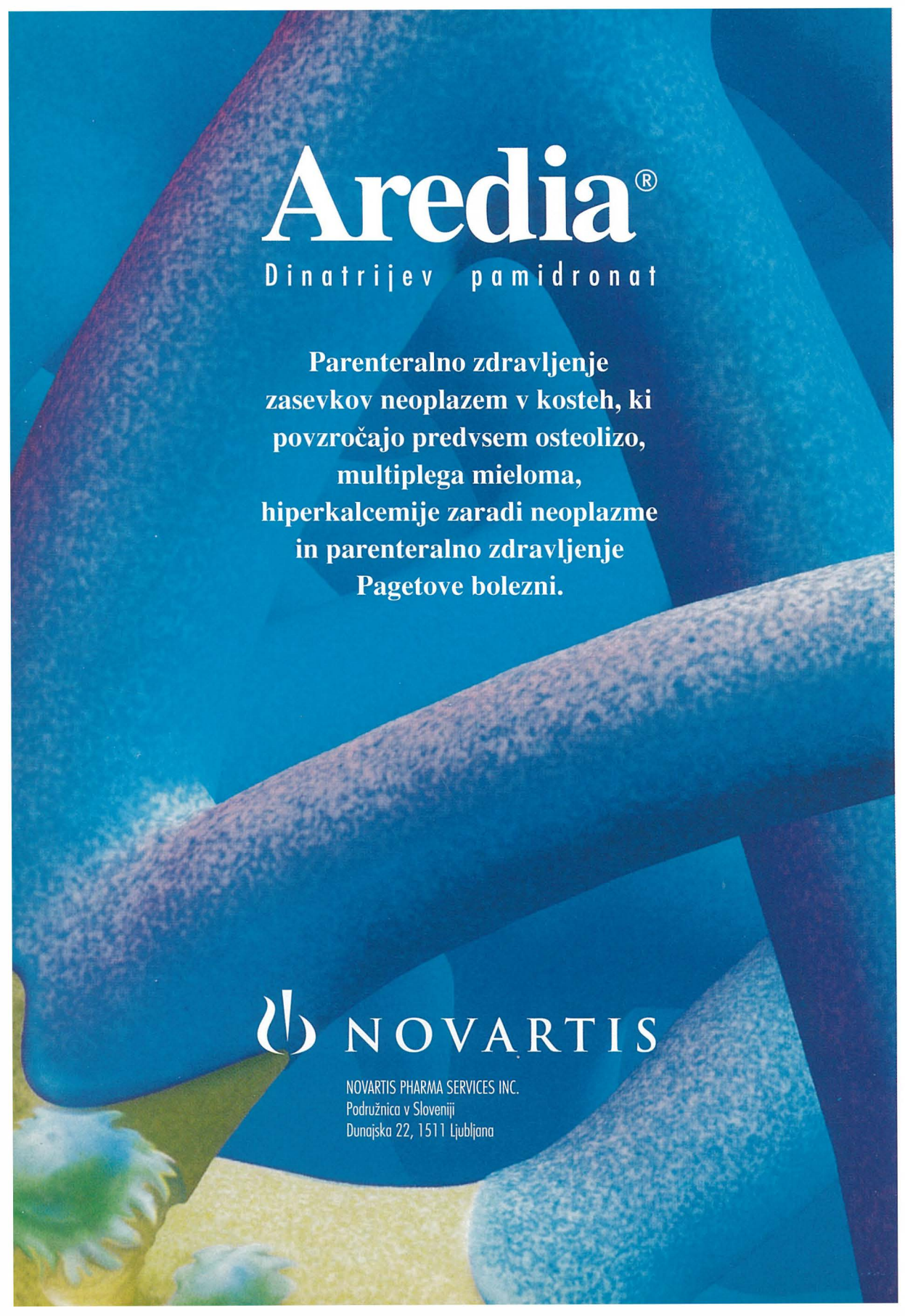
RADIOLOGY AND ONCOLOGY

March 2000
Vol. 34 No. 1
Ljubljana

ISSN 1318-2099


Aredia®
Dinatrijev pamidronat
Parenteralno zdravljenje zasevkov neoplazem v kosteh, ki povzročajo predvsem osteolizo, multiplega mieloma, hiperkalcemije zaradi neoplazme in parenteralno zdravljenje Pagetove bolezni.
NOVARTIS
NOVARTIS PHARMA SERVICES INC.
Podružnica v Sloveniji
Dunajska 22, 1511 Ljubljana

RADIOLOGY AND ONCOLOGY

Editorial office
Radiology and Oncology
Institute of Oncology
Vrazov trg 4
SI-1000 Ljubljana
Slovenia
Phone: +386 61 1320 068
Phone/Fax: +386 61 1337 410
E-mail: gsersa@onko-i.si

March 2000
Vol. 34 No. 1
Pages 1-78
ISSN 1318-2099
UDC 616-006
CODEN: RONCEM

Aims and scope
Radiology and Oncology is a journal devoted to publication of original contributions in diagnostic and interventional radiology, computerized tomography, ultrasound, magnetic resonance, nuclear medicine, radiotherapy, clinical and experimental oncology, radiobiology, radiophysics and radiation protection.

Publishers
Slovenian Medical Association - Slovenian Association of Radiology, Nuclear Medicine Society, Slovenian Society for Radiotherapy and Oncology, and Slovenian Cancer Society
Croatian Medical Association - Croatian Society of Radiology

Affiliated with
Societas Radiologorum Hungarorum
Friuli-Venezia Giulia regional groups of S.I.R.M. (Italian Society of Medical Radiology)

Reader for English
Mojca Čakš

Key words
Eva Klemenčič

Secretaries
Milica Harisch
Mira Klemenčič

Design
Monika Fink-Serša

Printed by
Imprint d.o.o., Ljubljana, Slovenia

Published quarterly in 700 copies

Bank account number 50101 679 901608
Foreign currency account number
50100-620-133-900-27620-978-515266/6
NLB - Ljubljanska banka d.d. - Ljubljana

Subscription fee for institutions $ 100 (16000 SIT), individuals $ 50 (5000 SIT)

The publication of this journal is subsidized by the Ministry of Science and Technology of the Republic of Slovenia.

Indexed and abstracted by:
BIOMEDICINA SLOVENICA
CHEMICAL ABSTRACTS
EMBASE / Excerpta Medica

This journal is printed on acid- free paper

Radiology and Oncology is available on the internet at: http://www.onko-i.si/radiolog/rno.htm

RADIOLOGY AND ONCOLOGY

Ljubljana, Slovenia
March 2000
Vol. 34 No. 1

ISSN 1318-2099
UDC 616-006
CODEN: RONCEM

CONTENTS

EXPERIMENTAL ONCOLOGY

Radiology and Oncology is covered in *Biomedicina Slovenica, Chemical Abstracts, and EMBASE / Excerpta Medica*

Radiol Oncol 2000; 34(1): 1-9.

Correlation of ^{18}F-FDG-PET and histopathology in patients with malignant melanoma

Karl H. Bohuslavizki[1], Susanne Klutmann[1], Karsten Neuber[2], Juliane Wedler[2], Jürgen Altenhoff[2], Sabine Kröger[1], Ralph Buchert[1], Christian Bleckmann[1] and Malte Clausen[1]

Departments of [1]Nuclear Medicine and [2]Dermatology, University Hospital Eppendorf, Hamburg, Germany

Background. *Preliminary reports suggest that PET using ^{18}F-FDG may be a valuable diagnostic tool in patients with advanced malignant melanoma. Therefore, the aim of this study was to correlate PET lesions with histological findings in staging of malignant melanoma.*

Patients and methods. *A total of 82 patients with malignant melanoma underwent 107 PET examinations for primary staging or therapy monitoring. After an intravenous injection of 370 MBq ^{18}F-FDG whole-body images were acquired on an ECAT EXACT 47 (921) with an axial field-of-view of 16.2 cm (Siemens, CTI). Tumor-suspicious PET findings, results of physical examination, and tumor-suspicious lesions by conventional imaging, i.e. ultrasound, CT, and MRI, were evaluated histologically and correlated with each other on a lesion-by-lesion basis.*

Results. *PET detected 124 lesions with an increased focal tracer uptake. In addition, physical examination and conventional imaging revealed 65 tumor-suspicious lesions. In total, 189 tumor-suspicious lesions were evaluated histologically. ^{18}F-FDG-PET was true-positive in 115/189 lesions and false-positive in 9/189 lesions. In 21 out of 65 PET-negative lesions, biopsy could not confirm melanoma tissue. In contrast, in 44 out of 65 PET-negative lesions further biopsy revealed malignant melanoma tissue. Sensitivity and specificity of ^{18}F-FDG-PET for the detection of malignant melanoma tissue were 72% and 70%, respectively. Negative and positive predictive values of ^{18}F-FDG-PET imaging were 32% and 93%, respectively. With respect to anatomical localization, two thirds of false negative/positive PET lesions were located in the skin or mucosal area.*

Conclusions. *^{18}F-FDG-PET is a valuable diagnostic tool in order to prove tumor-suspicious lesions in malignant melanoma. However, for exclusion of skin metastases an accurate physical examination of patients with malignant melanoma by a dermatologist is indispensable. With respect to very aggressive treatment modalities of advanced malignant melanoma ^{18}F-FDG-PET may help to select the appropriate treatment protocol for each individual patient.*

Key words: melanoma-diagnosis-pathology; tomography, emission-computed; ^{18}F-FDG-PET; neoplasm staging; histology; treatment strategy

Introduction

Cutaneous malignant melanoma is one of the most common malignancies with a twofold to threefold increasing incidence over the last 40 years.[1] The most important prognostic factor is tumor staging at the time of diagnosis.[2] According to the recommendations of the American Joint Commission on Cancer (AJCC) the clinical stage is divided into four groups. Clinical stages I and II are defined for primary malignant melanomas limited to the site of the origin without any evidence of a tumor spread elsewhere. In case of palpable local lymph node involvement or disseminated disease, the patients are classified as clinical stage III and IV, respectively. At the time of the first presentation, in nearly 80 % of all patients with the clinical stage I or II with, a mean 5-year survival rate of 85 % was noted.[2] However, one third of the latter patients will have clinically undetectable lymph node metastases which, if left untreated, will significantly worsen the survival rate.[3,4] Thus, an accurate tumor staging is a prerequisite for selecting the adequate treatment protocol.

Conventional imaging, *i.e.* computed tomography, magnet resonance imaging, and ultrasound are valuable and well-established diagnostic tools in pretherapeutic staging.[5-8] However, these imaging modalities allow an identification of morphologic changes only, whereas tumor tissue in normal-sized lymph nodes can not be detected by definition.[9] Moreover, morphologically orientated imaging permits a screening of a pre-selected body area only. Since malignant melanomas are known for their aggressive lymphatic and hematogenic spread potency[3,7], one single non-invasive imaging modality with simultaneous imaging of the whole-body would significantly facilitate pre-therapeutic management in these patients. A number of radiotracers have therefore been suggested, *i.e.* ^{67}Ga-citrate,[10] ^{123}I-benzamide, ^{123}I-α-methyltyrosine,[9] and ^{99m}Tc-labelled antimelanoma-antibodies,[11] though a great number of false-negative findings were reported for all of these radiotracers.[9,12] In contrast, initial experiences demonstrated the clinical potency of positron emission tomography (PET) using 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) for the detection of both local and systemic spread of metastatic malignant melanoma.[1,13-22] Thus, within its geometric resolution of about 4-6 mm (FWHM), PET is able to detect tumor tissue independent of morphological changes due to an increased rate of glycolysis in malignant transformed cells. Since the early detection of malignant melanoma metastases increases patients' survival rate,[23,24] PET imaging might be a valuable diagnostic tool in detecting melanoma metastases.

Therefore, the aim of this study was to determine the clinical value of ^{18}F-FDG-PET in patients with malignant melanoma by comparing PET to findings of both clinical examination and morphological imaging, and to correlate these findings with histological results on a lesion-to-lesion basis.

Patients and methods

Patients

A total of 82 patients (37 female, 45 male) aged from 28 to 80 years with histologically proven malignant melanoma were investigated. The primary tumors were located in the skin area of the head and neck region in 17 patients, in the upper extremities in 6, in the lower extremities in 18, on the chest wall in 3, on the back in 29, and on the abdominal wall

Received 29 December 1999

Accepted 10 January 2000

Correspondence to: Dr. Karl H. Bohuslavizki, Department of Nuclear Medicine, University Hospital Eppendorf, Martinistr. 52, D-20246 Hamburg, Germany: Phone: +49 40 42803 4047; Fax: +49 40 42803 6775; E-mail: bohu@uke.uni-hamburg.de

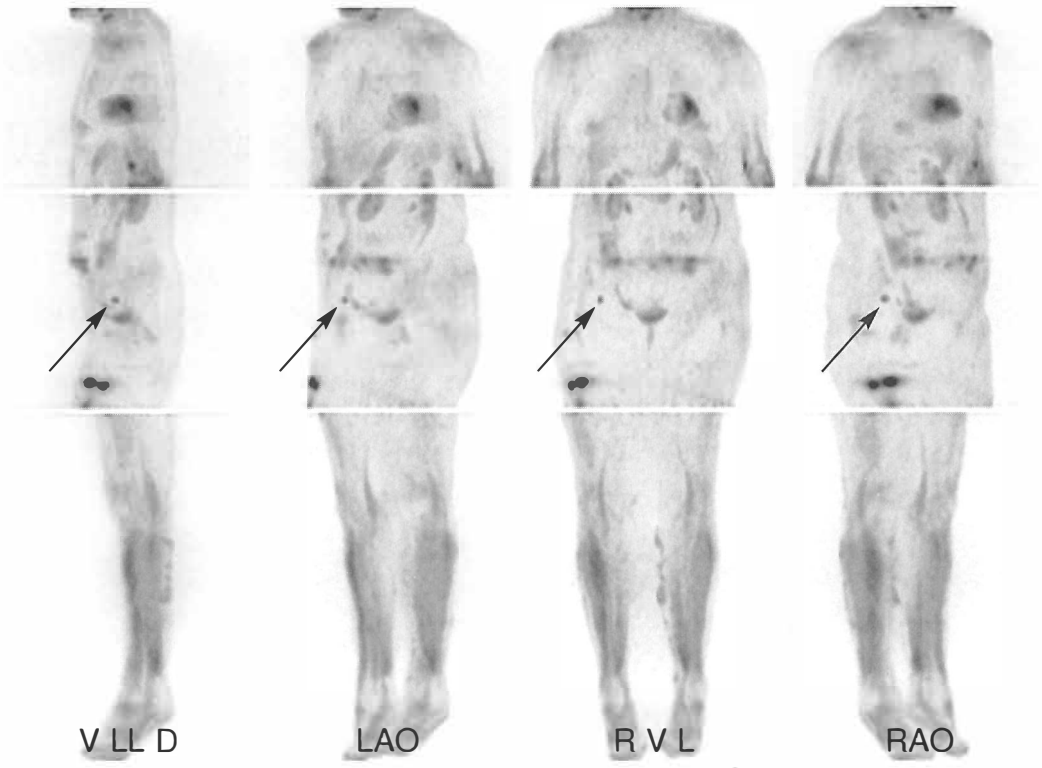


Figure 1. Maximum intensity projections (MIPs) from the left lateral (V LL D), anterior (R V L), right anterior oblique (RAO), and left anterior oblique (LAO) views of a patient (58/f) with a primary malignant melanoma of the right lower extremity. The patient was clinically suspected for inguinal lymph node metastases. Note increased ^{18}F-FDG uptake of both the primary tumor and the lymph node metastasis (arrow). Since no distant metastases were detected by ^{18}F-FDG-PET the patient was classified stage IIIB.

in 2 patients. Unusual sites of the primary malignant melanoma were the vulva and the retina in one and two patients, respectively. The anatomic site of the primary tumor was unknown in the remaining 4 patients.

A description of the Clark level[25] was available for 57 patients with the following distribution: level I, no patient; level II, 2 patients; level III, 13 patients; level IV, 34 patients; level V, 2 patients. Two patients were classified Clark level II/III, level III/IV and level IV/V, respectively.

Moreover, the classification results of the primary lesion thickness according to the Breslow scheme[26] were available for 59 patients. There were 10 patients with thin lesions (0,75 mm or less), 38 with intermediate lesions (0,76-3,99 mm), and 11 patients with thick lesions of 4 mm or greater.

PET scanning

The patients fasted for at least 12 hours prior to PET-scanning in order to minimize blood insulin levels and glucose utilization of normal tissue.27 Whole-body emission images were acquired without attenuation correction 60 min after i.v. injection of 370 MBq ^{18}F-FDG using an ECAT EXACT 47 (921) scanner (Siemens/CTI) with an axial field-of-view of 16.2 cm.

The patients were placed in the PET gantry feet first with both arms folded over the

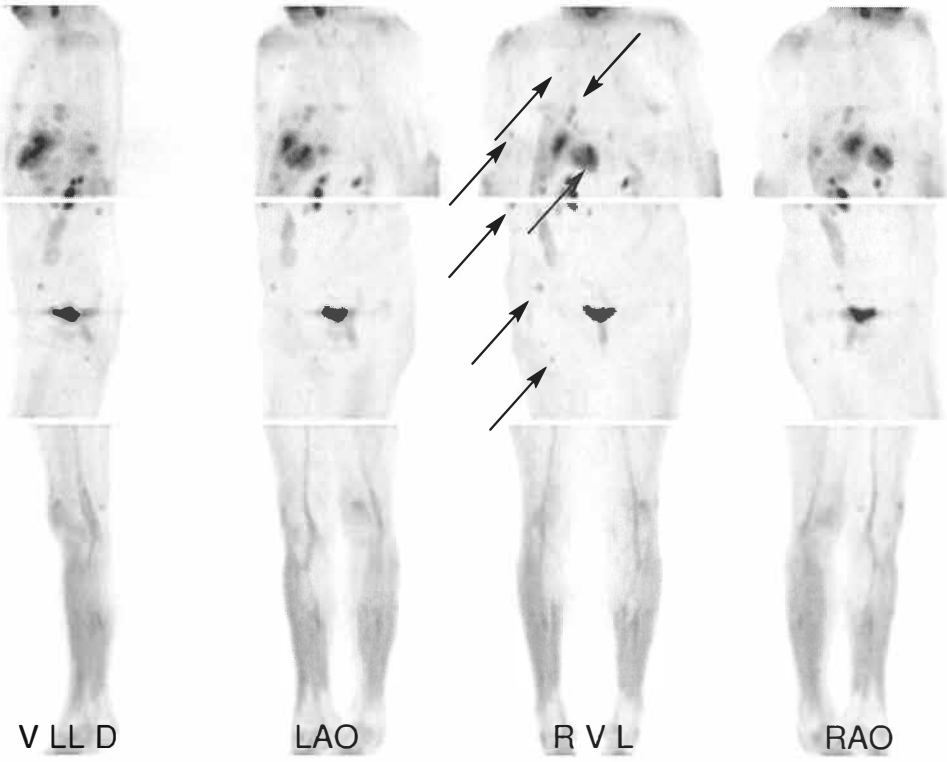


Figure 2. MIPs in same views as in figure 1 of a patient (69/f) after surgical treatment of a malignant melanoma located at the right leg. Note multiple metastases in [18]F-FDG-PET (arrows). However, a total of six metastases located within the skin area could not be identified by [18]F-FDG-PET imaging but by an accurate clinical examination.

abdomen. Images were acquired for 4 min per bed position covering the feet up to the middle of the femurs. Then, the patients were repositioned in the gantry head first, and the second set of images was acquired from the brain down to the waist. Prior to the third acquisition set from the waist down to the lower extremities, the patients were asked to empty the bladder in order to decrease urine activity. Emission data were reconstructed by filtered back projection using a Hanning filter with a cut-off frequency of 0.4 of Nyquist frequency. Thus, transaxial spatial resolution was approximately 12 mm. PET-images were printed on transparency film (Helios 810, Sterling) using a linear gray scale with highest activity displayed in black. Images were displayed with an upper threshold of five times of the mean activity in the lung. Standardized documentation included both 20 transversal and 20 coronal slices with a slice thickness of 13.5 mm each, and maximum-intensity-projections (MIPs) in the anterior, left lateral, right-anterior-oblique, and left-anterior-oblique view as published previously.[28]

Evaluation

Two independent nuclear medicine physicians interpreted PET images visually. All tumor-suspicious findings were evaluated histologically. Moreover, all patients underwent physical examinations as well as morphological imaging, i.e. chest X-ray, CT scans of the chest, brain and abdomen or MRI. Additional tumor-suspicious lesions of conventional

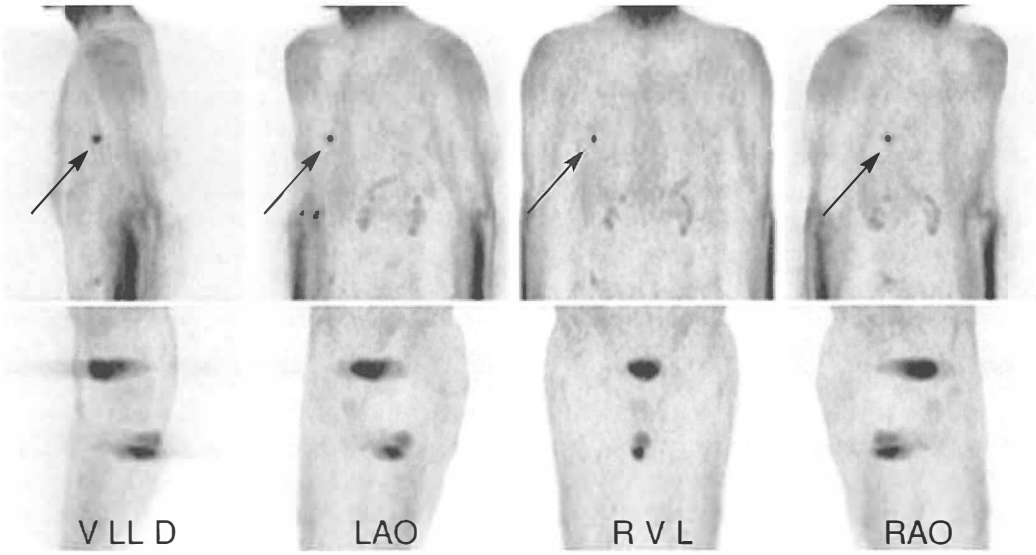


Figure 3. MIPs in same views as in figure 1 of a patient (56/m) after the excision of a malignant melanoma of the right leg. Patient showed up with a tumor-suspicious lesion in the right lung in postsurgical X-ray of the chest. ^{18}F-FDG-PET revealed an increased glucose metabolism within the lesion, and histology confirmed malignant melanoma metastasis. Thus, the patient was classified as stage IV.

imaging or physical examination were evaluated histologically, too. The results of histology and tumor-suspicious lesions were correlated on a lesion-by-lesion basis with special respect to anatomical localization.

Results

In a total of 82 patients PET detected 124 lesions with an increased focal tracer uptake. Following physical examination and conventional imaging, 65 additional lesions were recorded to be suspicious for malignant melanoma metastases. Thus, a total of 189 lesions were evaluated histologically.

PET was true-positive in 115/124 lesions (Figure 1) and false-positive in 9/124 lesions. In 21/65 PET-negative lesions, biopsy did not confirm melanoma tissue, whereas histology was positive for melanoma metastases in the remaining 44 lesions (Figure 2). Thus, for the detection of malignant melanoma tissue PET had an overall sensitivity of 72% and an overall specificity of 70%. The negative predictive value of ^{18}F-FDG PET was 32%. In contrast, the positive predictive value was 93%.

PET findings were either false-positive or false-negative in a total of 53 lesions. With regard to anatomical localization, 33 out of these lesions were located in the skin or within mucosal areas. Two lesions were situated in the lungs and three in the mesenterium. Five lesions were located in the axillary lymph nodes. The remaining 10 lesions were located in different regions of the body, *e.g.* the lung, the mediastinum. Thus, with respect to anatomical localization, almost two thirds of either false-positive or false-negative PET findings were located in the skin or within mucosal areas. If false-positive or false-negative skin or mucosal lesions were not taken into account, PET remained false-negative in 11 lesions with histologically proven melanoma tissue. Thus, in this subgroup, the sensitivity of PET scanning in melanoma patients increased to 91%.

Discussion

Initial studies assessed the clinical utility of ^{18}F-FDG-PET for the detection of metastatic malignant melanoma. Gritters and coworkers[3] studied 12 patients with a total of 52 biopsy- or CT-diagnosed melanoma lesions. All patients underwent additional ^{18}F-FDG-PET. Their initial data demonstrated the potential role of ^{18}F-FDG-PET for the detection of metastatic malignant melanoma, especially in untreated extrathoracic lesions. Steinert and coworkers[22] examined 33 patients with primary diagnosis or known relapse of malignant melanoma. In their patients, ^{18}F-FDG-PET showed a sensitivity of 92% for the detection of malignant melanoma lesions. Moreover, the specificity was 77% without further clinical information and 100% with clinical information. Corresponding findings were demonstrated by Holder and coworkers[16] who recommended ^{18}F-FDG-PET as a primary strategy imaging modality in the staging of melanoma patients.

In this study, a total of 82 patients with malignant melanoma underwent 107 ^{18}F-FDG-PET examinations. In 115 out of 124 lesions with pathological focal increased ^{18}F-FDG uptake, PET was true-positive. Moreover, in 9 PET-positive lesions, histology could not confirm malignant melanoma tissue. In contrast, in 44 out of 65 lesions detected by clinical examination or morphological imaging, only histological evaluation revealed malignant melanoma tissue. However, 21 out of 65 PET-negative lesions were true-negative. In accordance with the findings of other investigations the results of this study show that whole-body ^{18}F-FDG-PET is an accurate imaging modality in patients with malignant melanoma in order to screen the whole-body for the presence of metastases.

However, in this study, ^{18}F-FDG-PET had a sensitivity of 72% and a specificity of 70% only. It is remarkable that 53 lesions were either false-negative or false-positive in ^{18}F-FDG-PET. Two thirds of these lesions were located within the skin or mucosal areas. Thus, the skin and mucosal areas might be problematic regions for the detection of malignant melanoma metastases with ^{18}F-FDG-PET. One possible cause of false-positive results is the fact that ^{18}F-FDG is excreted via the urine. Thus, the contaminations of the skin, predominantly at the lower extremities might be interpreted as tumor-suspicious lesions of the skin. Moreover, it is known that patients treated with interferon alpha and interleukin-2 exhibit cutaneous inflammatory infiltrations at the injection site,[24] which may cause false-positive results in ^{18}F-FDG-PET. However, the majority of false PET findings were false-negative. The limited impact of ^{18}F-FDG-PET for the detection of skin and mucosal metastases might be due to physiological and technological reasons. First, tumor lesions located within the regions of high physiological ^{18}F-FDG uptake, *i.e.* the brain or the kidneys, might not be identified by ^{18}F-FDG-PET imaging. Second, the detection of small skin/mucosal lesions with diameters of less than 5 mm might be limited by geometrical resolution of ^{18}F-FDG-PET. Moreover, PET-images in this study were reconstructed by filtered back-projection. As a consequence, melanoma metastases in borderline areas, *i.e.* the skin, can hardly be differentiated from non-malignant transformed tissue. This problem might be solved by time-consuming iterative reconstruction algorithms. Moreover, the high number of false-negative PET lesions located within the skin and mucosal area underlines the necessity of an accurate and careful physical examination of the patient in daily clinical patient management. With these limitations in mind, whole-body ^{18}F-FDG-PET is a suitable imaging modality in order to prove tumor-suspicious lesions in malignant melanoma. However, for exclusion of skin metastases an accurate physical examination by a dermatologist is still indispensable.

Any diagnostic test should, in principle, not only be judged with respect to its statistic data, e.g. sensitivity, specificity, negative and positive predictive value, and accuracy, but rather in the light of its effect on treatment strategy. The therapeutic approach in malignant melanoma mainly depends on the extent of the disease. In clinical stages I (pT1/T2 N0 M0) and II (pT3/T4 N0 M0), excision of the primary malignancy is the golden standard. In the last few years elective lymphadenectomy was abandoned since its additional value for the patients' survival rate was demonstrated in retrospective patient studies only,[29,30] but not in randomized prospective patient studies.[30] If patients show up with regional lymph node metastases or in-transit-metastases but no distant metastases (stage IIIb), the therapeutic approach includes therapeutic lymphadenectomy. However, 10-year survival-rate decreases from 97% in patients staged pT1N0M0 to 19% in patients staged N1 or N2 and MO melanoma.[31] The primary treatment goal in patients with M1 malignant melanoma (clinical stage IV) is the reduction of tumoral masses in order to prolong patients' life expectancy as well as to improve the quality of life.[32] In principle, there are three therapeutic options: surgery, external radiotherapy, and chemotherapy. In case of isolated metastases, an operative treatment has proved to be helpful in the prolongation of patients' life expectancy. Most studies demonstrated life prolongation in case of total resection of all tumoral masses only.[33] Thus, 10-year survival-rate was expected to be as low as 3% in these patients with advanced malignant melanoma.[31] However, there is no well-established, standardized systemic treatment protocol for patients with distant metastases. Thus, the treatment strategy itself is still under clinical investigation and is the focus of several patient studies. There is now evidence that patients with stage IV malignant melanoma benefit from an aggressive chemotherapy with interleukin-2 and interferon alpha. These authors report of 5-year survival-rate of up to 10%.[24,34]

Thus, in addition to sensitivity and specificity of high-resolution ultrasonography of 70% and 90%,[35] respectively, even patients with advanced malignant melanoma may benefit from the detection of metastases by ^{18}F-FDG-PET due to several reasons. First, patients' survival rate decreases with an increasing number of involved lymph node regions.[36] Second, prognosis of patients is better with an early detection of metastases and with small tumor masses at the time of detection.[36] Third, ^{18}F-FDG-PET has been proved superior in the detection of lung metastases (Figure 3) as compared to conventional, well-established computed tomography.[37,38] Last, 18F-FDG-PET offers the advantage to image the whole body in one single procedure which is especially important because in malignant melanoma often unexpected, aberrant metastatic spread is found. Thus, ^{18}F-FDG-PET has already been suggested for the staging of malignant melanoma.[22]

Conclusions

^{18}F-FDG-PET is a valuable diagnostic tool in order to prove tumor-suspicious lesions in malignant melanoma. However, for exclusion of metastases physical examination by dermatologist and conventional imaging are indispensable. With respect to very aggressive treatment modalities of advanced malignant melanoma ^{18}F-FDG-PET may help to select the appropriate treatment protocol for the individual patient.

References

1. Macfarlane DJ, Sondak V, Johnson T, Wahl RL. Prospective evaluation of 2-[18F]-2-deoxy-D-glucose positron emission tomography in staging of regional lymph nodes in patients with cutaneous malignant melanoma. *J Clin Oncol* 1998; **16**: 1770-6.

2. Sober AJ, Koh HK. Melanoma and other pigmented skin lesions. In: Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci, A. S., Kasper DL, editors. *Harrison's principles of internal medicine*. 13 ed. New Yoork: McGraw-Hill, Inc; 1994. p. 1867-71.

3. Gritters LS, Francis IR, Zasadny KR, Wahl RL. Initial assessment of positron emission tomography using 2-fluorine-18-fluoro-2-deoxy-D-glucose in the imaging of malignant melanoma. *J Nucl Med* 1993; **34**: 1420-7.

4. Zartman GM, Thomas MR, Robinson WA. Metastatic disease in patients with newly diagnosed malignant melanoma. *N Nucl Med* 1987; **35**: 163-4.

5. Ginaldi S, Wallace S, Shalen P, Luna M, Handel S. Cranial computed tomography of malignant melanoma. *AJR* 1981; **136**: 145-9.

6. Shirkhoda A, Albin J. Malignant melanoma: correlating abdominal and pelvic CT with clinical staging. *Radiology* 1987; **165**: 75-8.

7. Silverman PM, Heaston DK, Korobkin M, Seigler HF. Computed tomography in the detection of abdominal metastases from malignant melanoma. *Invest Radiol* 1984; **19**: 309-12.

8. Patten RM, Shuman WP, Teefey S. Metastases from malignant melanoma to the axial skeleton: a CT study of frequency and appearance. *AJR* 1990; **155**: 109-12.

9. Steinert H, Boni R, Huch-Boni RA, Capaul R, von Schulthess GK, Westera G. 123I-alpha-methyltyrosine scintigraphy in malignant melanoma. *Nuklearmedizin* 1997; **36**: 36-41.

10. Bekerman C, Hoffer PB, Bitran JD. The role of Gallium-67 in the clinical evaluation of cancer. *Sem Nucl Med* 1985; **15**: 72-103.

11. Bockisch A, Oehr P, Biltz H, Hotze A, Kreysel HW, Biersack HJ. The clinical value of radioimmunodetection (RID) using antimelanoma antibodies. *Tumordiag und Ther* 1991; **12**: 112-6.

