RM, Larrimer NR. Phase II study of platinum and mitoxantrone in metastatic prostate cancer: a Southwest Oncology Group Study. *Eur J Cancer* 1992, **28A**, 477–478.
7. Moore MR, Troner MB, De Simone P, Birch R, Irwin L. Phase II evaluation of weekly cisplatin in metastatic hormone resistant prostate cancer: a Southeastern Cancer Study Group Trial. *Cancer Treat Rep* 1986, **70**, 541–542.
8. Qazi R, Khandekar J. Phase II study of cisplatin for metastatic prostatic carcinoma. An Eastern Cooperative Oncology Group Study. *Am J Clin Oncol* 1983, **6**, 203–205.
9. Canetta R, Bragman K, Smaldone L, Rozencweig M. Carboplatin: current status and future prospects. *Cancer Treat Rev* 1988, **15**(Suppl.), 17–31.
10. Allen JC, Walker R, Luks E. Carboplatin and recurrent childhood brain tumors. *J Clin Oncol* 1987, **5**, 459–463.
11. Slack NH, Brady MF, Murphy GP. Investigators in the National Prostate Cancer Project: a reexamination of the stable category for evaluating response in patients with advanced prostate cancer. *Cancer* 1984, **54**, 564–574.
12. Miller AB, Hoogstraten B, Stequet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981, **47**, 207–214.
13. Yagoda A. Response in prostate cancer: an enigma. *Sem Urol* 1984, **1**, 311–319.
14. Schmidt JD, Johnson DE, Scott WW, *et al.* Chemotherapy of advanced prostatic cancer. Evaluation of response parameters. *Urology* 1976, **7**, 602–610.
15. Citrin DI, Cohen AF, Harberg J, Schlise S, Hangen C, Benson R. Systemic treatment of advanced prostatic cancer. Development of a new system for defining response. *J Urol* 1981, **125**, 224–227.
16. Stamey TA, Yang N, Hay AR, Mc Neal JE, Freihe FS, Redwine E. Prostate specific antigen as a serum marker for adenocarcinoma of the prostate. *N Engl J Med* 1987, **317**, 909–916.
17. Ercole CJ, Lange PH, Mothisen M, Chion RK, Reddy PK, Vassella RL. Prostatic specific antigen and prostatic acid phosphatase in monitoring and staging of patients with prostatic cancer. *J Urol* 1987, **138**, 1181–1184.
18. Matzkin H, Lewyshon O, Ayalon D, Braf Z. Changes in prostate-specific markers under chronic gonadotropin-releasing hormone analogue treatment of stage D prostate cancer. *Cancer* 1989, **63**, 1287–1291.
19. Hudson MA, Behuson RR, Catalona WS. Clinical use of prostate specific antigen in patients with prostate cancer. *J Urol* 1989, **142**, 1011–1017.
20. Scher HI, Curley T, Geller N, *et al.* Trimetrexate in prostatic cancer: preliminary observations on the use of prostate-specific antigen and acid phosphatase as a marker in measurable hormone-refractory disease. *J Clin Oncol* 1990, **8**, 1830–1838.
21. Hryniuk W, Bush H. The importance of dose intensity in chemotherapy of metastatic breast cancer. *J Clin Oncol* 1984, **2**, 1281–1288.
22. Frei EF, Canellos GP. Dose: a critical factor in cancer chemotherapy. *Am J Med* 1980, **69**, 585–594.

Eur J Cancer, Vol. 29A, No. 15, pp. 2096–2100, 1993.
Printed in Great Britain

0959–8049/93 $6.00 + 0.00

Radioimmunoscintigraphy Using [^{111}In]Antimyosin Fab Fragments for the Diagnosis and Follow-up of Rhabdomyosarcoma

C.A. Hoefnagel, Ö. Kapucu, J. de Kraker, A. van Dongen and P.A. Voûte

Between 1987 and 1992, 39 radioimmunoscintigraphic studies using ^{111}In-labelled antimyosin Fab fragments were performed in 27 patients with rhabdomyosarcoma (RMS), 2 patients with leiomyosarcoma (LMS) and 1 with alveolar soft tissue sarcoma. 21 patients were children aged 3–14 years. These patients, who had histologically proven myosarcoma, were examined scintigraphically to search for local recurrences or metastases and to determine the response to treatment. The results of immunoscintigraphy were compared with histopathological parameters and other imaging modalities. The sensitivity of antimyosin scintigraphy in this series was 82% and the specificity was 73%. Although the technique appears to be not highly specific for RMS, it was found to be useful for the early detection of local recurrence and metastases, as well as for the evaluation of the response to therapy.
Eur J Cancer, Vol. 29A, No. 15, pp. 2096–2100, 1993.

