Diagnostic value of ^{99m}Tc-3PRGD$_2$ scintimammography for differentiation of malignant from benign breast lesions: Comparison of visual and semi-quantitative analysis

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Abstract

Objective: To compare the diagnostic value of visual and semi-quantitative analysis of technetium-99m-poly-ethylene glycol, 4-arginine-glycine-aspartic acid (^{99m}Tc-3PRGD$_2$) scintimammography (SMG) for better differentiation of benign from malignant breast masses, and also investigate the incremental role of semi-quantitative index of SMG. **Subjects and Methods:** A total of 72 patients with breast lesions were included in the study. Technetium-99m-3PRGD$_2$ SMG was performed with single photon emission tomography (SPET) at 60min after intravenous injection of 749±86MBq of the radiotracer. Images were evaluated by visual interpretation and semi-quantitative indices of tumor to non-tumor (T/N) ratios, which were compared with pathology results. Receiver operating characteristics (ROC) curve analyses were performed to determine the optimal visual grade, to calculate cut-off values of semi-quantitative indices, and to compare visual and semi-quantitative diagnostic values. **Results:** Among the 72 patients, 89 lesions were confirmed by histopathology after fine needle aspiration biopsy or surgery, 48 malignant and 41 benign lesions. The mean T/N ratio of ^{99m}Tc-3PRGD$_2$ SMG in malignant lesions was significantly higher than that in benign lesions (P<0.05). When grade 2 of the disease was used as cut-off value for the detection of primary breast cancer, the sensitivity, specificity and accuracy were 81.3%, 70.7%, and 76.4%, respectively. When a T/N ratio of 2.01 was used as cut-off value, the sensitivity, specificity and accuracy were 79.2%, 75.6%, and 77.5%, respectively. According to ROC analysis, the area under the curve for semi-quantitative analysis was higher than that for visual analysis, but the statistical difference was not significant (P=0.372). Compared with visual analysis or semi-quantitative analysis alone, the sensitivity, specificity and accuracy of visual analysis combined with semi-quantitative analysis in diagnosing primary breast cancer were higher, being: 87.5%, 82.9%, and 85.4%, respectively. The area under the curve was 0.891. **Conclusion:** Results of the present study suggest that the semi-quantitative and visual analysis statistically showed similar results. The semi-quantitative analysis provided incremental value additive to visual analysis of ^{99m}Tc-3PRGD$_2$ SMG for the detection of breast cancer. It seems from our results that, when the tumor was located in the medial part of the breast, the semi-quantitative analysis gave better diagnostic results.

Introduction

Breast cancer continues to be a major public health problem all over the world. An estimated 232,670 new cases of breast cancer were expected to be diagnosed among women in the US during 2014 and 40,430 deaths were estimated for breast cancer for the same year, which is expected to account for 29% of female cancer prevalence and 15% of all deaths [1]. Therefore, early detection of malignant breast lesions is necessary for proper management and treatment, which in turn results in a better and more favorable patients outcome [2, 3].

Mammography (MG) is a commonly used screening method to early detect breast cancer [4, 5], although its sensitivity is low in cases of dense breasts, Furthermore, approximately 10%-15% of palpable breast cancers are not visible on MG [6-8]. So, despite of its high sensitivity, it has a low specificity. Consequently, it would result in many unnecessary biopsies [6]. To overcome the above and in order to strengthen the diagnostic capacity of MG, other noninvasive imaging procedures such as ultrasonography, magnetic resonance imaging (MRI), scintimammography (SMG), and positron emission mammography can be used. In SMG, various radiopharmaceuticals have been used in the detection of breast cancer such as (explain acronym) thallium-201-chloride (^{201}TlCl$_2$), ^{99m}Tc-methoxyisobutylisonitrile (^{99m}Tc-MBI), ^{99m}Tc-tetrofosmin, and ^{99m}Tc-bombesin [9-14]. Among these radiopharmaceuticals, ^{99m}Tc-MBI has been very suc-

cessful. This tracer was originally dedicated for cardiac imaging and not specifically designed for tumor imaging [15, 16]. The exact mechanism by which it can detect malignancy is still largely unknown, which limits the evaluation of the biologic status of the tumor.