12. Boni R, Steinert H, Huch Boni R, Von Schulthess GK, Meyer J, Dummer R, et al. Radioiodine-labelled alpha-methyl-tyrosine in malignant melanoma: cell culture studies and results in patients. *Br J Dermatol* 1997; **137**: 96-100.

13. Damian DL, Fulham MJ, Thompson E, Thompson JF. Positron emission tomography in the detection and management of metastatic melanoma. *Melanoma Res* 1996; **6**: 325-9.

14. Boni R. Whole-body positron emission tomography: an accurate staging modality for metastatic melanoma. *Arch Dermatol* 1996; **132**: 833-4.

15. Hsueh EC, Gupta RK, Glass EC, Yee R, Qi K, Morton DL. Positron emission tomography plus serum TA90 immune complex assay for detection of occult metastatic melanoma. *J Am Coll Surg* 1998; **187**: 191-7.

16. Holder WD Jr, White RL Jr, Zuger JH, Easton EJ Jr, Greene FL. Effectiveness of positron emission tomography for the detection of melanoma metastases. *Ann Surg* 1998; **227**: 764-71.

17. Engel H, Steinert H, Buck A, Berthold T, Huch Boni RA, von Schulthess GK. Whole-body PET: physiological and artifactual fluorodeoxyglucose accumulations. *J Nucl Med* 1996; **37**: 441-6.

18. Kern KA. [^{14}C]deoxyglucose uptake and imaging in malignant melanoma. *J Surg Res* 1991; **50**: 643-7.

19. Reske SN, Bares R, Bull U, Guhlmann A, Moser E, Wannenmacher MF. Clinical value of positron emission tomography (PET) in oncologic questions: results of an interdisciplinary consensus conference. *Nuklearmedizin* 1996; **35**: 42-52.

20. Rinne D, Baum RP, Hor G, Kaufmann R. Primary staging and follow-up of high risk melanoma patients with whole- body ^{18}F-fluorodeoxyglucose positron emission tomography: results of a prospective study of 100 patients. *Cancer* 1998; **82**: 1664-71.

21. Paquet P, Hustinx R, Rigo P, Pierard GE. Malignant melanoma staging using whole-body positron emission tomography. *Melanoma* Res 1998; **8**: 59-62.

22. Steinert HC, Huch Boni RA, Buck A, Boni R, Berthold T, Marincek B, et al. Malignant melanoma: staging with whole-body positron emission tomography and 2-[F-18]-fluoro-2-deoxy-D-glucose. *Radiology* 1995; **195**: 705-9.

23. Reintgen DR, Balch CM, Kirkwood J, Ross M. Recent advances in the care of the patient with malignant melanoma. *Ann Surg* 1997; **225**: 1-14.

24. Richards JM, Gale D, Mehta N, Lestingi T. Combination of chemotherapy with interleukin-2 and interferon alfa for the treatment of metastatic melanoma. *J Clin Oncol* 1999; **17**: 651-7.

25. Clark WHJ, From L, Bernandino E, Mihm M. The histogenesis and biologic behavior of primary malignant melanomas of the skin. *Cancer* 1969; **29**: 705-26.

26. Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. *Ann Surg* 1970; **172**: 902-8.

27. Minn H, Leskinen-Kallio S, Lindholm P, Bergman J, Ruotsalainen U, Teršs M, et al. (18-F)fluorodeoxyglucose uptake in tumors: kinetic vs. stady-state methods with reference to plasma insulin. *J Comput Assist Tomogr* 1993; **17**: 115-23.

28. Bleckmann C, Buchert R, Schulte U, Lorenzen J, Bohuslavizki KH, Mester J, et al. Onko-PET: Bildanalyse mittels Monitor versus standardisierter Filmdokumentation. *Nuklearmedizin* 1999; **38**: 56-60.

29. Kaufmann R. Operative Therapie des primšren Melanoms. *Onkologe* 1996; **2**: 449-52.

30. Balch CM, Milton GW, Cascinelli NFSH. Elective lymph node dissection: pros and cons. In: Balch CM, Houghton AN, Milton GW, Sober AJ, Soong SJ, editors. *Cutaneous melanoma*. Philadelphia: Lippincott, J. B.; 1992. p. 345-66.

31. Hšffner AC, Garbe C, Biittner P, Orfanos CE, Rassner G, Burg G. The prognosis of primary and metastasizing melanoma. An evaluation of the TNM classification in 2495 patients and proposals for their revision. *Br J Cancer* 1992; **66**: 856-61.

32. Garbe C. Malignes Melanom. In: Seeber S, Schütte J, editors. *Therapiekonzepte* Onkologie. 3 ed. Berlin: Springer; 1998. p. 799-829.

33. Göhl J, Meyer T, Haas C, Altendorf Hofman A, Hohenberger W. Ist die chirurgsche Therapie von Fernmetastasen maligner Melanome sinnvoll? *Langenbecks Arch Chir* 1996; **113(Suppl)**: 122-6.

34. Hoffmann R, Müller I, Neuber K, Lassmann S, Buer J, Probst M, et al. Risk and outcome in metastatic malignant melanoma patients receiving DTIC, cisplatin, BCNU and tamoxifen followed by immuntherapy with interleukin 2 and interferon alpha2a. *Br J Cancer* 1998; **78**: 1076-80.

35. Blessing C, Feine U, Geiger L, Carl M, Rassner G, Fierlbeck G. Positron emission tomography and ultrasonography. A comparative retrospective study assessing the diagnostic validity in lymph node metastases of malignant melanoma. *Arch Dermatol* 1995; **131**: 1394-98.

36. Stachelmann WK, Rapaport DP, Soong SJ. Prognostic clinical and pathologic features. In: Balch CM, Houghton AN, Sober AJ, Soong SJ, editors. *Cutaneous melanoma*. St. Louis: Quality Medical Publishing, Inc.; 1998. p. 26-42.

37. Hoh CK, Hawkins RA, Glaspy JA, Dahlbom M, Tse NY, Hoffman EJ et al. Cancer detection with whole-body PET using 2-[^{18}F]fluoro-2-deoxy-D-glucose. *J Comput Assist Tomogr* 1993; **17**: 582-9.

38. Hoh CK, Schiepers C, Seltzer MA, Gambhir SS, Silverman DH, Czernin J, et al. PET in oncology: will it replace the other modalities? *Semin Nucl Med* 1997; **27**: 94-106.

Radiol Oncol 2000; 34(1): 11-9.

Value of ^{18}F-FDG-PET in clinical management of patients with osteosarcoma

Karl H. Bohuslavizki[1], Susanne Klutmann[1], Jürgen Bruns[2], Sabine Kröger[1], Christian Bleckmann[1], Ralph Buchert[1], Dimitrij Dobrowolskij[2], Janos Mester[1], Malte Clausen[1]

Departments of [1]Nuclear Medicine and [2]Orthopedic Surgery, University Hospital Eppendorf, Hamburg, Germany

Background. *The aim of this study was to define the value of ^{18}F-FDG-PET in clinical management of patients with osteosarcoma based on current treatment regimen.*

Patients and methods. *A total of 18 patients (4 female, 14 male) aged from 14 to 63 years with primary osteosarcoma (n=6) or suspect for relapse of osteosarcoma (n=12) were investigated retrospectively. First, all patients underwent conventional diagnostic work-up, i.e. X-ray and MRI of the primary bone lesion, CT scan of the chest as well as conventional bone scan. In addition, whole-body PET-images were acquired on an ECAT EXACT 47 (921) with an axial field-of-view of 16.2 cm (Siemens, CTI) after intravenous injection of 370 MBq ^{18}F-FDG. All tumor-suspicious PET-findings were evaluated histologically. Results of histology, PET findings and conventional imaging were correlated on a lesion-by-lesion basis.*

Results. *^{18}F-FDG-PET clearly depicted all primary osteosarcomas in 6 patients and a relapse of osteosarcoma in two patients. In the remaining 10 patients histology could not confirm a relapse of osteosarcoma. Eight out of 18 patients showed further lesions with an abnormal ^{18}F-FDG-uptake. These lesions were predominantly located in the lung (n=5), in the skeleton (n=3), and in the inguinal region (n=1). Three of 8 patients had primary diagnosis of osteosarcoma and 5 were suspected to have tumor relapse. All lesions but the lesion of the inguinal region turned out to be metastases of osteosarcoma. However, therapeutic management must be taken into consideration when interpreting these encouraging results. Since the vast majority of patients are known to have micro metastases at the time of diagnosis, combined treatment consisting of neoadjuvant chemotherapy and surgical resection of the tumor is the standard treatment. Thus, ^{18}F-FDG-PET has no significant impact in primary diagnosis of osteosarcoma. However, there are several clinical settings in which patients might benefit from ^{18}F-FDG-PET since their treatment regimen might be altered, i.e. differentiation of tumor relapse versus post-therapeutic changes, differential diagnosis of lung masses in post-therapeutic follow-up and detection of disseminated metastatic spread after initial therapy.*

Conclusions. *^{18}F-FDG-PET had no significant impact in initial staging. Nevertheless, it might be helpful in several clinical settings following neoadjuvant chemotherapy and surgical treatment of the primary tumor.*

Key words: osteosarcoma, diagnosis, pathology, tomography, emission-computed, treatment outcome

Introduction

Osteosarcoma is the second most common malignancy of the skeleton after multiple myeloma. Its incidence is estimated to be about 2-3/10^6 with a characteristic occurrence between 5-25 years of age and a second peak incidence in the fifth and sixth decades.[1,2] The disease may be divided into two categories: primary and secondary osteosarcoma. The primary osteosarcoma predominantly affects the metaphyseal portion of the extremity bones. However, its fundamental nature has yet been unknown. In contrast, the secondary osteosarcoma is often related to Paget's disease, fibrous dysplasia or is associated with retinolastoma.[1,3] The majority of secondary osteosarcomas are located in the truncus, craniofacial or even extraskeletal.[1]

According to the clinical stage of the UICC from 1997, the patients were divided into six groups based on TNM-stage and histological grading (Table 1).[1,4] At the time of primary diagnosis, as much as 75% of all patients were classified as clinical stage IIb that defines a histological grade three to four of osteosarcoma extended to the periost, but with no evidence of lymph node and distant metastases.[1] However, in 85-90% of these patients, occult metastases must be presumed which are predominantly located in the lungs (about 80%).[1] Moreover, osteosarcoma frequently metastasizes to secondary bone sites, which occurs in nearly 20% of all patients with occult metastases. The prognosis of osteosarcoma was poor prior to the development of effective chemotherapy.[3] The therapeutic management of osteosarcoma was improved by applying more potent and more aggressive chemotherapy. Therefore, accurate staging and re-staging procedures have become more and more important in the diagnosis of osteosarcoma. In this context, positron emission tomography (PET) using ^{18}F-fluorine-deoxyglucose (^{18}F-FDG) has become the focus of ongoing research, *i.e.* determining the metabolic rates of sarcoma[3,5-8], monitoring the neoadjuvant therapy response[9] and differentiating active sarcomas from post-treatment changes.[10-13] Since the management of osteosarcoma has been significantly improved by the introduction of a reliable staging system,[1,4] the diagnostic and therapeutic outcome might benefit from metabolic imaging using ^{18}F-FDG.[14]

Therefore, the aim of this study was to define the impact of ^{18}F-FDG-PET on staging and re-staging of patients with osteosarcoma based on current treatment regimen.

Materials and methods

Patients

A total of 18 patients (4 female, 14 male) aged from 14 to 63 years with primary osteosarcoma (n=6) or suspect for relapse of osteosarcoma (n=12) were investigated retrospectively. The majority of the patients had tumor suspicious lesions on the lower extremities (n=7) or lumbar vertebras (n=8). The remaining three patients had tumor-suspicious lesions located on the upper extremities. First, all patients underwent conventional clinical work-up. Then, ^{18}F-FDG-PET was performed.

Clinical work-up

As part of the routine clinical work-up, all patients underwent morphological imaging, *i.e.* conventional X-ray and MRI of the primary lesion, CT scan of the chest as well as conventional bone scan. A biopsy of the tumor-suspicious bone lesion was performed in all patients.

Received 13 December 1999
Accepted 27 December 1999

Correspondence to: Dr. Karl H. Bohuslavizki, Department of Nuclear Medicine, University Hospital Eppendorf, Martinistr. 52, D-20246 Hamburg, Germany; Phone: +49 40 42803 4047; Fax: +49 40 42803 6775; E-mail: bohu@uke.uni-hamburg.de

Table 1. Clinical stage and occurence of malignant bone tumors according to UICC from 1997

Clinical stage	TNM	Grading	Occurence
IA	T1, N0, M0	G1, 2	10 %
IB	T2, N0, M0	G1, 2	
IIA	T1, N0, M0	G3, 4	< 5 %
IIB	T2, N0, M0	G3, 4	75 %
III	Not defined	0	0
IVA	any T, N1, M0	G1-4	< 1 %
IVB	any T, any N, M1	G1-4	10 %

PET scanning

The patients fasted for at least 12 hours prior to PET scanning in order to minimize blood insulin levels and glucose utilization of normal tissue. Whole-body emission images were acquired without attenuation correction 60min after intravenous injection of 370 MBq ^{18}F-FDG using an ECAT EXACT 47 (921) scanner (Siemens/CTI, Knoxville, USA) with an axial field-of-view of 16.2 cm. Patients were placed in the PET gantry feet first with both arms folded over the abdomen. Images were acquired for 4 min per bed position covering the feet up to the middle of the femurs. Then, the patients were repositioned in the gantry head first, and the second set of images was acquired from the brain down to the waist. Prior to the third acquisition set from the waist down to the lower extremities, patients were asked to empty the bladder in order to decrease urine activity. Emission data were reconstructed by filtered back projection using a Hanning filter with a cut-off frequency of 0.4 of Nyquist frequency. Thus, transaxial spatial resolution was approximately 12 mm in reconstructed images. PET-images were printed on transparency film (Helios 810, Sterling) using a linear gray scale with the highest activity displayed in black. Images were displayed with an upper threshold of five times of the mean activity in the lung. Standardized documentation included both 20 transversal and 20 coronal slices with a slice thickness of 13.5 mm each, and maximum-intensity-projections (MIPs) in the anterior, left lateral, right-anterior-oblique, and left-anterior-oblique view as published previously.[15]

Evaluation

Two independent nuclear medicine physicians interpreted PET-images visually. All tumor-suspicious PET-findings were biopsized and evaluated histologically. The results of histology, PET-findings and conventional imaging were compared on a lesion-by-lesion basis.

Results

A total of 8 patients showed an increased uptake of ^{18}F-FDG in the area of the tumor-suspicious lesion. This included all patients (n=6) suspected for primary osteosarcoma and two patients suspected for local relapse of osteosarcoma.

In 3 out of 6 patients suspicious for primary osteosarcoma, an increased ^{18}F-FDG-uptake was the only pathologic activity seen within the PET-image. In contrast, 3 out of these 6 patients showed additional lesions with an abnormal ^{18}F-FDG-uptake. These lesions were located in the lungs or in the skeleton, each in one of the first two patients. Moreover, one patient showed up with pathological lung uptake as well as with an additional focus site in the left inguinal region.

In 2 out of 12 patients suspected for tumor

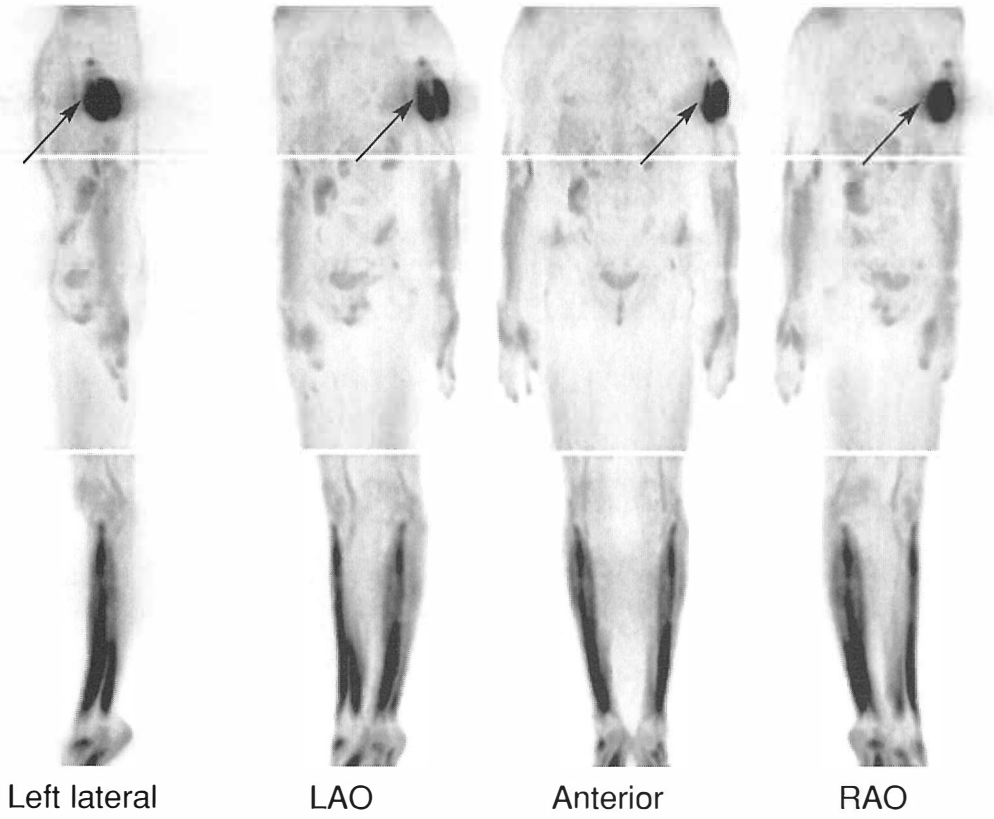


Figure 1. Maximum intensity projections of the truncus and the extremities of a patient with primary osteosarcoma of the left humerus. Note focal accumulation of ^{18}F-FDG at the primary tumour without any evidence of metastatic spread. However, the patient underwent adjuvant chemotherapy since microscopic metastatic foci must be presumed. Thus, ^{18}F-FDG-PET had no influence on treatment strategy.

relapse, ^{18}F-FDG-PET showed a focal increased uptake of ^{18}F-FDG at the tumor-suspected site of the bone. In 10 out of these 12 patients, PET was negative concerning the detection of a recurrent osteosarcoma. However, 5 out of 12 patients suspected for tumor relapse revealed further tumor-suspicious lesions with pathologic focally increased glucose metabolism. These lesions were located in the lungs (three patients) and in the skeleton (two patients). These 5 patients included one patient with positive ^{18}F-FDG-PET at the site suspected for tumor relapse, and also four patients with negative ^{18}F-FDG-PET concerning the detection of a tumor relapse.

Further evaluation of PET findings revealed that ^{18}F-FDG-PET clearly depicted all primary osteosarcomas in 6 patients (Figure 1, Figure 2) and a relapse of osteosarcoma in 2 patients. Thus, sensitivity of ^{18}F-FDG-PET was as high as 100 % for the detection both, of the primary tumor site and of relapsed osteosarcoma. In the remaining 10 patients, histology could not confirm a relapse of osteosarcoma. Thus, specificity of ^{18}F-FDG-PET was also 100%.

As far as metastases are concerned, ^{18}F-FDG-PET was true-positive in 2 out of 6 patients with histologically proved primary osteosarcoma (Figure 2). Thus, lung metastases were proved by the subsequent CT of

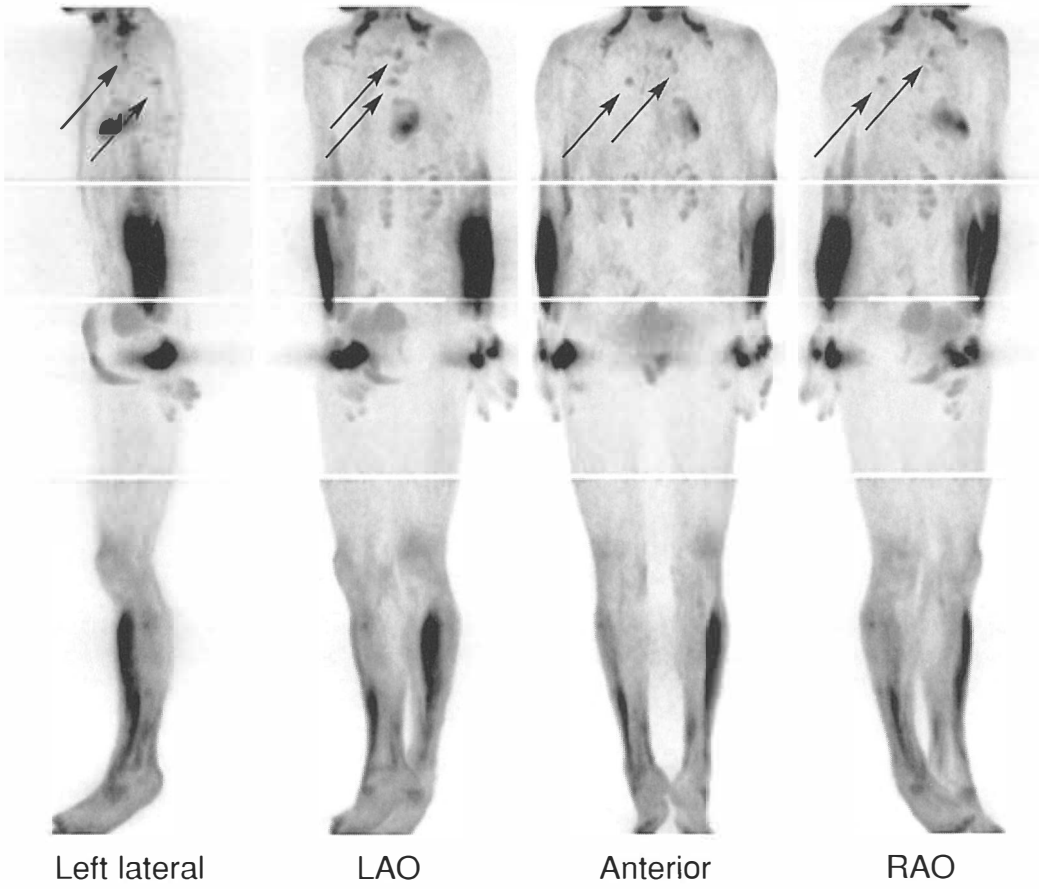


Figure 2. Maximum intensity projections of a patient with suspected relapse of osteosarcoma of the right tibia. Note focal accumulations of ^{18}F-FDG in both lobes of the lung without any evidence of local recurrence. This patient was staged as IVB. The patient underwent chemotherapy.

the chest and biopsy and a second site osteosarcoma was confirmed histologically, each in one of the two patients. Moreover, ^{18}F-FDG-PET was true-positive concerning the detection of lung metastases in one patient, but also false-positive in detecting inflammatory lymph nodes of the left inguinal region in the same patient. When comparing ^{18}F-FDG-PET to bone scintigraphy and CT of the chest, conventional imaging also proved all but one lesion each. Thus, based on PET findings, 3 out of 6 patients with primary osteosarcoma were classified being clinical stage IVB.

The further evaluation of metastatic lesions suspected by ^{18}F-FDG-PET revealed a clinical stage IVB in 5 patients suspected for relapse of osteosarcoma (Figure 3).

Discussion

Despite the fact that osteosarcoma represents only 0.1% of all tumor diseases, it is the second most common primary bone malignancy[1,2] after multiple myeloma. The pretherapeutic diagnostic work-up usually starts with a conventional X-ray of the tumor-suspi-

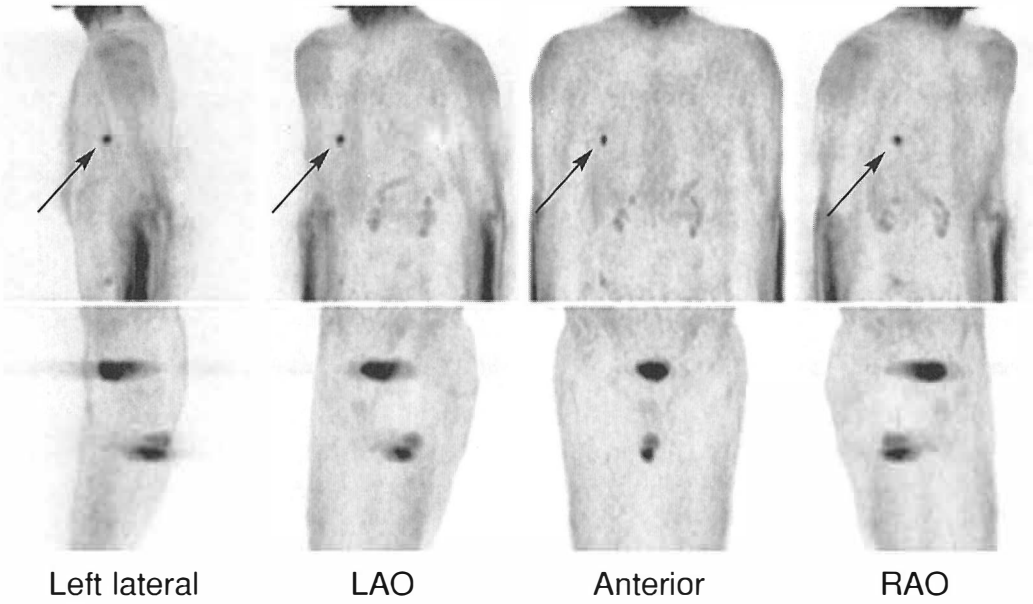


Figure 3. Maximum intensity projections of the truncus of a patient after therapy of an osteosarcoma of the right tibia and a newly diagnosed lung mass visualized by conventional X-ray in follow-up study. Note focal accumulation of ^{18}F-FDG in the right apical lobe confirming viable tumor tissue. Due to PET-findings the patient underwent surgery of the metastases and subsequent chemotherapy.

cious bone and subsequent biopsy.[16,17] MRI is performed in order to define the degree of penetration of the tumor into surrounding soft tissue as well as to estimate the local tumor infiltration into bone marrow.[14,17,18] Usually, CT of the chest and conventional bone scan are performed[19] since the metastases of osteosarcoma are known for their hematogenous route with predilection sites in the lungs and in the skeleton.

The standardized therapeutic management of osteosarcoma includes neoadjuvant chemotherapy followed by wide resection of the primary tumor.[20,21] Nowadays, limb-sparing procedures are more frequently performed than amputations.[22-24] However, as compared to ablative surgery procedures, limb sparing surgery has a 3-5 fold increased risk of local recurrence, which significantly worsens the prognosis.[25,26] Since both, the disease free survival rate and overall survival rate were shown to be higher, aggressive neoadjuvant chemotherapy was included into the routine therapeutic management of osteosarcoma.

The outcome of osteosarcoma has also been improved by the introduction of reliable staging systems.[27,28] Therefore, apart from conventional, well-standardized imaging procedures, radionuclide imaging using ^{18}F-FDG-PET became the focus of ongoing research by assessing its potential utility in sarcoma patients.[29] Nieweg and coworkers[8] examined 22 patients with malignant soft-tissue sarcomas. They found a sensitivity of 100% for the detection of the tumor. However, ^{18}F-FDG-PET seemed to be inappropriate in differentiating benign lesions from soft-tissue sarcomas of low or intermediate malignancy grades. Jones and coworkers[9] investigated the impact of ^{18}F-FDG-PET in treatment monitoring of soft-tissue and musculoskeletal sarcoma in nine patients. Their results suggested that ^{18}F-FDG-PET might be beneficial in this

special clinical setting. Garcia and coworkers[10] who found ^{18}F-FDG-PET helpful in differentiating active musculoskeletal sarcomas from post-treatment changes reported corresponding results. Moreover, ^{18}F-FDG-PET was investigated for differentiating various types of bone lesions by calculating the metabolic rate of glucose consumption.[30] However, a correlation between the metabolic rate and the biologic aggressiveness of bone tumors could not be shown.

In this study, the impact of ^{18}F-FDG-PET was defined in staging and re-staging of patients with osteosarcoma. It was shown that all primary osteosarcomas were detected by ^{18}F-FDG-PET revealing a sensitivity of 100%. Moreover, ^{18}F-FDG-PET was helpful in differentiating post-therapeutic changes from tumor relapse. As far as metastases were concerned ^{18}F-FDG-PET detected a hematogenous spread of the osteosarcoma in more than 50% of all patients investigated. However, the therapeutic management of patients with osteosarcoma must be taken into consideration when interpreting these encouraging results. The great majority of patients were classified as clinical stage IIB according to UICC at the time of initial diagnosis. However, only 10-15% of these patients can be reliably presumed to be free of distant metastases. In contrast, in 85-90% of these patients, hematogenous metastatic spread must be presumed, especially to the lungs. Thus, standardized treatment of osteosarcoma includes surgery of the primary bone tumor as well as the treatment of potential metastatic spread, *i.e.* neoadjuvant chemotherapy according to the Cooperative Osteosarcoma Study Group. Performing this treatment protocol, disease-free and overall survival rates after 4 to 5 years in patients with no detectable metastases increased from 20% in case of ablative surgery alone to 80% in case of additional neoadjuvant chemotherapy.[1,3,31] Since the detection of hematogenous spread has no clinical impact on influencing therapeutic management of patients with primary diagnosis of osteosarcoma at all, no clinical impact in incorporating ^{18}F-FDG-PET in this clinical setting can be expected.

However, there are some clinical settings in which ^{18}F-FDG-PET might be helpful to delineate further treatment regimen. First, ^{18}F-FDG-PET might be helpful in differentiating postoperative changes from tumor tissue in case of surgically treated osteosarcoma with prosthetic devices, since MRI is hampered due to technical reasons. Second, post-treatment follow-up consists of X-ray of the chest in half-year-intervals for the duration of about eight years post surgery. In case of newly diagnosed lung masses, ^{18}F-FDG-PET might be helpful to differentiate benign from malignant lesions.[32] This is especially important since the treatment of lung metastases is still potentially curative. Third, ^{18}F-FDG-PET might be helpful in the detection of hematogenous spread after the therapy of osteosarcoma. It was reported that patients with disseminated metastatic spread benefited from chemotherapy.[33] One third of all investigated patients showed a partial remission or a stable disease after combined chemotherapy. Thus, the detection of distant metastases significantly influences further therapeutic regimen.

Conclusions

In our series, ^{18}F-FDG-PET has no significant impact in initial staging of osteosarcoma. However, it may be helpful in several clinical settings following neoadjuvant chemotherapy and surgical treatment of the primary tumor, e.g. differentiation of tumor relapse versus posttherapeutic changes especially at the site of prosthesis, differential diagnosis of lung masses in posttherapeutic follow-up, and detection of disseminated metastatic spread after therapy.

References

1. Winkler K, Bielack S. Osteosarkom. In: Seeber S, Schütte J, editors. *Therapiekonzepte Onkologie.* Berlin: Springer; 1998. p. 830-54.

2. Campanacci M. *Bone and soft tissue tumors.* New York: Springer; 1986.

3. Krane SM, Schiller AL. Hyperostosis, neoplasms, and other disorders of bone and cartilage. In: Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL, editors. *Harrison's principles of internal medicine.* New York: McGraw-Hill, Inc; 1994. p. 2193-201.

4. Unni KK. Osteosarcoma of the bone. In: Unni KK, editor. *Bone tumors.* New York: Churchill Livingstone; 1988. p. 107-33.

5. Eary JF, Mankoff DA. Tumor metabolic rates in sarcoma using FDG-PET. *J Nucl Med* 1997; **39**: 250-54.

6. Eary JF, Conrad EU, Bruckner JD, Folpe A, Hunt KJ, Mankoff DA, et al. Quantitative [F-18]fluorodeoxyglucose positron emission tomography in pretreatment and grading of sarcoma. *Clin Cancer Res* 1998; 4: 1215-20.

7. Lodge MA, Lucas JD, Marsden PK, Cronin BF, O'Doherty MJ, Smith MA. A PET study of ^{18}FDG uptake in soft tissue masses. *Eur J Nucl Med* 1999; **26**: 22-30.

8. Nieweg OE, Pruim J, van Ginkel RJ, Hoekstra HJ, Paans AM, Molenaar WM, et al. Fluorine-18-fluorodeoxyglucose PET imaging of soft-tissue sarcoma. *J Nucl Med* 1996; **37**: 257-61.

9. Jones DN, McCowage GB, Sostman HD, Brizel DM, Layfield L, Charles HC, et al. Monitoring of neoadjuvant therapy response of soft-tissue and musculoskeletal sarcoma using fluorine-18-FDG PET. *J Nucl Med* 1996; **37**: 1438-44.

10. Garcia JR, Kim EE, Wong FCL, Korkmaz M, Wong WH, Yang DJ, et al. Comparison of fluorine-18-FDG PET and technetium-99m-MIBI SPECT in evaluation of musculoskeletal sarcomas. *J Nucl Med* 1996; **37**: 1476-79.

