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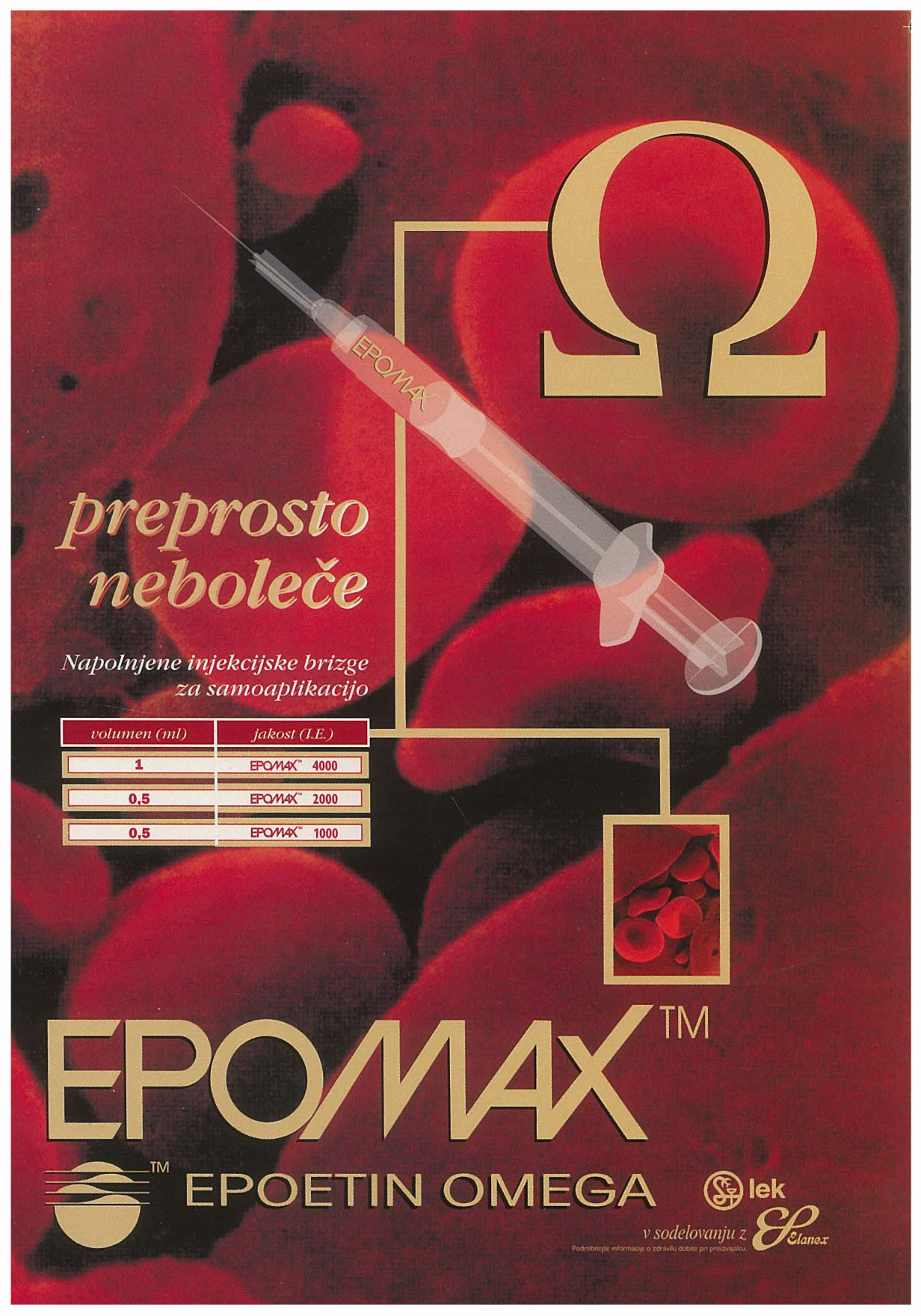

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Pharmaco-mechanical technique for selective thrombolysis in peripheral vessels

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Department of Radiology, Medical University Pécs, Hungary

Purpose. *The thrombus destroying power of selective low-dose fibrinolytic treatment was intended to be amplified with the mechanical force of the pulse-spray injector. The treatment time was expected to be shortened and the incidence of complications to be reduced.*

Methods. *Seventeen patients with arterial occlusion on the lower extremities underwent selective pulse-spray thrombolysis. All were treated according to a standard thrombolytic protocol. Streptokinase (Kabikinase®) 5.000-20.000 IU/h, or urokinase (Ukidan®) 20.000-40.000 IU/h and heparin 500-1.000 IU/h in physiological saline were administered with pulse-spray injector (Angiodynamics®) till the total dissolving of the thrombus.*

Results. *Between 1995-1997, 17 patients were successfully treated with an average treatment time of 12 hours,. No major complication occurred during this period. Surgical operation in the first 6 months after the treatment was needed in two cases because of reappearance of the clinical symptoms.*

Conclusion. *The pharmacomechanical selective thrombolysis with the pulse-spray technique is a reliable, relatively rapid and safe method for recanalization of occluded native arteries.*

Key words: arterial occlusive diseases-drug therapy; thrombolytic therapy-methods

Introduction

The percutaneous intraarterial selective thrombolysis is a radiologically controlled minimal invasive therapy used mainly in the cases of arterial occlusions of the extremities. Since 1959, when a research group under the leadership of Fletcher introduced for the first time the fibrinolytic treatment with streptokinase, a great progress has been achieved in this field.[1,2]

The method of drug activation of the fibrinolytic system through an intraarterial catheter first applied by Dotter in 1974 has been routinely used in the radiologic departments since the middle of the 1980's. In 1989, Bookstein and his co-workers introduced the pulse-spray pharmacomechanical treatment method.[3] The thrombus removing power of the selective low-dose fibrinolytic treatment was boosted by the mechanical force of a pulse-spray injector incredibly increasing the effective surface of drug and clot interaction.

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After an appropriate selection, patients with lower extremity arterial occlusion have been undergoing selective intraarterial thrombolysis with pulse-spray infusion in our angiographic department since the end of 1995.

Material and methods

Patient selection: For the performance of selective thrombolysis there are various indications and contraindications (Table 1). Appropriate selection and monitoring of the patients are of significant importance for a successful treatment. According to etiology, occlusions may be classified in the following three groups;

- arterial embolization- usually from the left side of dilated heart, after cardiac arrhythmic attack due to an aortic aneurism. Patients were usually admitted in the hospital in an acute stage of clinical manifestation, 1-2 hours after the embolization;
- arterial thrombembolization without a preexisting arterial disease - mainly of iatrogenic origin, usually with good response to thrombolytic treatment, solved within a few hours;
- arterial thrombembolization following a preexisting vessel disease - the most frequently presented form of disease in the lower extremities.

An intimal damage caused by the diabetic or atherosclerotic disease leads to an increase of the surface contact activity and, by destabilizing the homeostasis, activates the chain reaction of coagulation. All the patients who underwent selective thrombolysis in our department were classified into this group (Table 2). They had acute or subacute clinical signs of severe limb ischemia with a preceding history of claudication. All the patients with proper indication for pulse-spray selective thrombolysis were treated in our angiographic department. No one of the patients was excluded from this study. Seventeen patients underwent pulse-spray thrombolysis of acute arterial occlusion which was caused by atherosclerosis obliterans and by diabetic angiopathy in 12 and in 5 cases, respectively.

Table 2. Patients and results in selective pulse-spray thrombolysis

Patients		17
male/female		10/7
Average age		59 (39-83)
Main risk factors	AS*	12
	DM*	5
Fontaine stage III		7
stage IV		10
Therapeutic succes		17
Roecclusion within 6 monts		2

AS* - Atherosclerosis obliterans; DM* - Diabetes mellitus

Table 3. Treatment protocol in selective pulse-spray thrombolysis

1. Baseline angiogram
2. Introduction of guide wire
3. Infusion catheter selection
4. Thrombolytic agents infusion
5. Control angiography
6. Catheter removal
7. Postprocedure management, aftercare

Catheterization technique

In our angiographic department, a well-tested treatment protocol was used (Table 3). The main steps of the treatment protocol are schematically presented in Figure 1;

- pulse- spray technique - the jet spray of the lytic infusion causes a significant change in the intrathrombotic microstructure and improves the effectiveness of the method enlarging the surface for the action of the urokinase or streptokinase (Figure 2). The pulse- spray infusion was performed with an infusion pump through the catheter without blocking the end hole.

Table 1. Indications and contraindications for selective pulse-spray thrombolysis

Indications
acute and chronic native artery occlusion
acute and chronic graft occlusion
reoclusion after percutan angioplasty
Contraindications
Absolute
Active internal bleeding
Cerebrovascular accident, disease or surgical intervention within the last 2 months
Relative
Surgical operation or parenchymal organ biopsy within the last 2 weeks
Recent major trauma
Uncontrollable hypertension
Gastrointestinal ulcer
Pregnancy/postpartum
Embolization from a distant source

– infused lytic drugs - in arterial selective thrombolysis, the drug dose administered with the pulse-spray technique was lower than the ones indicated in systemic infusion protocols. The reports of early clinical successes in literature show significant preference of urokinase to streptokinase.[4-9] However, this observation has not been proved yet although it was explained in randomized trials with pharmacokinetic mechanisms of the drugs.[10] In our intraarterial treatments, 20000-40000 IU/hour urokinase (Ukidan®), or 5000-20000 IU/hour streptokinase (Kabikinase®) and 500- 1000 IU/hour heparin were administrated through the catheter.

Postprocedure management: In order to prolong the patency rate of the reopened vessels, a post- procedural care and change in lifestyle was proposed to all the patients. To prevent puncture site bleeding, false pulsating aneurysms and extensive haematoma 20-30 minutes after manual compression, a sand sack was placed on the bandage of the puncture site for 6 hours. Care was taken that the patients made exercises to increase blood flow in the affected extremity in order to prevent local or venous thrombosis due to the transient stasis under the compression and slow flow because of bed rest. The patients were kept in bed for the next 24 hours. Diuretics, if any, were excluded from the treatment in the ward, and, in cases of diabetes mellitus, the concentration of serum glucose was kept steadily within the normal limits. The 3rd-5th day after the treatment a daily dose of 2 x 50 to 2 x 100 mg of sodium pentosan polysulphate (SP 54®) was administered i.v. (occasionally s.c.). Smoking was prohibited to the patients while life-long physical exercises, low-fat and low-cholesterol diet were recommended to them. Control examinations (physical and Doppler-flowmonitoring) and laboratory tests (hematocrite, serum cholesterol, serum triglycerid, plasma fibrinogen) were made at the discharge from the hospital, 30 days and 3 months after the therapy and, later on, in 6 month intervals for life time.

Results

Selective thrombolysis was considered clinically successful in the cases in which the ischemic signs disappeared or significantly improved for a period of more than 30 days

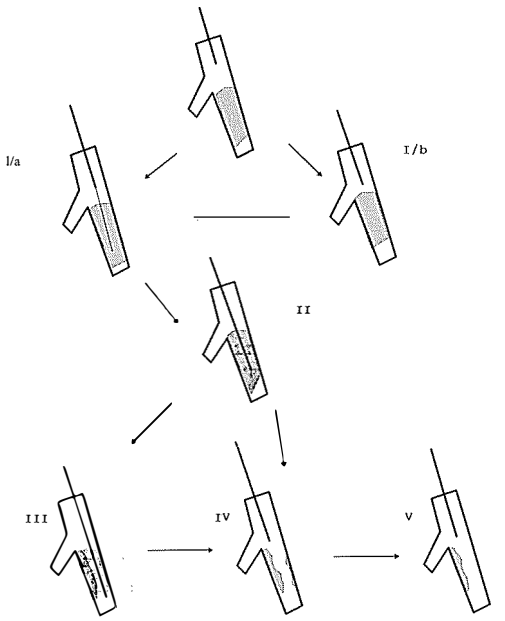

Figure 1. Main steps of treatment procedure. After the baseline angiogram a guide wire is introduced into the thrombus (I/a). If this is not possible the treatment starts with continuous selective thrombolytic infusion (I/b). If possible the infusion catheter is positoned in the thrombus leaving a thrombus plug at the distal end. After excluding the chance of accidental subintimal position pulse-spray lysis is initiated (II). After 4-6 hours of treatment if the control angiography shows reopening of the vessel (III), the pulse-spray technique may be converted to continuous infusion (IV) till the total dissolving of the thrombus. In cases of small persistent wall irregularities (V) percutaneous transluminal angioplasty is also performed.

after the therapy. In all 17 patients, the occluded arteries of the lower extremity were successfully reopened. In two of the treated cases, a surgical treatment was performed within the first 6 month after the therapy because of the recurrence of clinical symptoms. The first patient had diabetes mellitus for more than 15 years. After successful lytic treatment of the occlusion in the distal part of the popliteal artery, an amputation of the third left foot finger (previous amputation of the 4th and 5th finger on the same side in his clinical history) was carried out. The other patient did not adhere to the proposed post-procedure drug therapy and lifestyle changes. He underwent a successful transluminal endarterectomy of the popliteal artery 4 months after our treatment. Occult bleeding, allergic reactions, myocardial spasm, or distal embolization with an occurrence rate of 0.5-5%, which were often reported in literature,[4,11-15] were not observed during our procedures in spite of the careful control with laboratory tests, angiography and instrumental flow measurements.

Discussion

After the thrombolytic treatment we performed, in three of our cases, a percutaneous transluminal angioplasty on the chronic stenotic plaques detected by the control angiography and made visible only after solving the thrombotic deposits from the surface of the vascular wall.

To our opinion, a continuous selective infusion cannot provide an optimal concentration of the lytic enzymes in the thrombus where the activation of the plasminogen is required. The pharmacomechanical thrombolysis, which has appeared after a vast number of experimental research trials includes,

- shooting tiny jets of thrombolytic agent into the mass of thrombotic deposits;
- maceration of the thrombus;
- supplying in this way a larger surface for the action of the lytic enzyme,
- providing a simultaneous destruction of the majority of fibrin in the treated segment of the occlusion.

The maceration of thrombembolism is due to the jet effect of the infusion, i.e. its mechanical power. The duration of this process and the total thrombolysis as reported in literature has really a broad scale.[4,5,13,14,16,17] The explanation of this fact seems to be found in a multifactorial reason

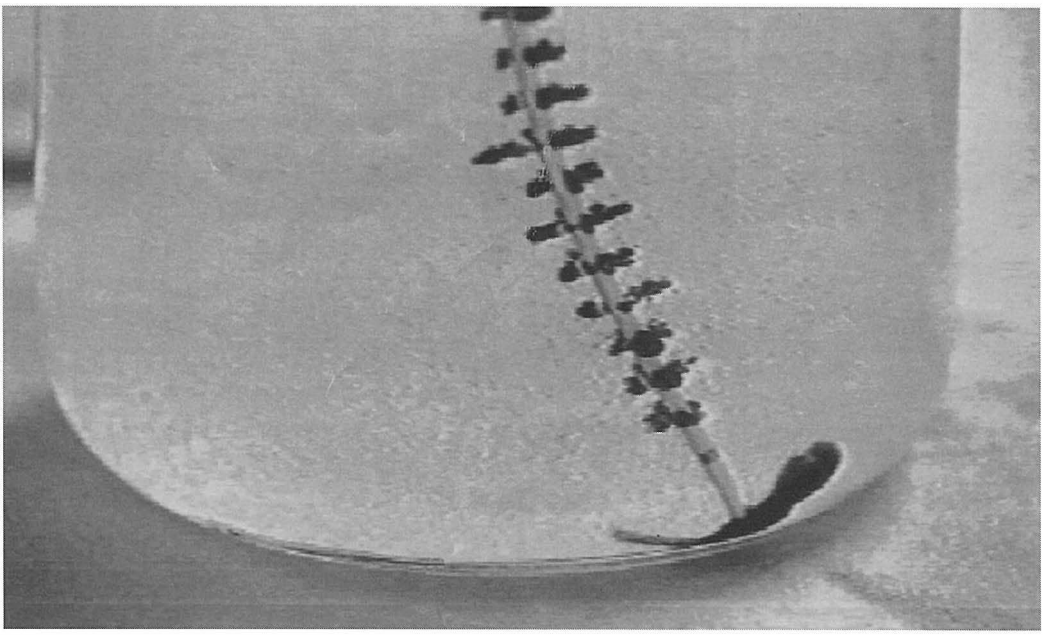

Figure 2. The pulse-spray system. Using the mechanical force of an automatic pump on the 1 cc syringe a bolus (0,1 - 0,5 ml) of saline solution containing 10% thrombolytic agents is infused in the thrombus with high pressure through the pressure responsive slit orifices of the catheter. The frequency of the jet spray varies from 6 to 120 seconds.

theory. The average treatment time in our study was 12 hours and was calculated from the treatment times ranging from 6 to 27 hours. These differences were mainly due to the site and size of the thrombus, its underlying disease and duration of ischemic conditions. It has been observed that chronic limb ischemia seems to be more resistant to thrombolysis,[18] but it should not considered as a relative contraindication.[13,14]

After an appropriate selection of patient, premedication and catheter technique, the next important step to achieve a really good and long-term clinical success is the establishment of a proper thrombolytic treatment plan and the monitoring of the patients. The role of partial thrombin time (PTT), thrombin time (TT) and fibrinogen serum concentration in „holding the treatment in hands" is discussed in the literature, and fibrinogen level under 1 g/l is often associated with occult bleeding.[1,10,11,14,17] This is especially significant in patients with clinical history of ulcer, cerebrovascular accident, recent major trauma, or surgery, especially around the pancreas and the prostate gland.

With the use of the pulse-spray technique, new requirements emerged. In order to reopen the occluded vessel as quickly as possible often thrombolysis of the whole length of the thrombus was attempted from the very start of the procedure.[19,20] An occasional distal embolization should be further treated with thrombolytic infusion or percutaneous thrombus aspiration. Other teams start the lytic therapy from the distal end of the thrombus and go further in proximal direction.[21] In the majority of our cases, most of these techniques proved to be non beneficial because of the hardness of the occlusion. Even in those cases, when the guide wire was easily introduced into the thrombus we suggest that, because of thehigh pressure, a short thrombotic plug left behind at the dis-

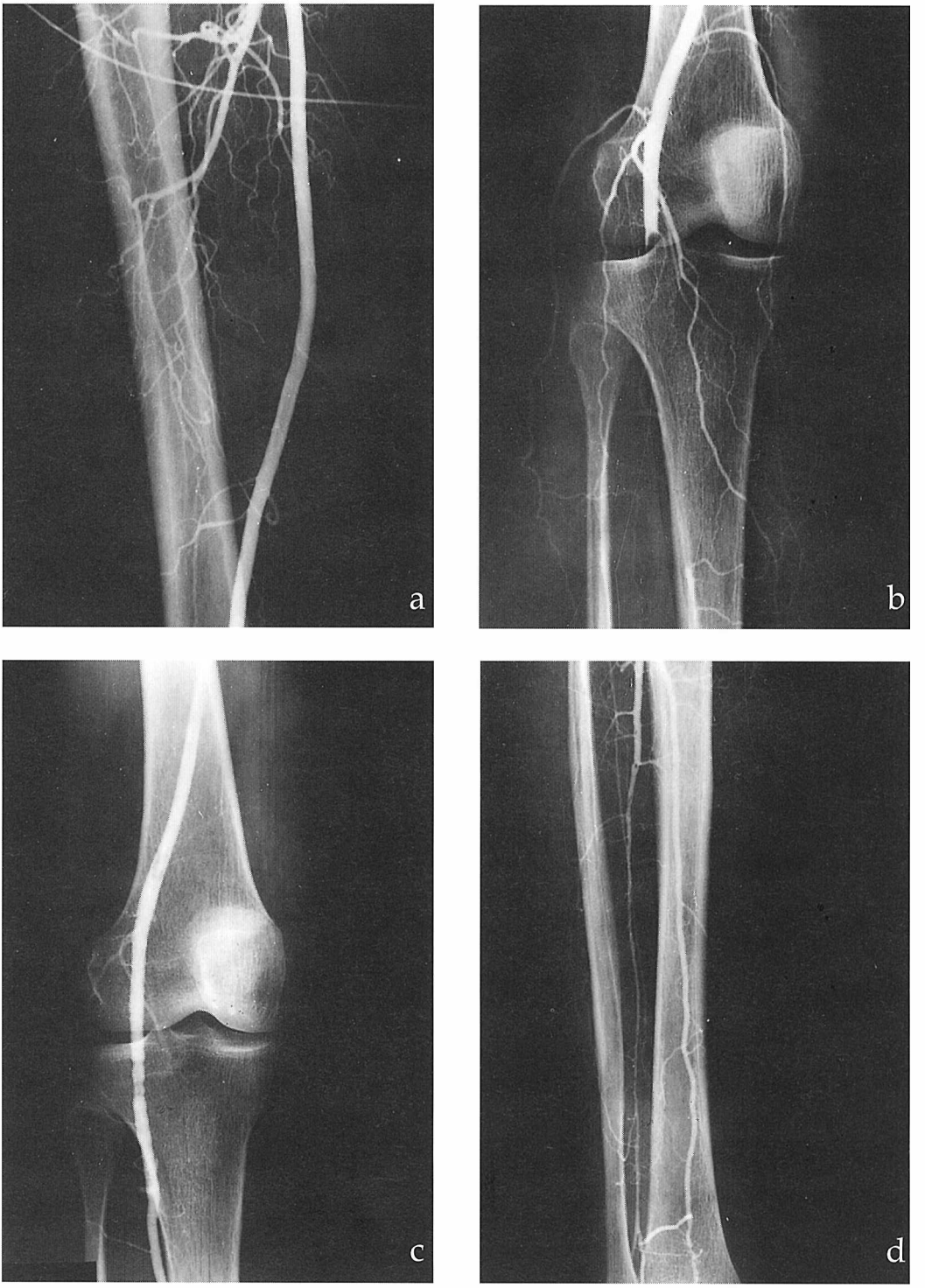
a
b
c
d

Figure 3 a-d. Case report. E. K. 63 year old female patient with a clinical history of diabetes mellitus for 10 years, complicated by hypertension in the last 3.5 years. Trigliceride and cholesterol level were slightly elevated. Complains started a week before her arrival in the department and increased in the last 2 days. The clinical picture was Fontaine IV. The right leg under the knee joint was livid, cyanotic and there was no pulse in the popliteal artery and distaly to that.
Selective angiography was performed with a cross-over technique, which demonstrated a total occlusion of the right popliteal artery (a, b). Pulse-spray infusion was initiated after positioning the catheter in the thrombus. The result of 1 hour control angiography was encouraging, so, pulse-spray technique was continued for further four hours. After nine hours of treatment, the control angiography showed total recanalization (c, d). The clinical picture at discharge from the hospital was Fontaine I- II. Three years after the procedure, strictly adhering to the postprocedure protocol, the patient had no significant increase of ischaemic symptoms.

tal end might be useful for the profilaxis of embolization (Figure 3).