Integrins αvβ3, are an important member of the transmembrane receptor family composed of noncovalently associated αv and β3 subunits. The extracellular region of α subunit can specifically recognize arginine-glycine-aspartic acid (RGD) in order to mediate the adhesion of integrin and extracellular matrix [17, 18]. Recently, a series of RGD peptide-based multimodality probes were developed for noninvasive imaging of integrin αVβ3 expression and several radioactive probes have been used in clinical investigations [19-22]. ^{99m}Tc-3(poly-(ethylene glycol),(PEG)$_4$-arginine-glycine-aspartic acid (^{99m}Tc-3PRGD$_2$) is a newly developed RGD-based radiotracer targeting the integrin αvβ3 receptor, which is an essential process for tumor growth and sprea-ding [23]. It has already been demonstrated that ^{99m}Tc-3PR-GD$_2$ allows specific imaging of αvβ3 expression and that the uptake of ^{99m}Tc-3PRGD$_2$ correlates with αvβ3 expression in tumor xenografts [24]. Biodistribution of ^{99m}Tc-3PRGD$_2$ in normal subjects showed low activities in the intestine, spleen, lungs and heart. Therefore, it could provide better quality of images [25]. Previously, our group applied ^{99m}Tc-3PRGD$_2$ SMG for noninvasive differentiation of breast cancer and compared the diagnostic value of this novel tracer with ^{99m}Tc-MBI by receiver operating characteristic (ROC) curve analysis. The tracer demonstrated an impressive image quality with high sensitivity in detecting breast cancer and showed higher tumor to non-tumor (T/N) ratios than ^{99m}Tc-MIBI [26, 27]. However, there has been no investigation regarding the comparison of visual and semi-quantitative analyses of ^{99m}Tc-3PRGD$_2$ SMG for the detection of primary breast cancer. In this study, we compared the visual and semi-quantitative indices for the detection of breast cancer and also investigated the incremental role of semi-quantitative index of ^{99m}Tc-3PRGD$_2$ SMG additive to visual analysis by ROC analysis.

Subjects and Methods

Subjects

Between October 2012 and August 2013, seventy-two consecutive female patients (22-74 years old, mean age: 47.6±11.4 years) with a suspected breast lesion on physical examination and/or suspicious mammographic findings that required fine needle aspiration biopsy (FNAB) were enrolled. Diagnosis was made by FNAB or by surgery. The ^{99m}Tc-3PR-GD$_2$ SMG findings were compared with the final histopathology diagnosis.

Inclusion criteria for entry into the study were: sex, not pregnant, suspicious breast lesion, recommendation for biopsy after MG, and informed consent of the patients. Exclusion criteria were: recurrent lesions, previous mastectomy, a medically unstable condition due to severe arrhy-thmias, heart failure or recent surgery, FNAB within 1 week prior to SMG, and pregnancy.

This study was approved by the Ethics Committee of China-Japan Union Hospital of Jilin University.

^{99m}Tc-3PRGD2 SMG
Radiolabeling and quality control procedures for 3P4-RGD$_2$ were performed as described previously [28]. 3P4-RGD$_2$ was radiolabelled with 749±86MBq ^{99m}Tc and thereafter administered via a single intravenous bolus injection in the contralateral arm to the affected breast, followed by a 10mL saline flush. The effective radiation dose to the body of ^{99m}Tc-3PRGD$_2$ SMG was 2.93±0.28mSv [29], SMG was performed at 60min after intravenous injection. Patients were in the supine position with raised arms during imaging. Single-photon emission tomography (SPET) was performed using a double-head γ camera (Precedence, Philips Healthcare, Eindhoven, The Netherlands), equipped with low-energy parallel hole collimators. The matrix was 128×128 pixels, and the photopeak was centered at 140keV with a symmetrical 20% window. Imaging was performed using 6° angular steps in a 20s time frame. Distance between breast and detector was minimized.

