therefore unclear. Reasonable evidence exists that radiolabeled amino acids have supplemental value in the evaluation of treatment and the detection of recurrence.

What about the diagnostic value, or level 3? The evidence seems considerable that radiolabeled amino acids provide better diagnostic information than does FDG. However, nearly all studies have used amino acid imaging in addition to CT or MRI, and the likelihood that these excellent anatomic modalities will be used less or differently seems low. Therefore, PET or SPECT studies will be added to the diagnostic evaluation. Finally, for nearly all these issues, many level 4 and 5 issues have not been proven—for example, the issue of whether these studies can replace, diminish, or change the current practice of biopsy, surgery,

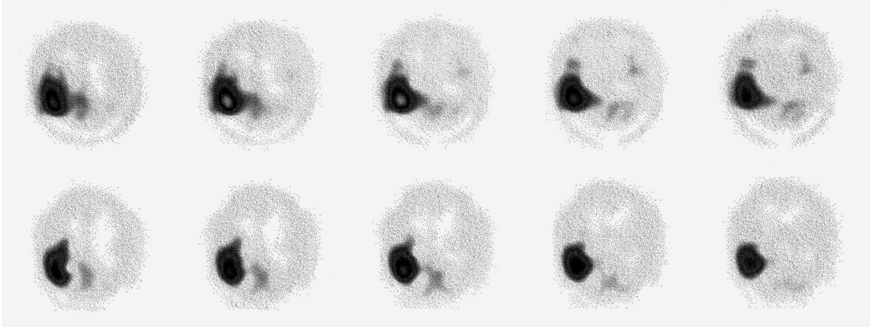

FIGURE 5. IMT SPECT (top row) and MET PET (bottom row) images of brain of patient with glioma show similar uptake and tumor delineation. Resolution of MET PET was converted to SPECT resolution.

TABLE 3
Clinical Studies Using TYR PET

Study	Year	No. of patients	Tumor type	Sensitivity (%)	Remarks and findings
Pruim (*56*)	1995	22	Primary brain	92	Specificity 67%; no correlation with grade
Heesters (*85*)	1998	10	Primary brain	—	PSR within original tumor volume unchanged after radiotherapy
Braams (*89*)	1996	11	Oral cavity	83	In nodal staging, better than MRI or CT; specificity 95%
Kole (*100*)	1997	13	Breast cancer	100	For primary tumor; visually less uptake than for FDG in fibrocystic disease
Ginkel (*107*)	1999	17	Sarcoma	82	Partial vs. complete remission distinguished after chemotherapy; specificity 100%
Plaat (*106*)	1999	21	Sarcoma	—	Correlation of PSR with Ki-67, not with grade
Kole (*57*)	1999	25	Sarcoma	—	FDG better for grading; TYR better for correlation with proliferation
Kole (*111*)	1998	10	Nonseminoma	20	
Kole (*119*)	1997	22	Various types	94	Chondrosarcoma not visualized
Que (*120*)	2000	10	Cervix	80	Interfering bone marrow and intestinal uptake present

PSR = protein synthesis rate.

and chemo- or radiotherapy, and the issue of whether these studies result in better treatment and survival of patients.

Head and Neck Cancer

Management of head and neck cancer, usually with both surgery and radiation therapy, critically depends on accurate assessment of the extent of local invasion and the presence of nodal metastases. Detecting occult metastases in clinically negative patients (N0) is important in selecting patients for neck dissections and radiotherapy. An accurate metabolic method to detect lymph node involvement therefore contributes to nodal staging. Such a method may also detect recurrences despite posttherapy scarring or edema, because these may require additional treatment.

Tumor Detection, Staging, and Grading. The Finnish group from Turku has extensively used MET PET to study patients with head and neck cancer. The group has shown good uptake (using standardized uptake values [SUVs] and transport rate analysis) of MET in these cancers. The largest study ($n = 47$) had a 91% sensitivity for detection of selected primary tumors that were larger than 1 cm (*86*). To our knowledge, no explicit study of tumor staging using MET has been published. No relationship with tumor grade could be assessed (*86–88*). In a small study using TYR PET, Braams et al. (*89*) found an 83% lesion-based sensitivity and a specificity of 95%, similar to FDG PET (Table 3; Fig. 6). Undetected metastases were either small (<5 mm) or in the vicinity of salivary glands with interfering physiologic uptake. The investigators suggested TYR PET to be superior to CT or MRI. A somewhat lower performance for IMT was reported by Flamen et al. (*90*), who found a 91% sensitivity for the primary tumor but only a 56% lesion-based sensitivity for metastases (Table 4).