INTRODUCTION

RHABDOMYOSARCOMA (RMS) is a tumour arising from primitive mesenchymal tissue, that mimics normal striated muscle [1]. Pathologically, the tumour is thought to develop from primitive rhabdomyoblasts or pluripotential mesenchymal cells. It is the most common soft tissue sarcoma in children, representing approximately 10% of all pediatric tumours. In children, the primary tumour site is distributed over the head and neck region, the pelvis, the orbit and the limbs. There are two peaks in the incidence, one at the age of 2–6 years and a second at the age of 18 years [2].

By histopathological classification nearly all of these tumours are of the embryonal type. RMS may become markedly enlarged before ever being discovered. Like all soft tissue sarcomas, RMS may infiltrate local structures and may invade both the lymphatics and the blood stream. Metastases to regional lymph nodes are common. Haematogenous metastatic sites are the lungs, bone and bone marrow. Bone scintigraphy is regarded as useful for the detection of RMS skeletal metastases [3]. Wein-

Correspondence to C.A. Hoefnagel at The Netherlands Cancer Institute. The authors are at the Dept. of Nuclear Medicine, The Netherlands Cancer Institute Plesmanlaan 121, 1066 CX Amsterdam and Werkgroep Kindertumoren, Emma Kinderziekenhuis/Academic Medical Center, Amsterdam, The Netherlands.
Revised 1 Mar. 1993; accepted 8 June 1993.

blatt and Miller [4] reported a sensitivity of 76% and a specificity of 85% in their series of 46 cases. Based on studies in only 17 patients, Quddus *et al.* [5], however, concluded that radiography may be a more sensitive indicator of disease. Soft tissue sarcoma may also be imaged with [^{67}Ga]citrate [6] and [^{201}Tl]chloride [7], but these tumour-seeking radiopharmaceuticals are not specific for RMS.

The pathological diagnosis of RMS relies upon the immunohistochemical demonstration of myosin and myoglobin in the tumour tissue. Although immunohistochemistry helps to confirm the diagnosis by identifying cross striations in tumours, which would otherwise by difficult to find, a negative reaction does not exclude it [8].

Recently, antimyosin monoclonal antibodies or fragments labelled with indium-111 (^{111}In) have been introduced in nuclear cardiology, and are successfully used for the detection of myocardial infarction [9, 10], myocarditis [11], heterologous cardiac transplant rejection [12] and anthracycline cardiotoxicity [13]. They have also been used for imaging rhabdomyolysis [14].

Radiolabelled antimyosin monoclonal antibodies could provide a specific tool for the scintigraphic detection of myosin-containing tumours, assuming that there is damage of the tumour cell membrane for the antibody to pass through and bind to intracellular myosin or for the myosin to be exposed. After Hoefnagel *et al.* [15] had demonstrated the feasibility of [^{111}In]antimyosin imaging in children with RMS, there were several preliminary reports of the use of this technique in RMS, leiomyosarcoma (LMS) and soft tissue sarcoma [16–21]. The aim of this study was to evaluate the sensitivity and specificity of antimyosin scintigraphy in children with RMS and to see to what extent the results contributed to the clinical management of this disease.

PATIENTS AND METHODS

Patient population

Between January 1987 and January 1992, 39 antimyosin scans were performed in 30 patients with histologically proven myosarcomas at The Netherlands Cancer Institute and in the Academic Medical Center, both in Amsterdam. 15 patients were female and 15 were male and their mean age was 9.4 years: 21 were children with ages ranging 3–14 years, the others were 15–31 years old.

27 patients were classified as RMS. 22 were of the dense subtype, 2 of the loose (both thyroid) and 2 of the alveolar subtype.

3 other patients, 2 with LMS and 1 with alveolar soft part sarcoma, were also investigated.