Visual analysis of ^{99m}Tc-3PRGD$_2$ SMG.
Two-experienced nuclear physicians who were not familiar with patients' clinical information like as history, physical examinations, or with the radiology findings interpreted the ^{99m}Tc-3PRGD$_2$ SMG individually on a workstation connected to a gamma camera with adjustments for contrast. The qualitative interpretation grades were as follows: Grade 1: no abnormal increased uptake. Grade 2: mildly increased uptake. Grade 3: definite focal increased uptake. Homogeneous uptake in both breasts was classified as grade 1. The ^{99m}Tc-3PRGD$_2$ SMG was considered positive for malignancy if the visual score was ≥2. Disagreements between reviewers were resolved by consensus, with a third experienced reviewer as referee.

Calculation of quantitative indices.
For quantitative analysis, regions of interest (ROI) were drawn around the tumor and around an area of normal breast tissue in the same breast on the lateral images and these ROI were used to determine the T/N ratios of ^{99m}Tc-3PRGD$_2$.

Calculation of quantitative indices.
All numerical results were expressed as mean ± SD. To compare these results, Student's t-test was used. The diagnostic accuracy of the semiquantitative analyses was evaluated using ROC curve analysis and the area under the curve. The SPSS 19 software (SPSS Inc., Malvern, Pennsylvania, USA) was used to determine optimal visual interpretation grade and cut-off values of quantitative indices for the detection of primary breast cancer with a statistical significance of observed differences at a P value less than 0.05.

Results

^{99m}Tc-3PRGD2 SMG and histopathology results

The imaging analysis results of ^{99m}Tc-3PRGD$_2$ SMG correlation with histopathology are detailed in Table 1. 42 of all 72 patients (42/72, 58.3%) were finally diagnosed as malignant breast lesions, and the rest 30 cases (30/72, 41.7%) were diagnosed as benign. Among the malignant breast lesions group, 42 patients (mean±SD: 58.6±12.4 years, range 22-70 years) had 48 malignant lesions with diameters ranging from 0.3cm to 7.5cm (mean±SD: 2.65±1.50cm). Invasive ductal carcinoma (IDC) was the most common (38/48 cases). There were also 6 ductal carcinomas in situ (DCIS), one invasive tubular carcinoma (ILC), one invasive cribriform carcinoma, one invasive papillary carcinoma and 1 medullary carcinoma. For the benign group, the mean age of 30 patients was 54.3±11.8 years (range 28-74 years). The histology confirmed that these 30 cases were with the 41 benign lesions ranging in diameter from 0.7cm to 8.1cm (mean±SD: 2.54±1.49cm). The histological types of benign breast lesions included 23 cysts, 13 fibroadenoma, 3 adenosis, 1 abscess and 1 lipoma (Table 1). Examples of positive and negative ^{99m}Tc-3PRGD$_2$ SMG are shown at Figure 1.

Comparison of ^{99m}Tc-3PRGD$_2$ SMG T/N ratio between malignant and benign lesions