Evaluation of Treatment. All the published studies have come from Finland. The largest study comprised 15 patients with 24 tumor sites (*88*). Of 9 sites in which uptake after radiotherapy remained high (SUV > 3.1), a complete response was found in none. In contrast, when posttreatment uptake was low (SUV < 3.1), most patients (70%) had a complete response. The pretreatment level of MET uptake was not associated with a histologic response (*91*). In a study published 3 y later, no relationship was found between initial MET uptake and overall survival, but posttreatment uptake appeared to have predictive value (*92*). However, Nuutinen et al. (*92*) found SUV ratios to have no predictive value before and during early treatment, because SUVs decreased by 30% in both relapsing and responding patients.

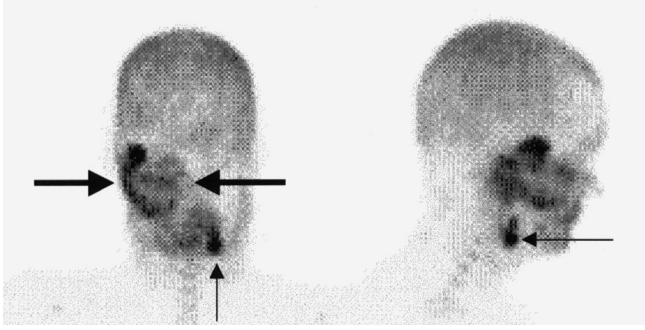

FIGURE 6. Coronal and sagittal projections of TYR PET study of patient with large, recurrent squamous cell carcinoma of right maxillary sinus extending into skull base show irregularly increased TYR uptake in tumor (thick arrows). Because of irradiation, uptake in both parotid glands and right submandibular gland has disappeared, and uptake in left submandibular gland is visible (thin arrows).

TABLE 4
Clinical Studies Using IMT Other Than in Brain Tumors

Study	Year	No. of patients	Tumor type	Sensitivity (%)	Remarks and findings
Flamen (*90*)	1999	11	Head and neck	91	For primary tumors; ~60% for nodal spread
Jager (*37*)	1998	20	Various types	—	Feasible in breast cancer, lung cancer, sarcoma, and lymphoma
Boni (*109*)	1997	7	Melanoma	37	For lesion detection
Jager (*110*)	2000	22	Carcinoid	43–60	Correlation with secretory activity
Jager (*42*)	2000	32	Sarcoma	100	88% specificity for differentiation between benign and malignant; correlation with proliferation
Jager (*96*)	2000	17	Lung cancer	94	For primary tumors; 60% for mediastinal lesions

Conclusion. Although we now know that MET is avidly taken up in head and neck cancer, nodal staging using MET has not been formally studied. Only one small study using TYR has suggested feasibility in nodal staging, and one study using IMT SPECT has suggested lower performance. MET uptake does not have prognostic meaning, and early monitoring of radiotherapy does not appear feasible. However, separation of complete responders from nonresponders may be possible with posttreatment imaging and may have clinical meaning.

For diagnostic performance in nodal staging, the accuracy (level 2) of amino acid PET methods is presumably sufficient, although evidence is limited and uncertainty remains about the detection of small lesions. However, the diagnostic value (level 3) does not seem to be better than for FDG PET (sensitivity, 70%–90%). On the basis of the limited data available, amino acid imaging does not appear clinically helpful.

Lung Cancer

Unfortunately, only a minority of patients with lung cancer can be cured. The most important factors determining survival are tumor resectability and the presence or absence of (mediastinal) metastases. For staging of metastases, CT and mediastinoscopy are currently the most important tools, but because of imperfect detection of mediastinal metastases many patients still undergo fruitless thoracotomies (estimated as high as 30%–50%).

All published studies have reported avid uptake of amino acids in primary lung cancer, both small cell and non–small cell. The intrinsic ability of tumors to concentrate MET, TYR, or IMT does not seem to be different. False-negative results are generally caused by a small tumor size in combination with technical factors such as the resolution of PET and SPECT devices.