Ten studies were performed prior to treatment; 29 studies were in patients who had received therapy, which consisted of surgery in 8, combination chemotherapy in 6, surgery and chemotherapy in 10 and multiple treatment modalities in 5. 5 patients were imaged both before and after therapy.

At the time of [^{111}In]antimyosin scintigraphy 15 patients had a primary tumour only, 5 had a primary tumour with metastases, 1 had a local recurrence, 4 a local recurrence with metastases and 5 patients had metastases only. In 4 patients single photon emission tomography was performed to confirm localisations in the thorax, pelvis and skull.

Imaging technique

The monoclonal antibody R11D10, directed against the heavy chain of cardiac myosin, coupled to diethylenetriamine-pentaacetate (DTPA) to allow labelling with ^{111}In, was used. Fab fragments of this antibody are commercially available as a two vial kit in sodium phosphate buffer together with a solution of sodium citrate (Centocor Europe, Leiden, The Netherlands). After mixing, 70–80 MBq [^{111}In]chloride were added to 0.5 mg of Fab–DTPA and incubated for at least 10 min. The labelling percentage, as tested by paper chromatography, was always >95%. Children received 0.25 mg antimyosin Fab fragments labelled with 37 MBq ^{111}In by slow intravenous injection.

Whole body scintigraphy was performed 24 and 48 h after administration, acquiring multiple planar images using a Siemens dual head gammacamera with medium energy collimators, with 20% windows set around the 171 and 245 keV photoenergy peaks and connected with a MDS A3 computer system. In 4 patients single photon emission tomography was performed using the same equipment, acquiring 60 views at 6° angle increments for 30 s/frame (five studies).

The scintigrams were interpreted by three physicians. The scintigraphic results were given a score on a 0–4 scale: 0 = negative, 1 = faint uptake, 2 = positive but less than liver, 3 = positive and equal to liver, 4 = positive and more than liver. Scintigraphic findings were correlated with available results of conventional radiology, ultrasonography, computed tomography and/or magnetic resonance imaging, as well as with immunohistochemistry.

RESULTS

The imaging results are listed in Table 1, together with the observed tumour localisations. No adverse reactions were observed. Normal tracer accumulation was seen in the kidneys, liver and, to a lesser extent, in bone marrow and the heart. [^{111}In]Antimyosin uptake into normal smooth and skeletal muscle was not apparent. [^{111}In]Antimyosin scintigraphy correctly identified tumour localisations of RMS in 22 of 26 cases, as well as 1 of 2 LMS. Minimal uptake (grade 1) was observed in 10 of these cases, grade 2 uptake in 11, and grade 3 in 2 patients. Very intense tracer accumulation (grade 4) was never observed.

Figures 1 and 2 show grade 2 uptake in primary RMS located in the right thorax aperture and in the left cheek, respectively.

Table 1. [^{111}In]*Antimyosin radioimmunoscintigraphy results in RMS/LMS*

	Positive scintigram	Negative scintigram	Total
Tumour +	23	5	28
Tumour −	3	8	11
Total	26	13	39
Sensitivity: 82%			
Specificity: 73%			
Sites:			
Primary tumour	7/10		
Tumour residue/local recurrence	8/9		
Metastases			
Bone	6/7		
Lymph nodes	7/8		
Bone marrow	0/1		
Lung	3/3		
Soft tissue	1/2		

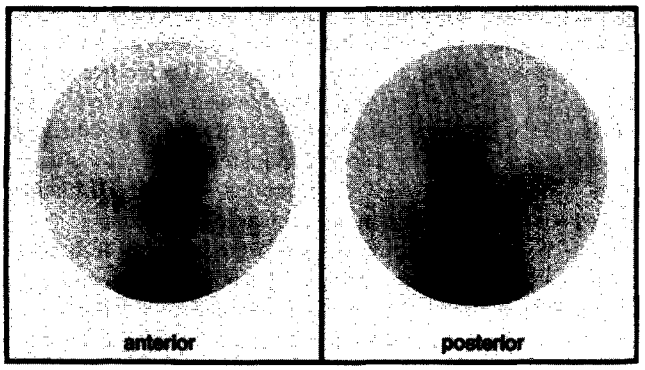


Fig. 1. [^{111}In]Antimyosin scintigram of a 3-year-old boy with a primary RMS in the right thorax aperture (grade 2 uptake).