Figure 2 showed the results of semi-quantitative indices of ^{99m}Tc-3PRGD$_2$ SMG between malignant and benign breast lesions. The T/N ratio between malignant normal breast lesions was significantly higher than that of benign to normal ratio (3.15±1.18 vs. 2.09±0.83; $P<0.05$).
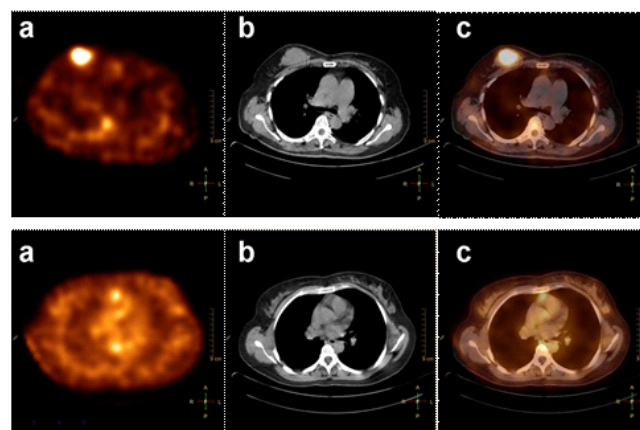

Figure 1. Examples of positive (up) and negative (down) ^{99m}Tc-3PRGD$_2$ SMG. (a) SPET, (b) CT and (c) fusion, images.

Visual and semi-quantitative analysis of ^{99m}Tc-3PRGD$_2$ SMG

The ROC analyses were performed to determine the optimal visual grade and cut-off values of T/N ratio for the detection of malignant breast lesions. When grade 2 and grade 3 were considered as malignant in the detection of primary breast ca-

Table 1. Scintimammography: results of imaging with ^{99m}Tc-3PRGD$_2$

Histology	No	Diameter(cm)	Visual	Semi-quantitative	Visual+semi-quantitative
Invasive ductal carcinoma	38	0.3-7.5	32/38TP 6/38FN	32/38TP 6/38FN	35/38TP 3/38FN
Invasive tubular carcinoma	1	1.2	1/1TP	1/1TP	1/1TP
Ductal carcinoma in situ	6	0.8-6.7	5/6TP 1/6FN	4/6TP 2/6FN	5/6TP 1/6FN
Invasive cribriform carcinoma	1	2.7	1/1FN	1/1FN	1/1FN
Invasive papillary carcinoma	1	2.7	1/1TP	1/1TP	1/1TP
Medullary carcinoma	1	1.9	1/1FN	1/1FN	1/1FN
Abscess	1	3.4	1/1TN	1/1TN	1/1TN
Adenosis	3	2-3.4	2/3TN 1/3FP	3/3TN	3/3TN
Cyst	23	0.7-5.0	17/23TN 6/23FP	17/23 TN 6/23 FP	19/23TN 4/23FP
Fibroadenoma	13	0.7-8.1	8/13 TN 5/13FP	9/13 TN 4/13FP	10/13 TN 3/13FP
Lipoma	1	2.2	1/1TN	1/1TN	1/1TN

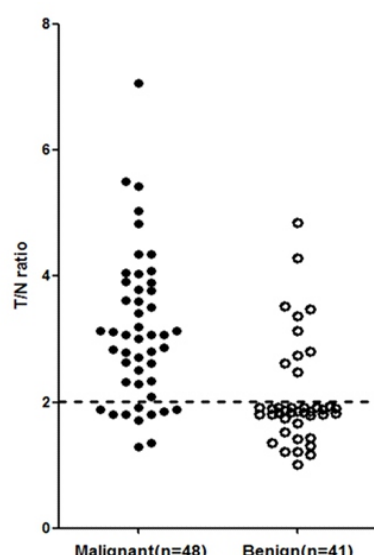TP: true positive; TN: true negative; FN: false negative; FP: false positive.

Figure 2. Shows the T/N ratio for ^{99m}Tc-3PRGD$_2$ in malignant and benign tumors. The T/N ratio between malignant normal breast lesions was significantly higher than that of benign to normal ratio (3.15±1.18 vs. 2.09±0.83; P<0.05).

ncer, the sensitivity, specificity and accuracy were 81.3%, 70.7% and 76.4%, respectively. The area under curve was 0.773 (95% confidence interval: 0.672-0.874). ROC analyses were performed to determine the optimal cut-off values of T/N ratios for the detection of malignant breast lesions. The optimal T/N ratio was 2.01. When T/N ratio 2.01 was used as cut-off point, the sensitivity, specificity and accuracy of ^{99m}Tc-3PRGD$_2$-SMM were 79.2%, 75.6%, and 77.5% respectively. The area under the curve (AUC) was 0.779 (95% confidence interval: 0.681-0.878) in semi-quantitative analysis.