Solitary Pulmonary Nodules. Because many patients initially present with a solitary pulmonary lesion, a method that can reliably predict or exclude malignancy would be of clinical benefit. Using MET PET in 24 patients, Kubota et al. (*93*) found a 93% sensitivity for the detection of malignancy at, however, a 60% specificity. Calculated from these data, the negative and positive predictive values are 86% and 76%, respectively. These figures are too low to reduce the need for invasive biopsies or surgery and therefore are unlikely to be clinically helpful, especially because surgery is the only chance of cure in lung cancer. However, a comparison with FDG PET (sensitivity and specificity, ~90%) is lacking, and experience with amino acids is limited. Difficulties in distinguishing malignant from nonneoplastic disease using MET were also reported by Nettelbladt et al. (*94*) using SUV and transport rate analysis ($n = 17$).

Staging. This issue has been addressed by few studies, the largest ($n = 41$) of which was performed by Yasukawa et al. (*95*). They showed that in the detection of mediastinal metastases, MET PET was superior to CT both in sensitivity (86% vs. 53%) and in specificity (91% vs. 84%). However, these figures were based on a retrospective cutoff point of 4.1 for the tumor-to-muscle ratio, showing that considerable MET uptake (tumor-to-muscle ratio, 2.9) was present in nonmetastatic nodes. This study provided no evidence that MET PET would be more clinically helpful than FDG PET. Nettelbladt et al. (*94*) found the correct mediastinal lymph node status using MET and FDG in 4 patients with and 10 patients without lymph node involvement. A SPECT study using IMT ($n = 17$) detected mediastinal metastases in 86% of involved patients but only 60% of all mediastinal metastases (Figs. 7 and 8). Lesions smaller than 1.5 cm were frequently not detected, and aspecific uptake was found in irradiated normal lung tissue (Fig. 3). Therefore, IMT SPECT did not appear clinically helpful (*96*).

Evaluation of Therapy. Despite treatment with radiotherapy, chemotherapy, or combinations of these, patient survival is extremely low. Evaluation of the effect of treatment is currently based on reduction in tumor size, as assessed by chest radiography or CT in addition to clinical parameters. Even when local control is achieved, many patients die of metastatic disease. The a priori relevance of imaging studies aimed at measuring therapy evaluation is therefore low. At the best, a prediction of ineffectiveness early during treatment, or of much viable tumor tissue after treatment, may

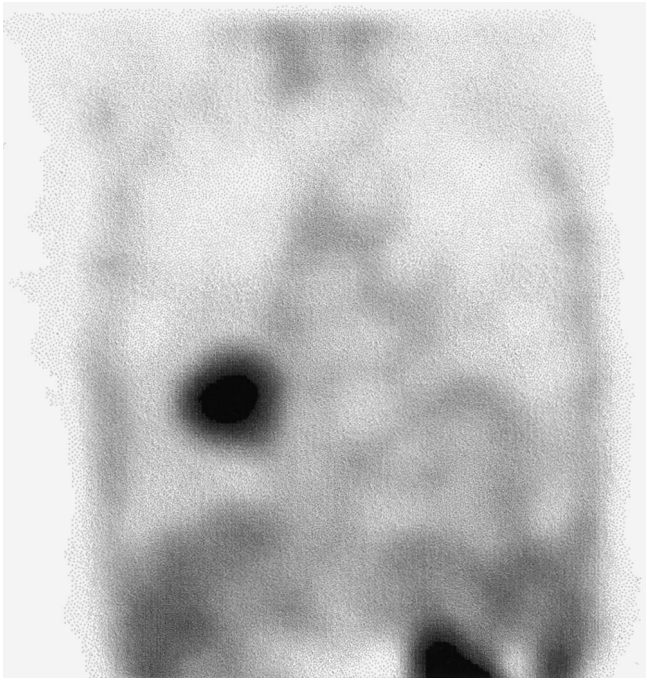

FIGURE 7. Coronal chest IMT SPECT section through 6-cm squamous cell carcinoma in right middle lobe of same patient as in Figure 3 shows high IMT uptake.

influence further treatment options. For example, chemotherapy may be changed or discontinued, or chemotherapy may be added after unsuccessful radiotherapy. However, accurate assessment of prognosis may be of value to individual patients.

As a prerequisite for successful treatment evaluation, Miyazawa showed that uptake of MET in lung cancer cells is representative of tumor growth, based on good correlations between MET uptake and DNA content, S phase, and S + G_2/M phase fractions (n = 24) (*97*). The main clinical description on the use of MET (n = 21) in lung cancer is from Kubota et al. (*98*). When MET uptake was reduced after chemo- or radiotherapy, patients were unlikely to experience local recurrence within 1 y. When MET uptake was not reduced after treatment, early recurrence was likely. However, assessment of tumor volume changes using CT better separated patients with local control from those likely to relapse, and MET PET therefore had no added value.