The patency rate of the reopened arteries is in strong dependence of a proper postprocedural management. It seems to be obvious that, without a proper aftercare, thrombolysis is not going to result in a satisfactory long-term patency, especially in cases of pre-existing progressive disease, although this assumption has not been proved in randomized studies on selective thrombolysis.

Conclusions

Till date, there has been a relative reluctance from the wide use of selective thrombolysis, mainly because of the long duration of the procedure, quite high radiation, lack of experience and a number of other reasons. The introduction of the pharmacomechanical method proved to be a great step forward in managing the occlusion with combined chemical and physical forces. The mechanical power of the jet spray multiplies the action of the lytic drug helping, on the one hand, its deeper penetration into the thrombus and, on the other, mechanically destroying the thrombus.

The selective thrombolysis with pulse-spray technique is a radiological treatment of occlusions which may be part of the complex treatment, including surgical procedures, or applied as a curative therapy alone.

With an appropriate selection of patients, a well-tested treatment protocol and proper post-treatment care of the patients both, the acute and subacute artery occlusions may be successfully treated and amputation rate significantly lowered.

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Spectral characteristics of the high-resolution liver CT data: detection of iron-overload and cirrhosis

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Background. *Statistical methods and Fourier analysis of CT values were used to detect the presence of liver cirrhosis and of iron-overload in the sets of high-resolution liver CT data.*

Subjects and methods. *Eleven liver cirrhosis patients, seven hemodialysed patients with iron-overload and 51 control individuals were included. CT unit SIEMENS SOMATOM DRH was used (kernel 2, 0.2 mm wide and 2 mm thick pixels). Square sample areas, 50 pixels wide, two per control and three per patient were collected. The areas were statistically analysed and decomposed in 100 linear fragments (50 lines and 50 columns of 50 pixels) for Fourier analysis. The mean and standard deviations of harmonic powers were calculated for each sample.*

Results. *The high-resolution CT data in the iron-overload patients differed from the controls by the increased mean density (≥79 HU) and reduced power of many harmonics ($p<0.01$). The high-resolution CT data of the cirrhotic patients showed the decreased mean density (<50 HU) and increased power of harmonics from 0.1 to 0.9 cycles/mm (wavelengths from 10 to 1.1 mm) ($p<0.01$).*

Conclusions. *Information extractable from the high-resolution liver CT data can distinguish between the normal and cirrhotic, or iron-overload livers.*

Key words: liver cirrhosis, liver diseases; iron overload; tomography, x-ray computed, Fourier analysis

Introduction

Ultrasound, CT and MR are dominant methods of liver imaging, mainly used for the detection of focal liver diseases. Conventional CT images with large pixels are unable to define CT characteristics of the cirrhotic liver. Fibrosis and altered liver architecture, as the main pathological characteristics of liver cirrhosis, do not substantially alter the mean density on these images[1], presumably due to the effect of partial volume. Each voxel of the conventional abdominal CT image contains parts of several liver lobules and the effect of partial volume thus disables the visualisation of detailed densitometric texture.

The present study was aimed to test whether the high-resolution CT values can distinguish

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normal liver images from the images of the cirrhotic or iron-overload livers. Statistical methods and Fourier analysis of CT values were used for this purpose.

Patients and methods

The control group consisted of 51 patients scheduled for abdominal CT examinations of kidneys, spine or other organs, without history or clinical signs of diffuse liver diseases. Eleven patients with a clinic diagnosis of liver cirrhosis were CT examined to exclude the possibility of liver neoplasm. All patients were in the condition of decompensated liver cirrhosis with ascitic fluid on this or any of the previous hospital stays. The iron-overload group consisted of seven hemodialysed patients with chronic renal failure and iron-overload (serum ferritin more than four times above normal). These patients were scheduled for the abdominal CT because of their renal or spine pathology.

A single, 2 mm thick liver CT image was taken through the central part of the liver on a SIEMENS SOMATOM DR-H CT unit before the contrast application. We used the abdominal kernel 2, 720 projections in four seconds with zoom factor 5.2. Pixels were 0.2 mm wide and 2 mm thick in all images.

CT data were stored and square areas wide 50 pixels (2500 pixels per square) were later transferred to a portable PC. A simple RS-232 adapter and XTALK program were used to connect the PC in the place of a DIGITAL control console. We used the command ABS/DIA/LIS from the measuring program of SOMATOM DRH to export the chosen area of CT values to the capture file on a portable PC. Files were edited in a DOS editor to discard textual information and, after that, the data were realigned to reconstruct the initial CT matrix.

From these data simple statistical measures were calculated.[2] Then, each sample area of CT values was decomposed in 50 columns and 50 rows (in total 100 linear segments of 50 pixels). These segments were analysed by the conventional Fourier analysis. Amplitudes of sine and cosine components were calculated for the harmonics ranging from 0. to 25. (from 0 to 2.5 cycles/mm). Amplitudes of the cosine and the sine components were used to calculate the power of each harmonic in the linear segment.[3] The mean harmonic power spectrum with standard deviations was calculated for each sample.

Observed parameters were tested by the calculation of ROC curves.[4] Numbers of true positive/negative and false positive/negative were used in 2•2 tables for the calculation of $\chi 2$ values.[2]

Results

Table 1 shows the statistical characteristics of the collected sets of CT data and calculated sets of contrast values.

The high-resolution (HR) CT data of the livers with iron-overload showed the increased mean density in comparison to the control samples with the decreased mean densitometric contrast value. Both characteristics are probably caused by the abundant and evenly distributed iron in liver tissue.

On the contrary, the HR CT data of the cirrhotic livers showed the reduced mean density and increased mean contrast value.

Table 2 shows the results of Fourier analysis. Mean powers with a standard deviation are shown only for the first 10 harmonics. Omitted harmonics include the 0. harmonic, whose power equals the mean densities already shown in Table 1. The powers of higher harmonics (from 11. to 25.) were similar in the controls and groups of patients and considered unimportant.

In comparison to the HR control CT data, the harmonic powers are increased in the group of the HR cirrhotic liver CT data and decreased in the group of the HR iron-overload liver CT data.

To test the described differences among the three groups of the HR CT data, elements of the ROC curves were calculated. Cut off points of

Table 1. Statistical characteristics of the high-resolution liver CT data

	Mean minimal value per sample (HU)	CT Mean maximal CT value per sample(HU)	Mean density (HU)	Standard deviation of CT values (HU)
Controls (102 samples of HR liver CT data)	-26.19	148.58	60.91	31.56
Cirrhotic liver patients (33 samples of HR liver CT data)	-75.27	156.73	45.04	44.08
Iron-overload patients (21 samples of HR liver CT data)	-5.81	146.76	71.87	29.85

Table 2. Results of the Fourier analysis. The power of the 0. harmonic equals the mean density from the Table 1. Undistinguishing harmonics in the range from 11. to 25. are omitted as unimportant

Harmonic	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Cycles/mm	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Wavelength(mm)	10	5	3.33	2.5	2	1.67	1.43	1.25	1.11	1
Controls (102 samples)										
Mean power (HU)	19.0	17.1	14.9	11.7	8.2	5.6	3.9	2.9	2.2	1.7
St. deviation (HU)	11.2	10.1	8.5	6.8	5.0	3.5	2.3	1.8	1.4	1.1
Cirrhotic liver patients (33 samples)										
Mean power (HU)	25.9	22.9	18.0	14.5	10.0	6.3	4.4	3.3	2.6	2.0
St. deviation (HU)	18.7	14.6	11.2	8.8	5.8	3.7	2.6	2.1	1.7	1.4
Iron-overload patients (21 samples)										
Mean power (HU)	16.1	14.5	13.3	10.1	7.0	5.0	3.5	2.5	1.8	1.4
St. deviation (HU)	9.1	8.1	8.1	5.4	4.0	2.9	2.1	1.5	1.1	0.9

the highest P^2 value and overall accuracy were selected as optimal.

The results of the comparison between the HR cirrhotic liver CT data and the control CT data are shown in Table 3.

The cirrhotic liver HR CT data can be distinguished from the controls by the decreased mean density (<50 HU, P^2=37.28, $p<0.0001$) and by the increased power of harmonics from 0.1 to 0.9 cycles/mm (wavelengths from 10 to 1.1 mm)($p<0.01$). Some harmonics showed an increased standard deviation of harmonic powers among linear segments ($p<0.01$).

The results of the comparison between the HR iron-overload liver CT data and the control CT data are shown in Table 4.

The iron-overload liver CT data can be distinguished from the controls by the increased mean density (>78 HU, P^2=30.44, $p<0.0001$), decreased power of many harmonics from 0.1 to 2.5 cycles/mm (wavelengths from 10 to 0.4 mm) ($p<0.01$).

Discussion

Spectral analyses proved to be a suitable technique for discriminating the ultrasound images of normal livers and steatotic ones. The reported study included six cases (two normal and four with steatosis).[5] The texture measure values were compared with the corresponding biopsy scores and the results indicated the ability of the spectral texture measure to discriminate the two conditions and to estimate the severity of histological change.

Table 3. ROC curve elements for the parameters distinguishing the cirrhotic liver HR CT data from the controls (P^2>6.63, p<0.01)

			Characteristics of HR CT data			Sensitivity	Specificity	Accuracy	χ^2
			Cirrhotic liver	Cut off point (HU)	Controls				
Mean density			<	50.0	≥	0.70	0.85	0.81	37.28
Cycles/mm		Wavelength (mm)			Fourier analyses				
Mean power (HU)	0.1	10	≥	25.0	<	0.48	0.88	0.79	20.45
	0.2	5	≥	23.0	<	0.52	0.92	0.82	31.52
	0.4	2.5	≥	14.0	<	0.48	0.81	0.73	11.57
	0.5	2	≥	10.0	<	0.48	0.84	0.76	14.83
	0.7	1.43	≥	4.4	<	0.55	0.74	0.69	8.84
	0.8	1.25	≥	3.5	<	0.45	0.86	0.76	14.88
	0.9	1.11	≥	2.4	<	0.55	0.72	0.67	7.49
St. dev. (HU)	0.1	10	≥	12.5	<	0.45	0.87	0.77	16.23
	0.2	5	≥	10.0	<	0.55	0.72	0.67	7.49
	0.4	2.5	≥	7.5	<	0.52	0.81	0.74	13.79
	0.5	2	≥	5.2	<	0.52	0.78	0.72	10.88
	0.9	1.11	≥	1.6	<	0.45	0.86	0.76	14.88

The same method was used to analyse trabecular patterns in digitised wrist radiographic images.[6] Authors found in the group of 68 patients that three spectral indices permitted quantification of the cancellous bone structure, and detection of the age related structural bone changes.

Another example is the reported study of the cross-sectional images of the posterior mandible.[7] In these images, useful diagnostic information was in the range of 0.12 to 0.6 cycles/mm for the contact radiographs and the 3 mm cross-sections images.

The idea of using high-resolution liver CT values was based on previous reports that high-resolution CT abdominal imaging proved useful in the detection of the adjacent organs' invasion by the stomach cancer.[8]

A CT slice thickness ranging from one to three millimetres is used in the chest CT imaging.[9] The high-resolution CT of the lungs correlates well with the pathologic findings.[10] It provides detailed visualisation of the lung parenchyma.[11] It is useful in differentiating similar patterns of abnormalities seen on chest radiographs, such as those seen in lymphangitic carcinomatosis and sarcoidosis, and in delineating the extent of co-morbid lung diseases, such as emphysema and asbestosis. The characteristics of the margins of metastatic pulmonary nodules noted on histopathologic examination correlated well with their high-resolution CT findings (z04),[12] while microscopic intravascular tumour emboli and lymphangitic tumour spread were difficult to detect.

Actual CT values or „pixel mapping" were found important in accurate diagnosing of small kidney angiomyolipomas.[13]

The images in iron-overload patients differed from the controls by the increased mean density (>79 HU) and reduced power and standard deviation of power of many harmonics. It is in accordance with the reported observation that the mean liver densities in patients with haemochromatosis are increased and in the range from 75 do 132 HU.[14] The reduced pow-

Table 4. ROC curve elements for the parameters distinguishing the HR iron-overload liver CT data from the controls (χ^2>6.63, p<0.01)

			Characteristics of HR CT data			Sensitivity	Specificity	Accuracy	χ^2
			Iron-overload	Cut off point (HU)	Controls				
Mean density			≥	79.0	<	0.38	0.98	0.87	30.44
Cycles/mm		Wavelength (mm)			Fourier analyses				
Mean power (HU)	0.1	10	<	16.0	≥	0.71	0.70	0.70	12.53
	0.2	5	<	14.0	≥	0.57	0.75	0.72	8.82
	0.4	2.5	<	10.0	≥	0.62	0.71	0.69	8.09
	0.7	1.43	<	3.5	≥	0.71	0.66	0.67	9.94
	0.7	1.43	<	4.4	≥	0.55	0.74	0.69	8.84
	0.8	1.25	<	2.4	≥	0.52	0.75	0.72	6.53
	0.9	1.11	<	1.9	≥	0.67	0.69	0.68	9.27
	1.0	1	<	1.3	≥	0.52	0.80	0.76	9.92
	1.1	0.91		1.2		0.67	0.68	0.67	8.68
	2.5	0.4		0.3		0.62	0.68	0.67	6.5
St. dev. (HU)	0.0	4	<	8.0	≥	0.57	0.78	0.75	11.02
	0.4	2.5	<	5.3	≥	0.71	0.66	0.67	9.94
	0.5	2	<	3.7	≥	0.67	0.74	0.72	12.66
	0.75	1.43	<	1.9	≥	0.67	0.68	0.67	8.68
	0.9	1.11	<	1.0	≥	0.57	0.76	0.73	9.5
		1							8.82
		0.91							8.09
	1.0	0.83		0.8					6.53
	.	0.77		.					7.00
	.	0.66	<	.	≥	-0.6	-0.7	-0.7	11.02
	.	0.62		.					9.30
	.	0.59		.					8.09
	2.3	0.55		0.4					6.50
		0.43							7.75
		0.42							6.53

ers of harmonics suggest reduced variability in the densities of adjacent pixels. Possible interpretation is that an evenly diffused iron-overload increases the mean density and simultaneously blurs the normal variability of the high-resolution liver CT data.

The high-resolution CT data of the cirrhotic liver in the present study differed from the controls by the low mean density (<50 HU), possibly because of the reduced partial volume effects, allowing visualisation of the infiltration of the fat in alcoholic liver steatosis.[15]

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Standardized image documentation in nuclear medicine

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There are no generally accepted standards for image documentation in every day nuclear medicine practice. This is a problem whenever hardcopies from other centers are to be re-interpreted or compared to present images of the same patient. In order to support image reading by a third party proposals of documentation necessary within images are elaborated. Examples of image documentation of the most frequently performed nuclear medicine studies are given, i.e. lung scintigraphy, thyroid scintigraphy, bone scintigraphy both in planar and in SPECT-technique, renal function scintigraphy, myocardial perfusion scintigraphy, and positron emission tomography. These examples are intended to stimulate discussion within the nuclear medicine community about the content of documentation necessary in nuclear medicine images.

Key words: nuclear medicine; diagnostic imaging; medical records-standards; minimal requirements

Problems of image documentation in nuclear medicine

There are no generally accepted standards for image documentation in every day nuclear medicine practice. This is a problem, whenever hardcopies from other departments/clinics/institutions have to be re-interpreted or compared to present images of the same patient. This problem is independent of the image quality itself. Even best image quality may be insufficient for interpretation, if essential information is missed. In consequence, incomplete image documentation may render interpretation difficult or even impossible. Thus, the main task of the working group „Standardized Image Documentation" of the German Society of Nuclear Medicine is to define guidelines for minimal requirements of nuclear medicine image documentation. This group has gained experience in this field for the last three years. Their proposals have been discussed on several national conferences and published in different journals.[1-4] The members of the working group are co-operating with corresponding working groups of various national nuclear medicine societies.

The examples presented below should be extensively discussed in order to maintain a consensus about them. These generally accepted standards could than support the manufacturers of nuclear medicine computers to implement the accepted requirements within their software. However, the standardization of image documentation should be

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considered as definitely independent of the ongoing standardization of nuclear medicine procedures. Furthermore, the elaborated proposals should not limit the definition of individual layout or individual extensions in image documentation.

Examples of images

The presented examples of typical nuclear medicine studies are partly representing the results of consensus conferences held as pre-congress meetings prior to the last three national congresses of nuclear medicine in Germany. The subcommittee considers them in a presented form as an initial point for an intended discussion within the European Nuclear Medicine Community. They are designed to cover the requirements of every day routine studies. The limitation of labeling to the minimum necessary for understanding of the image content by third parties is one of the declared intentions of the subcommittee. However, individual extensions of these minimal requirements by particular institutions should be permitted definitely.

Several data should be documented clearly on all nuclear medicine studies, i.e. patient name, date of birth, date of the study, and institution in which the study was performed. The injected amount of radioactivity and the tracer used are probably the most important study related data. The interpretation of the image should be supported by the display of the color bar used. Parameters derived from regions-of-interest should be supported by documentation of the underlying ROIs. The image sequence with the time of individual images should be documented for image sequences.

For most often performed types of studies the following specific requirements should be fulfilled in addition to the above mentioned basic requirements.

Lung

Thorough labeling of the views is mandatory in lung scintigraphy. Ventilation and perfusion studies should be clearly distinguished by appropriate labels (Figures 1 and 2). Documentation may be completed by denoting the side-related perfusion as well as the position of the patient both at the time of injection and during acquisition.

Thyroid

Labeling of the jugulum and of the Technetium-99m-uptake value in percent is essential in documentation of thyroid scintigraphy. It is recommended to complete the documentation by the matrix size, the display of the reference scale, and the time interval between injection and acquisition. It may be helpful to identify findings of palpation within the image (Figure 3).

Bone

Whole body bone scans should be documented at least in two directions both at two intensity levels, the first optimized for the ribs and the second for the spine (Figure 4).

Myocardium

For myocardial perfusion SPECT, the whole left ventricular myocardium has to be documented. Short axis, horizontal and vertical long axis slices should be presented both as stress and as rest images. An optimized arrangement of identical slices of the stress and resting study within the same image is recommended. Moreover, information on the position of the patient during acquisition and anatomical labeling of the slices are required. Pictograms or data about the type of the stress may support interpretation of the images as well (Figure 5).

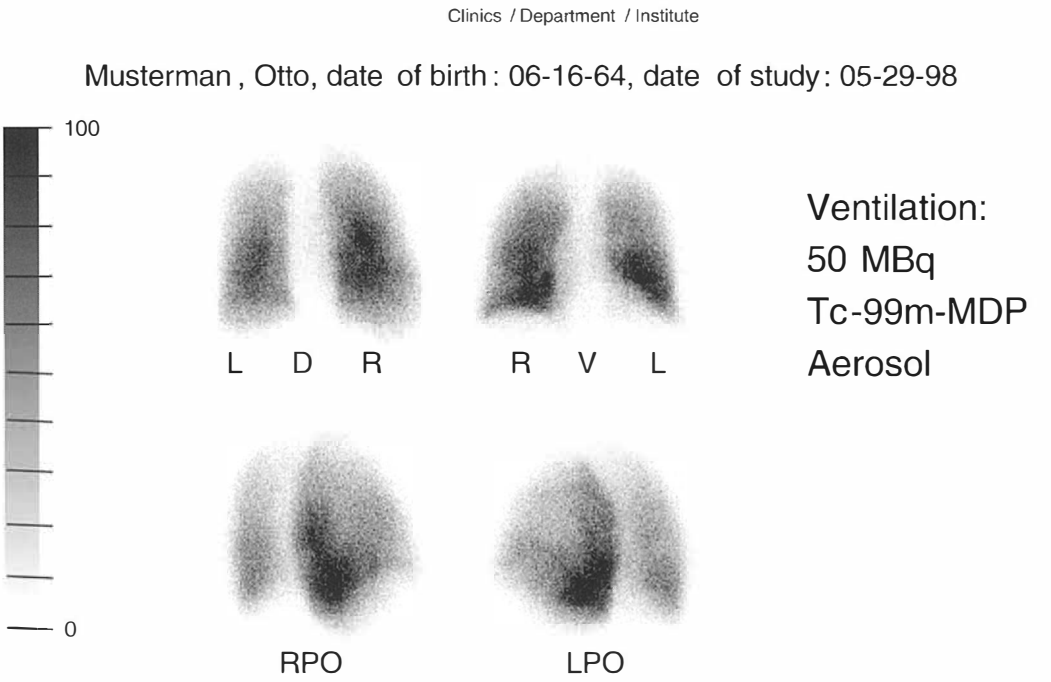

Figure 1. Ventilation scintigraphy of the lung.