Comparison of visual and semi-quantitative analysis for the detection of breast cancer

Figure 3 demonstrated the comparison of the diagnostic performances of visual and semi-quantitative analyses for the detection of malignancy. There were no statistical differences between visual assessment and quantitative analysis (P>0.05).
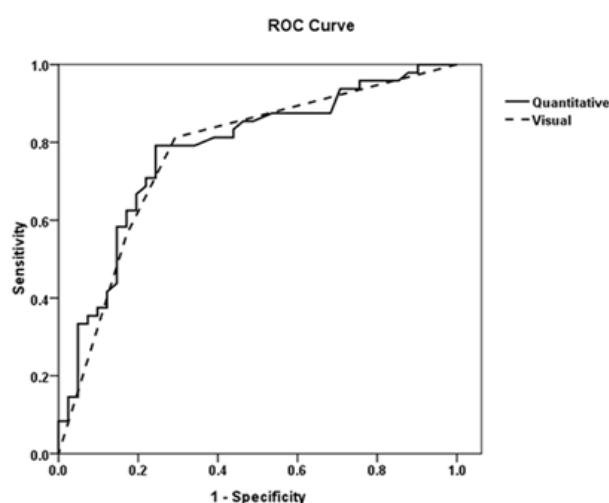

Figure 3. Comparison between visual and semi-quantitative analyses in the differential diagnosis of breast cancer and benign lesions using ROC analysis.

Incremental value of T/N ratio

The T/N ratio was added to visual grade for analysis of the incremental value for the detection of breast cancer. By adding the T/N ratio, one adenosis, 2 cysts and 2 fibroadenoma were accurately diagnosed. Furthermore, semi-quantitative analysis correctly diagnosed 3 IDC, which located in the medial part of the breast. Thus, when visual analysis was added to quantitative analysis, it showed 87.5% sensitivity, 82.9% specificity and 85.4% accuracy. The AUC (0.891, 95% confidence interval: 0.820-0.962) was significantly higher than those of visual analysis or semi-quantitative analysis, alone (P< 0.05).

Discussion

The ^{99m}Tc-3PRGD$_2$ is a new SPET radiopharmaceutical targeting integrin αvβ3 receptor for detecting tumors. In our previous studies, we have demonstrated that ^{99m}Tc-3PRGD$_2$ SMG was accurate in detecting breast cancer, of which sensitivity and specificity were 83%-89.3% and 73%-90.9%, respectively [26, 27]. However, no other study had investigated the comparison of semi-quantitative and visual analyses of ^{99m}Tc-3PRGD$_2$ SMG for the detection of primary breast cancer and evaluated the incremental value of semi-quantitative analysis.

The major finding of this study was that the optimal visual interpretation grade for the detection of primary breast cancer of ^{99m}Tc-3PRGD$_2$ SMG was grade 2. Furthermore the optimal cut-off value for malignant breast lesions was 2.01. The semi-quantitative analyses had the incremental diagnostic value when added to visual analyses.