Conclusion. The paucity of clinical data in lung cancer hardly permits a definitive conclusion. The criteria for diagnostic levels 1 and 2 seem to be fulfilled. However, when diagnostic value (level 3) is analyzed, neither in determining the nature of solitary pulmonary nodules nor in mediastinal staging do the current data show a clinical benefit over FDG PET. The value of radiolabeled amino acids in the evaluation of chemo- or radiotherapy, in itself already of questionable clinical value, has not been proven.

Breast Cancer

Only limited clinical results are available for breast cancer. MET uptake correlated well with the proliferation rate (S phase fraction) in primary and metastatic breast cancer lesions (n = 11), suggesting amino acids to be suitable for treatment evaluation (*99*). Kole et al. (*100*) studied the detection properties of TYR PET in 11 patients with breast cancer and 2 patients with only benign breast tumors. Visual uptake of FDG was better in malignant lesions, although uptake in fibrocystic diseases was less prominent using TYR. In contrast, Jansson et al. (*101*) found MET uptake to provide better tumor contrast, in comparison with FDG. In addition, they found early reductions in MET uptake (within 1–2 wk) after the onset of chemotherapy (n = 11). Similar findings were reported for breast cancer metastases (n = 8) (*102*). Uptake of IMT has been reported in four patients (*37*). These limited data permit only a level-1 or level-2 conclusion that amino acid studies are feasible in breast cancer. Clinical relevance remains to be defined.

Lymphoma

Traditionally, ^{67}Ga scintigraphy has been applied in the evaluation of masses after chemotherapy. Several amino acid studies have shown that MET accumulates strongly in most lymphomas, both of low and of high malignancy grades. In one of these studies (*103*) (n = 14), MET was more sensitive than FDG; in another (*104*) (n = 23), both tracers were similar. Uptake of MET did not appear to be related to grade. This finding contrasted with FDG uptake, which clearly increased in higher grading (*104*). Kinetic analysis of MET data in 32 patients, however, allowed separation of high-grade lymphomas from lymphomas of other grades (*105*), but final outcome was not related to MET uptake. Further research is necessary and appears most challenging in the evaluation of tissue after treatment. Significant competition from FDG PET and ^{67}Ga scintigraphy is to be expected.

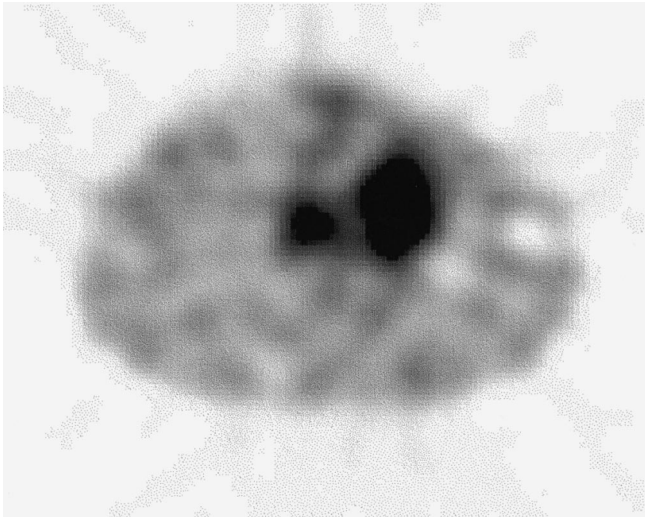

FIGURE 8. Transverse chest IMT SPECT image of patient with large cell carcinoma of left upper lobe and mediastinal metastasis shows avid uptake in both.

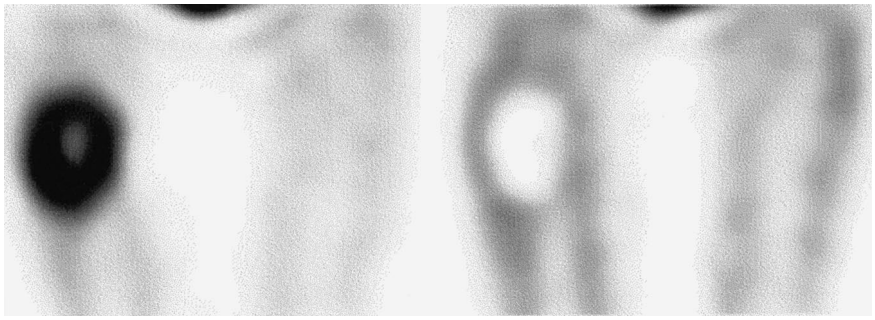

FIGURE 9. Coronal IMT SPECT sections through upper legs of patient with high-grade malignant fibrous histiocytoma, before (left) and after (right) regional hyperthermic cytostatic perfusion of leg, show disappearance of irregular intense IMT uptake after perfusion, in agreement with complete tumor necrosis.