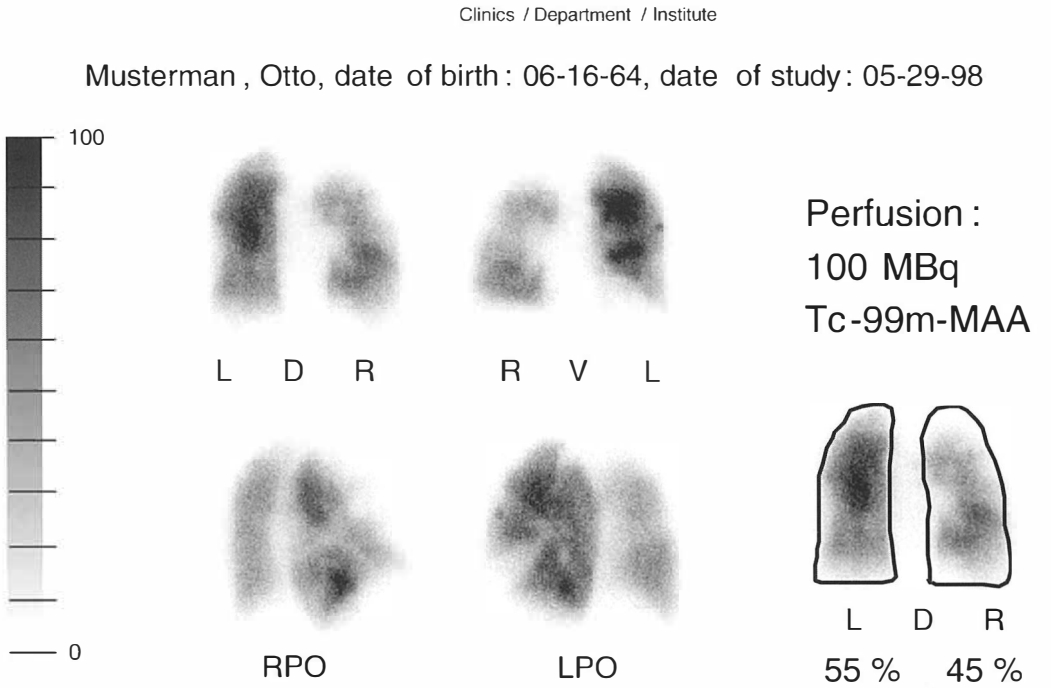

Figure 2. Perfusion scintigraphy of the lung.

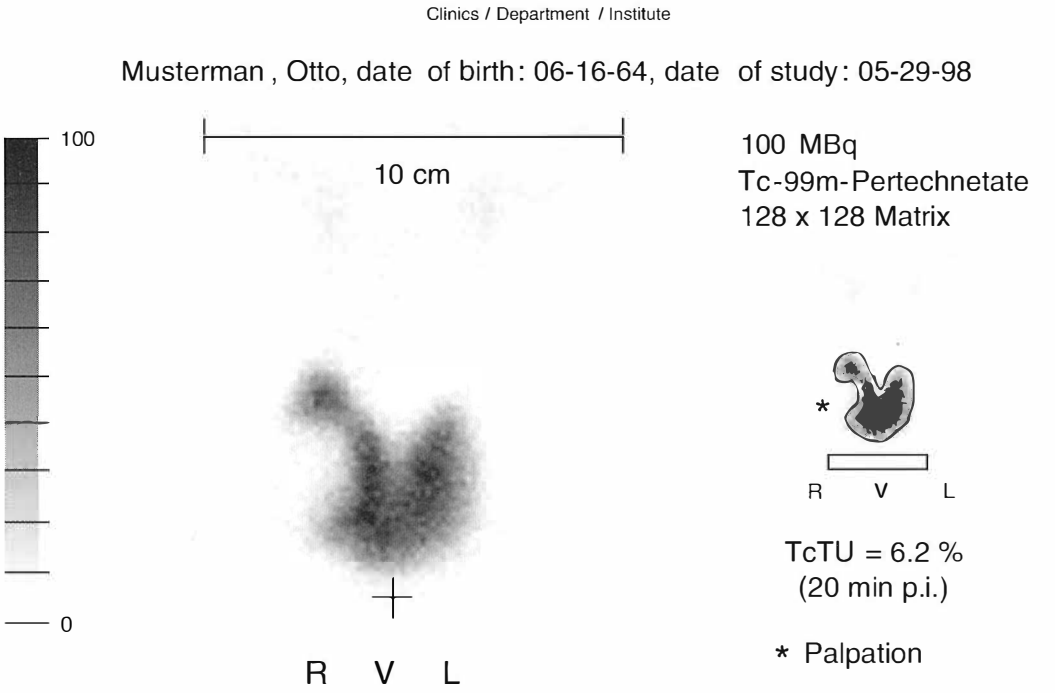

Figure 3. Quantitative thyroid scintigraphy.

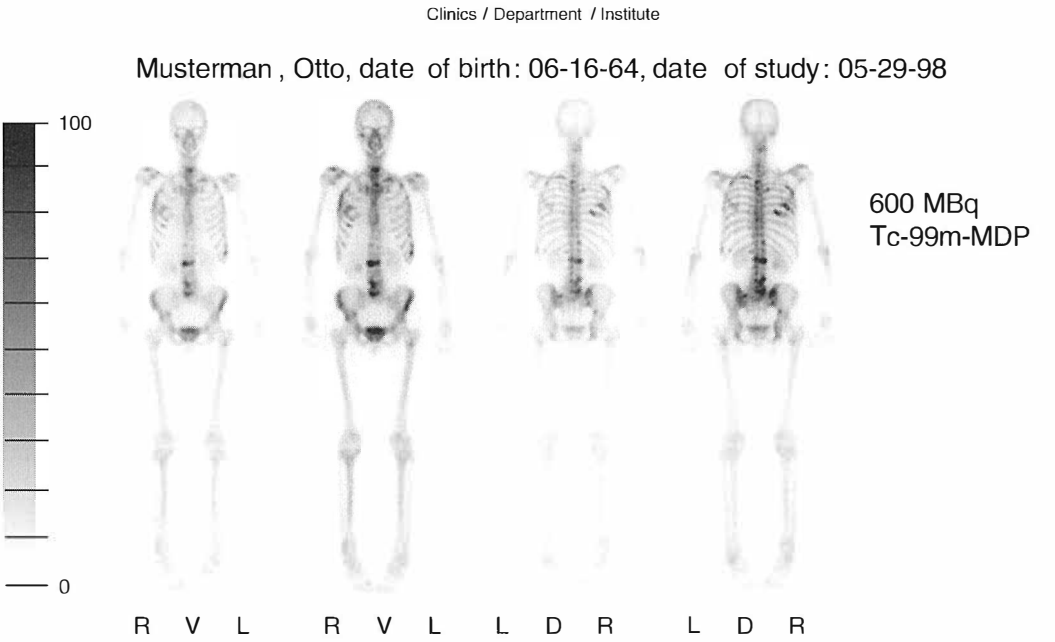

Figure 4. Whole body bone scan.

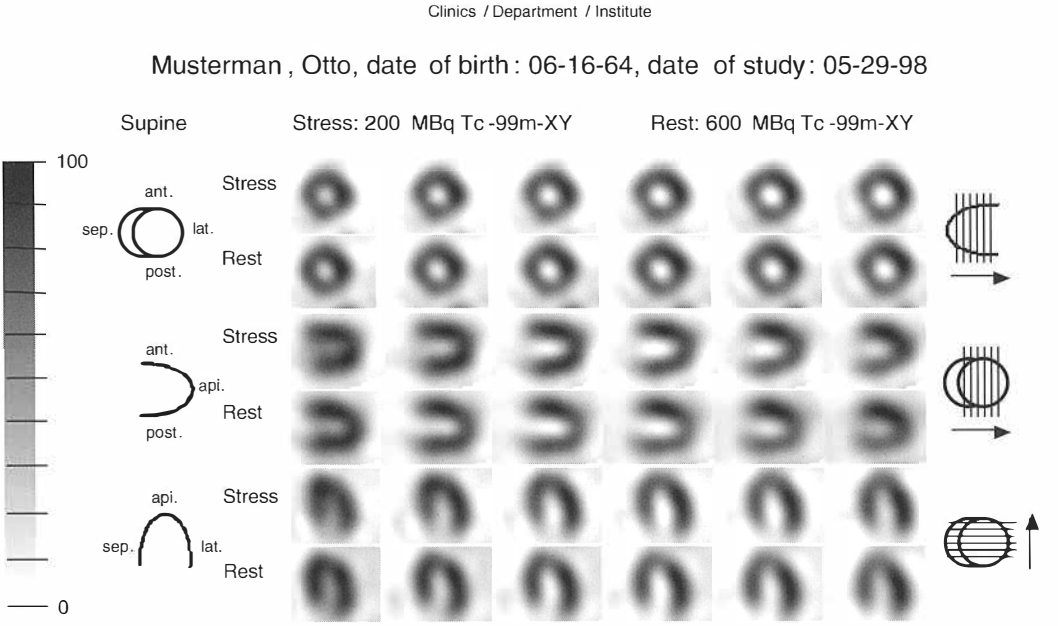

Figure 5. Myocardial perfusion scintigraphy.

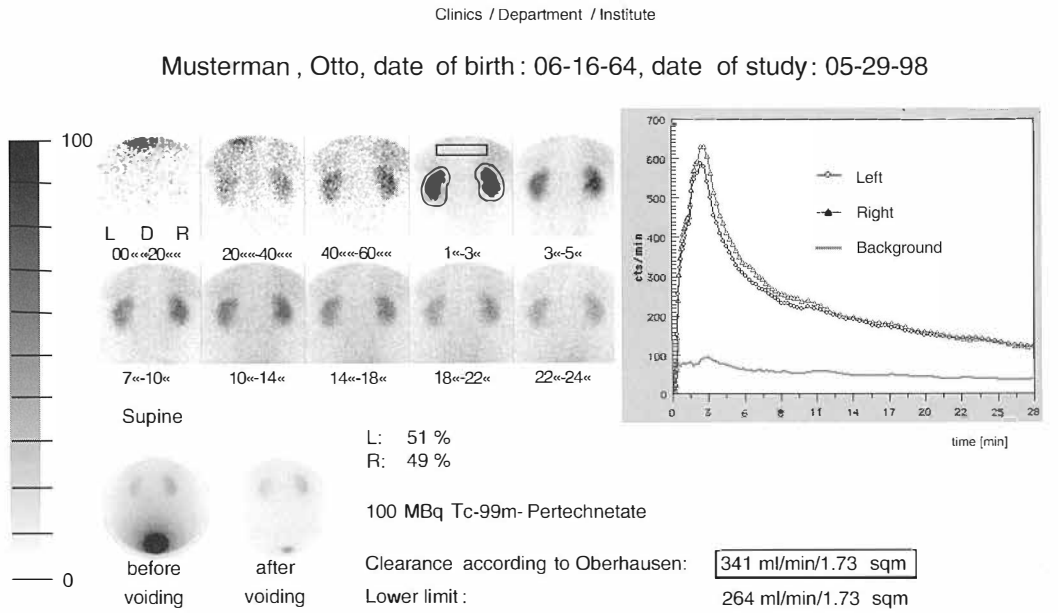

Figure 6. Renal function scintigraphy.

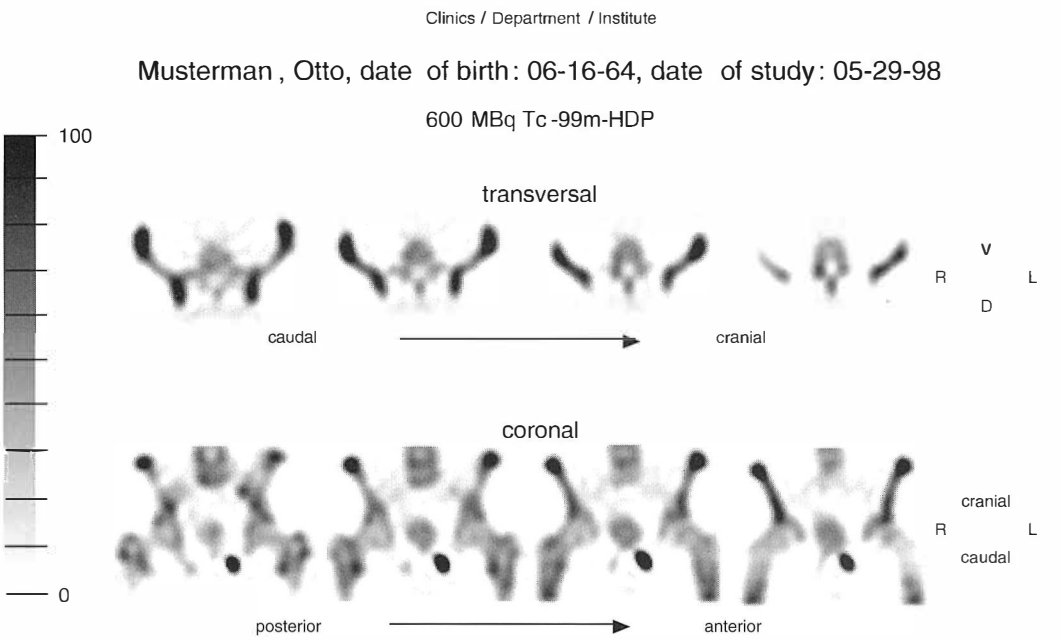

Figure 7. Transersal and coronal slices of bone scan in SPECT-technique.

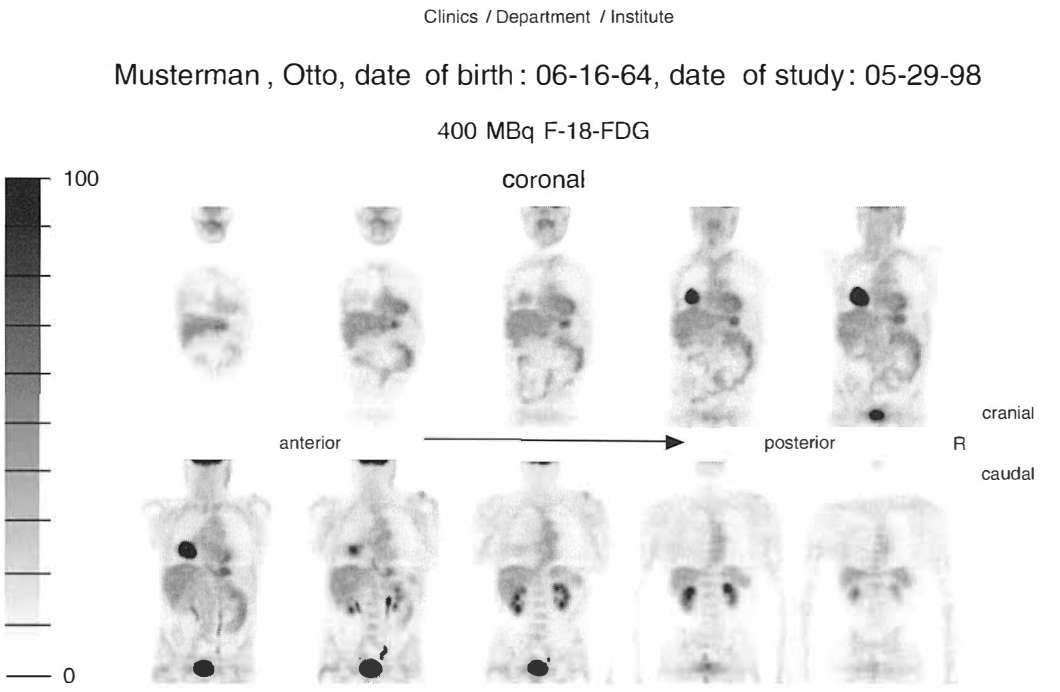

Figure 8. Coronal slices of positron emission tomography.

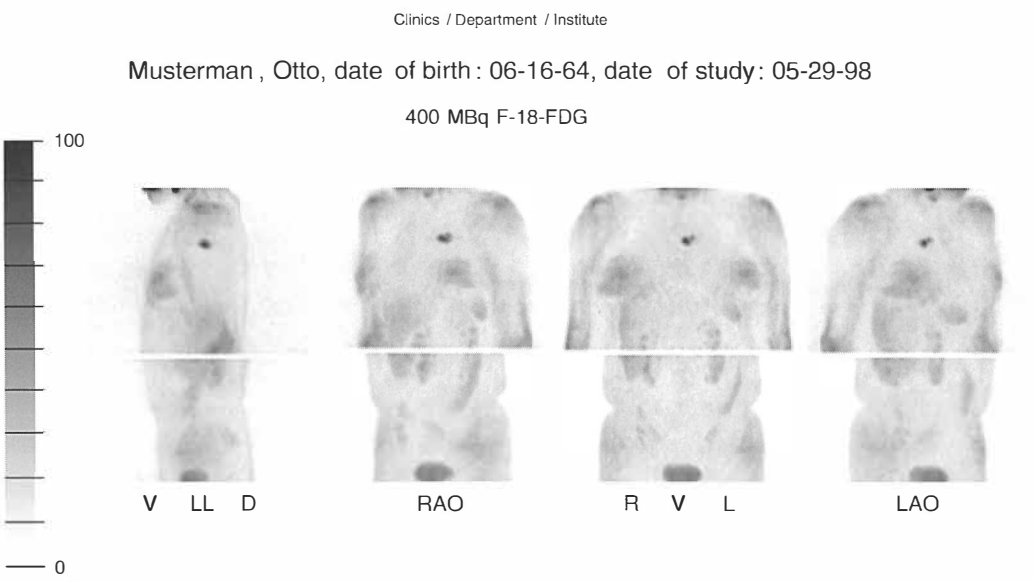

Figure 9. MIPs of positron emission tomography.

Function of the kidneys

Split renal function is the main data that should be documented within the image. Apart from numeric data on clearance, the method and age-dependent lower limits should be given in renal scintigraphy. After diuretics the documentation of the time of application, the curve and the effect of the diuretics in percent are essential (Figure 6). For the Captopril scintigraphy a separate documentation is recommended.

SPECT / PET

Regarding tomographic studies both the direction of the slice sequence and an anatomical labeling of the slices should be presented within the images (Figures 7 and 8). The interpretation of images may be supported by the use of pictograms. PET studies may be displayed as maximum intensity projections (MIPs) for overview (Figure 9).

Conclusion

Standardized image documentation in nuclear medicine is mandatory in order to ease image reading by a third party. Suggestions given above should be extensively discussed among nuclear medicine physicians in order to maintain a consensus about them.

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Detection of lymph node metastasis from osteosarcoma with 99mTc-MDP scintigraphy. Case report

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Osteosarcoma usually spreads via the blood stream, developing pulmonary and skeletal metastasis. Rarely, the disease spreads via the lymphatics. We are reporting a case when radionuclide bone imaging detects lymph node metastasis from osteosarcoma.

Key words: bone neoplasms - radionuclide imaging; osteosarcoma; lymphatic metastasis; technetium Tc 99 m medronate

Introduction

Osteosarcoma, as other malignant bone tumors demonstrates active bone turnover and therefore reveals intense uptake on bone scintigraphy.[1] Bone-seeking radiopharmaceuticals occasionally demonstrate intense uptake in soft tissue metastasis from osteosarcoma (lung, brain, renal, lymph nodes).[2-4] This tumor spreads usually via the blood stream with development of pulmonary and skeletal metastasis.

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Case report

The patient, male at the age of 14, first presented two years before this report. He had been complaining of painful, livid swelling in his left calf for 4 months until he was referred to us. He was seen by pediatric oncologist after radiography, bone scintigraphy, fine needle aspiration and open biopsy had been performed. Histology finding confirmed osteosarcoma of the left tibia.

Plain radiography of the left leg demonstrated a poorly-defined lesion located centrally in the left proximal metaphysis of the tibia, extending into the shaft. The lesion revealed a permeable pattern of destruction with proliferation, extensive sunburst spiculation, mineralization of the tumor, lumps, clouds and segments of ossified matrix. The

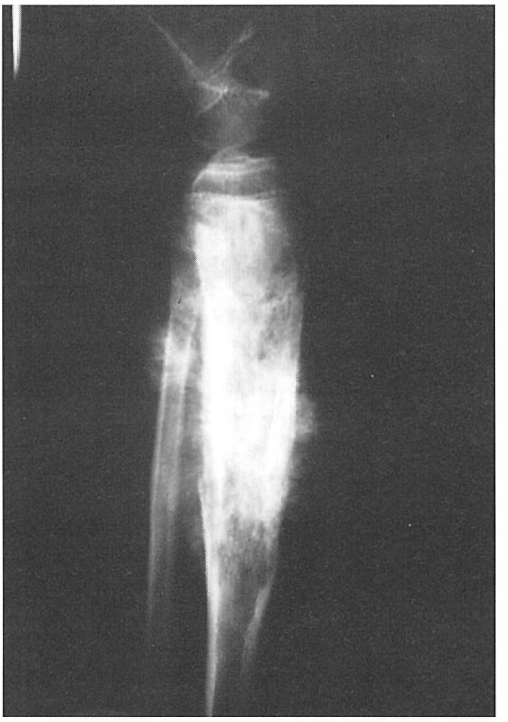

Figure 1. Pain radiography of the left tibia (in the period of the initial diagnosis).

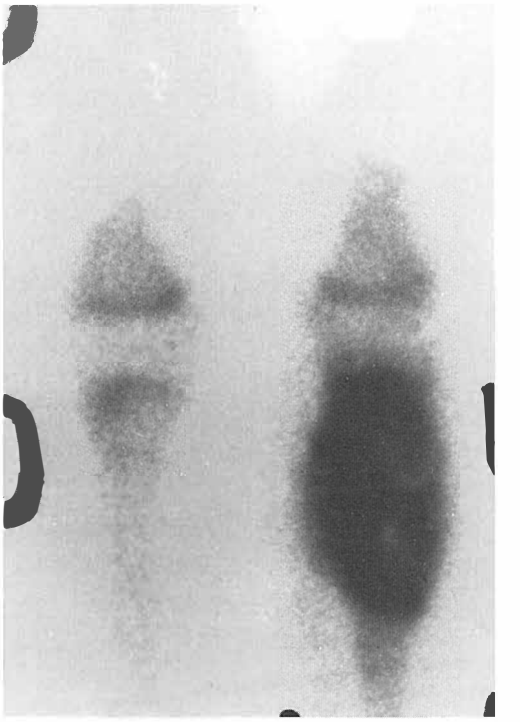

Figure 2. 99mTc-MDP bone scan of the primary tumor.

radiographic finding with these features strongly suggested high grade osteosarcoma (Figure 1).

Bone scintigram with 99mTc-MDP revealed inhomogenously increased uptake of bone radiotracer in the upper third of the left tibia with extension into the soft tissue adjacent to the bone (Figure 2).