The prevalence of breast cancer in our study group was 58.3%, which was higher than the study of Liu L. et al (2014). (41.8%) [26]. However, our sensitivity and specificity of visual analysis were not as good as in their group with low prevalence of breast cancer, because, in our study, there were 9 false negative cases in visual grade 1, and 12 false positive cases in grades 2 and 3. However, the low sensitivity, specificity and accuracy of visual analysis of ^{99m}Tc-3PRGD$_2$ SMG could be improved by adding semi-quantitative analysis. In our study, the semi-quantitative analysis identified exactly 3 true positives in visual grade1. Also, semi-quantitative analysis could reduce by 4 the false positive cases in visual grades 2 and 3. By adding semi-quantitative analysis to visual analysis, the sensitivity, specificity and accuracy of ^{99m}Tc-3PRGD$_2$ SMG improved to 87.5%, 82.9% and 85.4%, respectively, which is better than Liu's L. et al (2004) results. By adding semi-quantitative analysis to visual analysis, the AUC was also increased to 0.891, which is larger than the visual analysis alone or quantitative analysis alone. This difference was statistically significant [25]. Thus, visual and quantitative analysis of ^{99m}Tc-3PRGD$_2$ SMG should be combined to better diagnose breast lesions.

If the tumor was located in the medial part of the breast, which is always further away from the detector, the visual ana-

lysis alone could lead to a false negative result. In this case, the semi-quantitative analysis should be obtained for differentiated diagnosis of the breast mass. In our study, 3 patients with IDC located in the medial part of the breast were negative in by visual analysis. Finally, semi-quantitative analysis diagnosed them correctly. These findings suggest that the semi-quantitative analysis of ^{99m}Tc-3PRGD$_2$ SMG could reduce the number of unnecessary invasive breast biopsies if the quantified index is calculated.

In this study, tumor T/N ratios were different from our previous research, [27] which could be due to the fact that the cases enrolled in our study were different than before.

There are several limitations to this study that call for further investigation-research. First, the number of patients studied in this study was relatively small, in order to obtain objective cut-off values of T/N ratio. Second, our present study cohort included a relatively high proportion of malignant breast lesions (58.3%).

To our knowledge, this is the first study for the evaluation by visual and semi-quantitative analysis of ^{99m}Tc-3PRGD$_2$ SMG for the detection of malignant breast lesions. The optimum role of ^{99m}Tc-3PRGD$_2$ SMG in differentiating malignant from benign breast lesions has still not clearly defined. Despite some limitations, this study suggests the combined diagnostic role of visual and semi-quantitative analyses by ^{99m}Tc-3PRGD$_2$ SMG for differentiating malignant breast lessions, in agreement with previous studies [26, 27].

The optimal visual grade for diagnosis of breast cancer by ^{99m}Tc-3PRGD$_2$ SMG was grade 2. The optimal cut-off value for the detection of breast cancer was 2.01 for T/N ratio.

In conclusion, semi-quantitative and visual analysis statistically showed similar results. The semi-quantitative analysis provided incremental value additive to visual analysis of ^{99m}Tc-3PRGD$_2$ SMG for the detection of breast cancer. It seems from our results that, when the tumor was located in the medial part of the breast, the semi-quantitative analysis gave better diagnostic results.