11. Hicks RJ. Nuclear medicine techniques provide unique physiologic characterization of suspected and known soft tissue and bone sarcomas. *Acta Orthop Scand Suppl* 1997; **273**: 25-36.

12. Kole AC, Nieweg OE, van Ginkel RJ, Pruim J, Hoekstra HJ, Paans AM, et al. Detection of local recurrence of soft-tissue sarcoma with positron emission tomography using [^{18}F]fluorodeoxyglucose. *Ann Surg Oncol* 1997; 4: 57-63.

13. Lucas JD, O'Doherty MJ, Wong JC, Bingham JB, McKee PH, Fletcher CD, et al. Evaluation of fluorodeoxyglucose positron emission tomography in the management of soft-tissue sarcomas. *J Bone Joint Surg Br* 1998; **80**: 441-7.

14. Seeger LL, Gold RH, Chandnani VP. Diagnostic imaging of osteosarcoma. *Clin Orthop* 1991; **270**: 254-63.

15. Bleckmann C, Buchert R, Schulte U, Lorenzen J, Bohuslavizki KH, Mester M, et al. Onko-PET: Bildanalyse mittels Monitor versus standardisierter Filmdokumentation. *Nuklearmedizin* 1999; **38**: 56-60.

16. Priolo F, Cerase A. The current role of radiography in the assessment of skeletal tumors and tumor-like lesions. *Eur J Radiol* 1998; **27 (Suppl 1)**: S77-85.

17. Dominkus M, Kainberger F, Lang S, Kotz R. Primšr maligne Knochentumoren. *Radiologe* 1998; **38**: 472-82.

18. Sundaram M. The use of gadolinium in the MR imaging of bone tumors. *Semin Ultrasound CT MR* 1997; **18**: 307-11.

19. Focacci C, Lattanzi R, Iadeluca ML, Campioni P. Nuclear medicine in primary bone tumors. *Eur J Radiol* 1998; **27 (Suppl 1)**: S123-31.

20. Davis AM, Bell RS, Goodwin PJ. Prognostic factors in osteosarcoma: a critical review. *J Clin Oncol* 1994; **12**: 423-31.

21. Friedman MA, Carter SK. The therapy of osteogenic sarcoma: current status and thoughts for the future. *J Surg Oncol* 1972; 4: 482-510.

22. Kotz R, Salzer M. Rotation-plasty for childhood osteosarcoma of the distal part of the femur. *J Bone Joint Surg Am* 1982; **64**: 959-69.

23. Kotz R, Ritschl P, Trachtenbrodt J. A modular femur-tibia reconstruction system. 1986; **9**: 1636-52.

24. Windhager R, Millesi H, Kotz R. Resection-replantation for primary malignant tumours of the arm. An alternative to fore-quarter amputation. *J Bone Joint Surg Br* 1995; **77**: 176-84.

25. Glasser DB, Lane JM, Huvos AG, Marcove RC, Rosen G. Survival, prognosis and therapeutic response in osteogenic sarcoma. *Cancer* 1992; **65**: 698-708.

26. Picci P, Sangiorgi L, Rougraff BT, Neff JR, Casadei R, Campanacci M. The relationship of chemotherapy-induced necrosis and surgical margins to local recurrence in osteosarcoma. *J Clin Oncol* 1994; **12**: 2699-705.

27. Enneking WF, Spanier SS, Goodman MA. Current concepts review. The surgical staging of musculoskeletal sarcoma. *J Bone Joint Surg Am* 1980; **62**: 1027-30.

28. Enneking WF, Spanier SS. A system for the surgical staging of musculoskeletal tumors. *Clin Orthop* 1980; **153**: 106-20.

29. Kern KA, Brunetti A, Norton JA, Chang AE, Malawer MM, Lack E, et al. Prediction of tumor grad in human sarcomas by positron emission tomogrphy. *J Nucl Med* 1988; **29**: 181-6.

30. Kole AC, Nieweg OE, Hoekstra HJ, van Horn JR, Koops HS, Vaalburg W. Fluorine-18-fluorodeoxyglucose assessment of glucose metabolism in bone tumors. *J Nucl Med* 1998; **39**: 810-5.

31. Winkler K, Bieling P, Bielack S, Delling G; Dose C; Jurgens-H, et al. Local control and survival from the cooperative osteosarcoma study group studies of the german society of pediatric oncology and the vienna bone tumor registry. *Clin Orthop* 1991; **270**: 79-86.

32. Tse N, Hoh C, Hawkins R, Phelps M, Glaspy J. Positron emission tomography diagnosis of pulmonary metastases in osteogenic sarcoma. *Am J Clin Oncol* 1994; **17**: 22-5.

33. Fuchs N, Bielack S, Bieling P, Winkler K. Phase II study of Carboplatin (CB) and etoposide (VP 16) in pretreated disseminated osteosarcoma (OS). 7. Meeting of the European Musculo-Skeletal Oncology Society (EMSOS); Amsterdam, Oct 6-7; 1994.

Radiol Oncol 2000; 34(1): 21-5.

Combined modality treatment with organ preservation in invasive bladder cancer

Tanja Čufer

Institute of Oncology Ljubljana, Slovenia

Background. *The standard treatment for muscle-invasive bladder cancer is still radical cystectomy. However despite mutilating surgery half of the patients eventually develop metastatic disease and subsequently die of the disease. In view of these problems, a bladder-sparing approach using multi-modality treatment with transurethral resection (TUR), irradiation and chemotherapy has been tested in this disease. So far, the results published by five groups, showed that the survival rates of patients treated by multi-modality therapy with a bladder sparing approach, based on the response to initial TUR and chemotherapy or chemoradiotherapy, are comparable to cystectomy series, while also offering a 60% to 70% chance of maintaining a functioning bladder. The probability of survival with bladder preserved was found to be around 40% at 5-years. The best predictor of successful multi-modality treatment with bladder preservation seems to be a complete response to initial therapy and a close cystoscopic surveillance is obligatory to allow for cystectomy at earliest opportunity, if necessary.*

Conclusions. *Multimodality treatment with selective bladder preservation offers a chance for long term cure and survival equal to radical cystectomy in muscle invasive bladder cancer, while also offering a chance of maintaining a normally functioning bladder. It is expected, that the identification of biological factors with a predictive value for successful chemoradiation will allow for a better selection of patients who could benefit from this treatment in future.*

Key words: bladder neopasms-therapy, combined modality therapy; cystectomy, bladder preservation

Introduction

The standard treatment for muscle-invasive bladder cancer is radical cystectomy and bilateral pelvic lymph node dissection which offers a high pelvic cure rate (85% - 90%). However the muscle invasive bladder cancer is a systemic disease and half of patients eventually develop metastatic disease and subsequently die of the disease.[1]

Eradication from the bladder of muscle invading tumor is possible in some patients using conservative surgery alone, radiation therapy alone or systemic chemotherapy alone. However, each of these modalities alone gives only a 20% to 40% chance of loco-

Received 7 December 1999

Accepted 18 December 1999

Correspondence to: Assist. Prof. Tanja Čufer, Ph.D., M.D., Institute of Oncology, Zaloška 2, 1000 Ljubljana, Slovenia; Phone: +386 61 1314 225; Fax: +386 61 1314 180; E-mail: TCufer@onko-i.si

regional control of disease, which is poor when compared to radical cystectomy, although, due to high rate of metastatic spread of disease, similar survival rates can be demonstrated.[2] When two of these modalities are used together, higher rates of local control can be achieved.[2]

Combined modality treatment with bladder preservation

When organ preservation is considered a treatment option, the primary goal is the cure of patient and the secondary goal is sparing of the functional organ, without compromis-

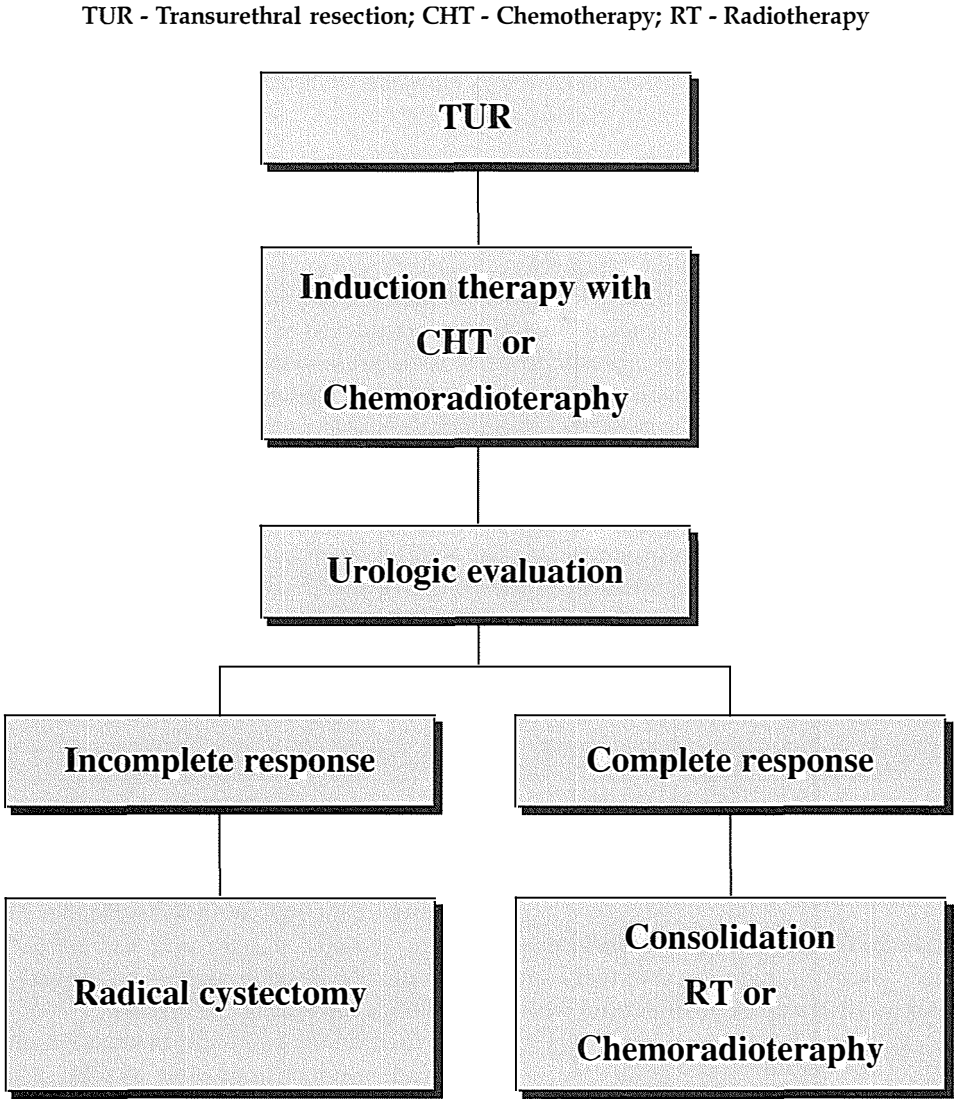


Figure 1. Algorithm for evaluation and treatment of muscle-invasive bladder cancer with a selective bladder preservation.

ing the survival. A multimodality approach using a combination of transurethral resection (TUR) followed by sequential or concomitant chemotherapy and radiotherapy seems to be such an option in the treatment of muscle invasive bladder cancer. With a combination of TUR and chemoradiation a much higher complete response rates (around 70%) can be achieved than by either therapeutic modality alone[2] and the overall survival rates, achieved by multimodality treatment are similar to the survival rates after radical cystectomy even though they have never been compared in randomized fashion.[2] Conservative surgery i.e. TUR reduces the dose of radiotherapy required for complete tumor eradication in bladder and chemotherapy addresses microscopic disease both locally and systemically. Over the last six years the argument for bladder preservation with a multimodality approach has been strengthened by reports from five centers: Massachusetts General Hospital (MGH),[3,4] the Radiation Therapy Oncology Group (RTOG),[5,6] the University of Paris,[7] the University of Erlangen[8,9] and the Institute of Oncology Ljubljana.[10,11] In all of the centers TUR was followed by cisplatin-based chemotherapy with subsequent or concomitant radiation (Figure 1). In four out of five centers i.e. MGH, RTOG, Paris and Ljubljana a selection for bladder preservation was based on urologic evaluation of response to induction TUR and chemotherapy alone[10,11] or chemoradiation[3-7] and in the absence of a complete response, radical cystectomy was performed before the bladder had received radical doses of radiation. The rate of complete response, obtained by multimodality treatment in these studies ranges from 53% to 80% which is more than complete response rates obtained with either therapy alone; a bladder preservation was possible in around 70% of patients; the overall survival at 4 to 5 years ranges from 52% to 62%, which is similar or perhaps even better as in any of the reported cystectomy series and the probability of survival with bladder preserved is around 40% at 5 years[3-11] (Table 1). The patients who have a complete response to initial therapy do much better. The overall survival of complete responders is over 70% at 5 years and is much better than the overall survival of the patients who did not respond completely and had an attempt of salvage cystectomy.[7,10,11]

The best predictor of successful bladder preservation seems to be a complete response to initial therapy. There are also other tumor or patient characteristics which were found to be independent prognostic factors such as

Table 1. Results of multimodality treatment with organ preservation

Series (References)	No. of patients	Bladder CR rates (%)	Bladder spared (%)	Overall survival (%)	Survival with bladder spared (%)
MGH (3,4)	106	70	58	52 (5-year)	43 (5-year)
RTOG (5,6)	91	75	60	62 (4-year)	44 (4-year)
University of Paris (7)	54	74	Not applicable	59 (3-year)	Not applicable
Institute of Oncology, Ljubljana (10,11)	105	62	71	58 (4-year)	45 (4-year)
University of Erlangen (8,9)	139	80	79	52 (5-year)	40 (5-year)

performance status, tumor stage, presence of tumor associated carcinoma in situ, completeness of TUR and tumor associated hydronephrosis.[3,4,7,8,10,11] Selection of patients, according to the response to induction therapy allows for prompt cystectomy if residual disease is found and a close cystoscopic surveillance, all patients treated by multimodality bladder sparing approach must be willing to go through, allows for cystectomy at the earliest opportunity, if necessary.

Following multimodality bladder preservation approach up to 40% of patients develop bladder recurrences[4,6,7,9,11] but most of them are superficial tumors which can be successfully treated by TUR and intravesical agents.

Many urologists are concerned that conserved irradiated bladder function poorly. In the large study of Erlangen of more then 200 patients with bladders preserved, only three required cystectomy[9] and in the update of MGH including induction by TUR and two cycles of chemotherapy followed by concomitant chemoradiation, no patient among 76 patients with bladders preserved had to undergo a cystectomy for multimodality treatment related morbidity.[12] The same group reported excellent tolerance in 21 women who were successfully treated by multimodality approach and bladder preservation, at a median follow up of 56 months all patients were continent and without dysuria and hematuria; bladder capacity and function remained unchanged in 91% of patients.[13] Similarly, the Paris group demonstrated no major impact of later complications on social and professional life of the patients treated with a multimodality bladder sparing approach.[14] Even though the percentage of the patients which reported urinary symptoms related to decreased bladder capacity was higher (40%) than in MGH group, they concluded that the quality of life after combined modality therapy with bladder sparing appears to be superior to that obtained in the best enterocystoplasty series. In addition, Lynch et al found no difference in urinary and rectal function between 72 post-radiotherapy patients, mostly males, and a similar control group of patients with no prior history of bladder or bowel disease.[15]

Conclusions

Multimodality treatment with selective bladder preservation offers a chance for long term cure and survival equal to radical cystectomy in muscle invasive bladder cancer, while also offering a 60% to 70% chance of maintaining a normally functioning bladder. The probability of survival with bladder preserved is around 40% at 5 years. The ideal candidates for such a treatment are patients with a clinical stage T2 disease without tumor associated hydronephrosis in which radical TUR is possible and in which complete response to induction therapy is achieved. Patients with more locally advanced tumors are less successfully treated using this approach, however, there are no data to suggest that patients with more advanced disease are in any way disadvantaged by preoperative chemoradiotherapy as and attempt for bladder conservation. Multimodality treatment with bladder preservation is now a reasonable alternative to radical cystectomy when undertaken by and experienced multimodality team of urologists, medical oncologists and radiation oncologists. The strategies that are expected to further improve the treatment results and quality of life of patients are the incorporation of promising new chemotherapeutic agents such as gemcitabine and paclitaxel and accelerated hyperfractionated radiation into treatment plan and the identification of biological characteristics of primary tumor with a predictive value for a successful chemoradiation , such as p53, which would allow for a better selection of patients who could benefit from multimodality treatment with selective bladder preservation.

References

1. Whitmore WF. Management of invasive bladder neoplasms. *Semin Urol* 1983; **1**: 4-10.

2. Shipley WU, Zietman AL, Kaufman DS, Althausen AF, Heney NM. Invasive bladder cancer: Treatment strategies using transurethral surgery, chemotherapy and radiation therapy with selection for bladder conservation. *Int J Radiat Oncol Biol Phys* 1997; **39**: 937-43.

3. Kaufman DS, Shipley WU, Griffin PP, Henery NM, Althausen AF, Efird JT. Selective preservation by combination treatment of invasive bladder cancer. *N Engl J Med* 1993; **329**: 1377-82.

4. Kachnic LA, Kaufman DS, Heney NM, Althausen AF, Griffin PP, Zietman AL, et al. Bladder preservation by combined modality therapy for invasive bladder cancer. *J Clin Oncol* 1997; **15**: 1022-9.

5. Tester W, Porter A, Asbell S. Combined modality program with possible organ preservation for invasive bladder carcinoma: Results of RTOG protocol 85-12. *Int J Radiat Oncol Biol Phys* 1993; **25**: 783-90.

6. Tester W, Porter A, Heaney J, Venner P, Whittington R, Byhardt R, et al. Neoadjuvant combined modality therapy with possible organ preservation for invasive bladder cancer. *J Clin Oncol* 1996; **14**: 119-26.

7. Housset M, Maulard C, Chretlen YC, Dufour B, Delanian S, Huart J, et al. Combined radiation and chemotherapy for invasive transitional cell carcinoma of the bladder. A prospective study. *J Clin Oncol* 1993; **11**: 2150-7.

8. Dunst J, Sauer R, Schrott KM, Wittekind, Altendorf-Hoffman A. Organ-sparing treatment of advanced bladder cancer: A 10-year experience. *Int J Radiat Oncol Biol Phys* 1994; **30**: 261-6.

9. Sauer R, Birkenhake S, Kuhn R, Wittekind C, Schrott KM, Martus P, et al. Efficacy of radiochemotherapy with platin derivatives compared to radiotherapy alone in organ-sparing treatment of bladder cancer. *Int J Radiat Oncol Biol Phys* 1998; **40**: 121-7.

10. Cervek J, Cufer T, Kragelj B, Zakotnik B, Stanonik M. Sequentional transurethral surgery, multiple drug chemotherapy and radiation therapy for invasive bladder carcinoma: Initial report. *Int J Radiat Oncol Biol Phys* 1993; 25: 777-82.

11. Cervek J, Cufer T, Zakotnik B, Kragelj B, Borstnar S, Matos T, et al. Invasive bladder cancer: Our experience with bladder sparing approach. *Int J Radiat Oncolo Biol Phys* 1998; **41**: 273-8.

12. Shipley WU, Winter KA, Kaufman DS, Lee WR, Heney NM, Tester WR, et al. Phase III trial of neoadjuvant chemotherapy in patients with invasive bladder cancer treated with selective bladder preservation by combined radiation therapy and chemotherapy: Initial results of radiation therapy oncology group 89-03. *J Clin Oncol* 1998; **16**: 3576-83.

13. Kachnic LA, Shipley WU, Griffin PP, Zietman AL, Kaufman Ds, Althausen AF, et al. Combined modality treatment with selective bladder conservation for invasive bladder cancer: Long-term tolerance in the female patient. *Cancer J Sci Am* 1996; **2**: 79-84.

14. Lynch WJ, Jenkins BJ, Fowler CG. The quality of life after radical radiotherapy for bladder cancer. *Br J Urol* 1992; **70**: 519-21.

15. Housset M, Fitreman C, Dufour B, Huart J, Chretien Y, Mejean A, et al. Quality of life after combined radiation and chemotherapy with organ sparing for invasive bladder cancer [abstract]. *Int J Radiat Oncol Biol Phys* 1998; 42 Suppl **1**: 287.

Radiol Oncol 2000; 34(1): 27-33.

Primary non-Hodgkin's lymphoma of bone: treatment and outcome

Gary M. Proulx[1], Ahmed Y. El-Agamawi[3], R.Jeffery Lee[1], James B. Orner[1], Myron Czuczman[2], Phillip McCarthy[2], Zale Bernstein[2], and Steve Bernstein[2]

[1]Department of Radiation Oncology and [2]Medical Oncology, SUNY at Buffalo School of Medicine and Biomedical Sciences and Roswell Park Cancer Institute, University of Buffalo, Buffalo, NY, USA, [3]Department of Oncology and Nuclear Medicine Al-Azhar University Cairo, Egypt

Background. *This study was performed to assess the characteristics, management and outcome of patients with primary Non-Hodgkin's lymphoma of the bone that were diagnosed and treated at Roswelll Park Cancer Institute.*

Patients and methods. *Eighteen patients were presented to us with the criteria, for diagnosis with histopathological and immunohistochemical confirmation, of Non-Hodgkin Lymphoma with adequate bone biopsy between 1970-1996. Twelve patients received combined treatment with anthracycline based chemotherapy and localized radiation, 5 received localized radiation alone and 1 had chemotherapy alone.*

Results. *There were fifteen patients with stage IEA, and three patients were staged IVEA. The histopathological examination revealed 13 patients with intermediate - grade diffuse, large cell, 2 patients were intermediate-grade diffuse mixed small and large cells, 2 patients were intermediate-grade diffuse small cleaved cell type, and 1 patient had an anaplastic large cell type. Five patients treated with combined treatment and one patient treated with localized radiation alone are without evidence of the disease at a median follow-up of 13 years. Six patients who had combined treatment with radiation and chemotherapy and 3 patients who had radiation alone died from progression of their disease. Two died from other causes, one with combined treatment with radiation and chemotherapy and the other with radiation alone. One patient with combined treatment was disease free at one year but was lost to follow -up.*

Conclusions. *This study suggests that patients presenting with early-stage primary Non-Hodgkin's Lymphoma of bone can be treated with curative intent with the combination treatment of localized radiation and systemic chemotherapy. However, confirmation needs to be verified in larger and prospective studies.*

Key words: lymphoma non-Hodgkin-drug therapy-radiotherapy, bone neoplasms, anthracyclins; treatment outcome

Received 4 December 1999
Accepted 29 December 1999

Correspondence to: Gary M. Proulx MD, Hematology & Oncology Associates at CNY, 5700 W. Genesee St., STE 10S, Camillus, N.Y. 13031; Phone: +1 315 631-2000; Fax: 1 315 631 2007; E mail: gproulx @hoacny.com

Introduction

Primary non-Hodgkin's lymphoma of the bone (PLB) is a rare form of lymphoma. With such rare neoplasms, information on treatment and outcome is difficult to obtain in a controlled, prospective manner.[1,2] Treatment is generally based on the extent of disease. Patterns of failure for early stage disease have been thought to be more local than distant. However, more recently, these patients are thought to have a risk of both local and distant failure. In fact, lymphoma presenting in bone may often be a sign of disseminated disease. As a result, initial treatment with curative intent has evolved from local irradiation alone to combination local radiation and systemic chemotherapy.[3-6] This study relates our experience in the diagnosis and treatment of this rare clinical entity.

Primary malignant lymphoma of bone presents both diagnostic and therapeutic problems.[7] The Non-Hodgkin's lymphomas encompass over 29 types of lymphoma. The group of cancers under the general term lymphoma is quite broad. Over the years, different classification systems have been used to differentiate lymphomas including Rappaport classification (used until the 70's) the National Cancer Institute Working Formulation and most recently, the National European-American Lymphoma Classification (REAL) system.[8-10] Additionally primary non-Hodgkin's Lymphoma of bone (PLB) constitutes approximately 5% of all extranodal non-Hodgkin's Lymphoma (NHL) and 7% of primary bone tumors.[11] With PLB being rare disease, there has been paucity of publications on its treatment and outcome while the clinicopathologic staging has changed several times over the last 30 years.

Patients and methods

Patients

Between January 1, 1970 and December 31. 1996, eighteen patients met the criteria for a histologically confirmed diagnosis of non-Hodgkin's Lymphoma of the bone (PLB) by biopsy of the bony lesion. These were then classified according to the Working Formulation and The Revised European-American Lymphoma (REAL) classification.[9,10] To have a diagnosis of PLB, patients needed a primary focus in a single bone, with or without locoregional adenopathy. However, multiple bony lesions were allowed if there was no evidence of lymphomatous involvement outside of bone.

Staging

All patients were staged according to the Ann Arbor Staging Classification.[13] Patients were evaluated with a complete history and physical examination. Staging investigations included a minimum of a chest X-ray with X-rays of the bone lesion(s) and /or a skeletal survey. Ten patients had computed tomography (CT) scans of the chest, abdomen, and pelvis; three patients had gallium scans, one patient had magnetic resonance imaging (MRI); all patients had a bone marrow aspirate and / or biopsy.

Treatment and assessment of the response

Twelve patients received combined treatment with anthracycline based chemotherapy and localized radiation, 5 received localized radiation alone using 6 Megavoltage with doses range from 2000 cGy to 5000 cGy in 200 cGy per fraction and 5 treatments per week, and 1 had chemotherapy alone as their initial curative treatment.

A complete response was defined as disappearance of all evidence of lymphoma documented by a normal physical examination,

Table 1. Patients and tumor characteristics

Patient	Gender	Age	Site	Stage	Histology
1	F	49	Ulna, Metatarsal	IVE	DSC
2	M	44	L-spine	IE	DLC
3	F	68	Sacrum	IE	ALC
4	M	24	Femur	IE	DLC
5	M	21	Tibia	IE	DMC
6	M	64	Ileum	IE	DLC
7	M	24	Femur	IE	DLC
8	M	35	Femur	IE	DLC
9	M	29	Scapula	IE	DLC
10	F	68	L-spine	IE	DLC
11	F	57	T-spine	IE	DLC
12	M	32	Humerus	IE	DLC
13	M	84	Clavicle, C-spine	IVE	DLC
14	M	70	Sternum	IE	DMC
15	F	87	L-spine	IE	DLC
16	F	27	Ileum	IE	DLC
17	F	73	L-spine	IE	DSC
18	F	41	Femur, Ileum	IVE	DLC

M = male; F = female; DLC = diffuse large cell, DSC = diffuse small cell, DMC = diffuse mixed small and large cell, ALC = anaplastic large cell.

blood tests, and radiographic imaging. A partial response was defined as a decrease in more than 50% in size with residual radiographic abnormalities, which are sometimes considered as fibrosis or bone remodeling after treatment. No response was defined as less than a 50% reduction in size of known disease. Progressive disease was defined as an increase in the size of known disease or the appearance of new sites of involvement. Survival was measured from the date of diagnosis.

Results

Patients characteristics

Of the 18 patients, there were 8 females and 10 males. The mean and median age was 50 years and 49 years respectively (range 21 - 87). Fifteen patients were staged as IEA, 3 patients were staged IVEA. The most common presenting symptom was pain at the involved site. None of the patients presented with B symptoms. The histopathology included 13 patients with intermediate-grade diffuse large cell, (Diffuse large B-cell, in REAL) 2 patients were intermediate-grade diffuse mixed small and large cell, (Angioimmunoblastic T -cell lymphoma, in REAL) 2 patients were intermediate-grade diffuse small cleaved cell type, (Angiocentric lymphoma, in REAL) and 1 patient had an anaplastic large cell type (Anaplastic large cell (CD 30+), in REAL). Sites of involvement were long bone (7), flat bone (4), spine (6) and pelvis (5). Two patients had more than one bone involved. Patients and tumor characteristics are summarized in Table 1.

Outcome

Of the eighteen patients, 6 patients are without evidence of disease at a median follow-up of 13 years; 5 of these 6 patients received combined treatment with chemotherapy and radiation, while 1 patient had only localized radiation. Nine out of 18 patients died from progression of their disease; 2 were stage IVEA; 3 of these 9 had radiation alone as initial treatment, and the other 6 had combined modality treatment with radiation and chemotherapy. Of the remaining 3 patients (2 of whom had combined treatment with radiation and chemotherapy, and 1 with radiation alone), 2 died from other causes and were found to be free of disease; 1 patient was disease free at one year but was lost to follow-up. Table 2 outlines the treatment given and outcome after completion of treatment.

Discussion

Our series had similar patient and tumor characteristics as those with PLB reported in the literatures. The majority of patients however appear to have B cell tumors with inter-

Table 2. Treatment and outcome

Patient No.	Radiation cGy/#frxs	Chemotherapy Regimen x (#courses)	Initial Response	Status
1	3750/15	CHOP X 6	CR	ANED
2	4500/25	CHOP X 6	CR	DOD
3		CHOP X 8	CR	DWD
4	4000/20	MACOP- B X 6	CR	ANED
5	4000/20	CHOP X 3	CR	ANED
6	3600/18	CHOP X 6	CR	ANED
7	4400/22		CR	ANED
8	4000/20	CHOP X 6	CR	ANED
9	5000/25	CYTOXAN / PREDNISONE	CR	DOD
10	3200/16		CR	DOD
11	4000/20		CR	DOD
12	4000/20	CHOP X 6	CR	DOD
13	3000/10	CYTOXAN / PREDNISONE	CR	ANED*
14	3000/10	MCOP X 6	CR	DOD
15	2000/10		CR	DWD
16	4000/20	MCOP X 6	PR	DOD
17	3600/18		CR	DOD
18	3000/10	CYTOXAN	CR	DOD

cGy = centigray; frxs =fractions; ANED = alive without evidence of disease; ANED*= alive without evidence of disease but lost to follow-up at 1 year; DOD = dead of disease; DWD = dead without disease; CR = complete response; PR = partial response; MCOP = methotrexate, cyclophosphamide, vincristine, prednisone; CHOP = cyclophosphamide, doxorubicin, vincristine, prednisone; MACOP-B = methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin

mediate-grade mixed small and large cell lymphoma and the intermediate-grade diffuse large cell lymphoma.[12,14,15] A minority in literatures were a small non-cleaved (non-Burkitt's) lymphoma and an immunoblastic lymphoma[7] according to the Working Formulation. Our series had 13 patients with intermediate-grade diffuse large cell type; 2 patients with intermediate-grade diffuse mixed small and large cell; 2 with intermediate -grade diffuse small-cleaved cell; and 1 patients with an anaplastic large cell type.

The peak incidence of PLB is reported to be in the fifth decade with the median age 44 years and a male predominance. The most common symptom was localized bony pain and swelling with the majority of the cases involving the lower half of the body.[16,17]

Suryanarayan et al.,[18] studied the group of 31 children of PLB and reported the primary sites were the femur (9), tibia (8), with the remainder involving the upper half of the body. Twenty-one of the cases were classified as intermediate-grade large cell lymphoma. In our study, the age was ranging from 21 years to 87 years with the median age was 50 years. The most common presenting symptom was pain at the involved site. None of our patients had B symptoms and 12 patients had disease in the lower half of the body (4 flat bone, 6 spine).

Most pervious studies indicate the majority of the patients with PLB present with early stages. Vassallo et al.,[19] reported 7 of 8 cases presenting with stage IE disease, whereas 18 of 21 cases reported by Mendehall et al.,[20] presented with stage IE or IIE disease. Additionally two other studies, one by Baar et al.,[7] found 13 out of 17 patients with localized, stage IEA disease and the other by McIntyre et al.,[21] reported 18 out of 22 with stage IE disease. Similar to these studies, our series had 15 patients with stage IEA and only 3 patients with stage IVEA.

The rarity of non-Hodgkin's lymphoma of the bone and the misleading histologic features because of similarity to other small round cell tumors can cause considerable difficulty in diagnosing this entity. Additionally assessment of response after treatment is challenging because of the persistent radiologic abnormalities. Multiple bone biopsies are generally required as result. Hatori et al.,[22] concluded that flow cytometry, in conjunction with morphologic and other molecular techniques, can provide a rapid and accurate means of diagnosing of this disease. Ascoli et al,[23] found that clinical data, radiographic findings and cytohistological correlation led to a final diagnosis of primary non Hodgkin's lymphoma of the bone, confirmed by immunopositive staining for leukocyte common antigen CD45 and B-cell associated antigen CD20.

The best treatment of primary NHL of the bone remains unknown. All patients in our

series with stage IV and 9 patients with stage IEA received localized radiation and chemotherapy (with or without anthracycline based regimens). Our results with such treatment appear similar to that reported by others.