After the confirmation of osteosarcoma, chemotherapy with T6 protocol for solid tumors was commenced because the parents refused surgery. T6 induction and maintenance chemotherapy for solid tumors was used for 14 months after the initial diagnosis. Before the completion of chemotherapy (exactly one year after histologic confirmation of osteosarcoma) above-knee amputation was done. Three months after the surgery, enlarged lymph node in the left groin was noticed. CT scan of the left groin demonstrat-

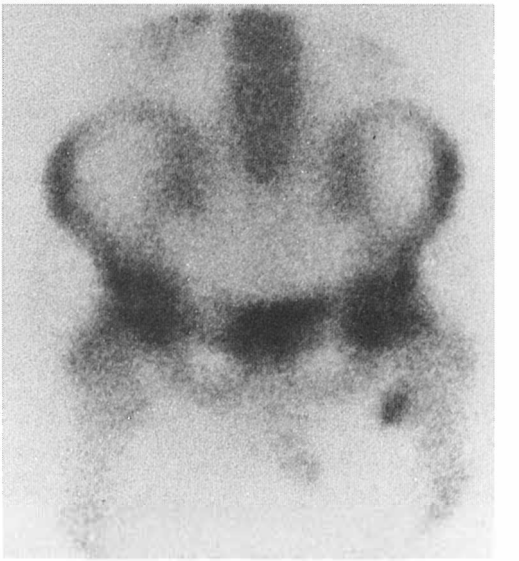

Figure 4. 99mTc-MDP bone scan after chemotherapy and surgery.

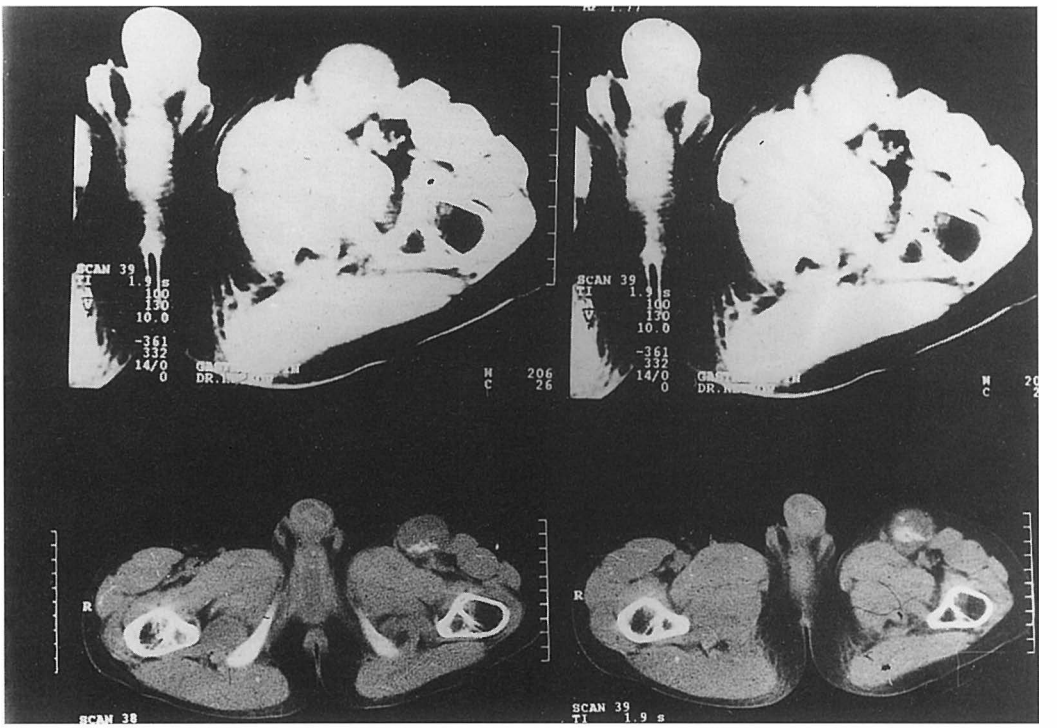

Figure 3. CT of the pelvis (region of the left groin).

ed the mass (lymph node) with punctate calcification (Figure 3). 99mTc-MDP bone scan did not show any focal hot spots throughout the skeleton, except an area of increased radiopharmaceutical activity located closely to the trochanter minor of the left femur, corresponding to the enlarged lymph node (Figure 4). The lymph node was removed and microscopic section (hematoxylin and eosin, x 400) revealed a metastatic deposition of osteosarcomatous tissue. There was a mesenchimal, osteoblastic stroma in which a deposition of osteoid and calcified osseous trabecullae (arrow) were found (Figure 5).

After the removal of the lymph node external beam radiotherapy of 50 Gy in the region of the left groin was applied. Recently after the termination of the radiotherapy, chest X-ray revealed pulmonary metastases. The child was in very bad condition and as he did not respond to radiotherapy at all, orthopedic surgeon, radiotherapist and pediatrician oncologist decided not to apply any other treatment. One month later (20 months after the onset of the disease) the patient died.

Discussion

The value of bone radionuclide imaging in the management of osteosarcoma is well established. Bone scan is quite useful in the delineation of the extent of the primary lesion, as well as in the follow up studies.[5-7] Local recurrence or skeletal metastasis are detected before they are radiographically apparent.

Osteosarcomatous lesions that spread via the lymphatics are very uncommon. Literature review revealed 2.7-11.4% of lymphatic

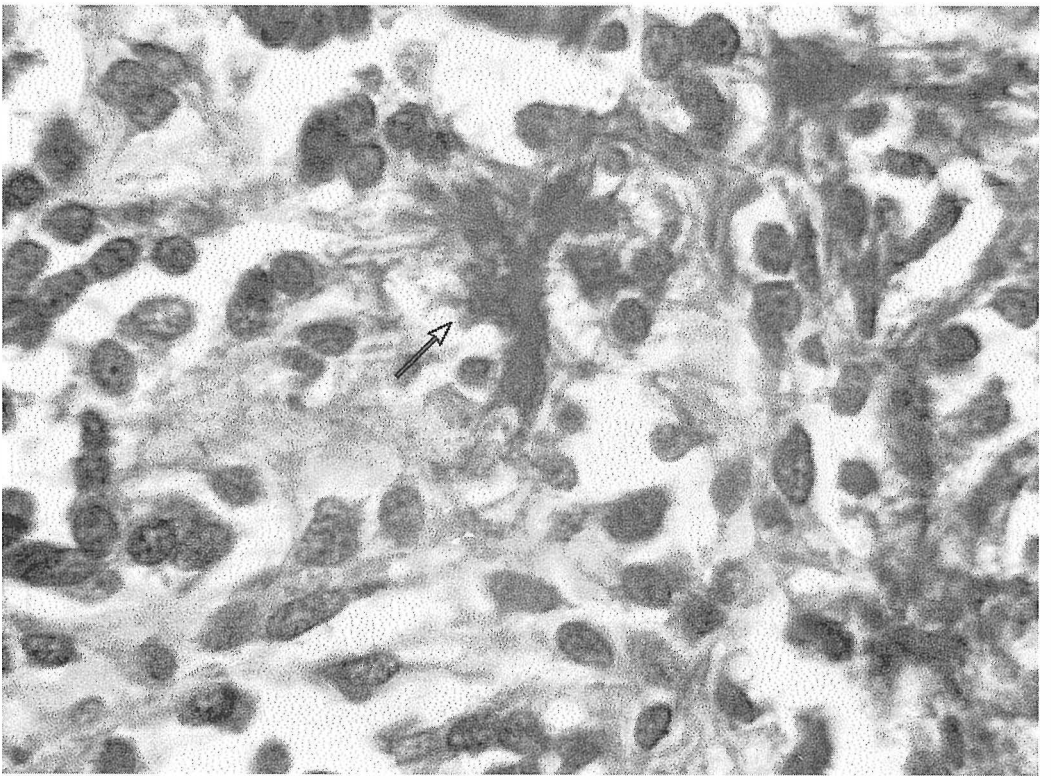

Figure 5. Microscopic section of the removed lymph node.

metastases in patients with osteosarcoma.[7] Its presence is a bad prognostic sign of the disease as none of these patients survived 5 years.[8]

Lymph node metastasis accumulate bone-seeking radiopharmaceutical avidly so 99mTc-MDP bone scan could be used in the assessment of any spreading (hematogenous or lymphatic) of osteosarcoma.

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A correlation of NK cytolitic test and BLT esterase test in determining activity of NK cells, stimulated by tumor target cells

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We examined the granule exocytosis in natural killer (NK) cells by measuring N-benzyloxycarboxy-L-lysine esterase activity. As a source of NK cells we used buffy-coat isolated NK cells or peripheral blood lymphocytes (PBL). The exocytosis was triggered by incubating cells with ionomycin/PMA or by NK cell susceptible tumour target cells K562. When we stimulated purified NK cells with tumour target cells, a close correlation (Corr=0.84) of cytolitic NK test results and BLT test results was obtained. We may conclude that BLT test can provide a valuable tool to discriminate further NK cell deficiency in patients with low cytolitic NK test results.

Key words: killer cells, natural; exocytosis, cell degranulation

Introduction

Secretory granules of lymphocytes may be important organelles, serving to concentrate and store different enzymes, cytolitic proteins and biologically active molecules. It is possible that exocytosis of granules plays an important role in cytolitic activity of cytotoxic T lymphocytes (CTL). Trypsin-like serine esterase (BLT esterase) was described as an easily detectable biochemical marker of granules.[1] It was shown that secretion of this enzyme is triggered by incubating CTL with antigen-bearing target cells or with immobilised anti-TCR monoclonal antibodies - in both cases the crosslinking of T-cell receptors (TCR) is required. The exocytotic process can be also triggered by activation of protein kinase C (with phorbol esters) and by simultaneous translocation of calcium through CTL plasma membrane with Ca^{2+} ionophores.[2] It has been shown that external Ca^{2+} is obligatory for both the TCR-triggered and for the phorbol ester/ Ca^{2+} ionophore - triggered exocytosis, because Ca^{2+} chelators (EDTA, EGTA) and Ca^{2+} channel blockers (nifedipine, verapamil) inhibit exocytosis.[3]

Natural killer cells (NK cells) are a subpopulation of lymphocytes, whose function has been defined to date by their ability to mediate the *in vitro* destruction of certain

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neoplastic and virally infected cells in a rapid, non-MHC restricted fashion. Because NK cells do not express antigen receptors they must recognise their targets in a way, that differs from T cells antigen recognition.[4,5] Nevertheless, the killing mechanisms of both cell types may be similar and they include granule exocytosis that releases enzymes, cytolitic proteins and biologically active molecules into areas bounded by the close apposition of effector and target cell plasma membranes.[6,7]

In our experiments, we provided an evidence that the BLT esterase, besides the use for monitoring CTL exocytosis, can also be used as a biochemical marker of NK cell granule exocytosis. We used BLT-esterase assay for monitoring NK cell exocytosis. As a source of NK cells we used buffy-coat isolated NK cells or buffy-coat isolated lymphocytes (PBL) as well. The exocytosis was triggered by incubating cells with ionomycin/PMA or by NK cell susceptible tumour target cells K562.

Materials and methods

Separation of human peripheral blood lymphocytes (PBL)

Buffy coats from normal blood donors were obtained with the kind co-operation of Transfusion Centre in Ljubljana. Buffy coats were diluted with two parts of sterile PBS and overlayered on Ficoll-Isopaque. Gradients were spun at room temperature for 20 min at 250 g. Cells (PBL) were subsequently harvested from above with a Pasteur pipette and washed three times. An aliquot from each sample was then suspended in trypan-blue solution for counting in a counting chamber.

NK cell isolation

Ficoll-isolated PBL cells were first incubated in nylon-wool columns in order to remove adherent cells (monocytes, B lymphocytes). Columns consisted of 10-ml plastic syringes, filled with 0.5 g of nylon wool. Each column was pre-washed with 30 ml of warm RPMI (37°C) and loaded with 1 ml of PBL suspension. Maximal cell load/column was 100 million cells. After 1 hr incubation at 37°C in a 5% CO_2 humidified atmosphere, non-adherent cells were gently washed out with 20 ml of warm RPMI (37°C). For further NK cell isolation, a commercially obtained preparation of polyvinylpyrrolidone-coated silica (Percoll, Pharmacia, Uppsala, Sweden) was first made isotonic for use with living lymphocytes. 1.5 M phosphate-buffered saline (PBS) was added to the stock solution of Percoll in a ratio of one part PBS to nine parts Percoll. For subsequent use, this stock (100%) was diluted with 0.15 M PBS or cell culture medium for cell separations. Discontinuous gradients were generated in 12 ml sterile plastic centrifuge tubes, which had been previously wetted with a small amount of serum to allow even flow of the Percoll. Non-adherent PBL cells, obtained from nylon-wool columns, were resuspended in 2 ml of 100% Percoll and successively less dense solutions layered carefully on top in 2 ml aliquots. Gradients for lymphocytes were run between 60% and 40%, with a 10% differential between layers. Once generated, gradients were spun for 10 min at 450 g. After centrifugation, distinct bands could be observed at the various interfaces. Gradients were harvested from above with a Pasteur pipette, and the cellular composition of each fraction was evaluated flow cytometrically. Cell preparations, containing more than 85% of NK cells (CD3-CD56+ cells) were considered NK cell homogenous and were used in subsequent experiments.

NK-enriched PBL cells

Ten ml of heparinised blood was diluted 1/2 with RPMI and layered on Ficoll-Isopaque.

Isolated PBL cells were further separated by Percoll centrifugation as described above. Low density cells were collected with a Pasteur pipette and used for NK test and BLT-E release assay.

Short-term cell culture with IL-2

Isolated cells were ($2x10^6$ cells/ml) cultured in a 24-well plate in the presence of 100 units/ml of recombinant human IL-2 (Sigma). After 18 hours of incubation (37°C in humidified 5% CO_2 atmosphere) the cells were recovered and used for subsequent studies.

NK test

Effector cells were tested against ^{51}Cr-labelled K562 target cells. K562 cells ($5x10^6$) in 0.5 ml medium were labelled by the addition of 100 (Ci sodium chromate ($Na_2{}^{51}CrO_4$, sp. act. 150-500 (Ci/mg) and incubated at 37°C for 1 hr. The cells were then washed three times in medium and resuspended to a concentration of 1×10^6 cells/ml in complete medium. Effector cells were prepared at a concentration of 5×10^6/ml and added to each well of the microtiter plate in a volume of 100 (l. Triplicate cultures were established in U-bottomed microtiter plates in a total volume of 100 (l, diluted in complete medium to obtain target/effector ratio (T/E) ranging from 1/4 to 1/64. Hundred µl of target cells were then added to effector cells. Plates were incubated for 4 h at 37°C in a 5% CO_2 humidified atmosphere, and released radioactivity was measured in a gamma counter. The percentage cytotoxicity was calculated by the equation: % cytotoxicity = (cpm experimental release - cpm spontaneous release) x 100 / (total radioactivity incorporated into cells). Spontaneous release was determined by incubating the target cells in medium alone. To compare the results at different E/T ratios, data are expressed in lytic units (LU). One LU represents the number of effector cells required for lysis of 20% of target cells. LU were determined from log-linear plots of the data points, and are reported at LU20/107 effector cells.[8]

BLT test

The amount of secreted BLT esterase was measured using 1 x 105 cells (PBL or NK cells) in 0.1 ml of cell culture medium in the 96-well microtiter plate in the presence of tumour target cells (K-562) or pharmacological stimuli - PMA (10 ng/ml) / A23187 (0.5 ug/ml). After 4-hr incubation at 37°C in a 5% CO_2 humidified atmosphere, cells were resuspended by gentle pipetting and centrifuged at 200 g for 5 min. Fifty µl aliquots of supernatant were used to assay BLT esterase activity. Total cellular content of the enzyme was determined using 0.1% Triton X-100 solubilized cells. Determinations were carried out in triplicate. Data are presented as specific percentage of enzymatic activity released which was calculated from the equation: % release = 100 x (E-S) / (T-S), where E represents the number of enzyme units in the supernatant of the experiment well, S the number of enzyme units in the supernatants of the well containing no stimuli, and T the total number of units of BLT esterase per well. The total BLT esterase content in target cells K-562 was always less than 5% of that in PBL or NK cells. For measuring BLT esterase activity, 0.05 ml of culture medium was mixed with 0.95 ml of BLT solution (0.2 mM BLT, 0.22 mM DTNB in PBS, pH 7.2). The mixture was incubated at 37°C for 20 min and the reaction was stopped by adding 5µl of 0.1 M PMSF, which was dissolved in dimethylsulfoxide. The solution was diluted by 1 ml of PBS and absorbency at 412 nm was measured in comparison to a blank solution (cell culture medium) that was treated exactly as experimental.

Flow cytometry

Flow cytometric analysis was performed by a fluorescence-activated cell sorter (FACSort, Becton Dickinson, Mountain View, CA, USA). A two-parameter analysis was performed to determine the proportion of CD3-CD56+ cells in samples.[9] Monoclonal antibodies (CD3-FITC, CD56-PE) were purchased from Becton Dickinson (Mountain View, CA, USA).

Statistical analysis

Correlation analysis was calculated by using a standard analysis package Quattro pro (Borland International, USA).

Results

For determining the time course of BLT-E secretion by NK cells, a secretion of Percoll-isolated NK cells was triggered by synergistic action of PMA and ionophore A23187 or NK cell susceptible tumour cells K562. Treatment of NK cells with PMA and A23187 results in faster release of granule enzyme than during incubation with K562 cells (Figure 1). When NK cells were incubated with K562 cells, BLT-E measurement reached stable values in 3-4 hours. Therefore a 4-hr incubation time was chosen for performing further experiments.

As a granule enzyme release is thought to be involved in NK cell cytotoxicity, we compared the influence of K562-induced NK cell triggering on BLT-E secretion and NK-cytotoxicity in healthy blood donors. In Figure 2 we present the data of experiments done on peripheral blood mononuclear cells. A poor correlation (Corr=0.54, SE =3.2) results most probably from low BLT secretion levels that are very close to a background release levels. Because NK cells represent only a minor part in PBL, BLT-E secretion results can be improved by using purified NK cells. Our results (Figure 3) demonstrate that detectable BLT-E secretion results can be achieved in experiments done on pure NK cells. Also a correlation (Corr = 0.84, SE=1.28) of NK cell cytotoxicity and BLT-E secretion results was improved substantially.

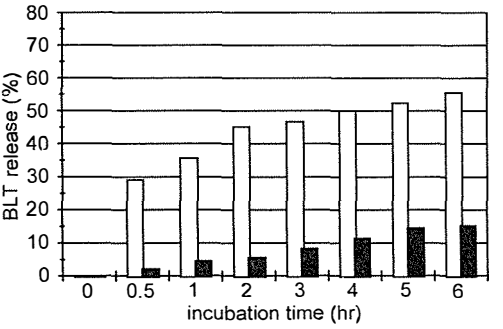

Figure 1. The time course of BLT esterase secretion by purified NK cells stimulated with PMA/A23187 (empty columns) or tumour target cells K562 (black columns). 1 x 105 purified NK cells were incubated in the presence of PMA/A2387 or tumour target cells K562. After incubation periods indicated on the abscissa, BLT esterase activity in the supernatant was measured. BLT esterase activity is presented as a percentage of maximal BLT release. Each point represent an average of at least four experiments, made in triplicates.

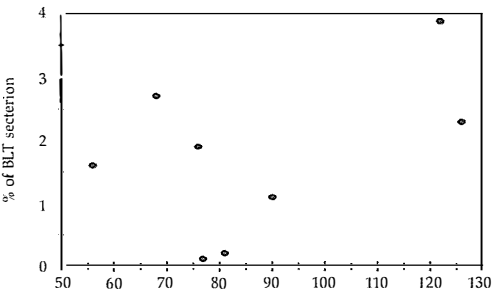

Figure 2. A correlation of NK cytolitic test and BLT-E test results in peripheral blood lymphocytes (PBL), stimulated with tumour targets. Peripheral blood lymphocytes of normal blood donors were stimulated with tumour target cells K562 at different effector/target ratios (E/T = 1/4 - 1/64). After 4 hour stimulation, supernatants were collected for cytolitic NK cell test and BLT-E release test as well. NK tests results are expressed in lytic units (LU) and BLT-E test results are expressed as percentages of maximal BLT-E release from effector cells. Each point represent an average of at least four experiments, made in triplicates.

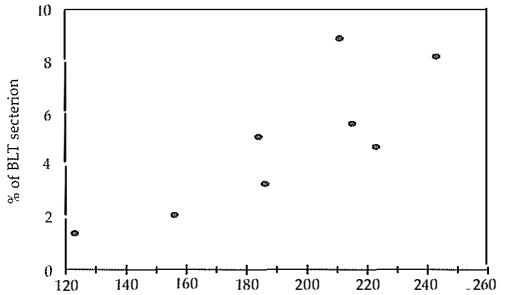

Figure 3. A correlation of NK cytolitic test and BLT-E test results in isolated NK cells, stimulated with tumour targets. Purified NK cells of normal blood donors were stimulated with tumour target cells K562 at different effector/target ratios (E/T = 1/4 - 1/64). After 4 hour stimulation, supernatants were collected for cytolitic NK cell test and BLT-E release test as well. NK test results are expressed in lytic units (LU) and BLT-E test results are expressed as percentages of maximal BLT-E release from effector cells. Each point represents an average of at least four experiments, made in triplicates.

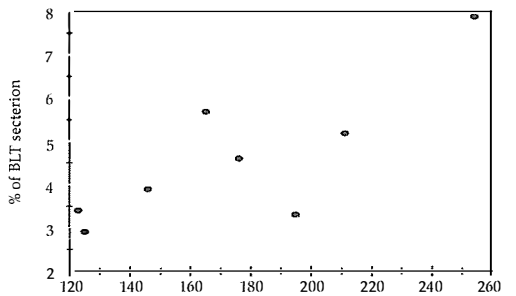

Figure 4. A correlation of NK cytolitic test and BLT-E test results in NK-enriched peripheral blood lymphocytes, stimulated with tumour targets. NK-enriched of normal blood donors were stimulated with tumour target cells K562 at different effector/target ratios (E/T = 1/4 - 1/64). After 4 hour stimulation supernatants were collected for cytolitic NK cell test and BLT-E release test as well. NK tests results are expressed in lytic units (LU) and BLT-E test results are expressed as percentages of maximal BLT-E release from effector cells. Each point represents an average of at least four experiments, made in triplicates.

Flow cytometrical evaluation of NK-enriched PBL revealed that 3-4 x enrichment of NK cell concentration was usually obtained, with NK cell content ranging from 40 to 65%. As shown in Figure 4, maximal NK lysis reached 250 LU while maximal BLT-E release was 10% of total BLT-E content of peripheral blood lymphocytes. The correlation of NK cell cytotoxicity and BLT-E secretion results(Corr = 0.79, SE=0.97) was close to correlation in experiments performed on purified NK cells.