Soft-Tissue Sarcoma

Intensive uptake of both IMT and TYR has been described for soft-tissue sarcoma (Tables 2 and 4) Both IMT and TYR uptake correlated with various histologic parameters of proliferation, such as Ki-67, mitotic index, and silver-staining nucleolar organizer region (*42,57,106*). Using IMT SPECT, benign and malignant tumors could be differentiated with high accuracy and minimal overlap. TYR (and possibly IMT) uptake appeared useful in the evaluation of regional cytostatic perfusion but could not replace histology (Fig. 9) (*107*). In comparison with FDG, uptake appeared to be less influenced by inflammatory tissue. Staging of sarcoma patients using radiolabeled amino acids has not been described. The fact that lymph node staging was especially improved may be of clinical benefit.

Melanoma

MET PET detected all lesions larger than 1.5 cm in 10 patients but missed smaller lesions (*108*). Tyrosine, as a precursor in melanin synthesis, should theoretically be interesting in melanoma detection. Studies using IMT in melanoma were performed in the 1970s. Although several small case descriptions exist on ocular melanoma, a recent study on detection of known melanoma lesions provided disappointing results for IMT total body scintigraphy (*109*). Only large lesions ($>$1.5 cm) were detected using SPECT.

Neuroendocrine Tumors

Because tyrosine is a precursor in catecholamine synthesis, uptake of IMT and TYR might be expected in pheochromocytomas, neuroblastomas, and carcinoid tumors. Jager et al. (*110*) reported uptake of IMT in roughly 50% of carcinoid lesions, and uptake correlated with both serotonin and catecholamine metabolism in these tumors. Because carcinoids have a high amino acid demand, further studies may be successful and contribute to improved staging and treatment evaluation.

Miscellaneous

TYR uptake was detected in only 20% of patients with metastatic nonseminoma (*111*). MET uptake was found in 78% (18/23) of bladder cancers but was not helpful in assessing response to chemotherapy (*112*), although on theoretic grounds the low urinary excretion of MET and TYR should provide adequate images of bladder tumors. For primary tumor detection or evaluation, clinical use is unlikely, because the bladder is easily accessible by other means (such as cystoscopy). MET may possibly play a role in nodal staging but has not been studied yet. MET uptake has been described in 7 of 7 ovarian cancers and in 14 of 14 uterine carcinomas (*113,114*). From our own experience, TYR has not proven valuable in nodal staging of cervix or vulva cancer (*90*). Interfering and unpredictable bowel uptake frequently interfered with visualization of small metastases. No data exist on the use of radiolabeled amino acids in colorectal cancer.

CONCLUSION

The theoretic and preclinical background of amino acid imaging is sound and supports clinical applications. No convincing evidence indicates that, for clinical applications, radiolabeled amino acids that are only transported into the cell are inferior to those that enter protein synthesis; arguments for the opposite also exist. The fact that amino acid imaging is less influenced by inflammation than is FDG PET may be advantageous; however, tumor specificity is not perfect.

In brain tumor imaging, the use of radiolabeled amino acids is established. The use of IMT SPECT appears to be equally as valuable as the use of PET methods. The diagnostic accuracy of amino acid imaging is adequate, and the diagnostic value is probably advantageous. However, the true therapeutic value and the final value in terms of patient outcome remain to be established.

Limited data in head and neck cancer and lung cancer suggest reasonable diagnostic accuracy but inferior diagnostic value in comparison with FDG PET. In most other tumors, the data do not yet permit definitive conclusions, but the general feasibility of amino acid imaging has sufficiently been shown. For nearly all tumor types, more research is required using larger patient series in well-defined clinical settings. In these continuing efforts, newer radiolabeled amino acids such as [^{18}F]fluoroethyltyrosine will also be of interest. In addition, these conclusions are especially based on MET, TYR, and IMT, because these have been most used. Their shared affinity for the L-transport systems may limit generalization to other classes of amino acids.

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