In cases of documented complete remission (7 RMS and 1 LMS) no pathological accumulations were found.

False negative results occurred in 5 patients. In 2 of these cases this may be attributed to the fact that chemotherapy had been started only days before the scintigram; in 2 other cases a tumour residue measuring only a few millimeters in diameter was missed, one of which was of a different tumour type (alveolar soft part sarcoma). The three false positive findings were due to wound healing after surgery in 2 cases and sinusitis in another patient.

The calculated sensitivity for all studies was 82% and the specificity was 73%. Excluding the 3 cases of tumour other than RMS, these figures hardly changed (85 and 70%, respectively).

Single photon emission tomography allowed a better delineation and localisation of tumours in the head, thorax and pelvis in 4 patients (five studies). This technique was particularly useful to discriminate between tumour and bladder/bowel activity in the pelvis, and to show the site and extension of intracranial lesions (Fig. 3a, b).

Comparing the imaging results with immunohistochemistry in 11 [^{111}In]antimyosin-positive cases, only four tumours stained positively for muscle myosin. A better correlation was found for desmin (nine of 11 positive) and vimentin (six of 11 positive).

In the 5 patients who were scanned both before and after chemotherapy, the scintigram was correctly converted from positive to negative in 3 cases (complete remission) and indicated considerable improvement in 2 patients attaining a partial remission (Fig. 4).

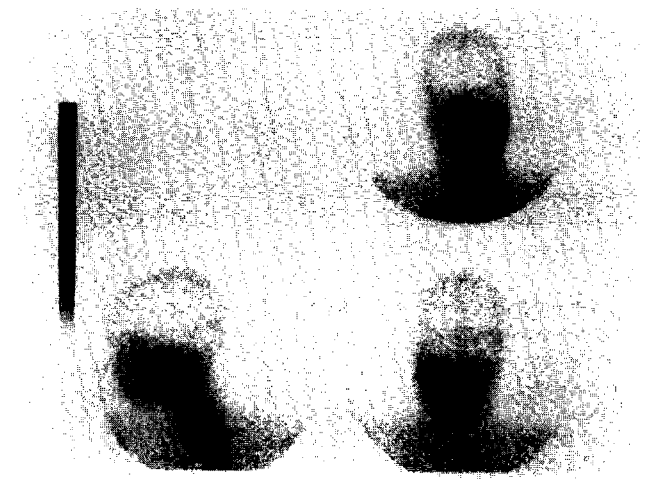


Fig. 2. Anterior, left-lateral and posterior view of [^{111}In]antimyosin scintigrams of a 4-year-old girl, showing a local recurrence of rhabdomyosarcoma in the left cheek (grade 2 uptake).

Although in most cases [^{111}In]antimyosin confirmed RMS localisations which were already documented by other techniques, scintigraphy added new information in 5 patients: two primary tumours of unknown localisation, as well as a clinically unsuspected recurrence were detected and subsequently confirmed by computerised tomography and histology; in 1 case iliac lymph nodes, not shown on computerised tomography were found and confirmed, and in 1 patient in remission of LMS the ultrasonographic finding of compression of the left atrium was substantiated by local [^{111}In]antimyosin accumulation.