Bibliography

1. Siegel R, Ma J, Zou Z et al. Cancer statistics, 2014. *CA: A Cancer for Clinicians* 2014; 64(1): 9-29.
2. National Institutes of Health Consensus Development Panel: National Institutes of Health Consensus Development Conference statement: adjuvant therapy for breast cancer, November 1-3, 2000. *J Nat Cancer Inst* 2001; 93(13): 979-89.
3. Buscombe J R, Cwikla J B, Holloway B et al. Prediction of the usefulness of combined mammography and scintimammography in suspected primary breast cancer using ROC curves. *J Nucl Med 2001*; 42(1): 3-8.
4. Gang M, Kim JI, Oh KO et al. Factors associated with mammography adherence among married Chinese women in Yanbian, China. *Asian Pacific J of Cancer Prevention* 2013; 14(12): 7207-13.
5. White A, Miller J, Royalty J et al. Clinical outcomes of mammography in the National Breast and Cervical Cancer Early Detection Program, 2009-2012. *Cancer Causes Control* 2015; 26: 723-32.
6. Kopans D B. The positive predictive value of mammography. *Americ J Roentgenol* 1992; 158(3): 521-6.
7. Orel S G, Kay N, Reynolds C et al. BI-RADS categorization as a predictor of malignancy 1. *Radiology* 1999; 211(2): 845-50.
8. Murphy I G, Dillon M F, Doherty A O et al. Analysis of patients with false negative mammography and symptomatic breast carcinoma *J Surg Oncol* 2007; 96(6): 457-63.
9. Lyra M, Vamvakas I. Dosimetry in scintimammography by ^{99m}Tc-MIBI, ^{99m}Tc-Tetrofosmin, ^{99m}Tc-(V)DMSA and ^{201}TlCl compared with X-rays mammography. *Hell J Nucl Med* 2009; 12(2): 184-8.
10. Berghammer P, Sinzinger H. The efficacy of ^{99m}Tc-MIBI scintimammography in the evaluation of breast lesions and axillary involvement. *Hell J Nucl Med* 2011; 14(1): 83-4.
11. Bombardieri E: Axillary lymph node metastases detection with nuclear medicine approaches in patients with newly diagnosed breast cancer. *Intern J Oncol* 1996; 8(4): 693-9.
12. Shariati F, Aryana K, Fattahi A et al. Diagnostic value of ^{99m}Tc-bombesin scintigraphy for differentiation of malignant from benign breast lesions. *Nucl Med Commun* 2014; 35(6): 620-5.
13. Piccolo S, Lastoria S, Muto P et al. Scintimammography with ^{99m}Tc-MDP in the detection of primary breast cancer.[J]. *Quart J Nucl Med (official publication of the Italian Association of Nuclear Medicine (AIMN) [and] the International Association of Radiopharmacology (IAR))* 1997; 41(3): 225-30.
14. Silov G, Erdogan Z, Ozdal A, et al. The value of ^{99m}Tc-tetrofosmin scintimammography in the assessment of P-glycoprotein in patients with breast cancer. *Hell J Nucl Med* 2013; 16(3): 218-22.
15. Larock M P, Cantineau R, Legrand V et al. ^{99m}Tc-MIBI (RP-30) to define the extent of myocardial ischemia and evaluate ventricular function. *Europ J of Nucl Med* 1990; 16(4-6): 223-30.
16. Kumita S I, Nishimura T, Uehara T et al. Evaluation of left ventricular function using gated planar myocardial imaging with ^{99m}Tc-MIBI. Kaku igaku. *The Japanese J Nucl Med* 1991; 28(28): 591-7.
17. Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. *Science* 1987; 238(4826): 491-7.
18. M D, Pierschbacher, Ruoslahti E. Variants of the cell recognition site of fibronectin that retain attachment-promoting activity. *Proc. Nat. Acad. of Sciences of U. S. A.* 1984; 81(19): 5985-8.
19. Haubner R H, Wester H J, Weber W A et al. Radiotracer-based strate strategies to image angiogenesis. *The Quarterly journal of Nucl Med : official publication of the Italian Association of Nuclear Medicine (AIMN) [and] the International Association of Radiopharmacology (IAR)* 2003; 47(3): 189-99.
20. Haubner R, Wester H J, Weber W A et al. Noninvasive Imaging of αvβ3 Integrin Expression Using ^{18}F-labeled RGD-containing Glycopeptide and Positron Emission Tomography. *Cancer Res*. 2001; 61(5): 1781-5.
21. Mcparland B J, Miller M P, Spinks T J et al. The Biodistribution and Radiation Dosimetry of the Arg-Gly-Asp Peptide F-18-AH111585 in Healthy Volunteers. *Nucl Med* 2008; 49(10): 1664-7.
22. Tore B G, Rimma D, Ariel S et al. Integrin receptor imaging of breast cancer: a proof-of-concept study to evaluate ^{99m}Tc-NC100692. *Journal of Nucl Med* 2006; 47(9): 1434-9
23. Zhu Z, Miao W, Li Q et al. ^{99m}Tc-3PRGD$_2$ for integrin receptor imaging of lung cancer: a multicenter study. *J Nucl Med* 2012; 53(5): 716-22.
24. Liu Z, Jia B, Shi J et al. Tumor uptake of the RGD dimeric probe ^{99m}Tc-G3-2P4-RGD$_2$ is correlated with integrin αvβ3 expressed on both tumor cells and neovasculature. *Bioconjugate Chemistry* 2010; 21 (21): 548-55.
25. Jia B, Liu Z, Zhu Z et al. Blood clearance kinetics, biodistribution, and