Discussion

A vast majority of studies on granule exocytosis has been performed on cytotoxic T clones, while NK cell clones have been studied less frequently.[10-13] For studying exocytosis we used purified NK cells instead of cloned cells. An advantage of purified cells is that they better represent an *in vivo* physiological situation while cloned cells may have altered many of their phenotypic characteristics during long term *in vitro* cultivation. A disadvantage of studies on purified NK cells may be, besides laborious isolation procedure, a weak exocytotic activity of purified NK cells. Because previous reports [11] indicated that IL-2 can enhance, in a cytokine concentration-dependant manner, NK cell-mediated cytotoxicity, we established a short-term (18 hours) culture of purified cells to obtain exocytotic activities well distinct from background values.

Using the assay system described, it is possible to study enzyme granule exocytosis of NK cells. Because BLT esterase was described by many authors as an easily detectable biochemical marker of cytolitic granules, we used BLT-E release assay to detect NK cell exocytosis. It was shown that BLT-E is released by immune cells, capable of cell-mediated killing - including CTL and NK cells as well. *In vivo*, CTL release cytotoxic granules after they recognise surface antigens presented by MHC I molecules on the surface of target cells (virus infected or tumour cells). *In vitro* a similar process can be triggered by incubating CTL with antigen-bearing target cells or with immobilised anti-TCR (CD3) monoclonal antibodies - in both

cases the crosslinking of T-cell receptors (TCR) is required. On the other hand, NK cell utilise, *in vivo* two distinct mechanisms to trigger exocytosis. One, termed antibody-dependent cellular cytotoxicity (ADCC), utilises NK cell surface Fc-receptors and antibodies directed against the target-cell antigens. The other involves direct interaction between NK cells and their target cells and utilises NK cell receptors that have not yet been fully characterised.[14,15] *In vitro*, a process of NK killing can be demonstrated by incubating NK cells with antibody coated (ADCC killing) or some tumour target cells (K562 cells).

The exocytotic process in both cell types (CTL and NK) can be also triggered *in vitro* by the activation of protein kinase C (with phorbol esters) and by simultaneous translocation of calcium through CTL plasma membrane with Ca^{2+} ionophores.[2] Our results demonstrate that PMA/A23187 very efficiently trigger BLT-E secretion of purified NK cells. In less than 2 hour incubation time almost half of the total BLT-E content is released from NK cells. On the other hand, when NK cells were incubated with K562 cells, BLT-E measurement reached stable values in 3-4 hours and, in that time less than one third of total BLT-E content was released.

Because PMA/A23187 triggering acts on CTL and NK cells, experiments should be done only with homogenous cell populations - CTL or NK cell clones or purified cells. On the other hand, K562 cells trigger NK cell exocytosis without influencing CTL exocytosis.[3] That would theoretically enable studies of NK cell exocytosis in mixed cell populations (e.g. patients PBL). Our results, when using PBL in BLT-release test, demonstrate very low BLT-E release. A possible reason lies in calculation of BLT-E release: an enzyme release is expressed as a percentage of specific release versus total enzyme cell content. Because a total cell enzyme content in PBL is a sum of total enzyme content in CTL and NK cells, NK cell release makes usually very low proportion of the sum. Furthermore, a calculation of BLT release can be greatly influenced by the proportions of CTL and NK cells in PBL.

Obviously the experiments are best to be performed by using isolated NK cells to obtain high BLT-E release/effector cell. On the other hand, to perform extensive clinical studies, NK cell isolation may not be appropriate method because considerable amounts of blood are required. As a compromise, we performed a simple NK-enrichment procedure by using Percoll gradient centrifugation. By using NK-enrichment procedure we were able to obtain PBL containing more than 45% of NK cells. Furthermore, to perform BLT-E release assay and NK-cytotoxicity test as well in most instances only 10 ml of blood was required i.e. the quantity that enables to perform more extensive clinical studies.

Our results confirm a close association between granule enzyme exocytosis and NK lytic process in healthy persons. A correlation between NK test and BLT-E release results increases with the purity of NK cells, used in the experiment. Because a granule enzyme exocytosis is supposed to be a part of a lytic process in NK cells, a BLT-E release assay could be used to further analyse the lytic process in NK cells. A described assay procedure is simple and requires only moderate blood volumes, so, it is possible to perform it in clinical studies. Its use might be important especially in patients with impaired NK cytotoxicity (cancer patients, immunodeficient patients) because a defect of NK cell cytotoxicity can be influenced also by the factors that do not include granule enzyme exocytosis (NK cell binding and recognition of target cells, cytokine stimulation, activation of NK lytic machinery).

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Micronuclei in cytokinesis-blocked lymphocytes of patients following iodine-131 radiotherapy

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The micronucleus assay in cytochalasin-B cytokinesis blocked peripheral blood lymphocytes in 10 patients with hyperthyreosis and various types of thyroidal carcinomas was investigated. Patients received 259 - 5180 MBq I-131 sodium iodide perorally. Micronucleus (MN) frequencies were measured before and after I-131 administration. Pre treatment MN results were regarded as controls. Our results show considerable variability concerning age and activity applied. Also, the number of MN and the number of binucleated cells with micronuclei (BNMN) showed Poisson regression adjust for within subject correlation overdispersion. Log dose alone was not significant. The interaction of time and dose was significant at higher doses,while the rate of MN changing was slower. Relative risk time was calculated for the lowest dose (259 MBq). By doubling the dose, the rate of daily increase in the number of MN and BNMN decreases by approximately 5% (Relative risk MN =0.955; Relative riskBNMN = 0.954).

Key words: hyperthyroidism-radiotherapy; thyroid neoplasms-radiotherapy; iodine radioisotopes-adverse effects; lymphocytes; micronucleus test

Introduction

Radioiodines are often used for experimental purposes, diagnosis and therapy in clinical practice. The ionizing energy of radioiodine affects not only the thyroid with its uptake points, but other tissues as well, especially lymphocytes during their circulation through and around the gland containing the radioisotope. Therefore, it seemed to be of interest to investigate the cytogenetic alterations in blood lymphocytes of patients treated with iodine-131.

In order to determine the genotoxic risk associated with the diagnostic and therapeutic exposure to iodine-131, we conducted a follow-up study on the frequency of micronuclei in cytochalasin-B blocked blood lymphocytes.

Until now the scoring of chromosome aberrations has been considered the most relevant method for the cytogenetic dosimetry. Nevertheless, in the last few years, the

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micronucleus assay in human peripheral blood lymphocyte cultures using the cytokinesis-block method has been demonstrated to be a fast and sensitive cytogenetic technique and has been receiving increased attention for biological monitoring of radiation exposure.[1,2]

Counting micronuclei in cells which have undergone one cell division after the clastogenic insult is deemed to be a simpler and more sensitive method than conventional chromosome aberration assay.[3] Micronuclei originate from acentric fragments or from whole chromosome aberration assay and may serve as a measure for both chromosome breakage and loss.[1,3]

The aim of this study was to identify the relation between the number and distribution of micronuclei, using diagnostic and therapeutic doses of radioiodine-131. The frequency of micronuclei in cultured and cytokinesis-blocked circulating lymphocytes was determined before the treatment and at several short intervals after it. The quantity of perorally administrated iodine-131 depends on what the clinical practice determines to be a sufficient therapeutical dose for either hyperthyroidism or thyroid carcinomas. The doses applied in our study ranged between 259 and 5180 MBq.

Materials and methods

Lymphocyte cultures and micronucleus test

Venous blood was taken from 10 patients, 2 males and 8 females between 22 and 83 years of age, with Basedow-Graves disease (hyperthyreosis) and thyroid carcinomas, treated with ^{131}I (Table 1). First we quantified the micronuclei in the samples taken before treatment, and then we repeated the measurement on samples taken at short intervals (day 1,2 and 4) after treatment. Duplicate lymphocyte cultures in F-10 medium (Gibco, GB) supplemented with 20% of new-born calf serum (Biological Industries, Israel), penicillin (10 000 IU/ml) and streptomycin (10 000 μg/ml) were set up. Lymphocytes were stimulated by phytohaemagglutinin (PHA-Murex, GB). The cultures were incubated for 72 hours at 37°C, and at 44 h after the initiation of cultures cytochalasin-B (Sigma, Germany) dissolved in dimethylsulphoxide was added to arrest cytokinesis. The cells were collected by centrifugation, and instead of hypotonic treatment, we used 0.9% sodium chloride for 5 minutes at room temperature, fixation in methanol: glacial acetic acid, 3:1. Air dried slides were stained with 5% Giemsa solution. Each sample was analyzed only for binuclear cytochalasin B blocked cells with well-preserved cytoplasm. In each sample 1000 binucleated cells were analysed. The slides were scored at 1000x magnification.

Statistical methods

For the statistical analysis we used Poisson regression adjusted for within subject correlation. The data were analysed using the SAS 612.PROC.GENMOD statistical package.

Results

Table 1 shows age, sex, diagnosis, the applied activity for each patient, the total number of micronuclei (MN) in cytochalasin B cytokinesis blocked peripheral human blood lymphocytes in 1000 binucleated cells, and the number of binucleated cells with micronuclei (BNMN) in patients before and after radioiodine application, in order to show the MN distribution among cells. In the group studied, there was a considerable variability concerning age and activity applied.

In some cases the cytogenetic study was not able to be carried out in all samples due to low stimulation of the cultured lympho-

Table 1. Dose, age, sex, diagnosis, total number of micronuclei (MN) in 1000 binucleated cells, number of binucleated cells with micronuclei (BNMN) in the hyperthyroidism and carcinoma patients treated with radioiodine-131

Subject	Sex	Age (years)	Diagnosis	Aplicated activity (MBq)	Pretreatment		Post-treatment (24 hours)		Post-treatment (48 hours)		Post-treatment (96 hours)	
					MN	BNMN	MN	BNMN	MN	BNMN	MN	BNMN
1	F	53	Hyperthyreosis	259	56	45	78	64	81	68	119	100
2	F	29	Hyperthyreosis	262	24	18	-	-	84	72	62	57
3	F	56	Hyperthyreosis	309	26	24	-	-	74	60	100	91
4	F	40	Toxic adenoma glandulaae thyroideae	1176	22	22	37	34	37	31	44	39
5	F	57	Papillary thyroid carcinoma	2960	22	18	28	23	32	29	32	30
6	F	67	Follicular thyroid carcinoma	2960	22	19	35	34	52	46	94	83
7	F	83	Papillary thyroid carcinoma	2960	70	56	69	57	62	53	-	-
8	M	22	Mixed follicular/papillary carcinoma metastases	3700	20	16	15	14	20	16	25	23
9	F	64	Papilary thyroid carcinoma	3700	25	24	12	12	29	26	19	17
10	M	71	Follicular thyroid carcinoma - distant metastases	5180	102	82	156	130	100	84	117	98

Table2. Results of statistical analysis

Variable	Parameter	Relative risk	Lower confidence limit	Upper confidence limit	p
1	MN time (day)	1.27494	1.04487	1.55566	0.0000
2	MN time x dose	0.95530	0.93518	0.97585	0.0000
3	MN age (10 years)	1.22708	1.09297	1.37763	0.0005
4	BNMN time (day)	1.29153	1.05895	1.57520	
5	BNMN time x dose	0.95426	0.93418	0.97477	
6	BNMN age (10 years)	1.21817	1.08966	1.36184	

cytes. We observed binucleated cells with 1-4 micronuclei (Figure 1)

Poisson regression adjusted for within subject autocorrelation was used to compare the variation in the frequency of MN and BNMN in the four sampling times. The results in Table 2 show that the number of micronuclei increased in time. For both MN and BNMN the models showed overdispersion. For MN the deviance was 473.5 and Pearson χ^2 515.7 with 33 degrees of freedom. For BNMN the deviance was 364.5 and Pearson,s χ^2 was 399.5 with 33 degrees of freedom. We have introduced a scaled in the final model to adjust for overdispersion (for MN scale was 3.7878 and for BNMN 3.3234).

Log dose alone was not significant. The interaction of time and dose was significant at higher doses, the rate of the MN changing was slower. A relative risk for time was calculated for the lowest dose (259 MBq). By doubling the dose, the rate of daily increase in the growth of number of MN and BNMN decreased for about 5% (Relative $risk_{MN}$=0.955; Relative $risk_{BNMN}$= 0.954).

The data obtained when patients were classified into five subgroups, according to the activity received (< 300 MBq, 1176 MBq, 2960 MBq, 3700 MBq, 5180 MBq) are shown graphically in Figure 2. These values show wide variability in the baseline frequency of BNMN among patients before treatment.

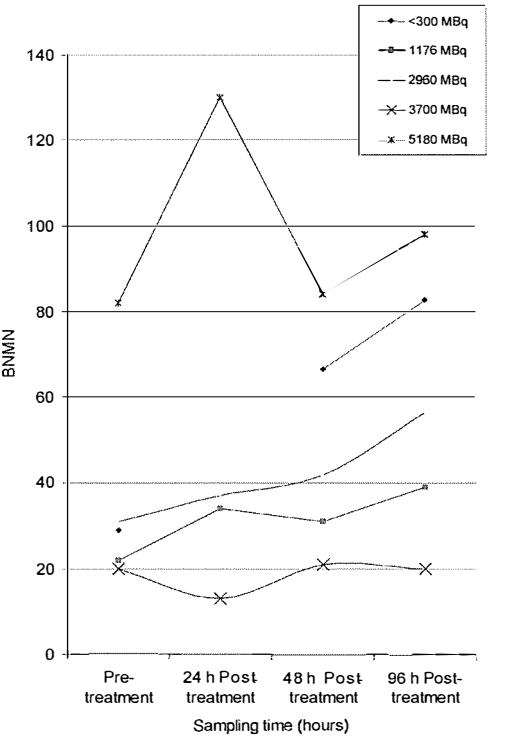

Figure 2. Frequency of BNMN before and after the treatment.

Discussion

A consequence of radiation-induced DNA damage is the formation of micronuclei, which appear similar to the main nuclei but are smaller and have a reduced DNA content.[4]

The use of micronucleus assay to evaluate radiation exposures resulting from internally deposited radioactive materials in people pre-

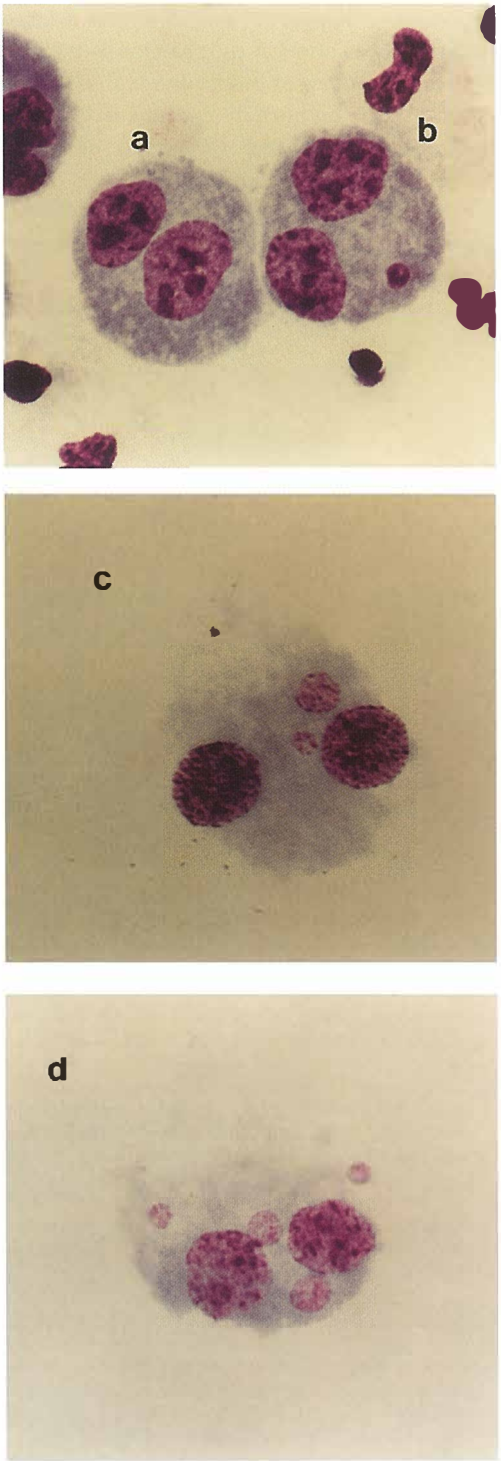

Figure 1. Photomicrograph of binucleate human blood lymphocyte without micronuclei (a) , with one micronuclei, (b), with two micronuclei (c) and with four micronuclei (d). Stained by 5% Giemsa (magnification 1000 x)

sents several specific problems. First, the deposition, distribution, and dose to individual cells are dependent on the radionuclides involved, the route of exposure, the metabolic state of the individual and the chemical and physical form of the material. Second, because of individual differences, it is impossible to make a reliable estimate of the radiation dose that the individual receives even if the exposure level, radionuclides involved, and their physical and chemical form are known.

For nonuniformly distributed internally deposited radionuclides, it is important to recognize that not only the radiation dose is nonuniform, but also the lymphocytes used to evaluate the exposure are nonuniformly distributed in blood, organs, lymph nodes and lymph follicles throughout the body.

It is well established that there is heterogenity in the repair of lesions along the DNA molecule.[5-8] The level of gene activity and repair at the level of the chromosome are critical factors that may influence the formation of chromosome aberrations and micronuclei.[9]

The obtained results indicate that the varied cell response to low doses depends on individual features of the patients, quite similar to those observed by Brown.[10] Moreover, it is important to notice a corresponding irregularity in the distribution of the incorporated radionuclides, not only in the doses, but in the lymphocytes irregularly distributed in blood, organs, lymph nodes and follicles. The obtained results as well as the referred literature [11,12,13] point out individual differences in reacting to exposure. As there is variation in the proliferation rate of the lymphocytes from different individuals, kinetic differences appear to remain a source of vari-

ability in the MN assay.[14] Keldsen and his coworkers[15] noticed considerable absorption differences between patients equally exposed to iodine-131 (here patients 6, 7 and 9). It is interesting to note that some patients have micronuclei control values within the range of those seen in healthy population.[16] This finding has been confirmed by the results of our study (patients 1, 2, 3, 6, 7, 8, and 10). It seems that the loss of lymphocytes is associated with big lesions.

The evaluation of damage immediately after the exposure is incomplete. The reason can be sought in the temporary mitotic delay as well as in the potential transfer of chromosomal instability from the exposed parental to daughter cells.[17] The comparison of all patients in the study brings forward big individual differences since the changes were noted before the exposure (patients 1, 7, and 10). Unable to determine the etiology of such changes, we came to the conclusion that the results obtained after the therapeutic doses of radiopharmaceuticals should be evaluated with respect to the results obtained before the exposure.

If we want to compare our results with the similar study of Gutierrez and coworkers,[2] it is evident that in their group of subjects there is a significant increase of 5% of MN per week, compared to pre-treatment, and after that MN is lower than in the pre-treatment period. The rate of increase was essentially lower than in our results. In the study by Gutierrez *et al.*, a time change was significant, but one could not observe the interaction between time and dose. *Log* dose and group, together, were significant.

Depending on age, our results point out an increase in MN by 20% for ten years.

Radiotherapy treatments are very toxic for lymphocytes, and could result in multilocus mutagenesis which could affect cloning efficiency of „hit" cells. Seifert *et al.*[11] calculated that each lymphocyte of a radiotherapy treated patient carried an average of six induced mutations. This group also observed a large individual variation in the reaction exposure. One of the causes of interindividual variation could be the polymorphism at the large number of loci involved in repair of DNA damage.[18] Another one is individual body size, which influences the dose to the target tissues. Finally, the differences in each individual's physical or chemical environment may be involved in the heterogeneity of response.

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Prognostic relevance of urokinase plasminogen activator and its inhibitors in patients with breast cancer

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Urokinase plasminogen activator (uPA) and its inhibitors, PAI-1 and PAI-2, play an important role in intercellular tissue degradation, thus promoting tumor cell invasion into the adjoining structures and metastasizing. Our study was aimed to assess a possible prognostic value of uPA, PAI-1 and PAI-2 in a retrospective series of 87 patients with breast cancer stage I-III, whose cytosols were stored in the archives of the Institute of Oncology in Ljubljana. The median follow-up was 35 months. The prognostic value of the established prognostic factors and uPA, PAI-1 and PAI-2 were evaluated by means of univariate statistical analysis and partial multivariate models. The obtained uPA values were very low and did not correlate with the disease-free survival, whereas PAI-1 and PAI-2 significantly influenced the time to the first recurrence. Patients with PAI-1 values above 5 ng/mg proteins had statistically significantly worse disease-free survival than the patients with lower PAI-1 values (58% vs. 85%). In the case of PAI-2, the situation was just the opposite: the patients with PAI-2 values exceeding 6.4 ng/mg proteins had statistically significantly better 3-year disease-free survival than the patients with lower values (90% vs. 60%). Both, PAI-1 and PAI-2 retained their independent prognostic value, irrespective of the addition of the established prognostic factors to partial multivariate models, and only with locally advanced disease the prognostic value of PAI-1 was greater than that of PAI-2.

Key words: breast neoplasms; urokinase; plasminogen activator inhibitor 1; plasminigen activator inhibitor 2; prognosis

Introduction

A number of extracellular proteolytic enzymes are expressed in the tumor tissue; these are involved in the invasion of tumor cells into the surrounding tissues as well as in distant dissemination process. The central role among proteolytic enzymes is attributed to the serine proteinase - urokinase plasminogen activator (uPA) and to uPA inhibitors types 1 and 2 (PAI-1 and PAI-2).[1,2,3]

A decade ago, researchers came to the idea that serine proteinases could be an indicator of the metastatic potential of tumors, and thus also a prognostic factor of cancer. In the last decade, a number of studies were carried out which were aimed to assess the prognos-

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tic relevance of uPA, PAI-1 and PAI-2 in breast cancer and other solid tumors.[4]

In his first study concerned with the prognostic value of serine proteinases, Duffy and co-workers determined uPA content in the tumor cytosols from patients with breast cancer, which were prepared for routine determination of hormone receptors.[5] The results of this study for the first time established a correlation between uPA and the prognosis in breast cancer patients. Several other authors later on also confirmed the same findings.[6-9] From the 90's on, a similar relevance was reported for PAI-1 determined in the cytosols from breast cancer patients.[10-14] The measured mean and cut-off values of uPA and PAI-1 differed from one study to another. The reason for this variability could be attributed to different tumor tissue preparation techniques and different buffers used. Because of those differences, a standard cut-off value that would delineate high values from low ones could not be determined. However, the results of investigations performed so far unequivocally speak in favor of the independent prognostic value of uPA and PAI-1.[6-14] High values of either of these two factors are associated with a higher risk of recurrence and a shorter survival. PAI-2 has not been sufficiently investigated yet. For the difference from uPA and PAI-1, high PAI-2 values were found to be associated with a favorable prognosis.[9,10] So far, the independent prognostic value of PAI-2 has been confirmed by one of the two studies performed.[9]

Our retrospective study was aimed to established whether the values of uPA, PAI-1 and PAI-2 determined in tumor cytosols prepared with phosphate buffer, which are otherwise used routinely for hormone receptor determination, correlate with the established prognostic factors, and whether they significantly influence the disease free survival of patients with breast cancer.