Ferreri et al.,[24] studied 20 patients with PLB, 12 patients with monostotic disease (stage I and II) and 8 patients with polyostotic disease (stage IV), all patients received adriamycin-containing chemotherapy in association with radiation therapy to 45 Gy. All patients with monostotic disease achieved complete remission (CR). After a median follow-up period of 50 months, 10 patients were alive and relapse-free, 1 other patient was alive following relapse and 1 patient died with relapse free.

The survival rate for the patients in this study were 92% at 50 months but the survival rate with the patients staged IV was 25% at 40 months. They concluded that the treatment of patients with early stage PLB with adriamycin- containing chemotherapy and whole bone irradiation to 45 Gy, prevented local relapse and produce more favorable outcome. Baar et al,[16] reported on 17 patients treated for early-stage PLB with combined modality treatment with chemotherapy and radiotherapy. They noted that 13 of 17 (76%) patients were disease free at a median of 29 months. Of these 13 patients, all patients received chemotherapy with anthracycline based regimen with 9 receiving adjuvant radiotherapy to the primary site of the disease. Of the 4 patients who relapsed, 2 received chemotherapy alone; 1 received radiotherapy alone; and 1 received chemotherapy plus radiation to the primary site. The authors concluded that combined treatment with chemotherapy and radiation improves outcome over single modality treatment of either radiation or chemotherapy for localized Non-Hodgkin's lymphoma of the bone.

One study reported by Tondini et al.,[25] demonstrated a 5-year relapse-free survival and total survival of 83% for patients with stage I and II disease with a short chemotherapy course of cyclophosphamide, doxorubicin, vincristine, and prednisone followed by locoregional irradiation.

In another study, Bacci et al.,[26] observed 30 patients treated for PLB over a 10- year period. Four patients were treated with radiation at a dose of 3000-4500 cGY in 15-23 fractions to the whole bone with the boost of 1000-1500 cGy in 5-8 fractions to the primary lesion and 26 patients were treated with radiotherapy plus adjuvant chemotherapy with one of two anthracycline-containing regimens. There were 3 systemic relapses in this group and local relapse was observed in one of the four patients treated with radiotherapy only. The overall disease-free survival was 88% at a mean follow-up period of 87 months.

In contrast to these very positive results, Mendenhall et al.,[20] described their experience with 11 patients who received combined -modality therapy, 9 patients who received radiotherapy alone and 1 patient who received chemotherapy alone. There were 13 relapses in 21 evaluate cases. Six of 9 patients who received radiotherapy alone relapsed either regionally or distantly and 6 of 11 patients who received combined chemotherapy and radiotherapy treatment relapsed distantly. The patient who received chemotherapy alone relapsed locally. Overall 5-year survival was 56%. In this series, no treatment modality demonstrated and clear-cut advantage in the management of PLB.

In summary, our experience would suggest that those patients presenting with early-stage primary Non-Hodgkin's Lymphoma of bone can be treated with curative intent and do best with combination treatment consisting of localized radiation and systemic chemotherapy. Patients with advanced stage disease, however, have a poor prognosis despite aggressive combination treatment and should be considered for treatment on protocols. However, because our small patient numbers and heterogeneity of patients dis-

ease definite conclusions can not be drawn but warrant future investigation in a multi-institutional setting to accrue enough patients for prospective, randomized studies.

References

1. Fairbanks RK, Bonner JA, Inwards CY, Strickler JG, Habermann TM, Unni K, et al. Treatment of stage IE primary lymphoma of bone. *Int J Radiat Oncol Biol Phys* 1993; **28**: 363-72.

2. Freeman G, Berg J W, Cutler S J. Occurrence and prognosis of extranodal lymphomas. *Cancer* 1972; **29**: 252-60.

3. Prestidge BR, Horning SJ, Hoppe RT. Combined modality therapy for stage I-II large cells lymphoma. *Int J Radiat Oncol Biol Phys* 1988; **15**: 633-9.

4. Kaminski M, Coleman N, Colby TV, Cox RS, Rosenberg SA,. Factors predicting survival in adults with stage I and II large-cell lymphoma treated with primary radiation therapy. *Ann Intern Med* 1986; **140**: 747-56.

5. Cabinallis F, Bodey GP, Freireich EJ. Management with chemotherapy only of stages I and II malignant Lymphoma of aggressive histologic types. *Cancer* 1980; **46**: 2356-9.

6. Mauch P, Leonard R, Skarin A, Rosenthal P, Cone S, Chaffey J, et al. Improving survival following combined radiation therapy and chemotherapy for unfavorable prognosis stage I-II non- Hodgkin's lymphoma. *J Clin Oncol* 1985; **3**: 1301-8.

7. Baar J, Burkes RL, Bell R, Blackstein M, Fernandes B, Langer F. Primary non-Hodgkin's lymphoma of bone. A clinicopathologic study: *Cancer* 1993; **4**: 1194-9.

8. Rappaport H, Winter WI., Hicks EB. Follicular Lymphoma: a re-evaluation of its position in the scheme of malignant lymphoma, based on a survey of 253 cases. *Cancer* 1956; **9**: 792-821.

9. The non-Hodgkin's lymphoma pathologic confirmation project. National Cancer Institute sponsored study of classification of non-Hodgkin's lymphoma. Summary and description of a working formulation for clinical usage. *Cancer* 1982; **49**: 2112-35.

10. Armitage, Weisenburger D. New approach to classification non-Hodgkin's lymphomas; Clinical features of the major histologic type. *J Clin Oncol* 1998; **16**(8): 2780-95.

11. Freeman C, Berg JW, Cutler SJ. Occurrence and prognosis of extranodal lymphomas. *Cancer* 1972; **29**: 252-60.

12. Liu ZJ, Wen JM. Pathology of primary non-Hodgkin's lymphoma of bone. *Chinese Journal of Oncology*. 1986. 8 (5): 352-4.

13. Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M. Report of the committee on Hodgkin's disease staging classification. *Cancer Res* 1971; **31**: 1860-1.

14. Ostrowski ML., Unni KK, Banks PM, Shives TC, Evans RG, O'Connell MJ, et al. Malignant lymphoma of bone. *Cancer* 1986; **58**: 2646-55.

15. Clayton F, Butler JJ, Ayala AG, Ro JY, Zornoza J. Non-Hodgkin's lymphoma in bone: Pathologic and radiologic features with clinical correlates. *Cancer* 1987; **60**: 2494-501.

16. Baar J, Burkes RL, Gospodarowicz M. Primary non-Hodgkin's lymphoma of bone. *Semin Oncol* 1999; **26(3)**: 270-5.

17. Salter M, Sollacio RJ, Bernreuter WK, Weppelmann B. Primary lymphoma of bone: The use of MRI in pretreatment evaluation. *Am J Clin Oncol* 1989; **12**: 101-5.

18. Suryanarayan K, Shster JJ, Donaldson SS, Hutchison RE, Murphy SB, Link MP. Treatment of localized primary non-Hodgkin's lymphoma of bone in children: Pediatric Oncology Group Study. *J Clin Oncol* 1999; **17(2)**: 456-9.

19. Vassallo J, Roessner A, Vollmer E, Grundmann E. Malignant lymphomas with primary bone manifestation. *Pathol Res Pract* 1987; **182**: 381-9.

20. Mendenhall NP, Jones JJ, Kramer BS, Hudson TM, Carter RL, Enneking WF, et al. The management of primary lymphoma of bone. *Radiother Oncol* 1987; **9**: 137-45.

21. McIntyre K, Maak G, Strauss J, Dickson B. Primary non-Hodgkin's lymphoma of bone [abstract]. *Proc Annu Meet Am Soc Clin Oncol* 1993; **12**: A 1293.

22. Hatori M, Ichinohasama R, Myers J, Schiller A, Kokubun S. Flow cytometrical and genotypic analysis of primary non-Hodgkin's lymphoma of bone. *Pathol Res Pract* 1997; **193(8)**: 557-64.

23. Ascoli V, Facciolo F, Nardi F. Cytodiagnosis of primary non-Hodgkin's lymphoma of bone. *Diagn Cytopathol* 1994; **11(2)**: 168-73.

24. Ferreri AJ, Reni M, Ceresoli GL, Villa E. Therapeutic management with adriamycin-containing chemotherapy and radiotherapy of mono-

stotic and polyosotic primary non-Hodgkin's lymphoma of bone in adults. *Cancer Invest* 1998; **16(8)**: 554-61.

25. Tondini C, Zanini M, Lombardi F, Bengala C, Rocca A, Giardini R, et al. Combined modality treatment with primary CHOP chemotherapy followed by locoregional irradiation in stage I or II histologically aggressive non-Hodgkin's lymphoma. *J Clin Oncol* 1993; **11**: 720-5.

26. Bacci G, Jaffe N, Emiliani E, Van Horn J, Manfrini M, Picci P, et al. Therapy for primary non-Hodgkin's Lymphoma of bone and a comparison of results with Ewing's sarcoma. Ten years experience at the Istituto Ortopedico Rizzoli. *Cancer* 1986; **57(8)**: 1468-72.

Radiol Oncol 2000; 34(1): 35-9.

A paraungual tumor? - No, just tungiasis

Rastko Golouh, Marko Špiler

Institute of Oncology, Ljubljana, Slovenia

Background. *Tunga penetrans is a burrowing flea that is prevalent in Central and South America, the Caribbean, tropical Africa, India and Pakistan. Tungiasis results from cutaneous infestation by gravid female flea, Tunga penetrans.*

Case report. *We report a case of tungiasis in a male who had been on a tourist expedition to Bolivia and Peru. The condition had manifested as a paraungual infiltration of a toe, that was clinically suspicious for tumor.*

Conclusions. *Microscopic examination of the lesion and travel history led to the diagnosis of tungiasis. This is the first case report of Tunga penetrans infestation in Slovenia.*

Key words: tungiasis; tunga penetrans; South America; travel; surgical pathology

Introduction

Tungiasis is an unusual zoonosis. In non-endemic countries, it might be misdiagnosed by unaware clinicians and it can even mimic a neoplastic process. For the pathologist, evidence of parasitic infestation in the skin specimen is easily identified. However, the exact typing of the parasite is more difficult.

Clinical history

The patient was a 46-year-old male who presented with a pea sized induration with a central black pit in paraungual skin of the first right toe of one month duration. Clinically, a tumor was suspected, and an excisional biopsy was performed.

Received 3 November 1999

Accepted 20 December 1999

Correspondence to: Prof. Rastko Golouh, PhD, MD, Institute of Oncology, Zaloška 2, 1000 Ljubljana, Slovenia; Fax: 386 61 1314 180.

Pathological findings

Grossly, the specimen consisted of a wedge excision of the skin measuring 8×6×3mm.

Microscopically, in the epidermis and dermis an organism is found, measuring 6 mm in the largest diameter and covered by a layer of parakeratotic cells (Figure 1).

After studying HE slides, the patient was interviewed again. It became evident that he had recently returned from Bolivia and Peru, having spent two months on trekking there. During this time, he often walked barefoot along the lake sandy beaches. On clinical reexamination, no other skin lesions were discovered.

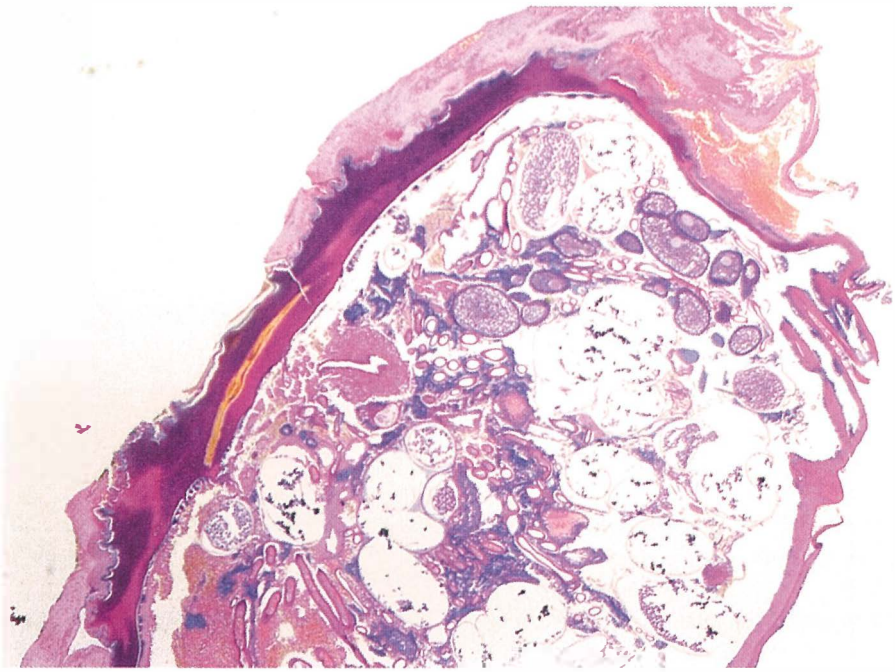


Figure 1. By exoskeleton supported body of the insect is localized in the epidermis and upper dermis, 20×.

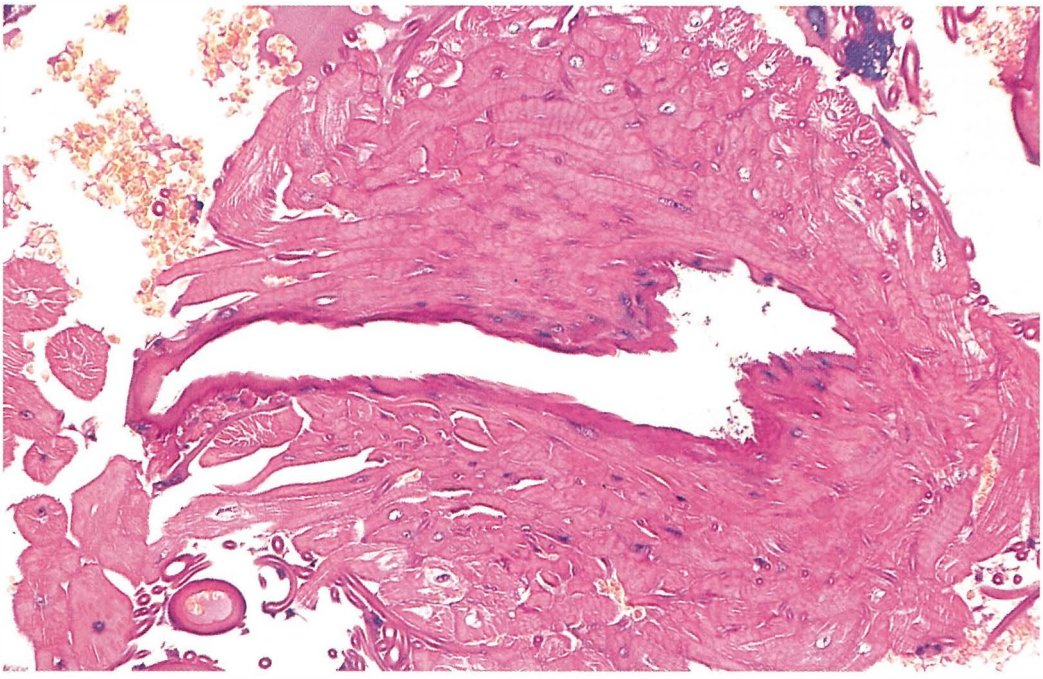


Figure 2. Well developed buccal apparatus with skeletal muscle fibers, 200×.

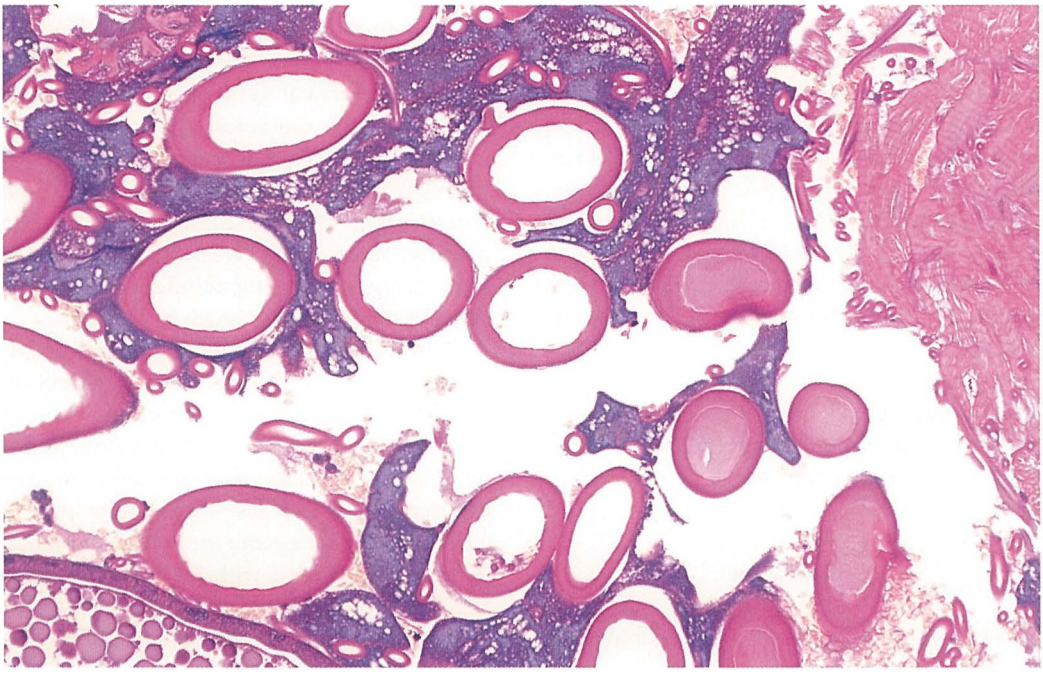


Figure 3. Inside the flea body are typical annular shaped respiratory organs - tracheids, 200×.

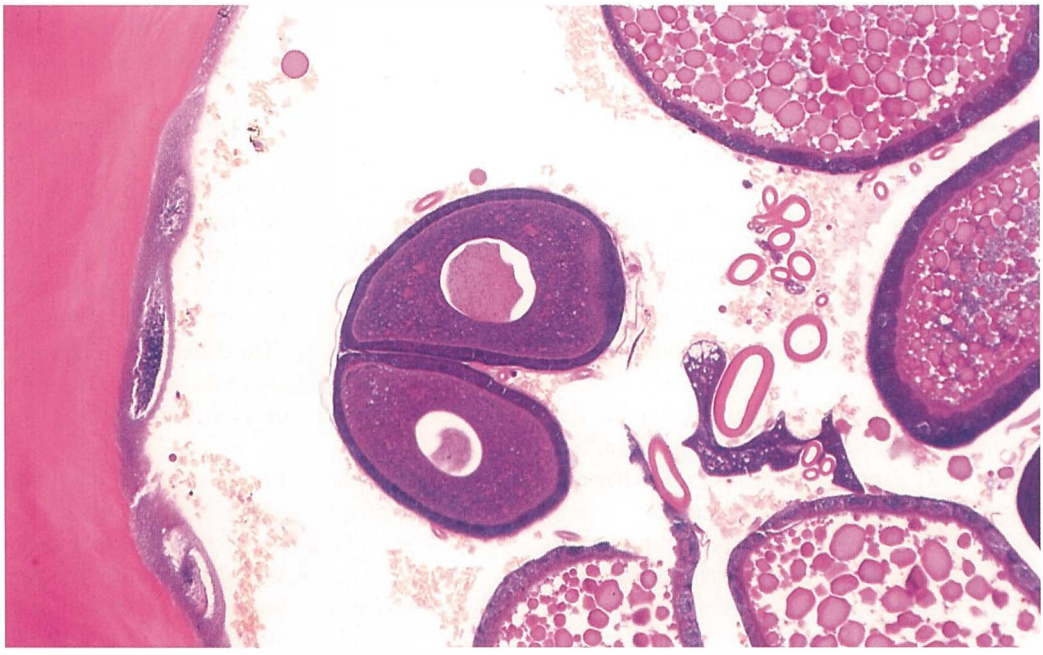


Figure 4. Eggs of Tunga penetrans in different stages of development are mostly oval with thickened wall and a pale center. Some of them superficially mimic Reed-Sternberg cells, 200×.

As we could not identify the parasite, we sent the frozen microscopic pictures using telepathology and Internet to the Armed Forces Institute of Pathology, Washington, and histological slides to Memorial Sloan-Kettering, New York, USA, for consultation. Both consultants (Peter L McEvoy, and Juan Rosai, respectively) independently agreed on the diagnosis of cutaneous tungiasis. Features of the parasite include an exoskeleton, some striated muscle (Figure 2), numerous tracheids - breathing tubes (Figure 3), and many developing eggs (Figure 4). All these features, together with rounded configuration of the organism, are typical of Tunga penetrans.

Discussion

The present case is the first case of tungiasis reported in Slovenia and neither clinician nor pathologist could state the correct diagnosis. The diagnostic dilemma was finally solved by consultation.

Tungiasis is an inflammatory condition of the skin caused by the flea Tunga penetrans. Tungiasis is prevalent in Central and South America, the Caribbean Islands, tropical Africa, the Seychelles, Pakistan and along the west coast of India. Typically, Europeans get infested after a visit to endemic countries. Therefore, it should not be too surprising that tungiasis was first reported in crewmen who sailed with Christopher Columbus on the ship Santa Maria and were stationed on Haiti in 1492.[1]

The life cycle of this hematophagous flea comprises four stages: adult, egg, larva, and pupa. After copulation the male flea dies and a merry widow begins a pattern of jumping up to 35 cm above the ground until she also dies or succeeds to burrow in the skin of a human being or animal, embedding its head in the dermis and its posterior segment parallel to the skin surface. Through this posterior segment, feces and eggs are extruded and respiration is accomplished. After invading the skin, her abdomen swells, the pregnant flea becomes spherical and eventually reaches about 1cm in diameter. Over 1-2 weeks, approximately 200 eggs are extruded. When the eggs are laid the female flea dies and shrivels up. The larvae then undergo two molting stages. Two weeks after hatching from its cocoon, the pupa undergoes development to the final adult stage in 7 days.[2]

Preferred habitats of Tunga penetrans include warm, dry, shady, and sandy soil. In humans, the unprotected feet and legs are most exposed to the infestation. When fleas attack they tend to concentrate on the toes, on areas between the toes, in the subungual or periungual areas, the instep and the ankle. Infestation is usually associated with local inflammation and frequently accompanied by intense irritation, tenderness, and even pain. Heavy infestation may lead to ulceration and fibrosis. Clinical features consist initially of pruritic, tender, or painful erythematous papule with a central black dot produced by the posterior part of the flea's abdominal segments. The fully developed lesion is a white pea-sized nodule with a central black pit or plug.[3]

Accurate parasite diagnosis depends on the identification of its ova and body parts. Microscopically, specific findings consist of oval eggs in different stages of maturation, measuring 0.05 to 0.1mm in diameter, tracheids and the presence of the flea, embedded in the epidermis with its head penetrating into the dermis. The differential diagnosis includes viral warts, myasis, scabies, tick bite, ingrown toenail [4] or even paraungual melanoma as in our case.

The complications of tungiasis, although rare, can be quite severe. Among others, gas gangrene and tetanus are the most dramatic.[4]

Treatment consists of removal of the flea by extraction with sterile needle of by curettage. A topical antibiotic ointment and sterile dressing is then applied. Tetanus prophylaxis should be considered.[5]

According to the literature, tungiasis is rare in Central Europe and only exceptional cases have been reported by Italian [6-11] and German [12-15] authors.

References

1. Hoeppli R. Early references to the occurrence of Tunga penetrans in tropical Africa. *Acta Trop* 1964; **20**: 143-53.
2. Connor DH. Tungiais. In Binford CH, Connor DH, eds. *Pathology of tropical and extraordinary diseases*; vol 2. Washington, DC. Armed Forces Institute of Pathology; 1976. p. 610-4.
3. Sanusi ID, Brown EB, Shepard TG, Grafton MD. Tungiasis: report of one case and review of the 14 reported cases in the United States. *J Am Acad Dermatol* 1989; **20**: 941-4.
4. Burke W, Jones BE, Park HK, Finley J. Imported tungiasis. *Int J Dermatol* 1991; **30**: 881.
5. Lowry MA, Ownbey JL. A case of tungiasis. *Military Medicine* 1996; **161**: 128-9.
6. D'Antuono A, Gatti M, Negosanti M, Passarini B, Pauluzzi P, Reggiani M. Tungiasis. A clinical case. *G Ital Dermatol Venereol* 1990; **125**: 259-61.
7. Veraldi S, Schinanchi R. Guess what? Tungiasis. *Eur J Dermatol* 1999; **9**: 57-9.
8. Fimiani M, Reimann R, Alessandrini C, Miracco C. Ultrastructural findings in tungiasis. *Int J Dermatol* 1990; **29**: 220-2.
9. Trevisan G, Puluzzi P, Kokelj F. Tungiasis: report of a case. *J Eur Acad Dermatol Venereol* 1992; **1**: 73-5.
10. Carabelli A, Di Vincenzo R, Vanotti P, Bertani E. La tungose. *Nouv Dermatol* 1992; **11**: 826.
11. Veraldi S, Camozzi S, Scarabelli G. Tungiasis presenting with a sterile pustular lesions on the hand. *Acta Derm Venereol (Stockh)* 1996; **76**: 495.
12. Baurle G, Stroothenke M. Tungiasis - eine "Urlaubsdermatose". *Hautarzt* 1981; **32**: 372-3.
13. Peschlow I, Schlenzka K, Merk G, Neumann HJ. Tropendermatosen aktuell Tungiasis, Ulcus tropicum, Leishmaniase Beobachtungen aus der Praxis. *Dermatol Monatsschr* 1983; **169**: 120-4.
14. Schuller-Petrovic S, Mainitz M, Bohler-Sommeregger K. Tungiasis - an increasingly frequent vacation dermatosis. *Hautarzt* 1987; **38**: 162-4.
15. Schmidt C, Ulrich G, Bersch W. Tungiasis - a middle European rare skin parasitosis. A case report. *Pathologe* 1993; **14**: 221-2.

Radiol Oncol 2000; 34(1): 41-7.

Cathepsin D and plasminogen activator inhibitor type 1 in normal, benign and malignant ovarian tissues: a preliminary report

Marina Šprem[1], Damir Babić[2], Marija Abramić[3], Duško Miličić[1], Ivan Vrhovec[4], Janez Škrk[4], and Maja Osmak[5]

[1]Department of Obstetrics and Gynaecology; [2]Department of Gynaecological and Perinatal Pathology, University Hospital and School of Medicine, Zagreb, Croatia; [3]Department of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia; [4]Institute of Oncology, Ljubljana, Slovenia; [5]Department of Molecular Genetics, Ruđer Bošković Institute, Zagreb, Croatia

Background. *The aim of the present study was to determine the concentration of cathepsin D (Cath D) and plasminogen activator inhibitor type 1 (PAI-1) in normal ovarian tissues, benign and malignant ovarian tumor tissues, and to asses relationship between Cath D and PAI-1 content, and some clinical and pathohistological parameters.*

Materials and methods. *Cath D contents and PAI-1 concentrations were determined (using immunoradiometric ELSA-Cath D assay and commercial IMUDIND[R] ELISA immunoassay, respectively) in 35 samples: 10 normal ovarii, 10 benign, 10 primary malignant and 5 metastatic ovarian tumors.*

Results. *The concentrations of Cath D were significantly higher in malignant (32.89±14.26 pmol/mg protein) and metastatic (31.42±10.24 pmol/mg protein), than in normal (13.68±4.03 pmol/mg protein) and benign (17.89±13.13 pmol/mg protein) ovarian tissues. There was no statistical differences in the concentrations of PAI-1 between normal, benign, malignant and metastatic tumor specimens. The concentrations of Cath D as well as PAI-1 did not correlate to the age of patients, menopausal status, parity, GOG risk group, clinical stage or pathohistological grading.*

Conclusion. *Concentrations of Cath D (but not PAI-1) were significantly increased in malignant and metastatic ovarian tumor tissues when compared to normal and benign ovarian tumor samples; they were independent from pathohistological and clinical parameters.*

Key words: ovarian neoplasms; cathepsin D, plasminogen activator inhibitor type 1

Correspondence to: Maja Osmak, Ph.D., Department of Molecular Genetics, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia. Phone: +385-1-4561-145; Fax: +385-1-4561-177; E-mail: osmak@rudjer.irb.hr

Introduction

Among gynaecological malignancies, ovarian cancer has been the major cause of death because they are usually presented in advanced clinical stage, with extensive intraab-

dominal metastasis and the unavoidable development of resistance to chemotherapy.[1,2]

Invasion and metastases are multi-step processes that require a complex cascade of interrelated events including extracellular matrix degradation, migration, proliferation and induction of neovascularisation.[3-5] These processes are complicated and only partially understood. Proteases associated with the invasive capacity of tumor cells are metalloproteinases (collagenases, gelatinases, stromelysin), cysteine proteases (cathepsin B, H, L), aspartyl proteases (cathepsin D) and serine proteases (urokinase type plasminogen activator and its activated product, plasmin).

Cathepsin D (Cath D) is a lysosomal acidic protease. It might be involved in tumor growth, invasion and metastasis by different mechanisms: a) by inactivating a secreted growth inhibitor, b) by releasing growth and angiogenesis factors from the extracellular matrix, c) by providing amino acids following phagocytosis of extracellular matrix, d) by degrading extracellular matrix and basal membranes, and e) by activating latent precursors from other proteinases involved in the invasive step of metastatic process.[5-7] Thus, Cath D may facilitate the spread of neoplastic cells and promote tumor invasiveness and metastatic potential through different mechanisms by acting at different levels of the metastatic process.

Positive correlation between Cath D level and aggressiveness of breast tumor, as well as shorter relapse free interval and overall survival of breast cancer patients [5,8,9], have stimulated the investigation on tumors of other origin. So, elevated levels of Cath D have been found in other tumors like melanoma, head and neck carcinoma, genital carcinoma.[6,10-12]

In the multi-step process of invasion, plasminogen/plasmin system plays an important role. Urokinase plasminogen activator (uPA) is a highly specific serine protease that converts plasminogen in plasmin.[4,13] Plasmin degrades fibronectin, laminin and other non-collagenous proteins of the extracellular matrix, and is able to activate latent collagenases. That leads to further degradation of extracellular matrix and invasion and metastasis. UPA activity is controlled by specific plasminogen activator inhibitors (PAI), which include plasminogen activator inhibitor type 1 (PAI-1), plasminogen activator inhibitor type 2 (PAI-2) and protease nexin.[14,15] PAI-1, a serine protease inhibitor (serpin), is the major inhibitor of PA in the plasma. High levels of PAI-1 in tumor extracts from breast, lung and gastric cancerous tissues appear to be highly significant and independent predictors for shorter overall survival.[14]

The importance of proteases in the regulation of ovarian carcinogenesis, invasion and metastases may further promote its clinical application in the detection of early stage of this disease, prognostic assessment of patients with ovarian cancer as well as in the development of new cancer specific treatment modalities. Limited information is available on the prognostic value of cathepsin D and plasminogen activator inhibitor type 1 regarding gynaecogolical malignancies. The aim of the present study was to determine the concentration of Cath D and PAI-1 in normal, benign and malignant ovarian tissues and to asses the relationship between Cath D and PAI-1 content and some clinical and pathohistological parameters.

Materials and methods

Tissue samples

The tissue samples were obtained from fresh specimens removed during surgical procedure at the Department of Obstetrics and Gynaecology of Medical School, University of Zagreb. The Ethics of Research Committee at the Medical School of the University of Zagreb approved the protocol.

Thirty-five tissue samples were analyzed: 10 normal ovarian tissues, 10 benign ovarian

tumor specimens, 10 primary ovarian carcinoma and 5 metastatic ovarian carcinoma samples.

After surgery, the samples were frozen in liquid nitrogen. For biochemical analysis, samples were they were prepared as previously described.[16] Briefly, each specimen was minced, homogenized in lysis buffer, and centrifuged for 45 min at 15000 g. Supernatant (tumor tissue cytosol) was used for biochemical studies.

Cathepsin D determination

Cath D content was measured in the cytosol of homogenized samples using a commercially available solid-phase two site immunoradiometric assay (ELSA-CATH-D, CIS Bio International, Gif-sur-Ivette, France). It detects precursors and mature form of Cath D. The values were normalized according the total protein assessed by Bradford's method[17], and expressed as pmol/mg protein.

Plasminogen activator inhibitor determination

The concentration of PAI-1 inhibitor was determined by immunoassay using a commercially available ELISA kit (IMUBIND^R, American Diagnostica Inc., Greenwhich, USA). It detects latent and active forms of PAI-1 and complexes. The concentrations of PAI-1 were expressed in ng/mg protein.

Statistics

The statistical significance of difference between the concentrations of cathepsin D and PAI-1 in the particular groups was tested by Kruskal-Wallis one way ANOVA test. Their relationship to pathohistological and clinical factors were calculated by Spearman's rank correlation test. Data are reported as mean ± SD. Differences were considered significant at $p<05$.