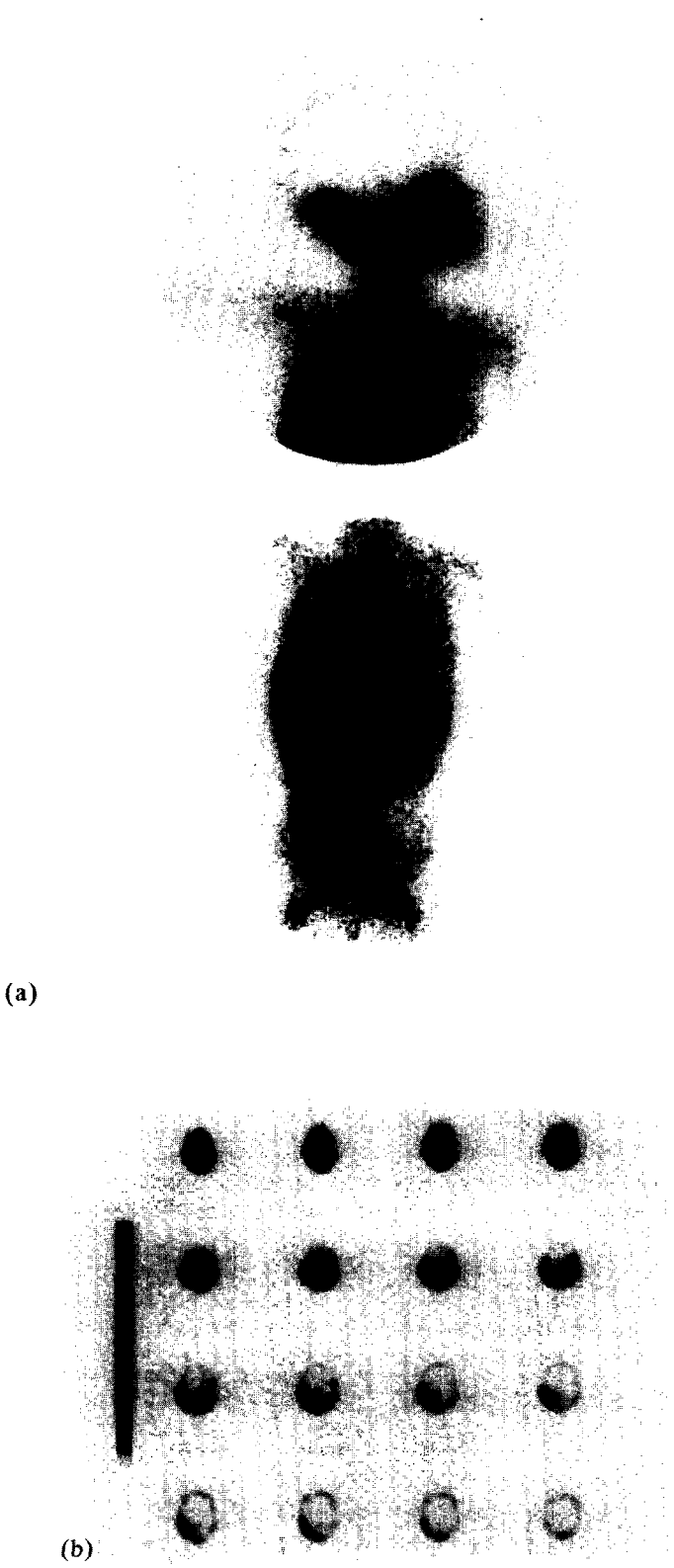
DISCUSSION

RMS is the most common soft tissue sarcoma in children, which can occur at multiple anatomical sites. Ten per cent of the patients already have metastases at the time of presentation. Reliable techniques to exclude metastases are essential for the planning of surgical management of this disease. RMS is also moderately sensitive to radiation therapy and chemotherapy [1]. Progress in the use of all three modalities has led to impressive improvements in survival. With improved survival rates the availability of non-invasive imaging modalities for follow-up has become more important for the early detection of recurrence and metastases. Antimyosin scintigraphy was originally developed for diagnostic imaging of myocardial disease. The fact that tumour cells may have a greater permeability of the cell membrane for ionic radiopharmaceuticals, in combination with the fact that RMS cells contain myosin, led to the hypothesis that this technique could potentially be used for the detection, staging and follow-up of RMS. After the feasibility of this approach was reported in 1987 [15], the present more extended study demonstrates that [^{111}In]antimyosin scintigraphy was successful in detecting RMS tumour localisations in 22 of 26 cases (85%), as confirmed by histopathology and other imaging techniques.

Previous publications of the use of [^{111}In]antimyosin Fab for tumour imaging in series of 8 and 20 patients with a variety of soft tissue tumours [17, 18] included only 4 cases of RMS and 9 of LMS. There were no children among these patients. Seregni *et al.* [20] demonstrated positive results in 7 of 9 children with primary rhabdomysarcoma. Also, the present study was performed predominantly in children with RMS. Combining the results of these four series, the overall sensitivity for RMS (40 patients) is 88% and for LMS (11 patients) 50%, i.e. in the case of RMS a relatively high sensitivity for radioimmunoscintigraphy.