Materials and methods

Our retrospective study was carried out in a series of 87 patients with operable and locally advancer breast cancer, who were admitted to the Institute of Oncology for the first time in 1994 or in the first two months of 1995, and operated on for cytologically confirmed breast cancer.

Deep-frozen tumor cytosols from those patients are stored in the cytosol bank of the Institute of Oncology in Ljubljana. The prepared cytosols are kept at a temperature of minus 20°C. The cooling was never discontinued.

Patients, tumors and treatment characteristics

The data on age, menopausal status, clinical tumor size, clinical lymph node status, pathological tumor size, pathohistological tumor type, malignancy grade, axillary lymph node involvement, hormonal receptor status, and primary treatment were derived from the patient record files stored in the archives of Institute of Oncology.

The stage of disease was classified according to UICC-WHO criteria (UICC, 1974). While the latter criteria were used for pathohistological tumor type determination, the grade of malignancy was assessed according to Scarf-Bloom-Richardson's classification.[10] The cut-off limit for positive estrogen and progesterone receptors was set at value > 10 fmol/mg proteins.

All the patients underwent a local radical treatment. All of them also had axillary lymphadenectomy performed. In the case that only conservative surgery was feasible, the patients were additionally irradiated to the area of the operated breast. Patients with positive axillary lymph nodes received adjuvant chemotherapy. The same was also given to the patients with negative axillary lymph nodes and presence of the established unfavorable prognostic factors (e.g. large and/or poorly differentiated tumor).

The mean age of patients at diagnosis was 52 years (range 29-75 years). Other characteristics of the patients and tumors are presented in Table 1.

Tissue preparation technique and the determination of urokinase system components

Immediately upon surgery, the removed tumor tissue was stored in liquid nitrogen. In the process of cytosol preparation, the frozen tumor was first ground with a microdysmembrator. The obtained powder was suspended in phosphate buffer (5 mM Na_2HPO_4, 1.7 mM KH_2PO_4, 1 mM monothioglycerol, 10% (v/v) glycerol, pH 7.4), and the suspension ultra-centrifuged at 100,000 x g for 45 min, at 4°C).

The total uPA concentration in the cytosol and extract was determined with IMUBIND® Tissue uPA ELISA Kit, while PAI-1 was determined with IMUBIND® Tissue PAI-1 ELISA Kit, and PAI-2 with IMUBIND® Tissue PAI-2 ELISA Kit (American Diagnostica Inc.)

Follow up

After completed primary therapy, the patients were subjected to regular follow-up examinations at the Institute of Oncology. The data on possible time and site of progression were derived from patients records.

The patients were followed up for 1-49 months (median follow up was 35 months).

Data processing

Interdependence of the urokinase system components with other primary tumor characteristics was determined on the basis of contingency tables and chi-square test. The influence of the component of urokinase system on the disease-free survival was presented by means of Kaplan-Meier's survival curves, and any differences in the survival analyzed with the log-rank test.[15,16] The multivariate regression analysis by Cox was used for the evaluation of independent prognostic value of urokinase system components.[17] Statistical analysis and graphic presentation of the results were done using „Statistica for Windows" and „BMDP" program packages.

Results

Urokinase system measurements

In 87 patients, the range of uPA values (concentrations) in the cytosol prepared with phosphate buffer was 0-1.83 ng/mg proteins, median 0.34 ng/mg proteins, the lower and the upper quarts being 0.15 and 0.53 ng/mg proteins, respectively.

In the same series of 87 patients, the range of PAI-1 levels in the cytosol prepared with phosphate buffer was 0.06-75.91 ng/mg proteins, median 6.02 ng/mg proteins, while the lower and the upper quarts were 3.77 and 8.93 ng/mg proteins, respectively.

The range of PAI-2 values in the cytosols prepared with phosphate buffer was 0.31-75.80 ng/mg proteins, median 3.35 ng/mg proteins, the lower and the upper quarts being 1.51 and 12.31 ng/mg proteins, respectively.

The influence of urokinase system components on disease-free survival

Within the median observation period of 35 months, the disease was found to recur in 28/87 patients (32%). Three patients (11%) presented with local recurrence, while 19 patients (68%) had distant metastases alone, and 6 patients (21%) had both. Three-year disease free survival of the whole group of patients was 69%. Disease free survival for the whole group of 87 patients is presented in Figure 1. We were trying to determine the cut-off values of uPA, PAI-1 and PAI-2, which

Table 1. Characteristics of 87 patients

		Number	(%)
Patients	Menopausal status		
	premenopausal	29	33
	postmenopausal	57	66
	unknow	1	1
	Tumor size		
	T1	8	9
	T2	54	62
	T3	13	15
	T4	12	14
	Nodal status		
	N0	54	62
	N1	26	30
	N2	7	8
	Stage (UICC- International Union against Cancer)		
	I	8	9
	II	60	69
	III	19	22
Tumors	Pathological tumor size		
	Tp1	1	1
	Tp2	13	15
	Tp3	57	66
	Tp4	14	16
	unknown	2	2
	Pathohistological tumor type		
	invasive ductal	76	88
	invasive lobular	8	9
	mucinous	1	1
	others	1	1
	unknown	1	1
	Differentiation grade (invasive ductal carcinoma)		
	GI	4	5
	GII	21	28
	GIII	48	63
	unknown	3	4
	Number of positive nodes		
	0	30	34
	1-3	26	30
	>3	31	36
	Estrogen receptors		
	≤10 ng/mg protein	43	49
	>10 ng/mg protein	44	51
	Progesterone receptors		
	≤10 ng/mg protein	60	69
	>10 ng/mg protein	27	31

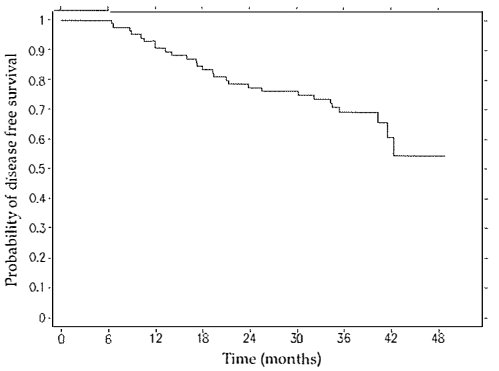

Figure 1. Relapse-free survival of 87 breast cancer patients.

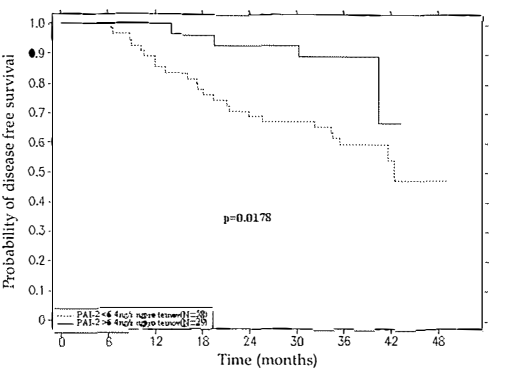

Figure 3. Relapse-frees urvival according to PAI-2.

could best differentiate patients with favorable prognosis from those with unfavorable one.

It has been found that the measured uPA values in the cytosols from our series of patients failed to correlate with the disease-free survival. The cut-off value of PAI-1 in our series of patients was 5 ng/mg proteins. The disease-free survival of patients with PAI-1 values exceeding 5 ng/mg proteins was statistically significantly worse than that of the patients with PAI-1 values under 5 ng/mg proteins (P = 0.0046) (Figure 2).

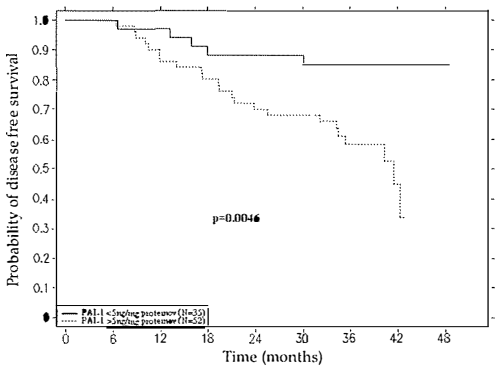

Figure 2. Relapse-free survival according to PAI-1.

The cut-off value of PAI-2 in our series of patients was 6.4 ng/mg proteins. The disease-free survival of patients with PAI-2 values exceeding 6.4 ng/mg proteins was statistically significantly better that that of the patients with PAI-2 values under 6.4 ng/mg proteins (p = 0.0178) (Figure 3).

Comparison of the influence of different prognostic factors on disease-free survival

Univariate analysis (log-rank test): The influence of menopausal status, clinical tumor size, clinical lymph node status, stage of the disease, pathohistological tumor size and grade of malignancy grade, pathohistological evidence of axillary lymph node involvement, presence of estrogen and progesterone receptors, as well as PAI-1 and PAI-2 content in the tumor on disease free survival was studied. It was found that disease-free survival was significantly influenced by 7/11 factors under study. The influence of clinical tumor size (operable cancers - T2 and T3 vs. locally advanced disease - T4) was also statistically significant. In our series, the patients with operable cancers presented with 73% 3-year survival, while those with locally advanced disease had only 20% 3-year survival rate ($p<0.00001$). Eight patients with tumors smaller than 2 cm (stage T1) were not included in the analysis.

Stage of disease was another prognostic factor that turned out to be statistically significant for the disease free survival. In our

series, 78% of patients with stage II at the time of diagnosis survived 3 years without evidence of disease, while only 32% of those with stage III were free of recurrence after 3 years ($p<0.00001$). Patients with stage I were too few (N=8) to be included into the statistical analysis. Both clinical and pathological status of the axillary lymph nodes exerted statistically significant influence on the disease free survival. While patients with non-palpable axillary lymph nodes survived 3 years without evidence of disease in 85%, those with palpable lymph nodes had only 45% 3-year disease-free survival ($p<0.00001$). After three years, the disease recurred in 42% of patients with pathologically positive axillary lymph nodes and in only 11% of those with negative pathological lymph node findings ($p=0.0053$). The number of involved lymph nodes was also statistically significant. Thus, the patients with 1-3 positive axillary lymph nodes had 80% disease-free 3 year survival, while in those with more than three positive lymph nodes this rate was only 41% ($p<0.00001$). Further, the recurrence of disease was significantly influenced by the content (presence) of estrogen receptors in the tumor. Patients with negative estrogen receptors had lower 3-year disease-free survival than those with positive estrogen receptors, i.e. 60% vs. 80% respectively ($p=0.0409$).

Both, PAI-1 and PAI-2 contents in the tumor significantly influenced the patients' survival. It turned out that the patients with PAI-1 tumor content exceeding 5 ng/mg proteins had statistically significantly worse disease-free survival than the rest of patients under study ($p=0.0046$). Thus, the disease recurred within 3 years in 42% of patients with PAI-1 content above the cut-off value, and in only 15% of those with PAI-1 content below the cut-off value (Figure 2).

With PAI-2, however, the situation was just the opposite. Patients with PAI-2 tumor content exceeding 6.4 ng/mg proteins had statistically significantly better disease-free survival than the rest of the patients under study ($p=0.0178$). Thus, 10% of patients with PAI-2 values >6.4 ng/mg proteins presented with recurrence within 3 years, as compared to the patients with PAI-2 values below 6.4 ng/mg proteins in whom the recurrence rate was as high as 52% (Figure 3). The results of univariate analysis are presented in Table 2.

Multivariate analysis: Independent prognostic value of PAI-1 and PAI-2 was studied by the multivariate Cox's regression model. Due to insufficient number of patients, we did not include into the model all the seven factors that had shown their prognostic value in the univariate analysis; instead, we made a few partial multivariate models. Thus the remaining five factors shown as statistically relevant by univariate analysis were added one by one to the basic two factors studied (PAI-1 and PAI-2) (Table 3).

When both inhibitors of plasminogen activator alone were included into the multivariate model, PAI-1 and PAI-2 turned out to be strong prognostic factors, PAI-1 being the more relevant of the two. If only these two factors were considered, the relative risk of recurrence would increase by 5.9-times in patients with PAI-1 exceeding 5 ng/mg proteins, and by 4.4-times in those with PAI-2 values below 6.4 ng/mg proteins.

PAI-1 and PAI-2 did not loose their prognostic value by inclusion of other prognostic factors into the model. A stronger prognostic value was established only for tumor size (operable vs. locally advanced cancers) and stage (stage II vs. stage III). Both inhibitors of plasminogen activator were shown to have a stronger prognostic value that clinical and pathological status of the axillary lymph nodes. In the multivariate model with PAI-1 and PAI-2, estrogen receptors lost their prognostic value.

Table 2. Univariante analysis of disease free survival (log-rank test)

Prognostic factor	Number	Number of relapses	p
premenopausal	29	10 (34)	0.8306
postmenopausal	56	18 (32)	
Tumor size*			
T2+T3**	67	20 (30)	<0.0001
T4	12	8 (67)	
Clinical nodal status			
palpable lymph nodes	54	9 (17)	<0.0001
nonpalpable lymph node	33	19 (58)	
Stage***			
II	60	14 (23)	<0.0001
III	19	14 (74)	
Pathological tumor size			
<20 mm	14	3 (21)	0.2692
≥20 mm	71	24 (34)	
Diferentiation grade****			
II	21	4 (14)	0.0980
III	48	19 (40)	
Pathological nodal status			
negative lymph nodes	30	5 (17)	0.0053
positive lymph nodes	57	23 (40)	
Estrogene receptors			
≤10 fmol/mg protein	43	18 (42)	0.0409
<10 fmol/mg protein	44	10 (23)	
Progesterone receptors			
≤10 fmol/mg protein	60	23 (38)	0.0978
>10 fmol/mg protein	27	5 (19)	
PAI-1			
<5 ng/mg protein	35	5 (14)	0.0046
>5 ng/mg protein	52	23 (44)	
PAI-2			
<6.4 ng/mg protein	58	24 (41)	0.0178
>6.4 ng/mg protein	29	4 (14)	

* 8 patients with tumor size <2cm (T1) were excluded from the analisis because the number was to small for statistical evaluation
** Difference between T2 and T3 was not statistically significant (log rank: p=0.1254)
*** 8 patients with stage I were excluded from the analysis because the number was to small for statistical evaluation
**** invasive ductal carcinoma; 4 patients with grade I were excluded from the analysis because the number was to small for statistical evaluation

Discussion

Our retrospective study was undertaken with the aim to show whether the components of urokinase system measured in tumor cytosols prepared with phosphate buffer for biochemical hormone determination had any prognostic value.

Our retrospective study has failed to show any correlation between uPA values and disease-free survival. Contrary to our results, the findings of three published retrospective

Table 3. Multivariate Cox regression analysis

Prognostic factor	RR*	95% CI**	p
PAI-1			
(<5 ng/mg protein *vs.* >5 ng /mg protein)			
>5 ng/ mg protein	5.89	2.21 - 15.66	0.0004
PAI-2			
(>6.4 ng/mg protein *vs.* < 6.4 ng /mg protein)			
<6.4 ng /mg protein	4.26	1.47 - 7.14	0.0079
PAI-1			
(<5 ng/mg protein *vs.* >5 ng /mg protein)			
>5 ng/ mg protein	7.79	2.83 -21.46	0.0001
PAI-2			
(>6.4 ng/mg protein *vs.* < 6.4 ng/ mg protein)			
<6.4 ng /mg protein	3.57	1.20 - 10.00	0.0218
Clinical tumor size			
(T2+T3 *vs.* T4)			
T4	8.34	3.39 -20.48	<0.00001
PAI-1			
(<5 ng/mg protein *vs.* >5 ng/ mg protein)			
>5 ng /mg protein	5.65	2.83 - 21.46	0.0006
PAI-2			
(>6.4 ng/mg protein *vs.* < 6.4 ng /mg protein)			
<6.4 ng mg/protein	4	1.41 - 12.5	0.0100
Clinical nodal status			
(palpable lymph nodes *vs.* nonpalpable lymph nodes)			
nonpalpable lymph nodes	3.75	1.67 -8.43	0.0014
PAI-1			
(<5 ng/mg protein *vs.* >5 ng/ mg protein)			
>5 ng mg/protein	5.77	2.13 - 15.63	0.0006
PAI-2			
(>6.4 ng/mg protein *vs.* < 6.4 ng /mg protein)			
<6.4 ng /mg protein	5.56	1.92 - 16.67	0.0018
Stage			
(II vs. III)			
III	6.96	3.14 - 15.40	<0.00001
PAI-1			
(<5 ng/mg protein *vs.* >5 ng/ mg protein)			
>5 ng /mg protein	5.25	1.95 - 14.12	0.0010
PAI-2			
(>6.4 ng/mg protein *vs.* < 6.4 ng /mg protein)			
<6.4 ng/ mg protein	5.26	1.75 - 14.29	0.0028
Pathological nodal status			
(negative *vs.* positive)			
positive	3.81	1.39 - 10.44	0.0092
PAI-1			
(<5 ng/mg protein *vs.* >5 ng /mg protein)			
>5 ng /mg protein	5.68	2.13 - 15.13	0.0005
PAI-2			
(>6.4 ng/mg protein *vs.* < 6.4 ng/ mg protein)			
<6.4 ng /mg protein	3.85	1.33 - 11.11	0.0128
ER			
(10 fmol/mg protein *vs.* > 10 fmol/mg protein)			
> 10 fmol/mg protein	-	-	n.s.***

* Relative risk; ** confidence interval; ***not significant

studies ascribe some prognostic value to uPA measured in cytosols.[11,18,19] The authors obtained different median values (0.4 - 0.52 ng/mg proteins) and different maximum values (3.2 - 4.4 ng/mg proteins), which were invariably higher than the values measured in cytosols during our retrospective study (median 0.34 ng/mg proteins, range 0-1.83 ng/mg proteins). This discrepancy could be explained by the fact that the cytosols from the archives of the Institute of Oncology were prepared with phosphate buffer alone, whereas the cytosols used in the three retrospective studies reported were prepared with buffers and addition of EDTA. It seems that the latter substance improves the extraction of proteins, and associated with that uPA, to such an extent that the measurements become more reliable and the analytical error lesser. Apparently, the uPA values determined in the tissue that has been processed according to the technique described are just high enough to allow for the determination of cut-off value which groups breast cancer patients by prognosis.

For the difference from uPA, PAI-1 and PAI-2 in our study correlated with disease-free survival. The univariate analysis has shown a statistically significant influence of PAI-1 and PAI-2 content in tumor cytosols on disease-free survival. Patients with higher PAI-1 values presented with recurrence more frequently than those with lower PAI-1 values. With PAI-2 the situation was just the opposite: the recurrence rates within three years in patients with higher PAI-2 values were lower. A similar influence of PAI-1 on disease-free survival was also established by univariate analysis in some other studies investigating the prognostic value of PAI-1. [6,7,9,11,14,20] Also the results of both studies on the prognostic value of PAI-2 are more or less consistent with our study.[9,10] While the French study -likewise ours - has confirmed the association of high PAI-2 levels with a favorable prognosis, Foeckens *et al.* in their study on 1012 patients failed to confirm a correlation between PAI-2 values and disease-free survival or overall survival. However, when their patients were grouped according to uPA tumor content, the patients with higher uPA values also had the cut-off value of PAI-2 determined, which distinguished the patients by prognosis. It has been found that the patients with higher uPA content had a better prognosis if they also had high PAI-2 values.[10]

Apart from PAI-1 and PAI-2, in our group of patients a statistically significant influence on the disease-free survival was also exerted by the established prognostic factors: clinical tumor size, clinical lymph node status, stage of the disease, pathological evidence of axillary lymph node involvement, and the presence of estrogen receptors in the tumor. In the evaluation of stage, stages II and III were compared, which meant a comparison between operable and locally advanced tumors, since a majority of stage III patients had locally advanced tumors. Thus the patients with locally advanced cancers and stage III had by all expectations worse disease-free survival. A statistically significant influence on the disease-free survival was also exerted by clinical and pathological lymph node status. Our univariate analysis has also shown that a worse prognosis was associated with negative estrogen receptors, while the presence of progesterone receptors in the tumor failed to provide prognostically relevant information. In our analysis menopausal status, pathological tumor size and grade of malignancy of invasive ductal cancers did not show prognostic value for disease-free survival. The reason for the absence of prognostic value of pathological size and grade of tumors could be attributed to a relatively small number of patients included, as well as to a small number of events in the groups of tumors smaller than 2 cm and in moderately differentiated tumors.

In multivariate models, both PAI-1 and PAI-2 showed an independent prognostic

value, the value of the former being somewhat higher. In our study, only clinical tumor size and stage were stronger prognostic factors than both inhibitors. In these two prognostic factors we actually used similarly formed groups, which are defined prevailingly by locally advanced disease, and thus providing a similar information. In our study, clinical and pathological lymph node status have shown a lower prognostic power than both inhibitors, while estrogen receptors have lost their independent prognostic value to PAI-1 and PAI-2. Thus, our study has revealed that PAI-1 and PAI-2 are strong independent prognostic factors, and that only locally advanced disease provides a more relevant information on the outcome of disease.

Independent prognostic value of PAI-1 for the disease-free survival of all breast cancer patients was also established by German[7,20], Dutch[6], and French[9] researchers. In all those studies, apart from pathological lymph node status, PAI-1 was found to be the strongest prognostic factor. Only the French study, which investigated not only PAI-1 but also the independent prognostic value of PAI-2, has confirmed that only pathological lymph node status has a stronger independent prognostic value.[9]

Our study has therefore established the value of PAI-1 and PAI-2 contents in tumor cytosols for the prognosis of disease in breast cancer patients. Based on the results obtained, we believe that in the cases when only cytosol which does not enable a reliable uPA determination is available, PAI-1 and PAI-2 can provide a sufficient information for foretelling the outcome of disease.