Results

The pathohistological characteristics of ovarian tumors that were entered into present study are given in Table 1. Their classification and histopathological grade, as well as clinical staging, was defined according to FIGO. Among the patients with benign ovarian tumors, those with cystadenofibroma serosum prevailed, while among the patients with primary malignant tumors, those with cys-

Table 1. Pathohistological characteristics of ovarian tumor specimens

Benign		Primary malignant		Metastatic carcinoma	
teratoma dermoides		cystadenocarcinoma serosum		breast cancer	2
cysticum	1	ovarii	5	carcinoma ventriculi	
cystadenofibroma serosum		cystadenocarcinoma mucinosum		(Krukenberg)	1
ovarii	8	ovarii	2	colorectal carcinoma	1
cysta serosa	1	cystadenocarcinoma		endometrial carcinoma	1
		anaplasticum ovarii	1		
		adenocarcinoma endometrioides			
		ovarii	1		
		cystadenocarcinoma mixtum			
		ovarii	1		

Table 1. Pathohistological characteristics of ovarian tumor specimens

Total	10	
Clinical stage		
I A	1	
IB	1	
IC	4	
III	2	
IV	2	
Tumor grade		
GI (well differentiated)		4
GII (moderately differentiated)		3
GIII (poorly differentiated)		3
Hystological type		
mucinosus	2	
serosus	5	
endometrioid	1	
mixed	1	
anaplastic	1	
Age (mean, range)		
<50	4	
>50	6	
Parity		
0	3	
≥1	7	
GOG(Gynecology Oncology group)		
low risk (stage IA and B, grade 1 or 2)		2
high risk (stage IC or II or grade 3)		8

tadenocarcinoma serosum were prevalent. (Table 1). Primary ovarian carcinomas were further classified as shown in Table 2. More than half of these malignant tumors belong, according to GOG, to high risk group.

The cytosol supernatant obtained after homogenization of ovarian tissue samples was analysed for the content of cathepsin D and plasminogen activator inhibitor type 1. Figure 1 shows the concentrations of cathepsin D in normal, benign and malignant ovarian specimens. Cath D levels were significantly higher in primary malignant (32.89 ± 14.26 pmol/ mg protein) and metastatic (31.42 ± 10.24 pmol/ mg protein) malignant ovarian carcinomas than in normal (13.68 ± 4.03 pmol/ mg protein) or benign (17.89 ± 13.13 pmol/ mg protein) tissue samples (p=0.003 for normal versus primary malignant tissues, p=0.035 for normal versus metastatic malignant tissues, p=0.028 for benign *versus* primary malignant tissues, p=0.029 for benign *versus* metastatic malignant tissues). There was no difference between the levels of Cath D in primary and metastatic ovarian tumor tissues (p=0.995), or between normal and benign tumor tissues (p=0.838).

Figure 2 presents the PAI-1 concentrations in normal, benign and malignant ovarian specimens. Although the mean concentration of PAI-1 was lower in normal (2.96 ± 1.49 ng/mg protein) than in malignant tumor tissues (8.80 ± 10.56 ng/mg protein), this difference was not statistically significant (p=0.170). Also, there was no statistically significant difference between the levels of PAI-1 in primary and metastatic ovarian carcinomas (p=0.864), or between normal and benign tumor tissues (p=0.948).

The concentration of cathepsin D as well as PAI-1 did not seem to correlate to the age of patients, their menopausal status, parity, or GOG risk group. Neither cathepsin D nor PAI-1 concentrations correlated with clinical staging or pathohistological grading.

Discussion

The major cause of death among gynaecological malignancies is ovarian cancer: over 70% of women are diagnosed with incurable advanced-stage disease, defined by widespread intraperitoneal metastases.[1,2] Tumor invasion is a complex, multistep sequence of events based on a cascade of coordinated cellular processes. The combined action of several proteolytic enzymes is involved in tissue

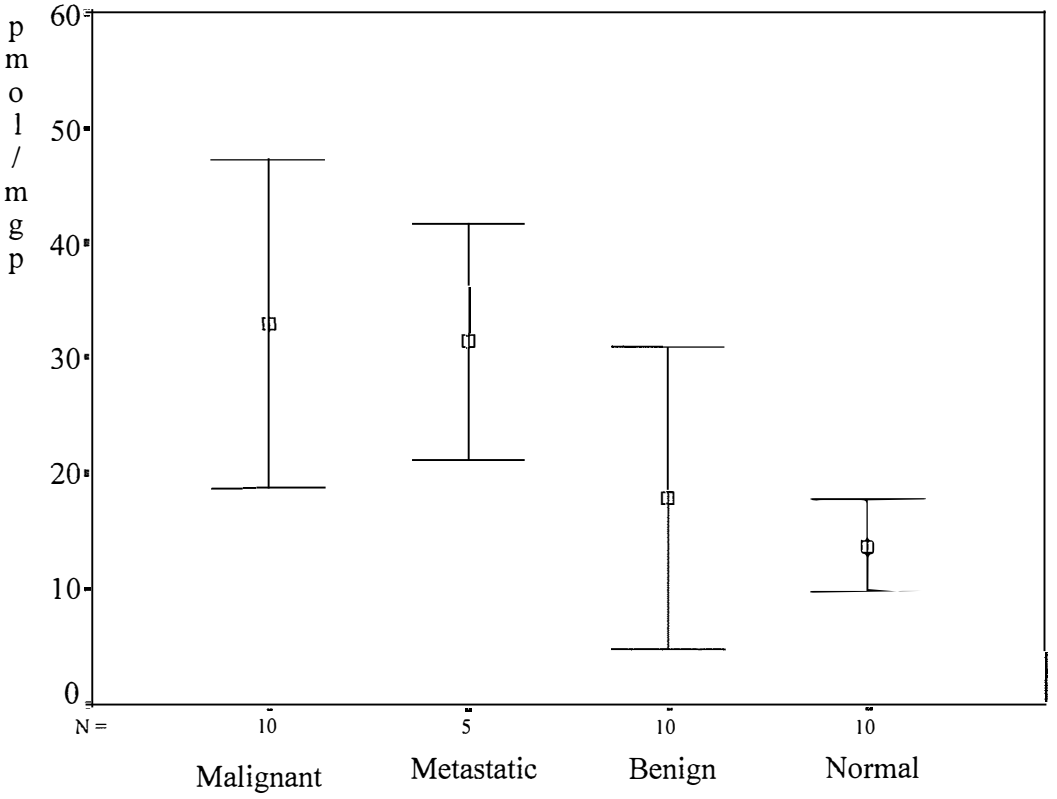


Figure 1. Concentrations of cathepsin D (mean ± SD) in primary and metastatic ovarian carcinomas, benign ovarian tumors and normal ovarian tissues. The number of samples per group are indicated.

degradation and remodeling in both, normal and tumor tissue.[3-5]

The collected evidence have proved that proteases and their inhibitors (including Cath D and PAI-1) may serve as prognostic factors in breast cancer, to predict the outcome of the disease.[5,8,9] Several tumor-associated proteases are important factors also in solid tumors of other origins, such as lung, gastric, colon, genital, head and neck, bladder, and kidney cancers.[4-6,10-12,18,19]

The aim of the present study was to determine and compare the levels of cathepsin D and plasminogen activator inhibitor type 1 in normal, benign, primary malignant and metastatic malignant tumor ovarian tissues. As mentioned in the Introduction, Cath D is a proteolytic enzyme that may be involved in invasion and metastasis through different mechanisms.[5-7]

In the present study, we have determined significantly higher concentrations of Cath D in primary and metastatic ovarian carcinomas than in normal or benign tumor samples (Figure 1), which was is in agreement with literature data.[12,20] It is important to point out that Cath D levels were similar in both, normal and benign tumor tissue samples, suggesting that malignant transformation of ovarian tissue was accompanied with the increased level of this protease. The concentration of Cath D was independent from the pathohistological parameters, such as hystological type or tumor grading, as well as clinical markers, such as clinical stage, age, parity and menopausal status.

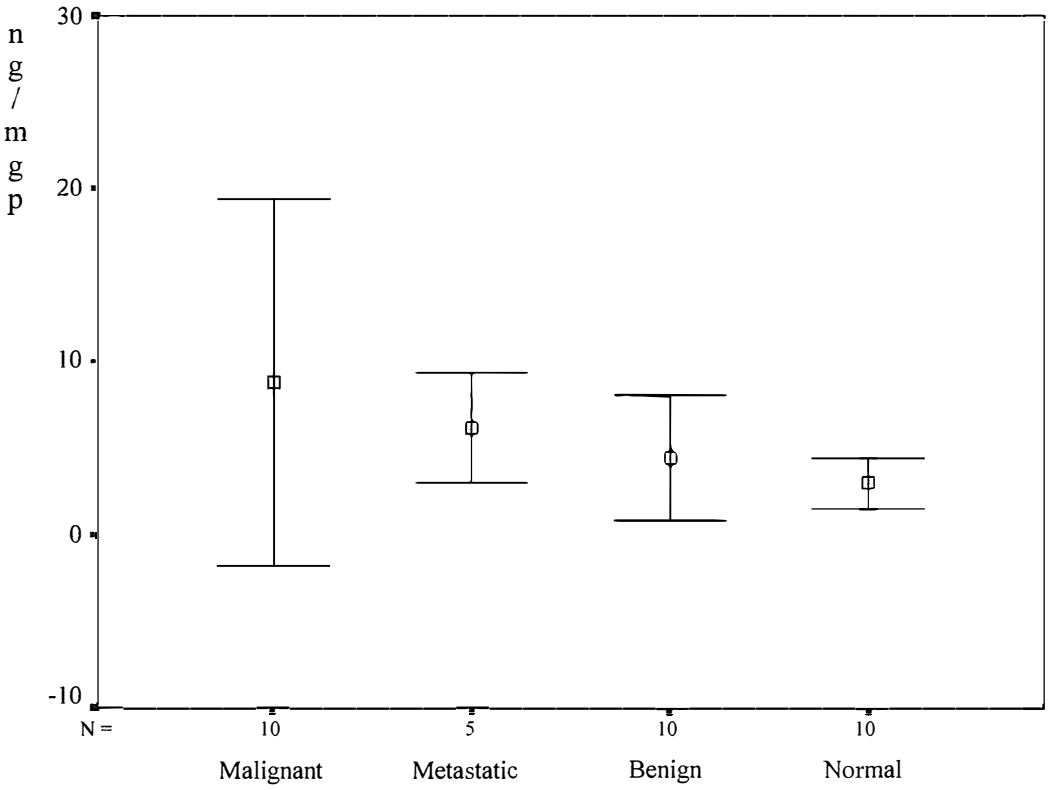


Figure 2. Concentrations of urokinase plasminogen activator inhibitor type 1 (mean ± SD) in primary and metastatic ovarian carcinomas, benign ovarian tumors and normal ovarian tissues. The number of samples per group are indicated.

Urokinase plasminogen activator has important role in the tissue degradation and in the invasiveness of tumor cells.[4,13] In spite of the fact that uPA activity is controlled by a specific inhibitor, such as PAI-1, the increased levels of both, uPA and PAI-1 were found in different tumors: breast cancer[20], gastric cancer[14] and bladder cancer.[21] Furthermore, increased levels of both, uPA and PAI-1 were associated with poor prognosis, and increased relapse rate and shorter survival.

The data in the literature concerning ovarian tumors and PAI-1 levels are contradictory. Some groups have found increased levels of PAI-1 in ovarian cancer[22], and even suggested that uPA and PAI-1 may predict the survival of patients with advanced ovarian cancer.[23] Others did not determine any increased levels of uPA and PAI-1 in ovarian tumors.[20] Our results concerning PAI-1 are closer to the data of Ruppert *et al.*[20] We did not find a significant increase of PAI-1 in malignant ovarian tumor tissues. However, the observed difference in absolute values for mean PAI-1 concentrations between normal and malignant ovarian tissues suggests that in order to draw important conclusions more samples should be examined.

In addition, comparing the levels of Cath D and PAI-1 in normal, benign and malignant tumor tissues, we have not found any correlation between these parameters.

In conclusion, Cath D overexpression in malignant tumor ovarian tissue may be relat-

ed to intraabdominal dissemination of ovarian tumor cells. In order to determine more precisely the diagnostic and prognostic values of Cath D and PAI-1 in ovarian cancer patients, we have started a clinical study with a higher number of patients with ovarian cancer and a longer follow up.

References

1. Pickel H. Prognostic factors in ovarian cancer. *CME J Gynecol Oncol* 1999; **4**: 8-12.

2. Friedlander ML. Prognostic factors in ovarian cancer. *Semin Oncol* 1998; **3**: 305-14.

3. Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell* 1991; **64**: 327-36.

4. Schmitt M, Janicke F, Graeff H. Tumor-associated proteases. *Fibrinolysis* 1992; **6**, Suppl 4: 3-26.

5. Sloane BF, Moin K, Lach TT. Lysosomal enzymes and their endogenous inhibitors in neoplasia. In: Pretlow TG, Pretlow TH (eds) *Biochemical and molecular aspects of selected cancers.* New York: Acad Press; 1993. Vol 2, p 411-66.

6. Leto G, Gebbia N, Rausa L, Tumminello FM. Cathepsin D in malignant progression of neoplastic diseases (review). *Anticancer Res* 1992; **12**: 235-40.

7. Rochefort H, Liaudet E, Garcia M. Alterations and role of human cathepsin D in cancer metastasis. *Enzyme Protein* 1996; **49**: 106-16.

8. Rochfort H. The prognostic value of cathepsin D in breast cancer. A long road to the clinic. *Eur J Cancer* 1996, **32A**: 7-8.

9. Westly BR, May FEB. Cathepsin D and breast cancer. *Eur J Cancer* 1996; **32A**: 15-24.

10. Zeillinger R, Eder S, Schneeberger CH, Ullrich R, Speiser P, Swoboda H. Cathepsin D and PAI-1 expression in human head and neck cancer. *Anticancer Res* 1996; **16**: 449-54.

11. Osmak M, Babić D, Abramić M, Vrhovec I, Miličić D, Škrk J. Cathepsin D content in malignant tumours of corpus uteri. *Eur J Cancer* 1997; **33**: 699-700.

12. Scambia G, Benedetti P, Ferradina G, BAttaglia F, Biaocchi G, Mancuso S. Cathepsin D assay in ovarian cancer: correlation with pathological features and receptors for oestrogen, progesterone and epidermal growth factor. *Br J Cancer* 1991; **64**: 182-4.

13. Aquirre Ghiso J, Alonso DF, Farias EF, Gomez DE, BAl de Kier Joffe E. Deregulation of the signaling pathways controlling urokinase production. Its relationship with the invasive phenotype. *Eur J Biochem* 1999; **263**: 295-304.

14. Pappot H, Gardsvoll H, Romer J, Pedersen AN, Hansen JG, Pyke C, Brunner N. Plasminogen activator inhibitor type 1 in cancer: therapeutic and prognostic implications. *Biol Chem Hoppe -Seyler* 1995; **376**: 259-67.

15. Rijken DC. Plasminogen activators and plasminogen activator inhibitors: biochemical aspects. *Bailliere Clin Haematol* 1995; **8**: 291-312.

16. Šimaga Š, Babić D, Osmak M, Ilić-Forko J, Vitale Lj, Miličić D, Abramić A. Dipeptidyl peptodase III in malignant and non-malignant gynaecological tissue. *Eur J Cancer* 1998; **34**: 399-405.

17. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein, utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248-54.

18. Nekarda H, Sievert JR, Schmitt M, Ulm K. Tumour associated proteolytic factors uPA and PAI-1 and survival in totally resected gastric cancer. *Lancet* 1994; **343**: 117-9.

19. Volm M, Mattern J, Koomagi R. Relationship of urokinase and urokinase receptor in non-small cell lung cancer to proliferation, angiogenesis, metastasis and patient survival. *Oncology Reports* 1999; **6**: 611-5.

20. Ruppert C, Ehrenforth S, Scharer I, Halberstadt E. Protease levels in breast, ovary, and other gynaecological tumor tissues-prognostic importance in breast cancer. *Cancer Detect & Prev* 1997; **21**: 452-9.

21. Hasui Y, Maratsuka K, Nishi S, Kitada S, Osada Y, Simiyosha A. The content of urokinase-type plasminogen activator and tumor recurrence in superficial bladder cancer. *J Urol* 1994; **151**: 16-20.

22. Casslen B, Bossmar T, Lecander I, Astedt B. Plasminogen activators and plasminogen activator inhibitors in blood and tumor fluids of patients with ovarian cancer. *Eur J Cancer* 1994; **30**:1302-9.

23. Kuhn W, Pache L, Schmalfeldt B, Dettmar P, Schmitt M, Janicke F, Graeff H. Urokinase (uPA) and PAI-1 predict survival in advanced ovarian cancer patients (FIGO III) after radical surgery and platinum-based chemotherapy. *Gynecol Oncol* 1994; **55**: 401-9.

Radiol Oncol 2000; 34(1): 49-57.

Antitumor effectiveness of bleomycin on SA-1 tumor after pretreatment with vinblastine

Maja Čemažar, Marija Auersperg, Gregor Serša

Institute of Oncology Ljubljana, Slovenia

Background. *In our previous study, vinblastine (VLB) was shown to increase the plasma membrane fluidity. This effect of VLB might be exploited for better transport of drugs through the plasma membrane. The aim of the present study was to determine whether pretreatment with VLB can increase the cytotoxic effect of BLM on intraperitoneal SA-1 tumors in mice.*

Materials and methods. *BLM and VLB were used as single agents or in various combinations, i.e. BLM injected 24 h before VLB or vice-versa, VLB injected 24 h before BLM. Cell and animal survival together with DNA histograms were the end-points used to determine the effect of these combined treatments.*

Results. *Both drugs, either as single treatment or in different combined therapy schedules reduced significantly the number of cells in peritoneal lavage, compared to control, saline treated animals. The combination of VLB, followed by BLM after 24 h reduced significantly the number of cells in peritoneal lavage, compared to the treatment in which BLM was followed by VLB or to the treatment with single drugs alone. Median survival time of mice treated with VLB alone, BLM alone and combination of both drugs was significantly prolonged compared to the control untreated mice. When VLB and BLM were combined, both treatment combinations were more effective than monochemotherapies with VLB or BLM. The best results were obtained when VLB was followed by BLM after 24 h. The DNA histogram of cells treated with VLB showed a decreased number of cells in S phase and an increased number of cells with DNA values greater than in G2M compartment compared to the control untreated cells. BLM in the dosage used in these experiments did not affect the progression of cells through cell cycle. Both combinations of VLB and BLM produced similar cell kinetic effect as VLB alone.*

Conclusion. *From these results we can conclude that the underlying mechanisms for enhanced antitumor effectiveness of BLM when VLB was given 24 h before BLM could be attributed predominantly to an increased membrane fluidity and possibly a cell kinetic effect of VLB.*

Key words: vinblastine; bleomycin; sarcoma experimental; fibrosarcoma

Received 15 December 1999
Accepted 24 January 2000

Correspondence to: Maja Čemažar, Ph.D., Department of Tumor Biology, Institute of Oncology, Zaloška 2, SI-1000 Ljubljana, Slovenia. Tel.: +386 61 323 063 ext. 29-33; Fax.: +386 61 133 74 10; E-mail: mcemazar@onko-i.si

Introduction

The design of presently used combined chemotherapeutic schedules is based on the data derived from preclinical studies, phase I and II clinical studies. However, little attention is paid to timing of drugs or possible interaction of drugs in a particular combined schedule. Both these factors could be crucial for the clinical effect of chemotherapy. The increasing knowledge and understanding of molecular mechanisms of drug-induced cytotoxicity form the basis for rational planning of clinical chemotherapy. In our previous work, we studied mechanisms of action of vinblastine (VLB), an antimitotic alkaloid.[1-3] VLB exerts cytotoxic activity against various tumors and is, at present, used mainly in combined chemotherapeutic schedules for treatment of testis tumors, Hodgkin's and non-Hodgkin's lymphomas, breast carcinomas, gastric carcinomas, squamous cell carcinomas, and many others.[4-8] In our previous study, we have demonstrated that, beside other effects, VLB also increases the plasma membrane fluidity and, consequently, its permeability.[1]

Our rationale for the use of VLB in combined chemotherapeutic schedules is that pretreatment with VLB could facilitate the transport into the cell of another chemotherapeutic drug with a hampered transport through the plasma membrane. One of such drugs is bleomycin (BLM); therefore, studies combining BLM and VLB could be very interesting. BLM is highly cytotoxic drug when present inside the cells.[9,10] It was shown that as little as several thousand molecules of BLM present inside the cell induce cell death.[10] BLM is a water soluble glycopeptidic antibiotic with limited antitumor effectiveness, used only in combined chemotherapeutic schedules in cancer treatment. The major reason for its limited antitumor effectiveness is the hampered transport of BLM through the plasma membrane. However, once inside the cell, BLM is highly cytotoxic inducing single and double strand breaks of DNA. Therefore, the cytotoxicity of BLM is dependent on its intracellular concentration and also upon the membrane permeability, which influences the uptake of the drug.[9,10] In our previous study, we already demonstrated that pretreatment with VLB significantly prolonged the survival of mice bearing SA-1 tumors treated with BLM, compared to the control groups.[11] We presumed that the observed antitumor effectiveness was due to the increased uptake of BLM and possibly also to the cell kinetic effects of both drugs. Therefore, in this study, we further elaborated the effect of pretreatment with VLB on i.p. SA-1 tumors treated with BLM. Cell and animal survival together with DNA distribution pattern were the end-points used for determining the effect of this combined treatment.

Materials and methods

Drug formulation

VLB (Vinblastine sulphate, Lilly France S.A.) was dissolved in 0.9% NaCl solution at a concentration 2.5 μg/ml. BLM (Bleomycin, Mack, Germany) was dissolved in 0.9 % NaCl solution at a concentration 500 μg/ml. Each animal was injected i.p. with 0.5 ml of the drug solution. According to Freireich *et al.*, the corresponding doses for VLB in humans would be 0.2 mg/m^2 (0.005 mg/kg) and for BLM 37 mg/m^2 (1.0 mg/kg).[12]

Animals

Inbred A/J mice were purchased from the Rudjer Bosković Institute (Croatia). Mice were maintained at a constant room temperature (22 °C) and natural day/night light cycle in a conventional animal colony. Before experiments, mice were subjected to an adaptation period of at least 10 days. Female mice in good condition, weighing 20-22 g, without

signs of infection, 10-12 weeks old, were included in the experiments.

Tumor model

Intraperitoneal (i.p.) SA-1 fibrosarcoma syngeneic to A/J mice was used in the study. The tumor was maintained i.p. as ascites by serial transplantation once a week. For induction of i.p. tumors, tumor cells from the donor mouse were harvested by peritoneal lavage with 4 ml of 0.9 % NaCl solution, washed and resuspended at a concentration of 6×10^5 cells/ml. Tumors were induced by i.p. injection of 3×10^5 viable SA-1 cells in 0.5 ml 0.9 % NaCl solution. Cell viability, determined by Trypan dye exclusion test, was over 95%.

Treatment protocol

Three days after tumor induction animals were randomly allocated into following groups: control (intraperitoneally treated with saline), VLB alone, BLM alone, VLB followed by BLM and BLM followed by VLB. The time interval between i.p. injection of the first and second drug was 24 h. In the case of monochemotherapy, 0.9 % NaCl was injected 24 h after the first drug. Each experimental group consisted of at least 6 mice and the data were pooled from 2-3 independent experiments.

Cell survival and flow cytometry

For the measurements, tumor cells were harvested by peritoneal lavage with 0.9 % NaCl 24 h after the completion of therapy *i.e.* 48 hours after the beginning of therapy. Tumor cells harvested from individual animals by peritoneal lavage were used for the determination of both, the cell survival and DNA distribution pattern. The effect of different treatments on cell survival was determined by counting the viable cells (Trypan dye exclusion test) in the peritoneal lavage of animals by means of hemocytometer. The results of cell survival were presented as a percentage of cells compared to the number of cells in the control animals. For flow cytometry measurements, cells were centrifuged, resuspended in citrate buffer solution and stored at -20 °C. Samples were then prepared according to the manufacturers protocol (CycleTEST PLUS, Becton Dickinson) and analysed using FACSCalibur flow cytometer (Becton Dickinson). The flow cytometer was operated at 488 nm and, after pulse shape analysis and gating on a cytogram of orthogonal vs. forward light scatter, a histogram of cell number against red (DNA-PI) fluorescence was recorded. Four different cell population were determined, cells in G_1, S, G_2M phases of cell cycle and cell with values of DNA greater than in G_2M compartment.

Animal survival assay

To determine the effect of BLM on the survival of mice pretreated with VLB, the mice were treated according to the treatment protocol and monitored for the day of death.

Statistical analysis

Data are represented as arithmetic means ± s.e.m.. The significance of the effect was determined using Student's t-test after one-way analysis of variance was performed; the levels of less than 0.05 were taken as indicative of significant differences. Survival curves were plotted by the Kaplan-Meier method. The differences between the survival curves were determined by Log Rank test.

Results

Cell survival

Cytotoxic effects of VLB and BLM as single treatments and in the combined treatment schedules on i.p. SA-1 tumors were deter-

Table 1. Comparison of the survival of mice with i.p. SA-1 tumors treated with combination of VLB and BLM and as a single drug treatment

		p compared to		
Group	Control	VLB	BLM	BLM-24h-VLB
VLB	<0.0001			
BLM	<0.0001	0.0051		
BLM-24h-VLB[1]	<0.0001	<0.0001	0.016	
VLB-24h-BLM[2]	<0.0001	<0.0001	0.003	0.0035

[1] BLM injected 24h before VLB

[2] VLB injected 24h before BLM

mined 24 h after the completion of therapy by counting the cells in the peritoneal lavage of the mice.

Both drugs, either as single treatment or in different combined therapy schedules reduced the number of cells in the peritoneal lavage significantly compared to the control, saline treated animals (Figure 1). VLB as single treatment was more effective compared to BLM; however, the difference in the mean value was not statistically significant.

The best results were obtained with combination of VLB followed by BLM after 24 h. The combination of pretreatment with VLB

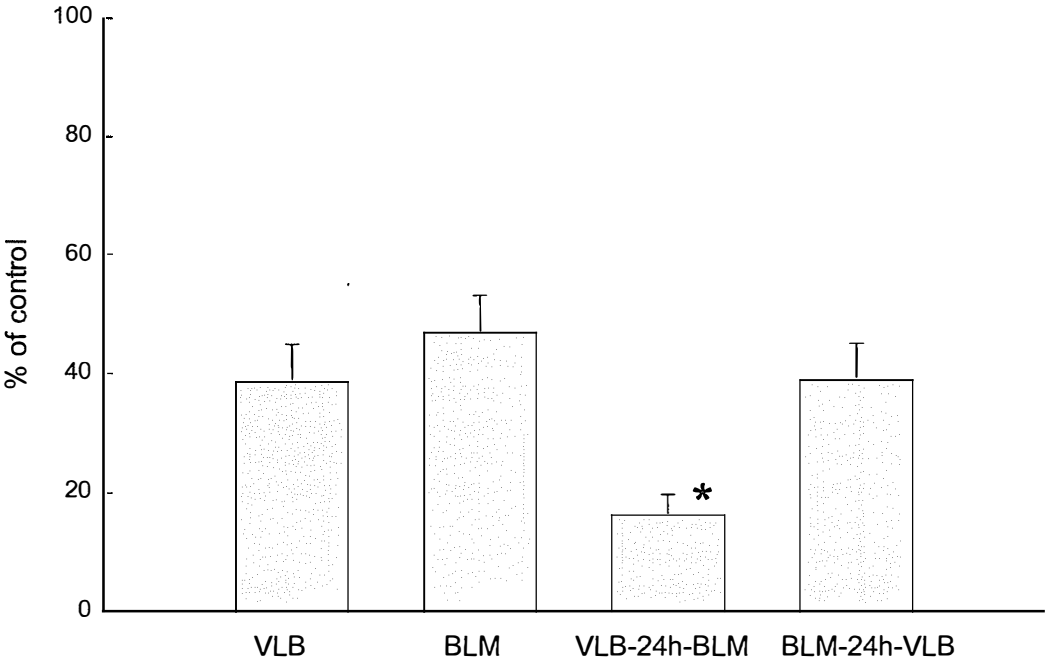


Figure 1. Number of cells in the peritoneal lavage of mice treated with VLB, BLM, VLB followed by BLM (VLB-24 h- BLM) and BLM followed by VLB (BLM-24h-VLB) presented as a percentage of number of viable cells in the peritoneal lavage of the control untreated mice. Bars are arithmetic means ± standard errors of the means. * $p<0.05$.

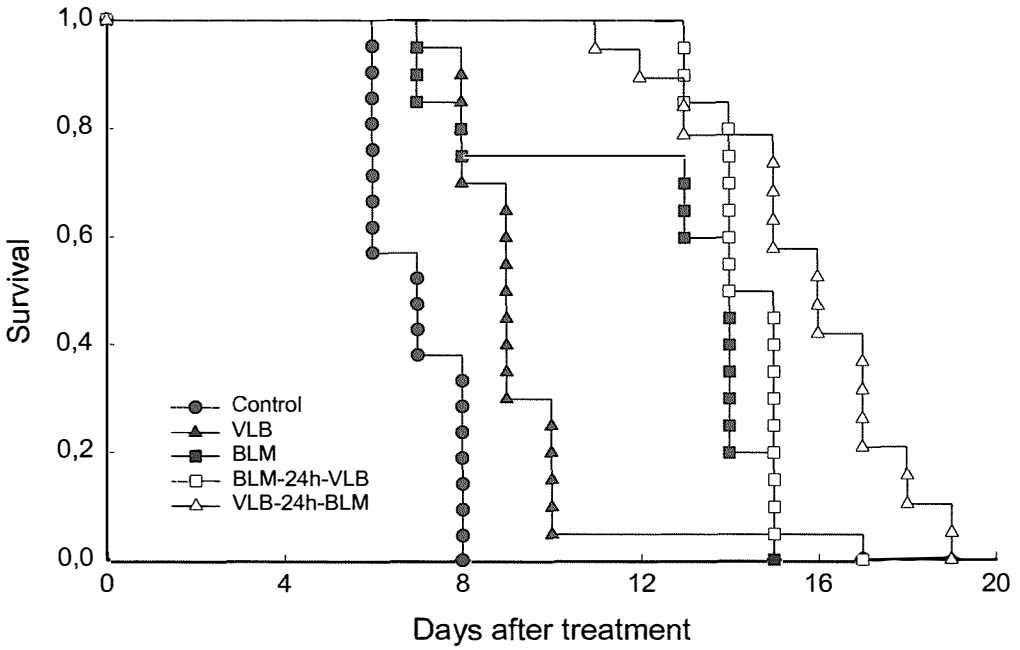


Figure 2. Survival of mice treated with VLB, BLM, VLB followed by BLM (VLB-24h-BLM) and BLM followed by VLB (BLM-24h-VLB). Survival curves were plotted by the Kaplan-Meier method.

followed by BLM reduced significantly the number of cells in the peritoneal lavage compared to the treatment in which BLM was followed by VLB or to the treatment with single drugs (Figure 1).

Animal survival

The antitumor effectiveness of VLB and BLM as single treatments and in the combined treatment schedules on i.p. SA-1 tumors were also determined on the survival of mice (Table 1, Figure 2). All treatments, VLB alone, BLM alone and combination of both drugs significantly prolonged median survival time of mice compared to the control untreated mice. When VLB and BLM were combined, both treatment combinations were more effective than monochemotherapies with VLB or BLM. The best results were obtained when VLB was followed by BLM after 24h. This treatment schedule resulted in the longest survival which was significantly better than in all other treatments (Table 1, Figure 1).

Flow cytometry

DNA histograms of the same samples that were used for measuring the cell survival were recorded by using FACSCalibur flow cytometer (Figure 3). The DNA histogram of cells treated with VLB showed a decreased number of cells in S phase and an increased number of cells with DNA values greater than in G_2M compartment groups compared to the control untreated cells.

BLM in the dosage used in these experiments did not affect the progression of cells through cell cycle. DNA histogram of the cells treated with BLM was in the same range as DNA histogram of the control untreated cells.