Immunoscintigraphy was unsuccessful in detecting existing tumours in 4 cases of RMS and 1 alveolar soft part sarcoma. A reason for these false negative results may be the fact that many tumours did not stain positively for myosin on immunohistochemistry, in concordance with the observations by Scupham *et al.* [8] who showed that better differentiated tumours stained more intensely than poorly differentiated RMS, or they may be attributed to the institution of chemotherapy prior to the scintigram, the extremely small tumour residue after surgery, or the tumour type.

Although the numbers of patients in complete remission of RMS are rather small, the specificity of [^{111}In]antimyosin scintigraphy was not as high as one would expect. In 2 cases false positive results were attributed to wound healing after recent surgery and in 1 patient the pathological accumulation was due to sinusitis, which disappeared after treatment with antibiotics.



(a)

(b)

Fig. 3. (a) Radioimmunoscintigraphy using [^{111}In]antimyosin Fab fragments in a 4-year-old girl, showing grade 2 uptake in lung metastases of rhabdomyosarcoma and detecting a local recurrence in the right temporo-occipital area of the skull, which was clinically unsuspected; (b) single photon emission tomography confirms this localisation and the intracranial extension of the lesion.

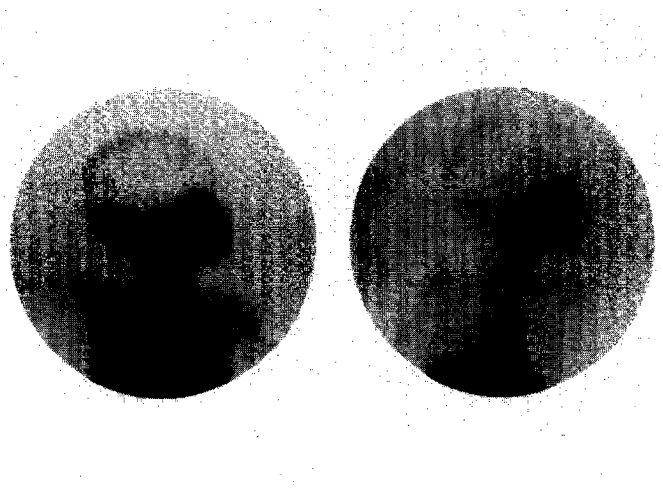


Fig. 4. Antimyosin radioimmunoscintigraphy in follow-up of RMS: scintigrams before (left) and after two cycles of combination therapy (right) demonstrate a marked decrease in pathological accumulation, indicating a favourable response.

In addition, the observation by Kairemo *et al.* [18] that other soft tissue sarcomas may also accumulate [^{111}In]antimyosin Fab, and our personal experience of positive [^{111}In]antimyosin Fab fragment imaging in 1 of 4 patients with breast carcinoma prior to surgery, suggest that this technique is not entirely specific for RMS. Considering the clinical role of [^{111}In]antimyosin imaging, it is concluded from this study that this technique is useful for the confirmation of RMS, the early detection of tumour recurrence and metastases, and to monitor the response to chemotherapy. The non-invasiveness of the procedure makes it an easily applicable parameter in the follow-up. It can, however, not be used to exclude disease, and due to the suboptimal specificity, positive findings need to be confirmed. Potential pitfalls of antimyosin imaging are wound healing, infection, contamination, bladder or bowel activity and residual activity in porth a cath reservoir or intravenous lines. Extrapolating these results to potential radioimmunotherapy, the limited intensity of the tumour uptake, e.g. in comparison to the concentration of [^{131}I]metaiodobenzylguanidine (MIBG) in neuroblastoma [22] is not a favourable dosimetric prerequisite.