It is presumed that a combination of both inhibitors, and perhaps also their combination with other components of the urokinase system or other proteinases might provide even better information for foretelling the outcome of disease, however, such an analysis would require a larger number of patients. It should be also necessary to establish the prognostic value of urokinase system components in individual subgroups of patients, distributed according to menopausal status, lymph node involvement, hormonal status, etc. Such an approach would enable us to detect the patients at an increased risk of recurrence within the prognostically more favorable groups. Nevertheless, such an analysis as well would require a considerably larger number of patients. Apart from that, it would be interesting to find out whether immunohistochemically determined components of the urokinase system would provide a similar information, or would such determination - likewise in the case of cathepsin D - undermine the beliefs about their prognostic value.[21] We plan to carry out a prospective study, which would touch upon at least some of the hypotheses presented here.

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Radiotherapy for choroidal neovascularisation of age-related macular degeneration

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Age-related macular degeneration caused by choroidal neovascularisation is an increasing problem in ophthalmology. The results of the therapy in the past were poor or associated with a number of side effects. Recently, some reports have shown a beneficial effect of low-dose irradiation. Therefore, we reviewed the data of our patients included in a pilot study to confirm the very preliminary data in literature. Fourty-three patients were irradiated with a linear accelerator (6 MV) at a total dose of 16 Gy. Six months after irradiation, 69% of our patients maintained or improved their visual acuity. We did not observe side effects or any acute or late sequelae within a median follow-up period of 12 months.

Key words: macular degeneration - radiotherapy, visual acuity

Introduction

Choroidal neovascularisation (CNV) is a major cause of severe loss of visual acuity in the patients with age-related macular degeneration.[1] Without therapy, the natural course of this disease would result in the loss of patient's sight.

In the past, laser photocoagulation was used as treatment method, but was, unfortunately, associated with further decrease in visual acuity.[2] In the treatment of CNV, interferon was used systemically, but, so far, the substance has not proved to be effective, moreover, it may be that it has severe side effects.[3]

Another approach is the surgical extirpation although the treatment results are not too encouraging.[4] Recently, some investigators have reported of a beneficial effect of low-dose irradiation of the subretinal neovascular membranes in the CNV.[5-13] The preliminary results are encouraging and the number of side effects is small.

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Patients and methods

Between September 1996 and July 1998, we treated 43 patients (25 women and 18 men) with radiotherapy for age-related macular degeneration. All patients gave their

informed consent before irradiation. The median age at the time of treatment was 74 years (range 60-88 years).

The right and the left eye were treated in 18 and 16 patients, respectively. In 9 patients, both eyes were involved. In all cases, the diagnose was set on the basis of visual examination (funduscopy) carried out by the ophthalmologist. All patients were diagnosed as having the exudative variety of macular degeneration. In 21 patients, angiography was used to determine the size of the neovascularisation.

Irradiation was carried out using a linear accelerator of 6 MV photons. A total dose per patient was 16 Gy and was given in normal fractionation with single doses of 2 Gy 5 times a week. When both eyes were involved, opposed portals were used and the dose was specifically adjusted to the middle of the scull. In case of unilateral neovascularisation, a single irradiation field was used. In all cases, we used a lens-sparing technique.

The median follow-up time was 12 months. Each patient was clinically examined before and immediately after the therapy and then every 3 months. All patients included in this study were questioned about their subjective experience of radiotherapy, treatment results and side effects.

Results

After the completion of irradiation, visual acuity improved in 5 patients (11%), in 35 patients (81%), it was the same as before irradiation and was worse in 3 patients.

Six months after the irradiation, 30 patients (69%) maintained their visual acuity while in 9 patients (21%), it was worse. Four patients were lost from the follow-up at the time of investigation.

Objective changes of visual acuity could be observed only to a limited extent (median difference 0.005). In 5 patients, the moist macular degeneration changed to a dry one without affecting the acuity. In 2 patients, the bleeding occurred again 3 and 7 months after the completed irradiation. As to the side effects, we have not observed any acute or late sequelae after irradiation.

In addition to the objective findings, the results of the interviews with the treated patients were the following results: 31 patients (72%) reported that irradiation had a beneficial influence on their acuity and 12 patients felt that irradiation did not improve their sight.

Discussion

Macular degeneration is an increasing problem in ophthalmology. Today, about 5% of the population in their sixties are affected.[1] The patients are handicapped by the loss of central vision and, therefore, their ability to read.

Often, both eyes are affected to various degrees and at different times.[14] Stage I shows a dry formation of senile membranes. A detachment of the pigment epithelium with retroretinar bleeding occurs within some weeks. In the exudative stage, disciform lesions are observed. They consist of choroidal neovascularisation and spread into the retroretinal space as a thin layer between the retina and choroid. At this stage, patients often report seeing only shadows. Regression of the exudative changes causes complete atrophy of the central part of the retina. The therapy is stage-dependant.[14]

In the early dry stage, no special therapy is recommended. The detachment of the pigment epithelium is treated with corticosteriods. Laser coagulation could be applied, but causes scars and decreases the acuity. [2,15-17] Neovascular membranes cannot be treated with laser. In these cases, radiotherapy can be applied as alternative treatment. Irradiation seems to be able to stop further progress of the disease.

Proliferating vascular cells have been

known to be relatively sensitive to low doses of radiation. Irradiation may prevent the proliferation of endothelial cells of newly formed subretinal capillaries and induce obliteration of the aberrant new vessels.[18] Some data that have appeared so far in the literature confirm the assumption that photon irradiation can be a beneficial tool in treating this disease. Chakravarthy *et al.* treated macular degeneration with a total dose of 10 or 15 Gy and recorded that the visual acuity was maintained or improved in 78% of all patients.[8] Hart and colleagues treated 41 patients with 10, 12 or 15 Gy and found no significant difference in the effect of treatment of 3 different dose regimes.[10] Bergking *et al.*[5] evaluated the patients in a study on doses ranging between 8 and 24 Gy. The first group received 8 Gy in a single fraction. In this group, only 30% had stable visual acuity. In the second group, 50% of patients having received a dose of 12 Gy had stable visual acuity after a follow-up of 18 months and in the third group having received a dose of 18 Gy, 40% of patients had stable visual acuity after the same follow-up period. In the group which had received 24 Gy, 80% of all patients had stable visual acuity.

Hence, it is possible that there is a relationship between total doses of irradiation and treatment results. But care should be taken not to exceed a total dose of 25 Gy in order to avoid an increase in side effects.

On the other hand, some data published in literature report of the positive treatment results using proton beam irradiation. Yonemoto *et al.*[13] reported that after a median follow-up of 11.6 months 58% of a total of 21 patients had an improved or stable visual acuity. So far, it has not been proved that, with regard to the treatment results, heavy particles such as protons are superior to normal photon therapy.

In our investigation, we recorded stable visual acuity objectively in 69% of all patients 6 months after irradiation. After interviewing the patients, it was estimated that the effect of treatment was beneficial in 72% of cases. As no acute or late sequelae and no changes in the number of stable diseases were observed within the median follow-up time of 12 months, we believe that low-dose irradiation can be a good treatment policy of the patients with progressive CNV. Nevertheless, the definitive role of radiotherapy in patients with CNV is still to be defined by a control phase-III study.

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Second malignancy after radiotherapy for seminoma

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A patient who developed a malignant peripheral nerve sheath tumor in the field of radiation 19 years after radiotherapy for stage I seminoma is presented. Data from recent population-based studies evaluating the risk of second malignancies in this group of patients is discussed. This case report illustrates the need for judicious evaluation of adjuvant radiotherapy in early stage seminoma patients.

Key words: seminoma-radiotherapy; radiotherapy-adverse effect; neoplasms, second primary; nerve sheath tumor; soft tissue sarcoma

Introduction

The role of adjuvant radiotherapy for stage I seminoma is controversial. Although the relapse free survival is greater than 95% with adjuvant therapy, there is no benefit in overall survival since patients who relapse are salvaged with treatment. Additional issues include the frequency of follow-up studies, maintenance of fertility, and the risk of radiation induced second malignancies. We report a patient who developed a malignant sarcoma in the radiation field 19 years following adjuvant radiotherapy for seminoma.

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Case report

A 45-year old man presented 19 years after left orchiectomy and radiotherapy for a stage I seminoma with severe pelvic pain and a dense left sciatic nerve palsy. Computer tomography (CT) showed a 8.5 cm enhancing heterogeneous mass extending to the superior aspect of the sacrum (Figure 1). An ultrasound-guided biopsy revealed a high grade malignant peripheral nerve sheath tumor. Staging chest and abdominal CT showed no additional lesions. The patient received 3000 cGy of external beam radiation. The original simulation films show the radiation fields (Figure 2), which includes the paraaortic, left iliac and pelvic region. Given this previous radiation treatment, it was now felt that neoadjuvant radiotherapy was not indicated. A curative surgical resection was attempted which included a modified left internal

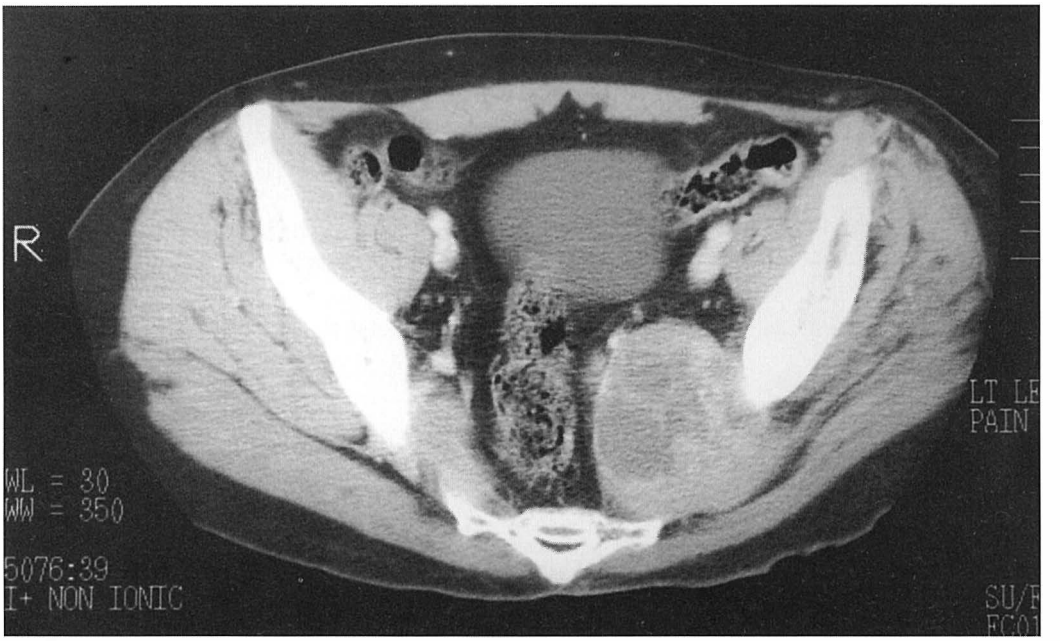

Figure 1. CT of the pelvis demonstrating a left pelvic mass. Tissue biopsy revealed a malignant peripheral nerve sheath tumor.

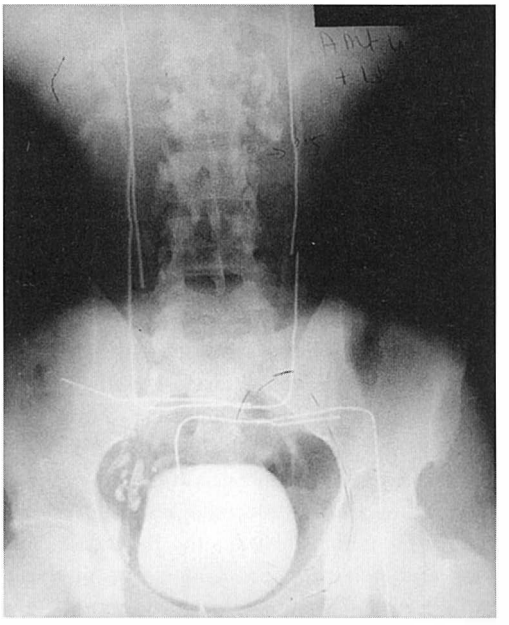

Figure 2. Original radiation ports for adjuvant radiotherapy. The soft tissue sarcoma occurred in the left pelvis within the radiation field.

hemipelvectomy with sacrifice of the sciatic nerve. Intraoperative margin assessment was negative for tumor, however, final pathology revealed a positive microscopic margin. The patient refused adjuvant radiation secondary to the risks of bladder and bowel toxicity. Ten weeks postoperatively the patient developed lung metastases. Treatment with Adriamycin was initiated.

Discussion

Iatrogenic carcinogenesis due to ionizing radiation is a well established observation. The probability of secondary malignancies increases with the dose. A threshold is not known, however, and doses as low as 1000 cGy have been associated with secondary malignancies.[1]

While radiation therapy is an integral part of the primary treatment for many cancers,

its role in stage I testicular seminoma remains controversial. Surveillance after orchiectomy may be a safe alternative to adjuvant radiotherapy, if one is prepared to accept a 15 to 20% recurrence rate. If all patients receive adjuvant radiotherapy, recurrences can be reduced to 2 to 4% which can be treated with systemic chemotherapy.[2] If one elects surveillance, 85% of patients are cured. The remaining 15% of patient will relapse and undergo retroperitoneal irradiation only (10%), chemotherapy only (3%), or combined chemoradiation (2%).[2] Therefore, with surveillance alone 1 to 2% more patients will require chemotherapy but the majority of patients will be spared radiotherapy. Young patients wishing to remain fertile may elect surveillance, whereas patients anxious about the higher relapse rate may opt for adjuvant radiotherapy.

The issue of radiation-induced malignancies in seminoma patients remains controversial.[3] While cases such as the one presented here support the entity of postirradiation malignancies in seminoma patients, population based studies have resulted in conflicting reports on the risk for cancer following adjuvant radiotherapy.[4,5] Whereas studies have found no evidence of an increased risk for second malignancies after adjuvant radiotherapy for seminoma, others have estimated the risk for postirradiation sarcoma in this group of patients at 0.003% to 0.8%.[1] The largest population based study on this subject has recently been published by the National Cancer Institute. It has documented a significantly elevated risk of second malignancies in seminoma patients treated with radiotherapy.[6] The cumulative risk of second malignancies at 25 years was 18.2 % for men with seminoma compared with 9.3 % in the general population.[6] The expected occurrence of connective tissue tumors in this group of patients was approximately 4 times higher in seminoma patients 20 years after initial treatment. The risk remained elevated throughout the follow-up period.[6] The median latency to tumor development was approximately 10 to 15 years.

Differences in treatment modalities, stage distribution, and surveillance for early stage seminoma patients weaken the conclusions of most studies examining radiation carcinogenesis. Unfortunately, the prognosis for patients who develop postirradiation sarcomas is poor, particularly for those located in the pelvis, with a 5 year survival rate of less than 10%.[1]

In conclusion, this case illustrates the need for the careful evaluation of risks and benefits of adjuvant radiotherapy in early stage seminoma patients.

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Sarcomas of the bladder: A case report with a review of the literature

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Sarcomas of the bladder are rare tumors with decisions about treatment and understanding of outcome based on limited reports from single institutions. Consequently, neither a standard staging system nor standard treatment exists for this disease.

A retrospective review of the medical records at our institution from January 1960 to December 1997 for sarcomas of the bladder and a review of the literature were made to better understand the natural history and treatment outcome of these tumors. We report on one case treated with curative intent and summarize the experience of other institutions.

If disease is localized to the bladder, then surgery alone, either by total or partial cystectomy or, alternatively, a multimodality approach using a combination of treatment with partial cystectomy and local radiation with chemotherapy to address risk of systemic disease can offer patients a good chance for cure

Key words: bladder neoplasms - drug therapy - radiotherapy - surgery; sarcoma

Introduction

Sarcomas are rare with an approximate incidence of only 7000 cases per year in the United States. Those involving the genitourinary tract, not including rhabdomyosarcomas, account for less than 5% of soft tissue sarcomas and only 1-2% of all genitourinary tumors. Bladder sarcomas, in particular, tend to occur at the extremes of life with a bimodal distribution of less than age sixteen and greater than age sixty.[1] Most patients present with painless gross hematuria with other common symptoms being urinary frequency, hesitancy and/or retention.[2,3-5] In adults , several histologic subtypes have been reported. The most frequent histology in adults appears to be leiomyosarcoma with other less frequent ones including malignant fibrous histiocytoma, angiosarcoma, fibrosarcoma, extraosseous osteogenic sarcoma and sarcoma NOS.[3,6-10] Rhabdomyosarcomas (RMS) are the most common pediatric blad-

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der sarcoma being clinically dissimilar from other soft tissue sarcomas and are generally managed according to national protocols. Although RMS occurs mainly in the pediatric population, forty cases were reported in a very early paper on bladder sarcomas in adults.[1]

No clear etiology exists for sarcomas of the bladder. Unlike bladder carcinomas which have several established risk factors (e.g. tobacco), sarcomas of the bladder have not been shown to have causal relationships to such factors. Very early reports have suggested that embryologic mesenchymal remnants are perhaps the origin of bladder sarcomas.[11] More recent literature has suggested a genetic link to sarcomas, including those of the genitourinary tract.[12-14] Also sarcomas arising as second malignancies have been reported from prior radiation treatment and chemotherapy treatment with the alkylating agent, cyclophosphamide.[15-18]

Its rarity prevents accruing enough patients to accurately assess treatment efficacies and to analyze characteristics of patients for prognostic indicators. Consequently, recommendations about treatment have been conflicting and based on small numbers from institutional experiences. We report a case diagnosed and treated at Roswell Park to add to the relatively few cases that have been reported and have reviewed the literature to assist in treatment decisions.

Case report

An 80 year old white male developed urinary frequency, urgency and painless gross hematuria. He underwent an intravenous pyelogram and cystoscopy. These studies revealed a mass within the bladder which was removed by partial cystectomy. The mass measured five centimeters in greatest dimension and the pathology showed it to be a leiomyosarcoma of intermediate grade. The remainder of his workup was negative for metastatic disease which included laboratory and radiographic studies. He received postoperative treatment using high energy photons to irradiate his whole pelvis to 4800 cGy to cover the bladder and its draining lymphatics and then boost treatment to the bladder only for an additional 800 cGy for a total dose of 5600 cGy. The radiation was given using 400 cGy per treatment with treatments given twice daily using an anterior-posterior field to the whole pelvis. This same treatment was used for the boost with the volume reduced to cover the bladder plus a margin. The patient remained disease free at one year follow up.

Discussion

Bladder sarcomas are rare tumors which makes treatment decisions and assessing treatment outcome difficult. Having a staging system that better characterizes bladder sarcomas would help with both treatment decisions and evaluating outcome. Currently, such a staging system is lacking. These tumors are presently staged according to the same systems utilized for bladder carcinomas, namely the Marshall-Jewet or American Joint Committee on Cancer (AJCC) TNM transitional cell bladder carcinoma systems. These staging systems are very effective with non-sarcomas of the bladder because they relate the close relationship between muscle invasion and risk of regional node and/or distant disease.[19] Bladder sarcomas, however, originate within the submucosal mesenchymal layer which would automatically stage them as invasive and place them at high risk for nodal and/or distant disease with a resulting poor prognosis. With the exception of RMS, this is not the case with sarcomas of the bladder which have a relatively low risk of regional nodal involvement. Using the AJCC soft tissue sarcoma staging system, on the other hand, would perhaps offer a better

approach since its predominate prognostic factor is grade.

However, tumor size would not adequately address bladder sarcoma. Swartz *et al.* have shown that the amount of bladder involvement is a prognostic indicator. We propose in this paper a staging system that

Table 1. Proposed staging system for sarcomas of the bladder

Histologic subtype
Leiomyosarcoma
Rhabdomyosarcoma
Other
Grade: low, intermediate, high
Size
Bulky (>30% bladder involvement)
Nonbulky
Pelvic sidewall involvement
Adjacent organ involvement
Regional nodal involvement
Distant metastasis

incorporates these apparent associated prognostic factors (Table 1) to allow better follow-up of patients and establish a multidisciplinary approach to bladder sarcomas. Specifically, mitotic activity, a major determinant of grade, has been shown to be an independent prognostic indicator and correlate well with developing metastases and overall survival.[20,21] Others include the extent of locoregional involvement and histologic subgrouping.

With respect to treatment and outcome of sarcomas of the bladder, surgery has remained the mainstay and improvements in outcome have been reported in the modern literature with survival as high as 63% at 5 years.[22] Sen *et al.* evaluated 13 patients at the Mayo Clinic between 1970 and 1985 with radical cystectomy with or without preoperative radiation.[23] They had five patients with leiomyosarcoma, three with rhabdomyosarcoma, and five with carcinosarcoma.

Two of the 5 patients with leiomyosarcoma were treated with preoperative irradiation. One (pathologic stage B1) of these two patients was treated with radical cystectomy and preoperative irradiation of 5000 cGy and died at sixteen months after developing local and distant disease. The other (PS B1) was treated preoperatively with 6000 cGy and is free of disease seventy-eight months after completion of treatment. Two of the five were treated with radiation alone with doses of 6500 cGy and 6000 cGy and died at eighteen months and forty-six months respectively after developing both local and distant disease. No clinical stage was given for either but neither was felt to be surgical candidates. One patient (PS A) had a radical cystectomy alone and is free of disease at eighty-six months. The authors conclude from their experience that those patients felt to be curative should be treated with radical cystectomy with or without preoperative irradiation regardless of histology and that with modern techniques for staging and treatment, patients have a much better prognosis today than patients described in the older literature.

Another series by Swartz *et al.* on leiomyosarcoma of the bladder reported on ten cases. Pathologic staging was not indicated in the paper so that no correlation of pathologic stage and treatment outcome can be made. One patient treated with partial cystectomy and postoperative radiotherapy (dose not given) for inadequate margins has remained alive without disease at 5 years of follow-up.[12] Three others treated with partial cystectomy alone with adequate margins are alive without disease at 6, 6 and 9 years. Only one patient was treated with definitive radiotherapy after an initial misdiagnosis of squamous cell carcinoma and recurred locally after 4 years. The other patients in the series had either radical cystectomy (n=3), partial

cystectomy with chemotherapy (n=1), ileal conduit (n=1), or biopsy alone (n=1), and all are dead of either postoperative complications or disease progression. The authors in this paper felt that if adequate margins can be obtained, then partial cystectomy is the treatment of choice, and that radical cystectomy should be performed only for more extensive lesions where adequate margins are not obtainable.