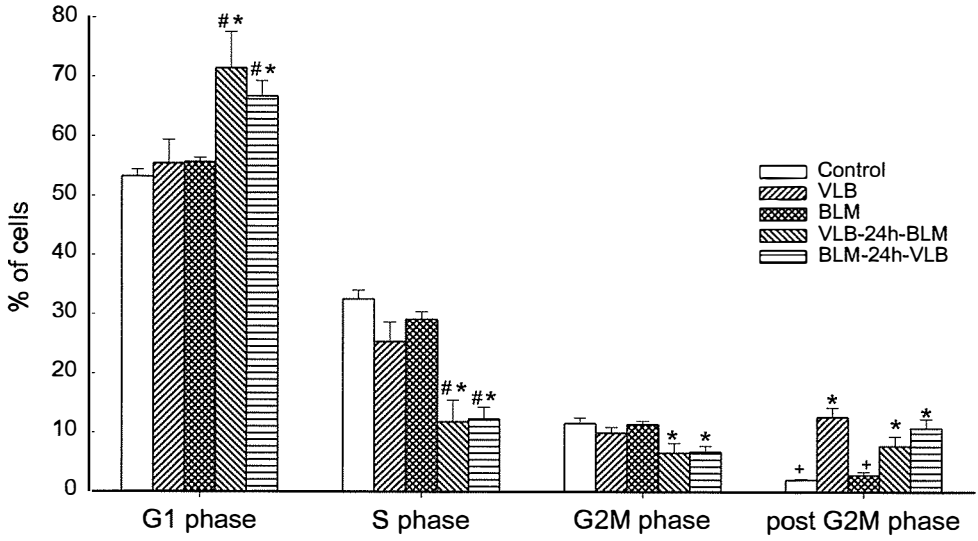


Figure 3. Percentage of cells in different compartment of cell cycle after the treatment of mice with VLB, BLM, VLB followed by BLM (VLB-24h-BLM) and BLM followed by VLB (BLM-24h-VLB). Bars are arithmetic means ± standard errors of the means. *p<0.05 compared to control and BLM treated animals, #p<0.05 compared to VLB treated animals, + p<0.05 compared to VLB treated animals and mice treated with combinations of VLB and BLM.

Both treatment combinations of VLB and BLM increased thge number of cells in G_1 phase, decreased the number of cells in S and G_2M phases of the cell cycle. In addition, both treatment combinations increased the number of cells with DNA values greater than in G_2M compartment, which was in the same range as the number of cells treated with VLB alone (Figure 3).

Discussion

Our study showed that pretreatment with VLB injected 24h before BLM enhanced antitumor effectiveness of BLM as determined by cell and animal survival of SA-1 tumor bearing mice. In spite of being widely used in various multidrug chemotherapy settings,[4,8,13,14] to our best knowledge, there is no studies exploring the role of timing of VLB and BLM.

VLB and BLM have different mechanisms of action. VLB interferes with the polymerization of tubulin, a protein which is involved in the formation of mitotic spindle microtubules and also an important component of cytoskeleton. In accordance with its effect on mitotic spindle microtubules, VLB blocks the cells in the metaphase of mitosis and thus acts as a cell synchronizing agent.[15] In addition, Madoc-Jones *et al.* reported on lethal action of VLB in interphase with the concentrations higher than those producing mitotic arrest.[17] The effect on cytoskeleton, however, might influence, beside the structures necessary for cell division, also the structures in the cell membrane. In our previous experiments using the same tumor model as in this study, we demonstrated that VLB increased plasma membrane fluidity as measured by electron paramagnetic resonance.[1] The same phenomenon was demonstrated in patients with squamous cell carcinoma and soft tissue sarcoma.[2,3] The results of both, experimental and clinical studies, suggested that VLB might be used for the enhancement of drug transport into the tumor cells. To prove this hypothesis, we tested the effectiveness of

VLB and BLM alone and in different combinations on the same tumor model as in the previous study.[11] The end-point used was animal survival. The results of that study showed that the best survival was obtained in mice treated with VLB 24 h before BLM. The observed effect was attributed to the increased plasma membrane fluidity and cell kinetic effect.[11] In the present study, we wanted to elucidate the mechanisms of this combination on the same tumor models, same drug combinations and at the same dosage of the drugs used by studying, beside animal survival, also cell survival and DNA distribution pattern. The animal survival experiments in the present study confirmed the results of previous experiments.[11] Again, the longest survival was obtained when VLB was given 24 h before BLM. In addition, this combined schedule also significantly reduced the cell survival compared to all three other treatments and to the control untreated tumors. In contrast, the survival of cells when BLM was followed by VLB did not significantly differ from the cell survival after treatment with single drugs. Therefore, the survival of cells did not match the survival of animals in this group. The survival of animals treated by BLM followed by VLB was better than the survival after monochemotherapies with VLB or BLM and worse than the survival of animals after treatment with VLB followed by BLM. One possible explanation for the discrepancy between the cell and animal survival after BLM followed by VLB is that cell death occurred later than 24 h after the completion of therapy, which was the time point for measuring cell survival in our experiments.

This increased effect of chemotherapy could be the result of either an increased plasma membrane fluidity or a cell kinetic effect caused by VLB or a combination of both effects. In our previous study, we found that VLB increases membrane fluidity of SA-1 tumor cells; we therefore assumed that this could be exploited to facilitate BLM uptake into the cells. To prove that an increased plasma membrane fluidity facilitates better accumulation of BLM in the cells, a measurement of BLM concentration in the cells after VLB treatment would be necessary. In our previous clinical studies using ^{99m}Tc labeled BLM (Tc-BLM), we showed that an increased accumulation of Tc-BLM was found in the tumors from approximately 24-48 h after the infusion of VLB.[8,16] In addition, our preliminary experiments using absorption spectroscopy for the quantification of cisplatin within the tumor cells showed a 4-fold increase of cisplatin after the pretreatment with VLB, compared to the treatment with cisplatin alone or when cisplatin was followed by VLB (Čemažar *et al.*, unpublished data).

A cell kinetic effect of VLB was proven by DNA single cell measurement. Cell kinetic effect of VLB seems to be dose dependent. Higher doses prolong the transition of cells through S phase, whereas lower doses, as used in the present study, increased the number of cells with DNA values greater than in G_2M phase of cell cycle.[17-19] BLM is reported to be the most effective in G_2M and G_1 and less in S phase of the cell cycle.[20] However, in the present study, the effect of BLM on the cell cycle was not demonstrated. One possible explanation for that could be high BLM dose used in our experiments which caused cell death in all phases of the cells cycle and therefore did not have the effect on the accumulation of the cells in a particular phase of the cell cycle. Both treatment combinations of VLB and BLM were effective in reducing cell survival which was reflected in DNA histograms as a decreased number of cells in S and G_2M phases of the cells cycle and a relative increase in the number of cells in the G_1 compartment of the cell cycle.

In conclusion, based on the known properties of VLB and BLM, we can assume that in the present study, predominantly an increased membrane fluidity and, possibly to a lesser extent, an accumulation of cells in

BLM-sensitive phases of cell cycle induced by VLB is responsible for the best effect of VLB and BLM combination in which VLB preceded BLM for 24 h. Our study also shows that understanding the interactions of agents in combined chemotherapeutic schedules could lead to a better planning and timing of drugs in clinical chemotherapy.

Acknowledgment

This work was supported by the Ministry of Science and Technology of the Republic of Slovenia. The authors thank Mira Lavrič, Karmen Zajc, Brigita Šturbej and Marjana Matič for their excellent technical assistance.

References

1. Serša G, Čemažar M, Šentjurc M, Us-Kraševec M, Kalebić S, Drašlar K, Auersperg M. Effects of vinblastine on cell membrane fluidity and the growth of SA-1 tumor in mice. *Cancer Lett* 1994; **79**: 53-60.

2. Šentjurc M, Schara M, Auersperg M, Jezernik M, Kveder M. Characterization of malignant tissues by EPR. *Stud Biophys* 1990; **136**: 201-8.

3. Šentjurc M, Schara M, Auersperg, M. Influence of vinblastine containing chemotherapy on tumor tissue membrane fluidity; an EPR study. *Arch Geschwulstforsch* 1988; **58**: 35-42.

4. Haskell CM. Drugs used in cancer chemotherapy. In: Haskell CM ed. *Cancer treatment*. WB Saunders Company; 1990. p. 69-70.

5. Van Tellingen O, Sips JMH, Beijnen JH, Bult A, Nooijen WJ. Pharmacology, bio-analysis and pharmacokinetics of the Vinca alkaloids and semi-synthetic derivates (review). *Anti-Cancer Res* 1992; **12**: 1699-716.

6. Auersperg M, Šoba E, Vraspir-Porenta O. Intravenous chemotherapy with synchronization in advanced cancer of oral cavity and oropharynx. *Z Krebsfotsch* 1977; **90**: 149-59.

7. Auersperg M, Us-Kraševec M, Lamovec J, Erjavec M, Benulič T, Porenta Vraspir O. Chemotherapy-a new approach to the treatment of verrucous carcinoma. *Radiol Iugosl* 1989; **23**: 387-92.

8. Auersperg M, Šoba E, Porenta O, Erjavec M, Us-Kraševec M, Bergant D, Furlan L. Rational scheduling of multidrug chemotherapy with synchronization in advanced squamous cell carcinoma of the oral cavity and oropharynx. In: Čupar I and Padovan I eds. *Currents concepts of head and neck cancers*. Jugoslavenska Akademija Znanosti i Umjetnosti; 1981. p. 286-99.

9. Lazo JS, Sebti SM, Schellens JHM. Bleomycin. In: Pinedo HM, Longo DL, Chabner BA, eds. *Cancer chemotherapy and biological response modifiers annual 16*. Elsevier Science B.V.; 1996. p. 39-47.

10. Tounekti O, Pron G, Belehradek J, Mir LM. Bleomycin, an apoptosis-mimetic drug that induced two types of cell death depending on the number of molecules internalized. *Cancer Res* 1993; **53**: 5462-9.

11. Čemažar M, Auersperg M, Serša G. Vinblastine increases antitumor effectiveness of bleomycin. *Radiol Oncol* 1997; **31**: 364-7.

12. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. *Cancer Chemother Rep* 1966; **50**: 219-45.

13. Jaffe N, LeeYY, Auersperg M, Us-Kraševec M, Porenta O, Gohde W, Lamovec J. Novel therapeutic strategies for the treatment of rhabdomyosarcoma and soft tissue sarcomas: Observations with arterial infusion chemotherapy. In: Maurer HM, Ruymann FB, Pochedly CE. eds. *Rhabdomyosarcoma and related tumors in children and adolescents*. Boca Raton, CRC Press; 1991. p. 243-60.

14. Pecorelli S, Wagenaar HC, Vergore IB, Curran D, Beex LVA, Wiltshaw E, Vermorken JB. Cisplatin (P), vinblastine (V) and bleomycin (B) combination chemotherapy in recurrent or advanced granulosa(-theca) cell tumors of the ovary. An EORTC gynaecological cancer cooperative group study. *Eur J Cancer* 1999; **35**: 1331-7.

15. Jordan MA, Thower D, Wilson L. Mechanism of inhibition of cell proliferation by Vinca alkaloids. *Cancer Res* 1991; **51**: 2212-22.

16. Auersperg A, Erjavec M, Us-Kraševec M. Accumulation of ^{99m}Tc-Bleomycin in human squamous cell carcinoma in vivo after synhronisation by vinblastine. *IRCS Medical Science: Cancer; Clinical Pharmacology and Therapeutics; Radiology and Nuclear Medicine* 1975; 3: 560.

17. Madoc-Jones H, Mauro F. Interphase action of vinblatine and vincristine: differences in their lethal action through the mitotic cycle of cultured mammalian cells. *J Cell Physiol* 1972; **72**: 185-96.

18. Auersperg M, Us-Kraševec M, Bešič N, Pogačnik A, Stanič K, Jezeršek B. Flow-cytophotometric DNA measurements for monitoring and planning chemotherapy in anaplastic thyroid carcinoma. In: Pimpl W, Galvan G, Kogelnik HD, Manfreda D, Niederle B, Schlag P, Waclawiczek HW eds. *Struma maligna*. Springer-Verlag; 1993. p. 107-13.

19. Auersperg M, Us-Kraševec M, Pogačnik A, Stanič K, Hočevar M, Jezeršek B. Flow-cytophotometric DNA measurements for planning chemotherapy in differentiated thyroid cancer *Radiol Oncol* 1993; **27 Suppl 6**:192S-7S.

20. Olive PL, Banath JP. Detection of DNA double strand breaks through the cell cycle after exposure to X-rays, bleomycin, etoposide and 125IdUrd. *Int J Radiat Biol* 1993; **64**: 349-58.

Radiol Oncol 2000; 34(1): 59-65.

Influence of hydralazine on interstitial fluid pressure in experimental tumors - a preliminary study

Blaž Podobnik and Damijan Miklavčič

Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia

Background. *Interstitial fluid pressure (IFP) has been recognised as the most important obstacle in macromolecular drug delivery to solid tumors. Our interest was to reduce differentialy tumor IFP with respect to IFP in surrounding and normal tissues in order to increase drug delivery to tumors as well to increase tumor blood flow and potentialy tumor tissue oxygenation. In this preliminary study we used hydralazine, a long-acting arterial vasodilator.*

Materials and methods. *Measurements of interstitial fluid pressure were performed in vivo on CBA mice bearing SAF tumors using wick-in-needle technique. Altogether eleven measurements were obtained on different animals with tumors of different size.*

Results. *IFP in tumors after hydralazine administration was significantly lower than initial values in corresponding tumors. On average tumor IFP decreased for 33% from initial value. On the contrary, no change in IFP in normal tissue was observed after hydralazine administration. Also, after injection of physiological saline instead of hydralazine there was no change in IFP neither in tumors nor in muscle. The results of our preliminary study on the effect of hydralazine on IFP in SAF tumor model is in accordance to previously reported studies. The decrease in tumor IFP was only observed in tumors, but not in muscle and surrounding subcutis.*

Conclusion. *Hydralazine is a vasodilator which is capable of decreasing tumor IFP, reproducibly and with favorably long lasting dynamics.*

Key words: sarcoma; experimental-drug therapy; hydralazine; extracellular space; interstitial fluid pressure; manometry

Introduction

Interstitial fluid pressure (IFP) has been recognised as the most important obstacle in macromolecular drug delivery to solid tumors.[1,2,3] IFP was also correlated with tumor blod flow.[4,5] Recent clinical study involving patients with cervical carcinoma[6] reported that tumors with high IFP were more likely to be hypoxic and less likely to regress completely after radiotherapy. Elevated IFP in solid tumors hinders fluid filtration from tumor vasculature which is the prime driving force for macromolecular transvascular flow. However, it is not clear at the moment how elevated IFP would affect tumor blood flow

Corespondence to: Damijan Miklavčič, DSc, Faculty of Electrical Engineering, Tržaška 25, 1000 Ljubljana. Tel: +386 61 176 84 56; Fax: +386 61 126 46 58; E-mail: damijan@svarun.fe.uni-lj.si

and oxygenation. Nevertheless, there is an obvious and increasing interest in modulating IFP in solid tumors thus by decresing it, facilitating macromolecular drug delivery like monoclonal antibodies into tumor tissue and possibly modyfing tumor blood flow and tissue oxygenation. Various vasoactive drugs have been used with variable success to modulate solid tumor IFP. Most of the drugs used (e.g. nikotinamide, angiotensin II, epinephrine, norepinephrin, nitroglycerin and hydralazine) have been reported to modulate tumor IFP.[7] In general, vasoconstricting agents resulted in increase of tumor IFP whereas vasodilating agents produced decrease in tumor IFP. Our interest was to reduce differentialy tumor IFP with respect to IFP in surrounding and normal tissues in order to increase drug delivery to tumors as well to increase tumor blood flow and potentialy tumor tissue oxygenation. In this preliminary study we used hydralazine, a long-acting arterial vasodilator. After i.v. administration we measured IFP in solid subcutaneous tumors (SAF anaplastic sarcoma) and subcutis close to tumor and/or muscle tissue in CBA mice.

Materials and methods

Animals and tumor model

All experiments were performed on 8 to 10 week old female CBA mice which were maintained under standard laboratory conditions with food and water *ad libitum*. The SAF (anaplastic sarcoma; 0.1 ml of crude tumor cell suspension) was transplanted subcutaneously under sterile conditions dorsolaterally on a right flank of the mice. Experiments were performed on tumors of different size ranging from 95 mm^3 to 800 mm^3. All experiments were performed at the Department of Tumor Biology, Institute of Oncology, Ljubljana in accordance with ethical provisions for research on animals.

Anesthesia

The treatment and measurements of animals which could cause discomfort or pain to animals were performed under general anesthesia. The mice were anesthetized with Isoflurane (Flurane-Isoflurane, Abbot Labs Ltd., UK; waporizer-Isotec 337C, Ohmeda, USA) gas anesthesia (1.5-2% of Isoflurane was mixed with NO_2, O_2 mixture; flow of NO_2 and O_2 was 0.6 l/min). Animals were anesthesied and placed on a heating pad (TCU 035,27S, Cheshire, UK) which maintained stable body temperature. Throughout the experiment rectal temperature and heating pad surface temperature were monitored. The rectal temperature was between 37 and 38 °C and the maximal surface temperature of heating pad was lower than 40 °C.

Drugs

Hydralazine (Hydrazinophthalazine, Sigma Chemical Co., St. Louis, MO) was dissolved in sterile saline (0.9% NaCl) prior to each experiment. A dose of 2.5 mg/kg was injected intravenously (i.v.) into retroorbital sinus.

Measuring technique and experimental protocol

IFP was measured by the wick-in-needle technique[8,3,9] using a 0.5 mm (25G) needle probe (Terumo Belgium) with a 2 mm sidehole about 3 mm from the tip. The needles were filled with two surgical thread fibers (5-0, Seide Silk). Prior to each experiment the measurement system was calibrated. All recordings of IFP were performed as two channel measurements, measuring IFP in tumor and in subcutis close to tumor or muscle. Needles were connected to pressure transducers (TSD104 and TSD104A, Biopac Systems Inc., CA-Goleta, USA) by a polyethylene tube and the entire system was filed with physiological saline (0.9% NaCl) which contained heparin (Krka, Slovenia) 72 u/ml for preventing blood clots to be formed. Special care was taken to

avoid trapping of air bubbles in a system during the filling. Saline in system was used as a conductor of pressure. Pressure transducers were connected via amplifier (DA100A, Biopac System Inc.) and data acquisition unit (MP100, Biopac Systems Inc.) on personal computer. The sampling frequency was 10 Hz.

During calibration of a measurement system zero reference pressure was obtained by placing the needles in a heparinesed physiological saline-filled beaker and calibration of pressure was done by elevating or lowering the beaker. At different levels (1cm and 30 cm) of liquid column (level 0 cm was equal to level of needle insertion into a tumor or muscle), output voltages of pressure sensors were measured and used for calibration. After the calibration one needle was inserted into the center of a tumor and the other one into subcutis or a muscle of a right hind limb. After that needles were slightly withdrawn to avoid compression of the tumor and muscle under the probe tip and left in place without external fixation. All measurements lasted for 2 hours or longer.

One of complete IFP measurements is given in Figure 1. After the initial equilibration period, compression/decompression (C/D) test was performed. This test allowed us to verify the continuum between the fluid phase in interstitium and needle lumen. By tightening the clamp on a polyethylene tube so as to inject a volume of approximately 0.2 µl into the tissue caused a sudden pressure rise (Figure 1). The pressure then declined, first rapidly and then more slowly, restabilized to the initial level within 30 seconds to 2 min. Withdrawal of the same amount of fluid by loosing the clamp gave a reverse response, a sudden fall in pressure with gradual return to the original level (Figure 1). This test was performed at the beginning and at the end of

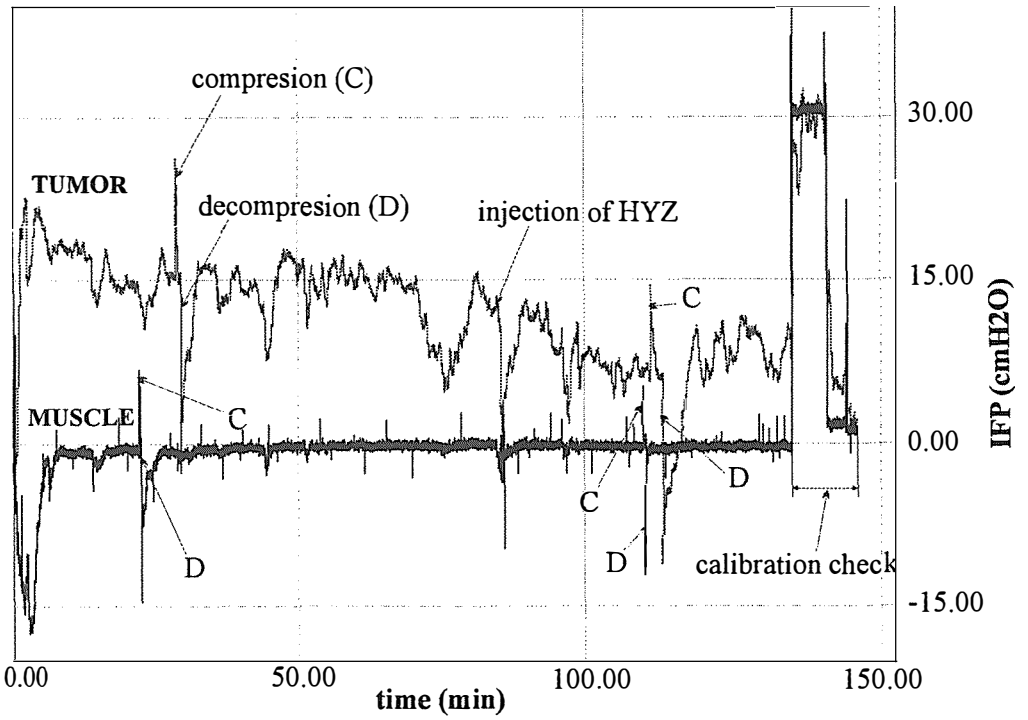


Figure 1. An example of IFP recording during the entire experiment. IFP in tumor and muscle tissue including compression (C) /decompression (D) test, injection of hydralazine (HYZ) and calibration chack are given.

each experiment and gave us the information about the quality and reliability of IFP measurements. Only results obtained in experiments where both tests were correct, were accepted and considered as reliable. Hydralazine was injected i.v. after a stable recording of IFP was obtained. The response of IFP to hydralazine was monitored for approximately one hour. After that period C/D test was performed again and needles removed from tissue. Measurement was finished with the calibration test in order to verify the calibration procedure performed prior to the beginning of the experiment.[10]

Data processing and statistical analysis

Initial IFP values in tumors and muscle or subcutis was determined as the mean value of IFP recording in the interval of app. 20 minutes duration after the first C/D test and prior to hydralazine injection. The values of IFP after hydralazine injection were determined as the mean value of IFP recording in the interval of app. 30 minutes duration starting at 10-15 minutes after hydralazine (or physiological saline) injection. All values are reported in tables and figures as mean±standard deviation. Statistical analysis of the data was performed with a paired t-test comparing measured values of IFP values in tumors before and after hydralazine injection, IFP in muscle tissue or subcutis before and after hydralazine injection after normality test was performed and fulfilled. Exact p-values are reported.

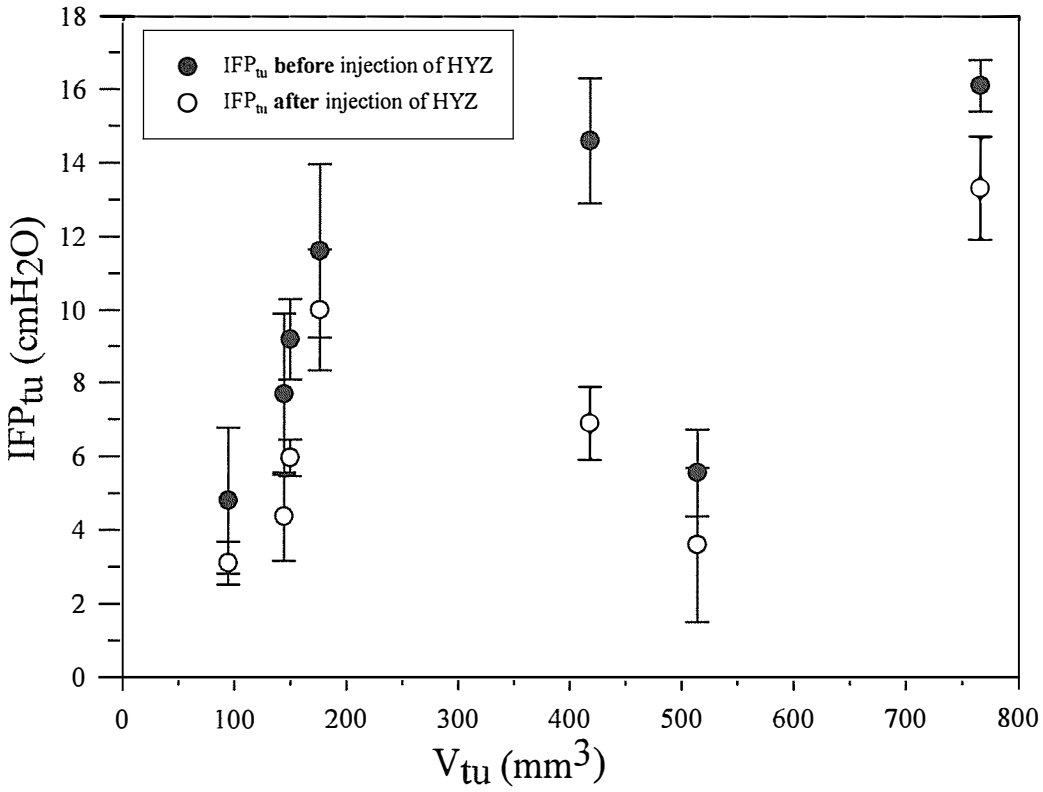


Figure 2. Picture shows interstitial fluid pressure (IFP) with standard deviation in SAF tumors respective on tumor volume. Black dots present IFP before injection of hydralazine (HYZ) and white dots present IFP after HYZ injection.

Table 1. Mean values of interstitial fluid pressure (IFP) with standard deviations in tumor (IFP_{tu}) and in muscle (IFP_{mu}) before and after injection of 2.5 mg/kg hydralazine (HYZ) with respect to tumor volume (V_{tu}). Decrease induced by HYZ in tumor and muscle IFP are given (ΔIFP_{tu} and (ΔIFP_{mu}, respectively)

V_{tu} (mm^3)	IFP_{tu} *BEFORE HYZ* (cmH_2O)	IFP_{tu} *AFTER HYZ* (cmH_2O)	ΔIFP_{tu} (cmH_2O)	IFP_{mu} *BEFORE HYZ* (cmH_2O)	IFP_{mu} *AFTER HYZ* (cmH_2O)	ΔIFP_{mu} (cmH_2O)
94.20	4.80 ± 1.9	3.10 ± 0.6	*1.70*	1.00 ± 0.3	0.90 ± 0.4	*0.10*
144.10	7.70 ± 2.2	4.36 ± 1.2	*3.34*	1.20 ± 1.5	0.77 ± 0.6	*0.43*
149.30	9.20 ± 1.1	5.96 ± 0.5	*3.24*	0.72 ± 1.1	0.79 ± 0.3	*-0.07*
175.80	11.60 ± 2.4	10.00 ± 1.7	*1.60*	0.37 ± 0.4	0.33 ± 0.7	*0.04*
417.80	14.60 ± 1.7	6.90 ± 1.0	*7.70*	***-0.20 ± 0.3**	***-0.15 ± 0.5**	***-0.05**
514.10	5.60 ± 1.2	3.60 ± 2.1	*2.00*	0.35 ± 0.2	0.40 ± 1.0	*-0.05*
765.80	16.10 ± 0.7	13.30 ± 1.4	*2.80*	***-0.10 ± 0.3**	***0.05 ± 0.3**	***-0.15**

*IFP values in subcutis

Results

Measurements of interstitial fluid pressure were performed in vivo on CBA mice bearing SAF tumors using wick-in-needle technique. Altogether seven measurements were obtained on different animals with tumors of different size in the range of 95 mm^3 to 800 mm^3. At the same time IFP in muscle or subcutis before and after hydralazine administration was measured. In addition four control measurements were performed, where instead of hydralazine physiological saline (80 to 100 µl, depending on mouse weight) was injected. We determined initial value of IFP in tumors and normal tissue (*i.e.* muscle or subcutis) during the stable recording of IFP signal (approximately 20-25 minutes before injection) and IFP after hydralazine or physiological saline injection during app. 30 minutes interval starting 10-15 minutes after injection as described in materials and methods section.

In general, immediately after needle insertion we recorded high negative IFP in tumor and in muscle or subcutis (Figure 1). Under control conditions when physiological saline was injected, the IFP returned rapidly and stabilized at a level around 0 cmH_2O for muscle and somewhere between 4 and 16 cmH_2O for tumor, depending on tumor size (Figure 2). After 10 to 15 minutes following hydralazine injection the IFP in tumor decreased on average for 33 % from initial level but there was no change of IFP in muscle or in subcutis (Table 1 and 2). The level which was observed lasted at least 30 minutes and then it slowly raised up towards the initial value.

Initial values of IFP in tumors based on measurements prior to any manipulations were between 4.8 and 16.1 cm H_2O with the mean±std value of 9.6±4.0 cm H_2O (n=11). The initial values of IFP in tumors were higher in larger tumors, as previously observed.[10] Initial values of IFP in muscle were between 0.3 and 1.6 cmH_2O and in subcutis between -0.1 and -0.2 cmH_2O with the mean±std value of 0.7±0.4 cm H_2O (n=9) and -0.15±0.07 cmH_2O (n=2), respectively.

The values of IFP in tumors and normal tissue (muscle and subcutis) after hydralazine administration are given in (Table 1) for each tumor and corresponding muscle or subcutis IFP measured at the same time. IFP in tumors after hydralazine administration was significantly lower than initial values in corresponding tumors (paired t-test: p=0.016). On aver-

Table 2. Mean values of interstitial fluid pressure (IFP) with standard deviations in tumor (IFP_{tu}) and in muscle (IFP_{mu}) before and after injection of 80-100 µl physiological saline (PHYS.SAL.) with respect to tumor volume (V_{tu}). Decrease induced by PHIS.SAL. in tumor and muscle IFP are given (ΔIFP_{tu} and ΔIFP_{mu}, respectively)

V_{tu} (mm^3)	IFP_{tu} *BEFORE PHYS. SAL* (cmH_2O)	$IFP_{tu\ AFTER}$ *PHYS. SAL* (cmH_2O)	$\Delta\ IFP_{tu}$ (cmH_2O)	IFP_{mu} *BEFORE PHYS. SAL* (cmH_2O)	$IFP_{mu\ AFTER}$ *PHYS. SAL* (cmH_2O)	$\Delta\ IFP_{mu}$ (cmH_2O)
94.50	9.41 ± 1.0	10.60 ± 0.9	*-1.19*	0.32 ± 0.2	0.50 ± 0.3	*-0.18*
136.00	14.00 ± 0.9	14.30 ± 1.2	*-0.30*	0.36 ± 0.4	0.30 ± 0.4	*0.06*
195.60	5.10 ± 1.0	5.70 ± 0.9	*-0.60*	1.60 ± 0.3	1.60 ± 0.3	*-0.00*
228.80	7.85 ± 1.0	7.60 ± 1.3	*0.25*	0.41 ± 0.2	0.45 ± 0.2	*-0.04*

age tumor IFP decreased for 33% from initial value. This decrease was between 14 to 53% in individual tumors (mean±std ΔIFP = 2.37±0.8 cmH_2O). On the contrary, no change in IFP in normal tissue was observed after hydralazine administration resulting in mean±std ΔIFP = 0.09±0.2 cmH_2O (p=0.376) (Table 1). In addition, injection of physiological saline instead of hydralazine produced no change neither in tumors with mean±std ΔIFP = -0.46±0.6 cmH_2O (p=0.223) nor in muscle with mean±std ΔIFP = -0.04±0.1 cmH_2O (p=0.490) (Table 2).

Discussion

The results of our preliminary study on the effect of hydralazine on interstitial fluid pressure in SAF tumor model is well in accordance to previously reported studies[4,7], both in the amplitude and the duration of response. The choice of hydralazine dose was based on previous studies, where comparable doses resulted in 50% deacrease in mean arterial blood pressure which occured within 10-15 minutes after hydralazine injection and lasted for at least 30 minutes.[4] In another study[7] both mean arterial blood pressure as well as tumor IFP were measured after 60µg of hydralazine injection (which corresponds to app. 3 mg/kg dose). The reduction of mean arterial blood pressure of 50% was obtained which is the same as in previously mentioned study[4] and approximately 50% reduction in tumor IFP was reported. In our study we observed an average of 33% (range: 14-53%) decrease in tumor IFP with respect to initial value. This response was noticed within 15 minutes after hydralazin injection and lasted for at least 30 minutes. The exact relation between mean arterial blood flow pressure and interstitial fluid pressure is not well known. In the same study[7] they developed a network model where they explored the role of mean arterial blood flow and tumor IFP. In addition, we did not observe any significant changes in IFP in muscle or subcutis, demonstrating a potentialy interesting differential effect. Other authors stipulated that observed decrease in tumor interstitial fluid pressure after hydralazine injection could be explained by the reverse steal-effect, which remains to be confirmed.