1. Raney RB, Hays DM, Tefft M, Triche JT. Rhabdomyosarcoma and undifferentiated sarcomas. In Pizzo PA, Poplack DG, eds. *Principles and Practice of Pediatric Oncology*. Philadelphia, JB Lippincott, 1989.
2. Voûte PA, Barrett A, Bloom HJG, Lemerle J, Neidhardt MK. *Cancer in Children. Clinical Management*. Berlin, Springer-Verlag, 1992.
3. Oates E, Sarno C. Imaging of pelvic rhabdomyosarcoma by bone scintigraphy. *Eur J Nucl Med* 1987, **13**, 297–299.
4. Weinblatt ME, Miller JH. Radionuclide scanning in children with rhabdomyosarcoma. *Med Pediat Oncol* 1981, **9**, 293–301.
5. Quddus FE, Espinola D, Kramer SS, Leventhal BG. Comparison between X-ray and bone scan detection of bone metastases in patients with rhabdomyosarcoma. *Med Pediat Oncol* 1983, **11**, 125–129.
6. Bekerman C. Childhood malignancies. In Hoffer PB, Bekerman C, Henkin RE, eds. *Gallium-67 Imaging*. John Wiley & Sons, New York, 1978, 145.
7. Ramanna L, Waxman AD, Waxman S, *et al.* Tl-201 scintigraphy in bone and soft tissue sarcoma: evaluation of tumor and mass and viability. *J Nucl Med* 1988, **29**, 854.
8. Scupham R, Gilbert EF, Wilde J, Wiedrich TA. Immunohistochemical studies of rhabdomyosarcoma. *Arch Pathol Lab Med* 1986, **110**, 818–821.
9. Khaw BA, Mattis JA, Melincoff G, Strauss HW, Gold HK, Haber E. Monoclonal antibody to cardiac myosin imaging of experimental myocardial infarction. *Hybridoma* 1984, **3**, 11–23.
10. Khaw BA, Yasuda T, Gold HK, *et al.* Acute myocardial infarct imaging with ^{111}In-labeled monoclonal antimyosin Fab. *J Nucl Med* 1987, **28**, 1671–1678.
11. Yasuda T, Palacios IF, Dec GW, *et al.* In-111 monoclonal antimyosin imaging in the diagnosis of acute myocarditis. *Circulation* 1987, **76**, 306–311.
12. Carrió I, Berná L, Ballester M, *et al.* In-111 antimyosin scintigraphy to assess myocardial damage in patients with suspected myocarditis and cardiac rejection. *J Nucl Med* 1988, **29**, 1893–1900.
13. Estorch M, Carrió I, Berná L, *et al.* Indium-111 antimyosin scintigraphy after doxorubicin therapy in patients with advanced breast cancer. *J Nucl Med* 1990, **31**, 1965–1969.
14. Krause T, Schümigen C, Hohenloser S, Kasper W, Meinertz T. Szintigraphie mit ^{111}In-markiertem antimyosin F(ab)$_2$ monoklonalem Antikörper und Tc-99m pyrophosphat bei Rhabdomyolyse. *Nucl Med* 1988, **27**, 38–39.
15. Hoefnagel CA, Voûte PA, de Kraker J, Behrendt H. Scintigraphic detection of rhabdomyosarcoma. *Lancet* 1987, **I**, 921.
16. Hoefnagel CA, de Kraker J, Voûte PA, Behrendt H. Tumor imaging of rhabdomyosarcoma using radiolabelled fragments of monoclonal anti-myosin antibody. *J Nucl Med* 1988, **29**, 791.
17. Cox PH, Verweij J, Pillay M, Stoter G, Schonfeld D. Indium-111 antimyosin for the detection of leiomyosarcoma and rhabdomyosarcoma. *Eur J Nucl Med* 1988, **14**, 50–52.
18. Kairemo KJA, Wiklund TA, Liewendahl K, *et al.* Imaging of soft tissue sarcomas with In-111-labeled monoclonal antimyosin Fab fragments. *J Nucl* 1990, **31**, 23–31.
19. Kairemo KJA, Lehtovirta P. Radioimmunodetection of uterine leiomyosarcoma with In-111 labeled monoclonal antimyosin antibody Fab fragments. *Gynecol Oncol* 1990, **36**, 417–422.
20. Seregni E, Maffioli L, Crippa F, *et al.* Radioimmunoscintigraphy of paediatric rhabdomyosarcoma. In Schmidt HAE, Höfer R, eds. *Nuclear Medicine—Nuclear Medicine in Research and Practice*. New York, Schattauer, 1992, 689–691.
21. Reuland P, Koscelniak E, Ruck P, Treuner J, Feine U. Application of an anti-myosin for scintigraphic differential diagnosis of infantil tumors. *Int J Rad Appl Instrum [B]* 1991, **18**, 89–93.
22. Hoefnagel CA, Voûte PA, de Kraker J, Marcuse HR. Radionuclide diagnosis and therapy of neural crest tumors using iodine-131 Metaiodobenzylguanidine. *J Nucl Med* 1987, **28**, 308–314.