radiation dosimetry of a kit-formulated integrin αvβ3-selective radiotracer [99m]Tc-3PRGD$_2$ in non-human primates. *Molecul Imag. and Biol.* 2011; 13(4): 730-6.

26. Liu L, Song Y, Gao S et al. [99m]Tc-3PRGD$_2$ Scintimammography in Palpable and Nonpalpable Breast Lesions. Molecular Imaging 2014; 13. 1535-3508.

27. Ma Q, Chen B, Gao S et al. [99m]Tc-3P4-RGD$_2$ Scintimammography in the Assessment of Breast Lesions: Comparative Study with [99m]Tc-

MIBI. *PloS one* 2014; 9(9): e108349.

28. Ma Q, Ji B, Jia B et al. Differential diagnosis of solitary pulmonary nodules using [99m]Tc-3P4-RGD$_2$ scintigraphy. *Europ J Nucl Med and Molec Imag* 2011; 38(12): 2145-52.

29. Guanghui C, Shi G, Tiefeng J et al. Pharmacokinetics and radiation dosimetry of [99m]Tc-3PRGD$_2$ in healthy individuals: A pilot study. *Nucl Science and Techniques* 2012; 23(6): 349-54.
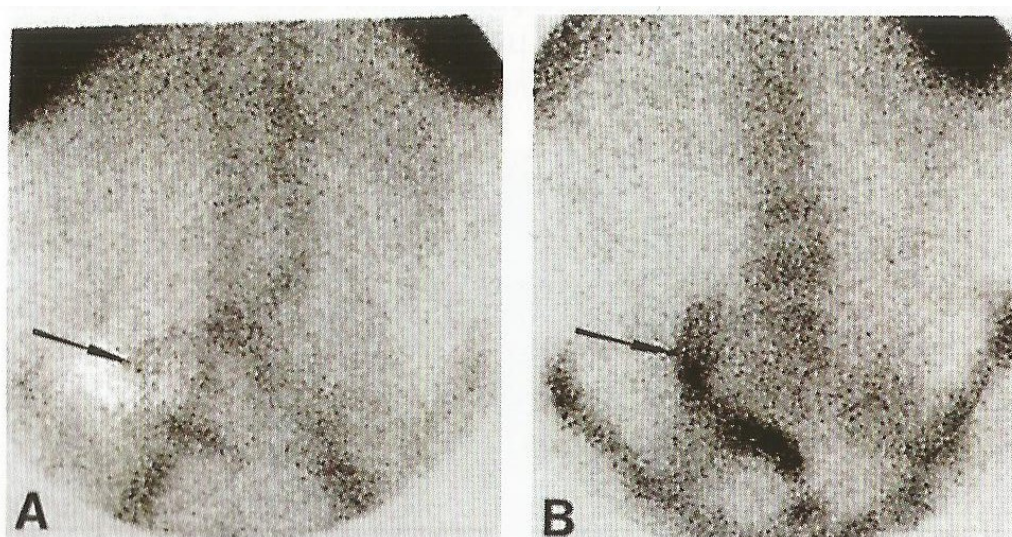
Ulcerative colitis examined with technetium-99m-HMPAO-white blood cells, at two minutes after injection (A). Becomes more positive at 30 minutes (B). From "Nuclear Medicine in 20 specialties" by Philip Grammaticos, 5th edition, p.395.