Our results on initial elevated interstitial fluid pressure and its dependence on tumor size are also well in accordance with our previous results[10] and the model we developed.[10,11]

In additional control experiments where we injected physiological saline only, no significant decrease in IFP was noticed after the

injection of phisiological saline apart from a short lived decrease in IFP which was present in both signals (in tumor and in muscle). This decrease was however a transient and IFP returned to the initial level within two to three minutes.

In conclusion, hydralazine is a vasodilator which is capable of decreasing tumor interstitial fluid pressure, reproducibly and with favorably long lasting dynamics. The decrease in tumor interstitial fluid pressure was only observed in tumors, but not in muscle and surrounding subcutis. Whether the response depends on tumor size and is accompanied by changes in tumor blood flow as suggested by other authors[4,7] and how would these affect tumor oxygenation remains to be determined.

Acknowledgements

This research was supported by The Ministry of Science and Technology of the Republic of Slovenia. Authors would like to express their gratitude to Maja Čemažar and Mira Lavrič, Simona Kranjc for their preparation of tumors and caring for the animals and to Jani Pušenjak for valuable directions in performing IFP measurements.

References

1. Jain RK. Transport of molecules across tumor vasculature. *Cancer Metas Rev* 1987; **6**: 559-93.
2. Jain RK. Transport of Molecules in the Tumor Interstitium: A Review. *Cancer Res* 1987; **47**: 3039-51.
3. Pušenjak J, Miklavčič D. Interstitial fluid pressure as an obstacle in treatment of solid tumors. *Radiol Oncol* 1997; **31**: 291-97.
4. Horsman MR, Christensen KL, Overgaard J. Relationship Between the Hidralazine-induced Cchanges in Murine Tumor Blood Supply and Mouse Blood Pressure. *Int J Radiat Oncol Biol Phys* 1992; **22**: 455-58.
5. Milosevic MF, Fyles AW, Hill RP. The Relationship Between Elevated Interstitial Fluid Pressure and Blood Flow in Tumors: A Bioengineering Analysis. *Int J Radiat Oncol Biol Phys* 1999; **43**: 1111-23.
6. Milosevic MF, Fzles AW, Wong R, Pintilie M, Kavanagh MC, Levin W, Manchul LA, Keane TJ, Hill RP. Interstitial Fluid Pressure in Cervical Carcinoma. *Cancer* 1998; 8**2**: 2418-26.
7. Zlotecki RA, Baxter LT, Boucher Y, Jain RK. Pharmacologic Modification of Tumor Blood Flow and Interstitial Fluid Pressure in a Human Tumor Xenograft: Network Analysis and Mechanistic Interpretation. *Microvasc Res* 1995; **50**: 429-43.
8. Fadnes HO, Reed RK, Aukland K. Intestitial Fluid Pressure in Rats Measured With a Modified Wick Technique. *Microvasc Res* 1977; **14**: 27-36.
9. Boucher Y., Rakesh KJ. Microvascular Pressure Is the Principal Driving Force for Interstitial Hypertension in Solid Tumors: Implications for Vascular Collapse. *Cancer Res* 1992; **52**: 5110-14.
10. Pušenjak J. Izdelava modela in merjenje tlaka medcelične tekočine v čvrstem tumorju. *Magistrska naloga* 1999; Ljubljana:Fakulteta za elektrotehniko.
11. Pušenjak J, Miklavčič D. Modeling of interstitial fluid pressure in solid tumors. *Journal of Simulation Practice and Theory* 2000 (accepted for publication).

Radiol Oncol 2000; 34(1): 1-9.

Korelacija ^{18}F-FDG-PET s histopatologijo pri bolnikh z malignim melanomom

Bohuslavizki KH, Klutmann S, Neuber K, Wedler J, Altenhoff J, Kröger S, Buchert R, Bleckmann C, Clausen M

Izhodišča. Preliminarna poročila kažejo, da bi lahko bil PET z uporabo ^{18}F-FDG pomembno diagnostično orodje pri bolnikih z napredovalim malignim melanomom. Cilj te študije je bil korelirati lezije prikazane s PET z histološkimi najdbami pri zamejevanju malignega melanoma.

Bolniki in metode. Pri skupno 82 bolnikih z malignim melanomom smo opravili 107 PET preiskav za primarno zamejitev ali spremljanje terapije. Po intravenozni aplikaciji 370MBq ^{18}F-FDG smo na ECAT EXACT 47 (921) posneli sliko celega telesa z aksialnim vidnim poljem 16.2 cm (siemens CTI). Histološko smo evaluirali na tumor sumljive PET izvide in klinične izvide in jih med seboj korelirali lezijo po lezijo.

Rezultati. S PET smo odkrili 124 lezij s povečanim fokalnim privzemom tracerja. Dodatno so klinični pregledi in konvencionalna slikovna diagnostika odkrili 65 za tumor sumljivh lezij. Skupno smo odkrili 189 za tumor sumljivih lezij in jih histološko ocenili. ^{18}F-FDG-PET je bil resnično pozitiven v 115 od 189 lezij in lažno pozitiven v 9. Pri 21 od 65 PET negativnih lezijah biopsija ni mogla potrditi melanomskega tkiva. Nasprotno pa je biopsija v 44 od 65 PET negativnih lezijah potrdila maligni melanom. Občutljivost in specifičnost ^{18}F-FDG-PET pri detekciji malignega melanoma je bila 72 oziroma 70%. Negativne in pozitivne predikcijske vrednosti ^{18}F-FDG-PET so bile 32 oziroma 93%. Glede na lokalizacijo sta bili 2/3 lažno negativnih oziroma pozitivnih lezij v koži in v mukozi.

Zaključki. ^{18}F-FDG-PET je dragoceno diagnostično orodje za dokazovanje za tumor sumljivih lezij malignega melanoma. Vsekakor pa je za izključitev kožnih metastaz nenadomestljiv klinični pregled bolnikov z malignim melanomom pri dermatologu. Glede na zelo agresivno zdravljenje napredovalega malignega melanoma lahko ^{18}F-FDG-PET pomaga pri izbiri pravega protokola za posameznega bolnika.

Radiol Oncol 2000; 34(1): 11-9.

Pomen ^{18}F-FDG-PET pri vodenju bolnikov z osteosarkomom

Bohuslavizki KH, Klutmann S, Bruns J, Kröger S, Bleckmann C, Buchert R, Dobrowolskij D, Mester J, Clausen M

Izhodišča. Namen te študije je bil ovrednotiti uporabnost ^{18}F-FDG-PET pri zdravljenju bolnikov z osteosarkomom, ki temelji na sedanjih režimih zdravljenja.

Bolniki in metode. Retrospektivno smo raziskali 18 bolnikov (4 ženske, 14 moških) starih med 14 in 63 let z primarnim osteosarkomom (n=6) ali sumom na relaps osteosarkoma (n=12). Pri vseh bolnikih smo najprej opravili konvencionalno diagnostiko (RTG in MRI primarne kostne lezije, CT toraksa in konvencionalni scintigram kosti). Dodatno smo napravili še PET celega telesa na ECAT EXACT 47 (921) z aksialnim vidnim poljem 16.2 cm (Siemens, CTI) po intravenozni aplikaciji 370 MBq ^{18}F-FDG. Vse za tumor sumljive izvide PET smo ocenili histološko. Izvide histologije, PET in konvencionalne diagnostike smo korelirali lezijo po lezijo.

Rezultati. ^{18}F-FDG-PET je jasno prikazal vse primarne osteosarkome pri šestih bolnikih in relaps osteosarkoma pri dveh bolnikih. Pri osmih izmed 18 bolnikov je prikazal še druge lezije z nenormalnim privzemom ^{18}F-FDG. Te lezije so bile v glavnem locirane v pljučih (n=5), okostju (n=3) in v ingvinalni regiji (n=1). Te lezije so se nahajale pri treh bolnikih s primarno diagnozo osteosarkoma in pri petih bolnikih z sumom na relaps osteosarkoma. Vse razen lezije ingvinalno so bile potrjene kot metastaze osteosarkoma. Pri interpretaciji teh vzpodbudnih rezultatov moramo upoštevati zdravljenje bolnikov. Ker vemo, da ima večina bolnikov ob diagnozi prisotne mikrometastaze, je standardno zdravljenje neoadjuvantna kemoterapija in kirurška resekcija tumorja. Tako ^{18}F-FDG-PET nima pomemnega vpliva pri primarni diagnozi osteosarkoma. Seveda pa je nekaj kliničnih situacij, pri katerih ima bolnik lahko korist od ^{18}F-FDG-PET, ker lahko le ta spremeni režim zdravljenja, na primer razlikovanje relapsa od sprememb po zdravljenju, diferencialna diagnoza pljučnih metastaz pri spremljanju bolnika in odkrivanje metastatske razširitve po inicialnem zdravljenju.

Zaključki. ^{18}F-FDG-PET nima pomembnega vpliva pri osnovni zamejitvi bolezni. Vseeno je lahko koristen v nekaterih kliničnih situacijah po neoadjuvantni kemoterapiji in kirurškem zdravljenju primarnega tumorja.

Radiol Oncol 2000; 34(1): 21-5.

Kombinirano zdravljenje raka sečnega mehurja z možnostjo ohranitve organa

Čufer T

Izhodišča. Radikalna cistekomija je še vedno standarden način zdravljenja mišičnoinvazivnega raka sečnega mehurja. Kljub hudi invalidnosti se pri polovici bolnikov v letih po posegu pojavijo oddaljeni zasevki, ki so vzrok njihovi smrti. Zaradi tega smo v zadnjih letih pri teh bolnikih preizkusili kombinirano zdravljenje z možnostjo ohranitve sečnega mehurja. Do sedaj objavljeni izsledki petih skupin kažejo, da je preživetje bolnikov zdravljenih z kombinacijo transuretralne resekcije (TUR), kemoterapije in obsevanja ter z ohranitvijo mehurja v primerih doseženega popolnega odgovora na zdravljenje, primerljivo s preživetjem bolnikov zdravljenih s cistekomijo. Ob tem okoli 60 do 70% bolnikov ohrani funkcionalen sečni mehur, petletno preživetje teh bolnikov pa je okoli 40%. Najpomembnejši napovedni dejavnik učinkovitega zdravljenja je popolen odgovor na TUR, kemoterapijo ali kemoradioterapijo. Skrben nadzor bolnikov z rednimi cistoskopskimi pregledi pa omogoča takojšnjo rešitveno cistektomijo.

Zaključki. Kombinirano zdravljenje raka sečnega mehurja z ohranitvijo organa omogoča pri določenih bolnikih podobno stopnjo ozdravitve in preživetja kot radikalna cistektomija, ob enem pa omogoča tudi ohranitev sečnega mehurja in njegove funkcije. Pričakujemo, da bo na podlagi bioloških dejavnikov v prihodnosti mogoče v naprej izbrati bolnike z večjo verjetnostjo odgovora na kemoradioterapijo, kar bo še izboljšalo rezultate kombiniranega zdravljenja in omogočilo ohranitev sečnega mehurja več bolnikom.

Radiol Oncol 2000; 34(1); 27-34.

Primarni ne-Hodgkinov lymphom kosti: zdravljenje in potek bolezni

Proulx GM, El-Agamawi AY, Lee RJ, Orner JB, Czuczman M, McCarthy P, Bernstein Z and Bernstein S

Izhodišča. Z raziskavo smo želeli ugotoviti značilnosti bolnikov s primarnim ne-Hodgkinovim limfomom kosti pa tudi kako smo jih zdravili na Roswelll Park Cancer inštitutu in kakšni so bili rezultati zdravlenja.
Bolniki in metode. V letih 1970-1996 smo preiskovali razširjenost bolezni in zdravili 18 bolnikov, ki so jim s kostno biopsijo histopatološko in imunohistokemično potrdili ne-Hodgkinov limfom kosti. 12 bolnikov je prejelo kombinirano kemoterapijo z antraciklini in so bili tudi lokalizirano-obsevani, 5 bolnikov smo samo obsevali in 1 je prejel samo kemoterapijo.
Rezultati. Ugotovili smo, da je 15 bolnikov imelo bolezenski stadij IEA in 3 bolniki stadij IVEA. Histopatološko pa je 17od 18 bolnikov imelo limfom srednje stopje malignosti, med njimi 13 bolnikov difuzni velikocelični, 2 difuzni mešani drobno- in velikocelični, 2 drobnocelični ter 1 od 18 bolnikov anaplastični velikocelični limfom. Srednja vrednost sledenja bolezni je bila13 let in 5 bolnikov, ki smo jih kombinirano zdravili ter eden, ki smo ga samo obsevali je še vedno brez znakov bolezni. 6 bolnikov, ki je prejelo kombinirano zdravljenje in 3 bolniki, ki so bili samo obsevani je umrlo. Pri dveh bolnikih je bila druga bolezen vzrok smrti, od njuju je bil eden kombinirano zdravljenjen in drug samo obsevan. O enem bolniku, ki je bil v popolni remisiji, po letu dni nismo več uspeli dobiti podatkov.
Zaključki. Raziskava nakazuje, da lahko bolnike z zgodnjimi stadiji primarnega ne- Hodgkinovega limfoma kosti uspešno zdravimo s kombinacijo kemoterapije in lokaliziranega obsevanja. Seveda pa so za dokončno ugotovitev potrebne večje randomizirane raziskave.

Radiol Oncol 2000; 34(1): 35-9.

Paraungvalni tumor? - Ne, samo tungiaza

Golouh R, Špiler M

Izhodišča. Tunga penetrans je zajedalska bolha, ki živi predvsem v Centralni in Južni Ameriki, Karibih, tropski Afriki, Indiji in v Pakistanu. Tungiaza nastane, ko se naseli v kožo gostitelja gravidna bolšja samica.
Prikaz primera. Opisujemo primer tungiaze pri pacientu, ki je bil pred tem na turističnem pohodu v Boliviji in Peruju. Bolnik je opazil paraungvalno infiltracijo kože palca na nogi. Ker je bila sprememba klinično sumljiva za tumor, so napravili ekscizijsko biopsijo.
Zaključki. Za pravilno diagnozo sta bili odločilni histološka slika in podatek o potovanju. Gre za prvi opisan primer tungiaze v Sloveniji.

Radiol Oncol 2000; 34(1): 67-73.

Radiol Oncol 2000; 34(1); 41-7.

Katepsin D in inhibotorji ter aktivatorji plasminogena tipa 1 v normalnem, benignem in malignem ovarijskem tkivu: Uvodno poročilo

Šprem M, Babić D, Abramić M, Miličić D, Vrhovec I, Škrk J, in Osmak M

Izhodišča. Študijo smo opravili z namenom, da bi določili koncentacijo katepsina D in aktivatorjev ter inhibitirojev plasminogena tipa 1 (PAI-1) v normalnem ovarijskem tkivu ter v tkivu benignega in malignega tumorja ter ocenili razmerje med vsebnostjo katepsina D in PAI-1 in še druge klinične ter patohistološke parametre.

Material in metode. V 35 vzrocih (10 vzorcev normalnega ovarijskega tkiva, 10 vzrocev benignega, 10 vzrocev malignega tumorskega tkiva in 5 vzorcev metastatskega ovarijskega tkiva) smo izmerili vsebnost katepsina D (izmerjeno z imunoradiometrično metodo ELISA-Cath D) in koncentracijo PAI-1 (izmerjeno s komercialno imunsko metodo IMUDINDR ELISA).

Rezultati. Koncentracija katepsinov D so bile bistveno višje v malignem (32.89 ± 14.26 pmol/mg protein) in metastatskem tkivu (31.42 ± 10.24 pmol/mg protein) kot pa v normalnem ovarijskem tkivu (13.68 ± 14.03 pmol/mg protein) ali tkivu benignega ovarijskega tumorja (17.89 ± 13.13 pmol/mg protein). Med koncentracijami PAI-1 v vzorcih normalnega tkiva in vzorcih benignega in malignega tumorskega tkiva ni bilo ugotovljenih statistično pomembnih razlik. Koncentracije katepsina D in PAI-1 tudi niso bile v korelaciji s starostjo bolnic, menpavzo, gravidnistjo, rizičnim GOG skupinam, kliničnemu stadije in patohistološko stopnjo tumorja.

Zaključki. Koncentracije katepsina D so bile pomembno večje v malignem in metastatskem ovarijskem tkivu, metdem ko koncentracije PAI-1 niso bile povečane. Koncentracije katepsinov niso bile odvisne od patohistoloških in kliničnih parametrov.

Radiol Oncol 2000; 34(1): 49-57.

Protitumorsko delovanje bleomicina na SA-1 tumorjih po predhodni terapiji z vinblastinom

M Čemažar, M Auersperg, G Serša

Izhodišča. V naši predhodni študiji smo ugotovili, da vinblastin (VLB) poveča fluidnost plazmaleme, kar bi lahko izkoristili za povečan transport zdravil preko plazmaleme. Namen naše raziskave je bil na mišjih intraperitonealnih tumorjih določiti, ali se poveča učinkovitost bleomicina (BLM) po predhodni terapiji z VLB.

Materiali in metode. Mišim smo injicirali samo VLB ali samo BLM ter obe zdravili v dveh kombinacijah: VLB injiciran 24 h pred BLM in BLM injiciran 24 h pred VLB. Učinkovitost terapije smo ugotavljali s preživetjem živali, štetjem celic, izoliranih iz peritonealne votline miši, ter DNA histogrami.

Rezultati. Obe zdravili, bodisi kot mono kemoterapiji ali v kombinaciji, sta povzročili značilno zmanjšanje števila celic v peritonealni votlini v primerjavi s kontrolno skupino. Ko smo injicirali VLB 24 h pred BLM je bilo število celic v izolatu peritonealne votline značilno zmanjšano v primerjavi z monokemoterapijama in s skupino, ko smo BLM injicirali 24 h pred VLB. Preživetje živali, zdravljenih samo z VLB ali samo z BLM ter obema kombinacijama zdravil, je bilo značilno podaljšano glede na kontrolno nezdravljeno skupino miši. Obe kombinaciji VLB in BLM sta bili tudi bolj učinkoviti kot monokemoterapiji. Najboljši rezultat pa smo dobili pri skupini, kjer smo injicirali VLB 24 h pred BLM. V primerjavi z DNA histogramom kontrolnih celic smo v DNA histogramu celic, ki so bile izpostavljene delovanju VLB, izmerili zmanjšano število celic v S fazi in povečano število celic z večjo količino DNA, kot jo imajo celice v G2M fazi celičnega ciklusa. Doza BLM, ki smo jo uporabili v naših poskusih, ni imela učinka na progresijo celic skozi celični ciklus. Obe kombinaciji VLB in BLM sta imeli podoben celično kinetični učinek kot sam VLB.

Zaključek. Glede na naše rezultate lahko zaključimo, da je mehanizem odgovoren za povečan učinek terapije, v kateri smo dali VLB 24 h pred BLM, predvsem povečana fluidnost celične membrane in verjetno tudi celično kinetični učinek VLB.

Radiol Oncol 2000; 34(1): 59-65.

Vpliv hydralazina na tlak medcelične tekočine v poskusnih tumorjih

Podobnik B in Miklavčič D

Izhodišča. Tlak medcelične tekočine (TMT) predstavlja glavno oviro pri vnosu makromolekul v čvrste tumorje. Naš namen je, znižati diferencialno tumorski TMT glede na TMT v okoliškem tkivu, da bi povečali vnos makromolekul, tumorski krvni pretok in s tem eventuelno izboljšati tudi oskrbo tumorskega tkiva s kisikom. V tej uvodni študiji je bil kot vazoaktivator uporabljen hidralazin (vazodilator z dolgotrajnim učinkom).

Materiali in metode. Meritve tlaka medcelične tekočine smo opravili s pomočjo igle s stenjem (angl. Wick-in-Needle - WIN technique) na miših tipa CBA s podkožno nasajenimi tumorji SAF. Skupno smo opravili enajst meritev na različnih živalih in v tumorjih različne velikosti.

Rezultati. TMT v tumorjih po vbrizgu hidralazina je bil značilno nižji, od začetne vrednosti TMT v teh istih tumorjih. V povprečju je padla vrednost TMT za 33% glede na začetno vrednost, medtem, ko v normalnem-zdravem tkivu po vbrizgu hidralazina ni bilo zaznati sprememb tlaka. Pri kontrolni skupini miši, kjer smo namesto hidralazina vbrizgali fiziološko raztopino sprememb TMT v tumorju in mišici nismo zaznali. Rezultati naše uvodne študije vpliva hidralizina na TMT v SAF tumorskih modelih so ponovljivi in se ujemajo z rezultati predhodnih študij. Znižanje TMT je opazno le v tumorjih medtem, ko v mišici in okoliškem tkivu teh sprememb ni bilo.

Zaključek. Rezultati potrjujejo, da je hidralazin vazodilator, ki značilno in za ustrezen čas zniža tlak medcelične tekočine v tumorjih.

Notices

Notices submitted for publication should contain a mailing address, phone and/or fax: number and/or e-mail of a ***Contact*** *person or department.*

Thoracic tumours

April, 2000

The ESO training course will take place in Nicosia, Cyprus.

Contact ESO office for Balkans and Middle East, N. Pavlidis, E. Andreopoulou Medical School, Department of Medical Oncology, University Hospital of Ioannina, 45110 Ioannina, Greece; or call +30 651 99394 or +30 953 91083; or fax +30 651 97505

Radiation oncology

April 2-6, 2000

The ESTRO teaching course on "Clinical Research in Radiation Oncology" will take place in York, United Kingdom.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759344; or fax +32 2 7795494; or e-mail martine.dansercoer@estro.be; or see Internet http://www.estro.be

Surgical oncology

April 5-8, 2000

The "10th Congress of the European Society of Surgical Oncology" will take place in Groningen, The Netherlands.

Contact the Conference Secretariat ESSO; Federation of European Cancer Societies, Avenue E. Mounier 83, B-1200 Brussels, Belgium; or call +32 2 775 0202; or fax +32 2 775 0200; or e-mail ESSO2000@fecs.be; or see Internet http://www.fecs.be

Oncology

April 6-7, 2000

The conference "26th Annual Diagnosis and Treatment of Neoplastic Disorders" will take place in Baltimore, Maryland, USA.

Contact the Conference Co-ordinator, Office of Continuing Medical Education, John Hopkins University School of Medicine, Turner 20, 720 Rutland Avenue, Baltimore, Maryland 21205-2195, USA; or call +1 410 955 2959; or fax +1 410 955 0807; or e-mail cmenet@jhmi.edu; or see Internet http://www.med.jhu.edu/cme

Oncology

April 6-8, 2000

The SASRO Annual Meeting will take place in St-Gallen, Switzerland.

See Internet http://www.sgsmp.ch/sas00-e.htm

Radiotherapy

April 9-13, 2000

The ESTRO teaching course on "Imaging for Target Volume Determination in Radiotherapy" will take place in Como, Italy.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; or see Internet http://www.estro.be

Oncology

April 11-14, 2000

The "Intensive Course in Oncology" will take place in Edinburg, United Kingdom.

Contact Ms. Jacqueline Lyons, Course Administrator, Lister Postgraduate Institute, 11 Hill Square, Edinburg EH8 9DR, U.K.; or call +44 131 650 2606; or fax +44 131 662 0580.

Radiobiology

April 14-16, 2000

The "International Symposium on Normal Tissue Reactions in Radiotherapy - Biological, Physical and Clinical Aspects of Early and Late Normal Tissue Response" will take place in Marburg, Germany.

Contact Dr. J.S. Zimmermann; call +49 6421 28 5854; or e-mail zimmermj@post.med.uni-marburg.de

Head and neck

May, 2000

The ESO training course "Head and Neck Pathology - Oncology" will take place in Ioannina, Greece.

Contact ESO office for Balkans and Middle East, N. Pavlidis, E. Andreopoulou Medical School, Department of Medical Oncology, University Hospital of Ioannina, 45110 Ioannina, Greece; or call +30 651 99394 or +30 953 91083; or fax +30 651 97505

Radiophysics

May 7-11, 2000

The ESTRO teaching course on "Dose and Monitor Unit Calculations for High Energy Photon Beams. Basic Principles and Application to Modern Techniques" will take place in Santorini, Greece.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; or see Internet http://www.estro.be

Brachytherapy

May 19-21, 2000

The "2nd Joint Meeting of the American Brachytherapy Society, GEC-ESTRO and GLAC will take place in Washington DC, USA.

Call +1 630 368 7896; or fax +1 7630 571 7837; or e-mail sansone@rsna.org

Clinical oncology

May 20-23, 2000

The 36th ASCO Annual Meeting will take place in New Orleans, Louisiana, USA.

Contact ASCO Office, 225 Reinekers Lane, Suite 650, Alexandria, VA 22314, USA; or call +1 703 299 0150; or fax +1 703 299 1044; or e-mail asco@asco.org; or see Internet http://www.asco.org

Radiation therapy

May 22-25, 2000

The 13th International Conference on the Use of Computers in Radiation Therapy will take place in Heidelberg, Germany.

Contact Ms. Karin Beinert, call +49 6621 422551; or fax +49 6621 422561; or e-mail iccr@dkfz-heidelberg.de; or see Internet http://www.dkfz-heidelberg.de/iccr/

Imaging, oncology and science

May 22-25, 2000

The congress "Imaging, Oncology and Science 2000 (IOS 2000) will take place in Birmingham, UK.

Contact IOS Secretariat, PO Box 2895; London, UK; or call +44 (0)20 7307 1410/20; or fax +44 (0)20 7307 1414; or e-mail ios@dial.pipex.com; or see Internet http://www.ios.org.uk

Breast cancer

June, 2000

The ESO training course will take place in New York, USA.

Contact ESO US Office, R. Boschi-Belgin, American-Italian Cancer Foundation (AICF), 872 Madison Avenue - 2B, New York - NY 10021, USA; or call +1 212 6289090; or fax +1 212 5176089; or e-mail aicfnyc@aol.com

Urological cancer

June, 2000

The ESO training course will take place in Athens, Greece.

Contact ESO office for Balkans and Middle East, N. Pavlidis, E. Andreopoulou Medical School, Department of Medical Oncology, University Hospital of Ioannina, 45110 Ioannina, Greece; or call +30 651 99394 or +30 953 91083; or fax +30 651 97505

Oncology

June 4-8, 2000

The ESTRO teaching course on "Molecular Oncology for Radiotherapy" will take place in Innsbruck, Austria.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; or see Internet http://www.estro.be

Radiotherapy

June 4-8, 2000

The ESTRO teaching course on "IMRT and Other Conformal Techniques in Practice" will take place in Amsterdam, The Netherlands.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; or see Internet http://www.estro.be

Computed tomography

June 8-11, 2000

The "5th Annual Computed Body Tomography for the Technologist 2000" will take place in Las Vegas ; Nevada, USA.

Contact Office of Continuing Medical Education, John Hopkins University School of Medicine, Turner 20, 720 Rutland Avenue, Baltimore, Maryland 21205-2195, USA; or call +1 410 955 2959; or fax +1 410 955 0807; or e-mail cmenet@jhmi.edu; or see Internet http://www.med.jhu.edu/cme

Oncology

June 24-26, 2000

The "International 4th Wolsberg Meeting on Molecular Radiation Biology/Oncology" will take place in Ermatingen, Lake Constance, Switzerland.

E-mail hans-peter.rodemann@uni-tuebingen.de

Ethics in oncology

June 25-28, 2000

The ESO Advanced Course on Ethics in Oncology will take place in Bled, Slovenia.

Contact Albatros Conferences and Tourism office, Ribenska 2, 4260 Bled, Slovenia; or call +386 64 741 101; or fax +386 64 741 031; or e-mail albatros@albatros-bled-sp.si

Scientific correspondence with Dr. Matjaž Zwitter, Institute of Oncology, Zaloška 2, 1000 Ljubljana, Slovenia; fax +386 61 1314 180; email mzwitter@onko-i.si

Computed tomography

July 20-23, 2000

The "8th Annual Advanced Topics in CT Scanning: The 2000 Edition" will take place in Lake Tahoe, NV, USA.

Contact Office of Continuing Medical Education, John Hopkins University School of Medicine, Turner 20, 720 Rutland Avenue, Baltimore, Maryland 21205-2195, USA; or call +1 410 955 2959; or fax +1 410 955 0807; or e-mail cmenet@jhmi.edu; or see Internet http://www.med.jhu.edu/cme

Radiophysics

August 27-31, 2000

The ESTRO teaching course on "Physics for Clinical Radiotherapy" will take place in Leuven, Belgium.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; or see Internet http://www.estro.be

Myelodysplastic syndromes

September, 2000

The ESO training course will take place in Patras, Greece.

Contact ESO office for Balkans and Middle East, N. Pavlidis, E. Andreopoulou Medical School, Department of Medical Oncology, University Hospital of Ioannina, 45110 Ioannina, Greece; or call +30 651 99394 or +30 953 91083; or fax +30 651 97505

Lung cancer

September 11-15, 2000.

The "9th World Conference on Lung Cancer" will be offered in Tokyo, Japan.

Contact Dr. Yoshihiro Hayata, The 9th World Conference on Lung Cancer, Tokyo Medical College Cancer Center, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan; or fax +81 3 3342 0893

Oncology

September 14-16, 2000

The "2nd International Symposium on Organ Sparing Treatment in Oncology" will take place in Bled, Slovenia.

Contact Ms. Natalija Bah, Cankarjev dom - Cultural and Symposium Centre, Prešernova 10, SI-1000 Ljubljana; or call +386 61 1767 132; or fax +386 61 217 431; or e-mail: natalija.bah@cd-cc.si

Radiation therapy

September 19-23, 2000

The 19th Annual ESTRO Meeting will take place in Istanbul, Turkey.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; web: http://www.estro.be

Colorectal cancer

October, 2000

The ESO training course will take place in Milan, Italy.

Contact ESO Office, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 0258317850; or fax +39 0258321266: or e-mail esomi@tin.it

Oncology

October 4-6, 2000

The 4th National Congress of Federation of Spanish Societies of Oncology (F.E.S.E.O.) will be offered in La Caruna, Spain.

Contact Technical Secretariat, Orzan Congres, S.L.; call +34 981 169 855; or fax +34 981 247 908

Radiation oncology

October 8-12, 2000

The ESTRO teaching course on "Evidence-Based Radiation Oncology: Principles and Methods" will be offered in Lleida, Spain.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; or see Internet http://www.estro.be

Radiobiology

October8-12, 2000

The ESTRO teaching course on "Basic Clinical Radiobiology" will be offered in Bratislava, Slovakia.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; or see Internet http://www.estro.be

Bioelectromagnetism

October8-12, 2000

The "3rd International Conference on Bioelectromagnetism" and "1st Slovenian-Croatian Meeting on Biomedical Engineering" will be offered in Bled, Slovenia.

Contact Prof. Damijan Miklavčič - president, Faculty of Electrical Engineering, University of Ljubljana, Tržaška 25, SI-1000 Ljubljana, Slovenia; or call +386 61 1768 264; or fax +386 61 1264 658; or e-mail 3rdICBEM@svarun.fe.uni-lj.si; or see Internet http://www.albatros-bled-sp.si

Radiation therapy

October 22-25, 2000

ASTRO Annual meeting will be held in Boston, Massachusetts, USA.

Contact American Society for Therapeutic Radiology and Oncology Office, 1891 Preston White Drive, Reston, VA 20191, USA; web site: www.astro.org

Radiophysics

November 9-12, 2000

The International Workshop on Monte Carlo Treatment Planning will be offered in Stanford, USA.

E-mail cma@reyes.stanford.edu; or see Internet http://www-radonc.stanford.edu/

Paediatric oncology

November 12-18, 2000

The training course under the auspices of the International Society of Paediatric Oncology will be held in Chandigahr, India.

Contact P.A. Voute, call +31 20 5665655; or fax +31 20 6912231

Paediatric oncology

November 16-20, 2000

The training course under the auspices of the International Society of Paediatric Oncology will be held in Sao Paulo, Brazil.

Contact P.A. Voute, call +31 20 5665655; or fax +31 20 6912231

Radiation morbidity

December 10-12, 2000

The ESTRO workshop on radiation morbidity will be held in Brussels, Belgium.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; or see Internet http://www.estro.be

Radiation therapy

January 30 - February 2, 2001

The International Meeting ICRO 2001 will take place in Melbourne, Australia.

Call +32 2 775 9342; or fax +32 2 779 5494; or e-mail info@isro.be

Radiophysics

September 18-23, 2001

The 6th ESTRO Meeting on Physics for Clinical Radiotherapy will be held.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; or see Internet http://www.estro.be

Radiation therapy

October 21-25, 2001

The ESTRO 20 / ECCO 11 Meeting will take place in Lisbon, Portugal.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; web: http://www.estro.be

Radiation therapy

November 4-7, 2001

ASTRO Annual meeting will be held in San Francisco, California, USA.

Contact American Society for Therapeutic Radiology and Oncology Office, 1891 Preston White Drive, Reston, VA 20191, USA; web site: www.astro.org

Radiation therapy

May 15-19, 2002

The 7th International Meeting on Progress in Radio-Oncology ICRO/÷GRO 7 will take place in Salzburg, Austria.

Contact Prof. D.H. Kogelnik, Salzburg, Austria; call +43 662 44823900; or fax +43 662 4482887; or e-mail d.kogelnik@lkasbg.gv.at

Radiation therapy

September 15-19, 2002

The 21st Annual ESTRO Meeting will take place in Prague, Czech Republic.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; web: http://www.estro.be

Radiation therapy

October 6-9, 2002

ASTRO Annual meeting will be held in New Orleans, Louisiana, USA.

Contact American Society for Therapeutic Radiology and Oncology Office, 1891 Preston White Drive, Reston, VA 20191, USA; web site: www.astro.org

Radiation therapy

October 19-23, 2003

The ESTRO 22 / ECCO 12 Meeting will take place in Copenhagen, Denmark.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; web: http://www.estro.be

Radiation therapy

October 19-23, 2003

ASTRO Annual meeting will be held in Salt Lake City, Utah, USA.

Contact American Society for Therapeutic Radiology and Oncology Office, 1891 Preston White Drive, Reston, VA 20191, USA; web site: www.astro.org

Radiation therapy

September 12-16, 2004

The 23rd Annual ESTRO Meeting will be held.

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 7795494; or e-mail info@estro.be; web: http://www.estro.be

Radiation therapy

October 3-7, 2004

ASTRO Annual meeting will be held in Atlanta, USA.

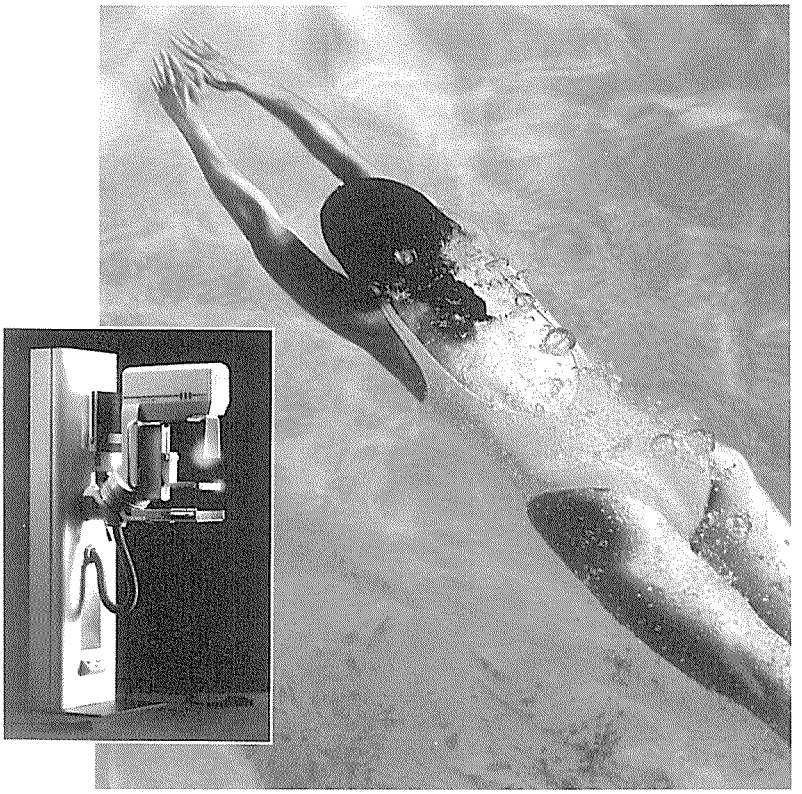
Contact American Society for Therapeutic Radiology and Oncology Office, 1891 Preston White Drive, Reston, VA 20191, USA; web site: www.astro.org

As a service to our readers, notices of meetings or courses will be inserted free of charge.

Please sent information to the Editorial office, Radiology and Oncology, Vrazov trg 4, SI-1000 Ljubljana, Slovenia.

SIEMENS

Rešitve po meri



Mammomat 3000 modular

Mammomat 3000 modular

- univerzalni sistem za vse vrste mamografije
- optimizacija doze in kompresije z OPDOSE in OPCOMP sistema
- modularna zgradba zagotavlja posodabljanje sistema
- servis v Sloveniji z zagotovljenimi rezervnimi deli in garancijo
- izobraževanje za uporabnike

SIEMENS d.o.o.
Dunajska 22
1511 Ljubljana
Telefon 061/1746 100
Telefaks 061/1746 135

FONDACIJA "DOCENT DR. J. CHOLEWA"
JE NEPROFITNO, NEINSTITUCIONALNO IN NESTRANKARSKO
ZDRUŽENJE POSAMEZNIKOV, USTANOV IN ORGANIZACIJ, KI ŽELIJO
MATERIALNO SPODBUJATI IN POGLABLJATI RAZISKOVALNO
DEJAVNOST V ONKOLOGIJI.

MESESNELOVA 9
1000 LJUBLJANA
TEL 061 15 91 277
FAKS 061 21 81 13

ŽR: 50100-620-133-05-1033115-214779

Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education - A Report for the First Quarter of 2000

In the first days of January 2000 a financial report concerning the state of affairs at the "Dr. J. Cholewa" Foundation for Cancer Research and Education was prepared and distributed to the members of the Boards of the Foundation by Mr. Slavko Fatur, the President of the Foundation. It was also reported that several new initiatives were put forward, to be discussed in the near future by the relevant members of the Foundation. It was further presented in the report, that the Foundation could finally be registered at the Department for Administrative Affairs at the City of Ljubljana Administrative Office, as required by the law and regulations of Republic of Slovenia. It is to be noted that regarding this matter the members of the Foundation greatly appreciate the help provided by Professor Verica Trstenjak, currently at the position of State secretary at the Ministry of Science and Technology of Republic of Slovenia. Additionally, after profound deliberations some new members are to be invited to join the Foundation. In the same way as the present members of the Boards of the Foundation, the new members represent the relevant strata of the Slovenian society, that take active interest and spend considerable part of their time dealing with the problems of cancer. In line with the present situation, the new members do not belong exclusively to the medical profession, but form a representative cross-section sample of the entire society in Slovenia.

The Foundation continues to support the regular publication of "Radiology and Oncology" international scientific journal, and the regular publication of the "Challenge ESO Newsletter". Both medical journals are edited, published and printed in Ljubljana, Slovenia. The Foundation also supported the publication of the Proceedings of the "14th Oncological Weekend" meeting, that was dedicated to the problems of lung and thyroid cancer. A contribution was given to support the publication of the Slovenian Dictionary of Medical Terminology and for the Proceedings of two meetings organised by the Nursing Association of Slovenia, and dedicated to the problems in oncology.

As already mentioned in the paragraphs of previous reports, the Foundation will concentrate its activities to further advance the research work in oncology in Slovenia. At least part of this research and educational work should be carried out in the facilities in Ljubljana and elsewhere in Slovenia. This new form of research shouldn't cover a period of more than one or two months. Special attention will be given to the requests coming from the regions of Slovenia outside Ljubljana. It will also continue to provide grants for the participation of Slovenian oncologists and others on various educational meetings abroad.

Some of the priorities listed above will be discussed at the forthcoming meetings of the Executive and Advisory Boards of the Foundation. These two meetings are to be held soon, probably at the end of February and in the first week of March 2000.

Borut Štabuc, MD, PhD
Andrej Plesničar, MD, MSc
Tomaž Benulič, MD

- *v svetu največ predpisovani sistemski antimikotik*
- *edini peroralni sistemski antimikotik za zdravljenje vaginalne kandidoze, ki ga je odobril FDA*

Skrajšano navodilo
Flukonazol je sistemski antimikotik iz skupine triazolov.

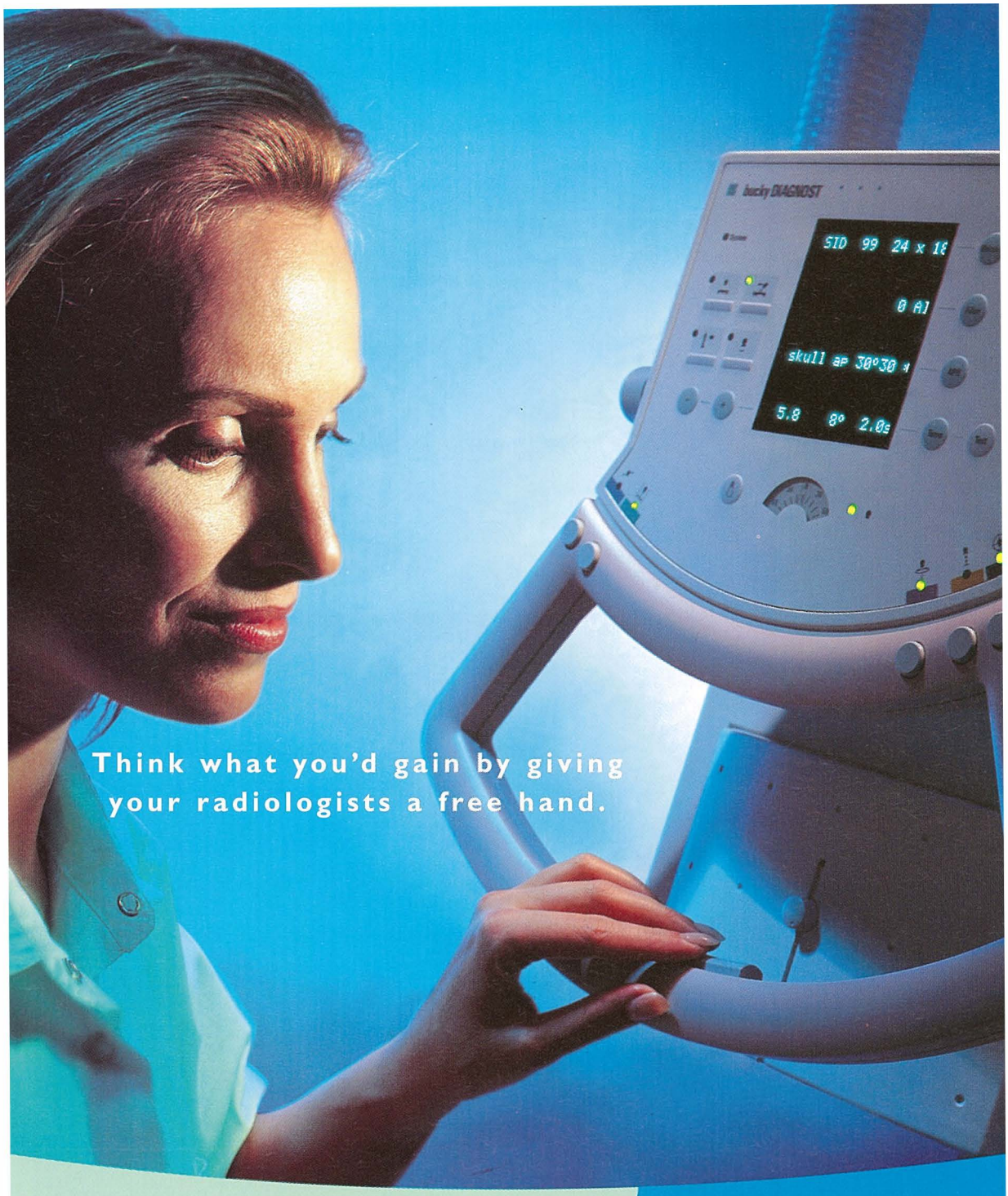
Odmerjanje pri različnih indikacijah:

vaginalna kandidoza	*150 mg v enkratnem odmerku*
mukozna kandidoza	*50 do 100 mg na dan*
dermatomikoze	*50 mg na dan ali 150 mg na teden*
sistemska kandidoza	*prvi dan 400 mg, nato od 200 do 400 mg na dan Največji dnevni odmerek je 800 mg.*
preprečevanje kandidoze	*50 do 400 mg na dan*
kriptokokni meningitis	*prvi dan 400 mg, nato od 200 do 400 mg na dan*
vzdrževalno zdravljenje	*200 mg na dan*

Kontraindikacije: Preobčutljivost za zdravilo ali sestavine zdravila. **Interakcije:** Pri enkratnem odmerku flukonazola za zdravljenje vaginalne kandidoze klinično pomembnih interakcij ni. Pri večkratnih in večjih odmerkih so možne interakcije s terfenadinom, cisapridom, astemizolom, varfarinom, derivati sulfonilureje, hidroklorotiazidom, fenitoinom, rifampicinom, ciklosporinom, teofilinom, indinavirom in midazolamom. **Nosečnost in dojenje:** Nosečnica lahko jemlje zdravilo le, če je korist zdravljenja za mater večja od tveganja za plod. Doječe matere naj med zdravljenjem s flukonazolom ne dojijo. **Stranski učinki:** Povezani so predvsem s prebavnim traktom: slabost, napenjanje, bolečine v trebuhu, driska, zelo redko se pojavijo preobčutljivostne kožne reakcije, anafilaksija in angioedem – v tem primeru takoj prenehamo jemati zdravilo. Pri bolnikih s hudimi glivičnimi obolenji lahko pride do levkopenije in trombocitopenije in do povečane aktivnosti jetrnih encimov. **Oprema in način izdajanja:** 7 kapsul po 50 mg, 28 kapsul po 100 mg, 1 kapsula po 150 mg. Na zdravniški recept. 1/99.
Podrobnejše informacije so na voljo pri proizvajalcu.

KRKA

Krka, d. d., Novo mesto
Šmarješka cesta 6
8501 Novo mesto



Are your radiographers' hands tied by equipment that slows them down? And what can you do about it? Nearly 70% of X-rays are made at a Bucky. A fact that has influenced the design of Philips' Bucky systems. It's led to technology without gadgetry, for uncomplicated, speedy work. Control units operated one-handed.

A display that eliminates trips to the generator. Tomography functions activated via a single button. All to speed patient throughput, for improved cost-efficiency. It's one way in which Philips Medical Systems is working with you to meet today's changing healthcare needs. For more information call 061 177 88 50.

website: www.philips.com/ms

PHILIPS

Let's make things better.

MEDILAB d.o.o.
DIAGNOSTIČNI RADIOLOŠKI CENTER

THE DAWN OF A NEW ERA IN MAMMOGRAPHY

This new screen film system provides the highest contrast images of any Kodak mammography system. The new components of this latest mammography system from Kodak are:

A new Kodak **Min-R 2190 Screen**

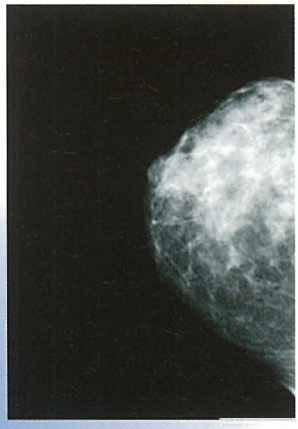
A redesigned **Kodak Min-R2 Cassette**

A compact Roomlight Film handling System for Mammography,

The Kodak **Miniloader 2000**

Benefits:

Improved resolution and contrast
Access to processed images



MEDITRADE d.o.o.
Generalni distributer.
Vse informacije na:
tel.: (061) 1894 600
http://www.meditrade.si
E-pošta: info@meditrade.si

LABORMED

laboratory & medical equipment

LABORMED d.o.o. · SLO-1215 Medvode · Zg. Pirniče 96/c
Telefon 061 / 621 098 · Telefax 061 / 621 415

Labormed v Sloveniji ekskluzivno zastopa naslednje firme:

KÖTTERMANN

laboratorijsko pohištvo, varnostne omare za kisline, luge, topila, pline in strupe, ventilacijska tehnika in digestoriji

EHRET

laminar flow tehnika, inkubatorji, sušilniki, suhi sterilizatorji in oprema za laboratorijsko vzrejo živali - kletke

DAKO

testi za aplikacijo v imunohistokemiji, patologiji, mikrobiologiji, virologiji, mono- in poliklonalna protitelesa…

GFL

laboratorijski aparati, omare in skrinje za globoko zamrzovanje

ROSYS ANTHOS

fotometri, avtomatski pralni sistem za mikrotitrine plošče

CHARLES ISCHI Pharma-Prüftechnik

specialna oprema za testiranje izdelkov v farmacevtski industriji; aparati za procesno kontrolo in kontrolo kvalitete

NOVODIRECT BIOBLOCK

kompletna oprema in pripomočki za delo v laboratoriju

ANGELANTONI SCIENTIFICA

hladilna tehnika in aparati za laboratorije, transfuziologijo, patologijo in sodno medicino

INTEGRA BIOSCIENCES

laboratorijska oprema za mikrobiologijo, biologijo celic, molekularno biologijo in biotehnologijo

CORNINGCostar

specialna laboratorijska plastika za aplikacijo v imunologiji, mikrobiologiji, virologiji, ipd., mehanske eno- in večkanalne pipete ter nastavki

BIOMERICA

hitri testi za diagnostiko, EIA / RIA testi

Advanced BIOTECHNOLOGIES

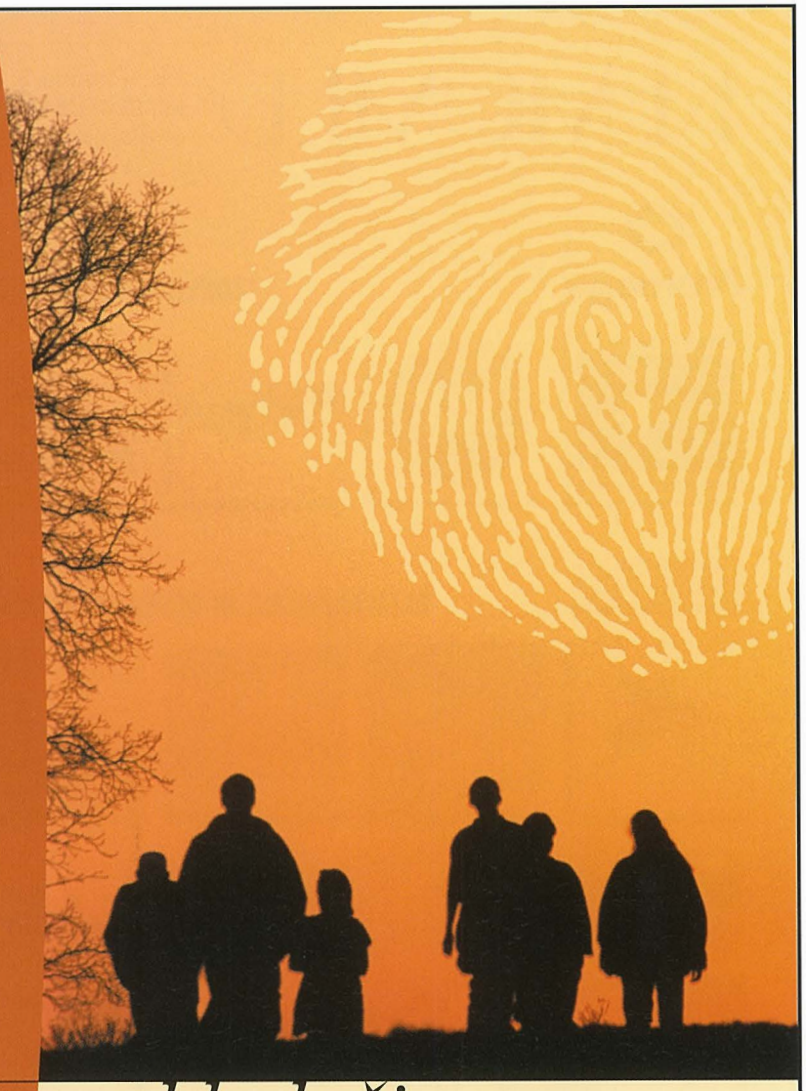
PCR tehnologija, potrošni material, reagenti, encimi

SVANOVA Biotech

Elisa testi za diagnostiko v veterini

HÜRNER

ventilacijska tehnika



zmaga nad bolečino

Podrobnejše informacije
dobite pri proizvajalcu.

Izdeluje:Bayer Pharma d.o.o.
Ljubljana, Celovška 135, Ljubljana
po licenci Grünenthal GmbH, Nemčija

Bayer
Bayer Pharma d.o.o.

GRÜNENTHAL

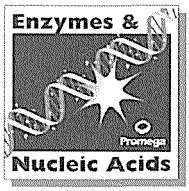
Tramal®
Tramadol
analgetik

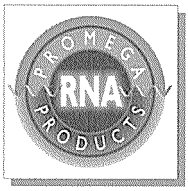
KEMOMED

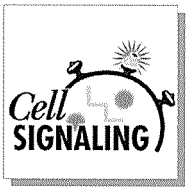
PE: Planina 3, 4000 Kranj
tel: 064/ 351 510, fax: 064/ 351 511
e-mail: kemomed@siol.net

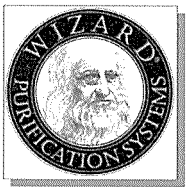
Promega

IZDELKI ZA MOLEKULARNO BIOLOGIJO



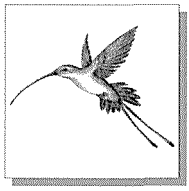
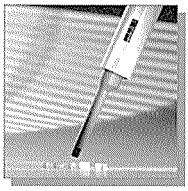
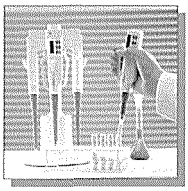
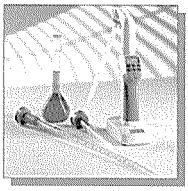
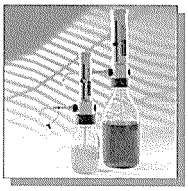






BIOHIT

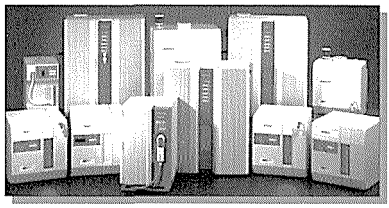
ELEKTRONSKE IN MEHANSKE AVTOMATSKE PIPETE

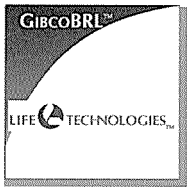






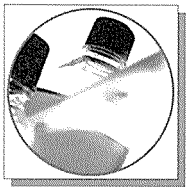
USF ELGA

Simply, the right solution!

LIFE TECHNOLOGIES™





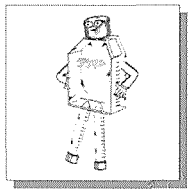
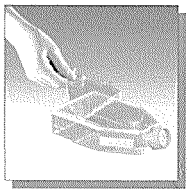
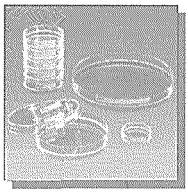
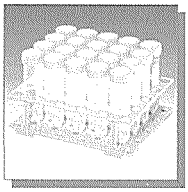
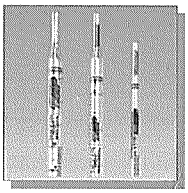


ČISTA VODA ZA LABORATORIJ

CELIČNE KULTURE

TPP®

PLASTIKA ZA CELIČNE KULTURE

Instructions for authors

Editorial policy of the journal *Radiology and Oncology* is to publish original scientific papers, professional papers, review articles, case reports and varia (editorials, reviews, short communications, professional information, book reviews, letters, etc.) pertinent to diagnostic and interventional radiology, computerized tomography, magnetic resonance, ultrasound, nuclear medicine, radiotherapy, clinical and experimental oncology, radiobiology, radiophysics and radiation protection. The Editorial Board requires that the paper has not been published or submitted for publication elsewhere: the authors are responsible for all statements in their papers. Accepted articles become the property of the journal and therefore cannot be published elsewhere without written permission from the editorial board. Papers concerning the work on humans, must comply with the principles of the declaration of Helsinki (1964). The approval of the ethical committee must then be stated on the manuscript. Papers with questionable justification will be rejected.

Manuscript written in English should be submitted to the Editorial Office in triplicate (the original and two copies), including the illustrations: *Radiology and Oncology,* Institute of Oncology, Vrazov trg 4, SI-1000 Ljubljana, Slovenia; (Phone: +386 61 132 00 68, Tel./Fax: +386 61 133 74 10, E-mail: gsersa@onko-i.si). Authors are also asked to submit their manuscripts on a 3.5" 1.44 Mb formatted diskette. The type of computer and word-processing package should be specified (Word for Windows is preferred).

All articles are subjected to editorial review and review by independent referee selected by the editorial board. Manuscripts which do not comply with the technical requirements stated herein will be returned to the authors for correction before peer-review. Rejected manuscripts are generally returned to authors, however, the journal cannot be held responsible for their loss. The editorial board reserves the right to ask authors to make appropriate changes in the contents as well as grammatical and stylistic corrections when necessary. The expenses of additional editorial work and requests for reprints will be charged to the authors.

General instructions• Radiology and Oncology will consider manuscripts prepared according to the Vancouver Agreement (*N Engl J Med* 1991; **324:** 424-8, *BMJ* 1991; **302:** 6772; *JAMA* 1997; **277:** 927-34.). Type the manuscript double spaced on one side with a 4 cm margin at the top and left hand side of the sheet. Write the paper in grammatically and stylistically correct language. Avoid abbreviations unless previously explained. The technical data should conform to the SI system. The manuscript, including the references may not exceed 15 typewritten pages, and the number of figures and tables is limited to 4. If appropriate, organize the text so that it includes: Introduction, Material and methods, Results and Discussion. Exceptionally, the results and discussion can be combined in a single section. Start each section on a new page, and number each page consecutively with Arabic numerals.

Title page should include a concise and informative title, followed by the full name(s) of the author(s); the institutional affiliation of each author; the name and address of the corresponding author (including telephone, fax and e-mail), and an abbreviated title. This should be followed by the *abstract page,* summarising in less than 200 words the reasons for

the study, experimental approach, the major findings (with specific data if possible), and the principal conclusions, and providing 3-6 key words for indexing purposes. Structured abstracts are preferred. If possible, the authors are requested to submit also slovenian version of the title and abstract. The text of the report should then proceed as follows:

Introduction should state the purpose of the article and summarize the rationale for the study or observation, citing only the essential references and stating the aim of the study.

Material and methods should provide enough information to enable experiments to be repeated. New methods should be described in detail. Reports on human and animal subjects should include a statement that ethical approval of the study was obtained.

Results should be presented clearly and concisely without repeating the data in the tables and figures. Emphasis should be on clear and precise presentation of results and their significance in relation to the aim of the investigation.

Discussion should explain the results rather than simply repeating them and interpret their significance and draw conclusions. It should review the results of the study in the light of previously published work.

Illustrations and tables must be numbered and referred to in the text, with appropriate location indicated in the text margin. Illustrations must be labelled on the back with the author's name, figure number and orientation, and should be accompanied by a descriptive legend on a separate page. Line drawings should be supplied in a form suitable for high-quality reproduction. Photographs should be glossy prints of high quality with as much contrast as the subject allows. They should be cropped as close as possible to the area of interest. In photographs mask the identities of the patients. Tables should be typed double spaced, with descriptive title and, if appropriate, units of numerical measurements included in column heading.

References must be numbered in the order in which they appear in the text and their corresponding numbers quoted in the text. Authors are responsible for the accuracy of their references. References to the Abstracts and Letters to the Editor must be identified as such. Citation of papers in preparation, or submitted for publication, unpublished observations, and personal communications should not be included in the reference list. If essential, such material may be incorporated in the appropriate place in the text. References follow the style of Index Medicus. All authors should be listed when their number does not exceed six; when there are seven or more authors, the first six listed are followed by "et al.". The following are some examples of references from articles, books and book chapters:

Dent RAG, Cole P. *In vitro* maturation of monocytes in squamous carcinoma of the lung. *Br J Cancer* 1981; **43:** 486-95.

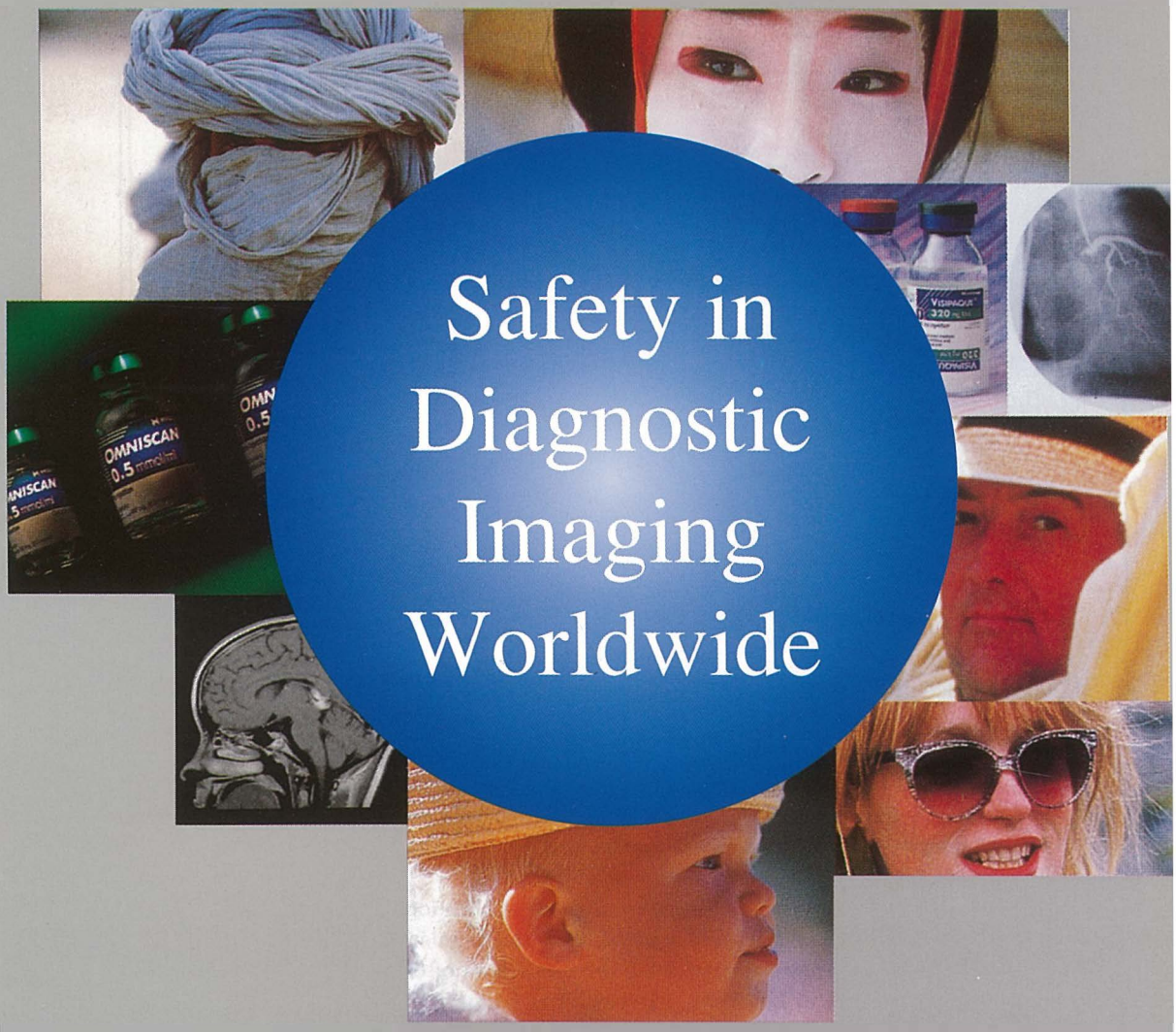
Chapman S, Nakielny R. *A guide to radiological procedures.* London: Bailliere Tindall; 1986.

Evans R, Alexander P. Mechanisms of extracellular killing of nucleated mammalian cells by macrophages. In: Nelson DS, editor. *Immunobiology of macrophage.* New York: Academic Press; 1976. p. 45-74.

Page proofs will be faxed to the corresponding author whenever possible. It is their responsibility to check the proofs carefully and fax a list of essential corrections to the editorial office within 48 hours of receipt. If corrections are not received by the stated deadline, proof-reading will be carried out by the editors.

Reprints: Fifty reprints are free of charge, for more contact editorial board.

For reprint information in North America Contact: International Reprint Corporation 968 Admiral Callaghan Lane, # 268 P.O. Box 12004, Vallejo; CA 94590, Tel: (707) 553 92 30, Fax: (707) 552 95 24.



Safety in Diagnostic Imaging Worldwide

Nycomed Imaging is proud of its role in providing for the early, accurate diagnosis of disease, thus improving patients' quality of life and prospect for effective treatment. The company is commited to the continuous development of inovative imaging product to enhance diagnostic procedures.

ZASTOPA
HIGIEA d.o.o., Trzin
tel.: (061) 1897 225
fax: (061) 1897 226

Nycomed Amersham

DISTRIBUCIJA
SALUS d.d. Ljubljana
tel.: (061) 1899 100
fax: (061) 1681 420