They did not feel that enough information exists on the role of either postoperative radiation or adjuvant chemotherapy with this disease to justify their use as part of initial curative treatment but rather could be utilized in the setting of recurrent and/or metastatic disease.

Eleven patients with leiomyosarcoma were reported by Ahleringer *et al.*[2,] 7 of whom had bladder as the primary site and 4 with prostate. Patients with nonbulky disease of the bladder were treated with surgical resection and given adjuvant chemotherapy and external beam irradiation if the margin or nodes were positive. Bulky disease, defined as greater than 30% of bladder involvement, received preoperative chemotherapy with or without postoperative irradiation. None of their patients had pathologically positive nodal disease. Six of the 7 bladder patients are alive without evidence of disease (range of 35-97 months) with 1 lost to follow-up. The authors concur with Swartz *et al.*[12] in suggesting that partial or subtotal cystectomy for smaller tumors to avoid urinary diversion is justified as long as negative margins are achieved, and that, based on the 3 patients treated for stage C prostate involvement, radiotherapy with doses of between 4500 cGy and 5000 cGy be used for microscopic residual disease after removal of bulky disease. They also further suggest adopting preoperative chemotherapy for cytoreduction using agents such as cisplatin and doxorubicin for bulky or transmural disease and postoperative chemotherapy for either responsive tumors to preoperative chemotherapy or pathologic 3A patients.

A series from Memorial Sloan Kettering reviewing their experience with urologic sarcomas between 1982 and 1989 found 6 cases of leiomyosarcomas of the bladder.[24] The authors discuss the local and distant recurrences as well as survival data according to histologic grade, size and margin status after surgery, but they make no distinction between the different subtypes of bladder sarcoma.

Thus no conclusions as to differences in outcome between leiomyosarcomas and other histologies can be made. No patient received irradiation as part of their treatment but two had treatment with surgery and chemotherapy. The 3 rhabdomyosarcomas were treated on a rhabdomyosarcoma protocol with multimodalities of surgery, chemotherapy and radiation. Two patients with small (< 2 cm) leiomyosarcomas, 1 of which was a low grade and the other a high grade tumor, underwent complete transurethral resection alone with local control reported with 7 years follow-up. As a group, when compared with other urologic sites, sarcomas of the bladder had the highest 5-year survival at 80%. The authors show that urologic sarcomas in general share similar prognostic indicators as with sarcomas at other sites; namely, with increasing grade, size and depth, both local recurrences and metastases increase.

Our review of the literature shows that varying modes of managing these rare tumor exists. This is not surprising since personal experiences and extrapolation of treatments tend to control management decisions when there is little data available to assist in the decision making process. The location of the tumor would seem to occur anywhere within the bladder by the published reports, although the trigone region appears to have the highest predilection.[25,26-29] This may account for the varying results that are achieved with using a partial resection alone.

Despite the differences that exist in the outcome between series, most series seem to suggest that a subtotal cystectomy as opposed to a radical cystectomy can achieve good results as long as adequate margins are achievable. More aggressive surgery appears to be indicated for large, bulky tumors. However, the addition of radiation for positive margins after partial cystectomy and/or chemotherapy also appears to offer good results and thus avoid the need for removing the bladder.[2] The results of Russo *et al.* and Suit *et al.* would suggest that, like sarcomas at other sites, a multimodality approach for urologic sarcomas may provide better functional outcome and durable local control.[24,30] The case we present in this paper, although with short follow-up, is a clear example of how properly selected patients can undergo conservative management with this disease involving the bladder, as has been shown with transitional cell tumors of the bladder, having disease controlled without the need for removing the bladder.[31] Adopting a standard staging system would allow for optimizing treatment in a multidisciplinary fashion by basing treatment decisions on prognostic factors and aid in the design of future treatment protocols.

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Epithermal neutron beam for BNCT at the JSI TRIGA reactor – modelling and experimental verification

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It has been reported that satisfactory thermal/epithermal neutron beams for Boron Neutron Capture Therapy (BNCT) could be designed at TRIGA research reactors, which are generally perceived as being safe to install and operate in populated areas. This contribution presents the most recent research activities in this field at the Jožef Stefan Institute TRIGA reactor, where an epithermal neutron beam for BNCT is being developed. Experimental verification of Monte Carlo simulation results proves the quality and wide applicability of the developed 3-D model, particularly of the reactor core and irradiation channels. Due to high attenuation of the epithermal neutron flux ($\Phi_{epi} = 4.1 \times 10^6$ n/cm^{2}s, two orders of magnitude below the therapeutic limit) the irradiation facility in the current stage of development is not appropriate for the clinical BNCT treatments. Furthermore, the contribution of the 2.5 mm air gap surrounding the facility is unacceptably high, thus making the relative gamma dose ($D\gamma/\Phi_{epi}$) almost 60-times higher than therapeutically recommended. Nevertheless, using gamma shielding of Pb or Bi and LiF or Li_2CO_3 (thermal neutron cut-off), the quality of the epithermal neutron beam would be significantly upgraded and become appropriate for in vitro studies of boron compound transport in malignant tumour cells or smaller laboratory animals.

Key words: boron neutron capture therapy; neoplasms-radiotherapy; nuclear reactors

Introduction

Boron Neutron Capture Therapy (BNCT)[1] is a bimodal treatment that offers the potential of a highly selective radiation effect - by α particles - while sparing normal tissues. Brain tumours, particularly glioblastoma multiforme (GBM), were chosen as the initial target for BNCT. GBM is an extremely lethal cancer, with no significant advances in therapy in the last two decades. Almost all patients die within two years, even with the best efforts using surgery, external beam therapy and chemotherapy.[1,2]

The central feature in effective BNCT is the selective delivery, concentration and build-up of the naturally occurring 10B isotope in tumour tissue, using one or more advanced drug delivery systems (DDS) such as monoclonal antibody carriers or liposomal deliveries.[3-5] As the tumour is irradiated with low energy neutrons (epithermal neutrons

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with 0.4 eV<E<10 keV become thermalized in the surrounding healthy tissue), there is a higher likelihood of the ^{10}B nucleus absorbing them than the nuclei of any other elements normally present in tissues. The boron nucleus become unstable and immediately splits into two recoiling ionizing particles, an particle (i.e., a helium nucleus) and a lithium nucleus:

$$n + {}^{10}B \rightarrow {}^{11}B^* \rightarrow {}^{4}He + {}^{7}Li + \gamma$$

These products of the BNCT reaction - $^{10}B(n,\alpha)^7Li$ - are very damaging to tissue, but of short range (the pathlength of particles is about one cell diameter, which is around 10 μm) and are confined to the immediate vicinity of the boron-containing compound which, hopefully, should be concentrated in the tumour. A major appeal of BNCT is that ^7Li and energetic α particles are produced by a fission reaction following neutron capture. These heavy particles carrying 2.79 MeV of energy, have a very high ionization density. Another advantage is that they can affect dividing and nondividing tumour cells alike - tumours are known to have a large number of viable but nonactive cells.

Up to the present NCT research in Slovenia has been carried out in a provisional irradiation system, consisting of a „dry cell" adjacent to the thermalizing column (Figure 1) at Jo_ef Stefan Institute (JSI) 250 kW TRIGA Mark II research reactor. The „dry cell" (originally a pool-type storage facility) is a unique advantage of the JSI TRIGA reactor, with approx. ground plan dimensions of 3x3 m^2 and additional neutron protection, currently refurbished for experimental purposes. The radiative field cross-section of the thermalizing column is approx. 60x60 cm^2, and is rather homogene due to the substantial distance from the core fuel elements. The irradiation field of the experimental set-up is approx. 10x10 cm^2, thus enabling irradiation of 9 foils with specimens at the time. The experimental set-up is mainly used for *in vitro* studies in a mixed neutron/gamma irradiation field, with the aim of determining the radiosensitivity of two cell lines: mouse B16F1 melanoma and human MCF7 breast carcinoma.[6,7]

Unfortunately, the contribution of fast neutrons (18.4%) as well as γ-rays (23%) to the total effective dose in the current experimental system is substantial.[6] Since this has a strong negative influence on the verification of ^{10}B or thermal neutron effects, as well as on the mortality of prepared cell cultures, the development of an irradiation facility with optimized beam properties (minimised fast neutron and gamma dose and maximised epithermal neutron flux) is indispensable. Hence Monte Carlo modeling, development as well as experimental verification of the epithermal neutron beam in the radial channel of the JSI TRIGA reactor is presented in this paper.

Methods

Monte Carlo modeling of the BNCT irradiation facility in the radial channel of the TRIGA reactor

The general purpose MCNP4B Monte Carlo code was used for modeling of the TRIGA reactor, together with the ENDF/B-V and ENDF/B-VI continuous cross-section libraries and S(α,β) scattering data from the ENDF/B-IV file.[8] n/γ transport was performed using geometry splitting, weight window (WWG) and direct statistical approach (DSA) variance reduction techniques.[8,9]

A detailed Monte Carlo model of the TRIGA Mark II research reactor has been developed, where all important details concerning the reactor core, graphite reflector, thermal and thermalizing column, irradiation channels and biological shielding were considered (Figure 1). The original TRIGA core

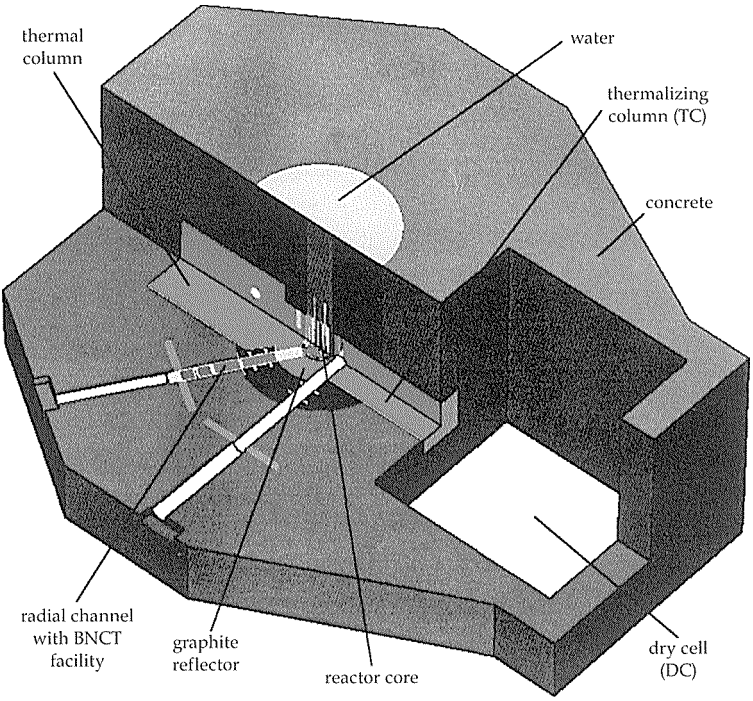

Figure 1. 3D Monte Carlo model of the JSI TRIGA Mark II research reactor.

configuration No. 147 was modified in such a manner that three fuel elements were positioned at the inlet of the radial channel. With this set-up (based on an MCNP TRIGA benchmark model[11]), which considered 50 fresh fuel elements with multiplication factor keff being 1.0100 ± 0.0017, the total neutron flux was enhanced by 40%, with a negligible change of neutron spectrum.[10]

On the basis of an extensive Monte Carlo pilot study of epithermal neutron filter and gamma shielding materials, the final configuration of the BNCT irradiation facility was elaborated, consisting of the following elements (Figure 2):[12,13]

- 30 cm Al + 40 cm Al_2O_3 (with a density of 2.3 g/cm^3, which can be achieved by pressing the Al_2O_3 powder (initial density is 1.6 g/cm^3) at 400-500 kg/cm^2) in a single piece, used as epithermal neutron filter,
- an additional 30 cm Al_2O_3 with 0.05 cm of Cd foil, used as a thermal neutron absorber,
- 15 cm Pb, used as a gamma shield and
- 15 cm Pb + 15 cm borated paraffin (90 vol. % $C_{30}H_{62}$ + 10 vol. % boric acid H_3BO_3), as additional neutron/gamma shield.

All the elements are cylindrically shaped and equipped with six small stainless steel wheels to enable unrestrained transport through the channel. In addition to numerous benefits offered by this design - easy handling and transportation of filter elements, as well as storage after irradiation - it has one major drawback: an approx. 2.5 mm air gap, leading directly from the reactor core to the irradiation point, thus allowing fast neutrons and gamma rays to stream through the gap and contributing significantly to the total dose. When the calculations were repeated using the MC model without the air gap, the fast neutron and gamma doses were reduced by more than 80%! The results of Monte

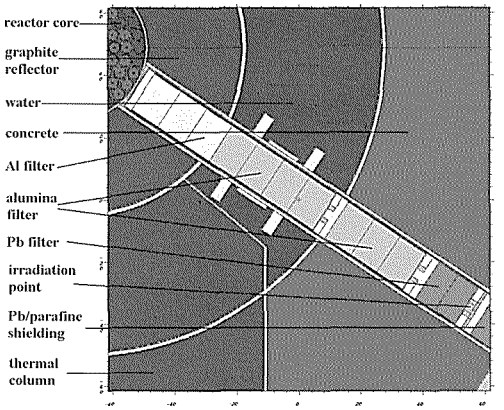

Figure 2. MCNP model of the BNCT facility inserted in the radial channel (units in cm).

Carlo calculations at the irradiation point (Figure 2) are presented in Table 1.

Experimental verification

Based on the results of MC calculations, the irradiation facility for BNCT treatment was manufactured. The details of elaboration were presented in more detail.[13] The measurements of neutron flux and mixed n/γ field dosimetry were performed with a set[13] of ^{115}In and ^{197}Au (n,γ) activation detectors for the thermal and epithermal range. A Cd cover (thickness 0.5 mm) was used in order to determine the cadmium ratio of the field. The integral fluxes in the fast neutron range were measured with the following threshold reactions: ^{115}In(n,n′)^{115m}In, ^{27}Al(n,p)^{27}Mg, ^{56}Fe(n,p)^{56}Mn, ^{64}Zn(n,p)^{64}Cu, ^{24}Mg(n,p)^{24}Na, ^{27}Al(n,α)^{24}Na and ^{19}F(n,2n)^{18}F. Pure metallic foils (In, Al, Fe, Zn and Mg) as well as Teflon™ (CF_2) were irradiated with Cd covers. Gamma dose was measured using a set of TLD detectors on the central axis of the channel. In order to absorb the radiation from electrons and soft X-rays from the outside and to affirm the reproducibility of the results, the set was confined in a 1 cm thick holder made of plexiglass. To obtain the neutron spectrum (Figure 3), the detector responses were adjusted with the SAND-II deconvolution code.[14] The systematic error of the experiment was estimated to be less than 3%. The standard deviation over the entire spectrum of detector saturated activities was less than 2%.

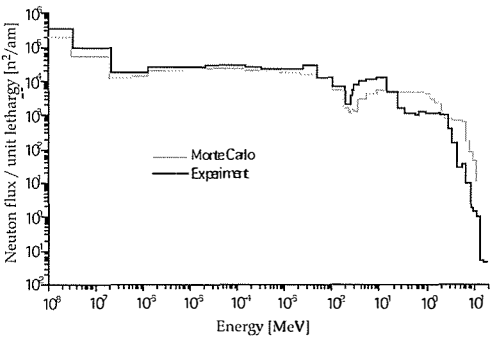

Figure 3. Calculated vs. measured neutron flux per unit lethargy.

Results

Experimental results confirmed 10% higher epithermal neutron flux Φ_{epi} (0.4 eV < E < 10 keV) at the irradiation point than MC calculated one. This remains two orders of magnitude below the recommended therapeutic limit of 10^9n/cm^{2}s, thus dictating quite long irradiation times.

Furthermore, the experiment confirmed a surplus of thermal neutrons (E<0.4 eV) (Φ_{term}/Φ_{epi} = 1.85 for Monte Carlo calculations results and 1.75 for the experiment). This can be attributed to thermal neutrons that arrive at the irradiation point from surrounding regions, *i.e.* the water and concrete of the reactor biological shield (those emerging from the neutron beam itself were cut-off with the 0.5 mm thick Cd absorber). The measured neutron spectrum (Figure 3) conforms the calculated one; discrepancies emerge only in the fast part of the spectrum (above 10 keV) but still remain within the 10% confidence interval of the Monte Carlo calculated total fast neutron flux.

Table 1. Results of MC calculations vs. experiment

Quantity[c]	Method		Trerapeutic
	Monte Carlo[a]	Experiment[b]	limit values
Φ_{nterm} (E<0.4 eV)	6.95e+6 (15)	7.1e+6 (+10)	/
$\Phi_{nepiter}$ (0.4 eV<E<10 keV)	3.75e+6 (12)	4.1e+6 (+20)	$>10^9$
Φ_{nfast1} (10 keV<E<300 keV)	3.86e+5 (17)	8.7e+5 (+10)d	/
Φ_{nfast2} (300 keV<E<20 MeV)	4.83e+5 (15)	/	/
J_{epiter}	2.55e+6 (12)	/	$>5*10^8$
D_{nfast}	7.04e+6 (13.8)	7.4e+6 (+15)	/
Dγ	6.07e+7 (11.7)	8.1e+7 (+45)	/
$D_{nfast}/\Phi_{nepiter}$	19	18	<5
$D\gamma/\Phi_{nepiter}$	162	197	<3
J_{epi}/Φ_{epi}	0.68	/	>0.5

[a] -( ) - relative errors in %

[b] - ( ) - discrepancy from Monte Carlo results in %

[c] - units: Φ_n and - [n/cm^{2}s], D_{nfast} and Dγ - [10^{-12} Gy s^{-1}], $D_{nfast}/\Phi_{nepiter}$ and $D\gamma/\Phi n_{epiter}$ - [10^{-13} Gy cm^2]

[d] - fast neutron flux, measured in a single energy group (10 keV<E<20 MeV)

To determine the influence of the air gap surrounding the filter configuration on the neutron and gamma dose, the profiles of the neutron and gamma fields were measured using photo-luminescence imaging plates (IP) with a 0.1 mm thick dysprosium screen, frequently used in the direct method of neutron radiography. The imaging plate is exposed with the neutron-activated Dy foil and later scanned with a laser reader. The gamma beam profile at the outlet of the radial channel is presented in Figure 4.

The experimentally measured gamma dose exceeds the calculated one by 33%, thus making the relative gamma dose ($D\gamma/\Phi_{epi}$ in units of 10^{-13} Gy cm^2) unacceptably high and almost 60-times higher than therapeutically recommended. The contribution of the 2.5 mm air gap is most clearly evident from Figure 5: the gap region is approximately 30-times more irradiated than the filter-covered region in relative PSL (Photo Stimulated Luminescence) units as a function of the spatial co-ordinate.

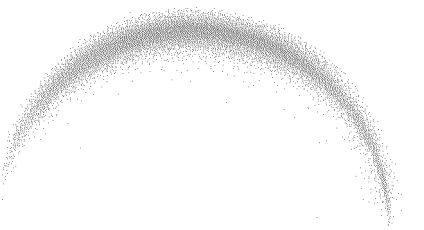

Figure 4. The profile of the gamma profile field at the outlet of the channel ($P_{reactor}$ = 2kW, T_{irrad} = 500s).

Discussion

Experimental verification of the BNCT irradiation facility at the JSI TRIGA reactor proves the quality and wide applicability of the developed 3-D Monte Carlo model, particularly of the reactor core and irradiation channels. The model can easily be extended for the purposes of Prompt Neutron Gamma Activation Analysis (PNGAA), Proton Recoil Spectrometry and

many other activities. It is accurate enough to enable agreement with the experimentally obtained results within the confidence intervals of the Monte Carlo calculations.

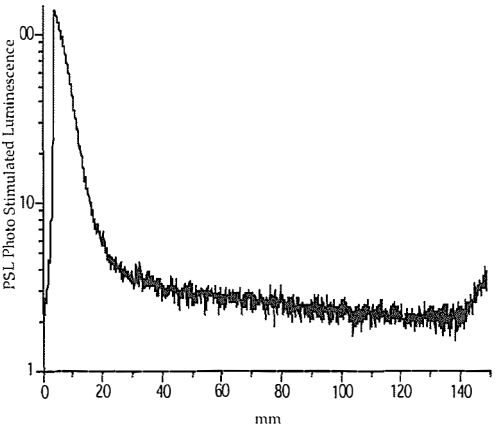

Figure 5. Numerical profile of the gamma field in relative PSL units (vertical cross-section of Figure 4).

In the current stage of development, the irradiation facility is not appropriate for clinical BNCT treatments. Due to high attenuation of the epithermal neutron flux, we were obliged to alter the initial design of the facility so as to move the irradiation point from the outlet of the radial channel to the interior immediately behind the lead gamma filter. This makes our radiation facility appropriate for *in vitro* studies of novel techniques of boron entrapment in malignant tumour cells (i.e. by application of electroporation[15,16]) or *in vivo* irradiation of smaller laboratory animals. Using stop (or shelter) made of Pb or Bi (gamma shielding) and LiF or Li_2CO_3 (thermal neutron cut-off), the influence of the air-gap on the irradiation point would be significantly reduced, thus increasing the quality of the epithermal neutron beam.

This work represents the first stage of the BNCT research project in Slovenia leading, hopefully, towards further development of a clinical irradiation facility for BNCT treatment of human patients in the thermalizing[17] or thermal column of the Jožef Stefan Institute TRIGA reactor.

Acknowledgements

The authors would like to express special thanks to dr. Kenneth W. Burn from ENEA, Bologna, Italy for extensive help with the variance reduction method DSA for the Monte Carlo calculations, and to dr. Anthony R. Byrne, Jožef Stefan Institute, Ljubljana, for a thorough review of this paper.

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Radiol Oncol 1999; 33(1): 1-8.

Farmakološko-mehanska tehnika za selektivno trombolizo na perifernem žilju

Hadjiev J, Horváth L, Mezõfi B, Szalay G

Namen: Pri selektivnem fibrinolitičnem zdravljenju z nizkimi dozami smo nameravali še mehansko izboljšati učinek uničevanja trombusov z uporabo pulzne razpršilne brizgalke. Predvidevali smo, da bomo obenem skrajšali čas zdravljenja in omejili število zapletov po zdravljenju.
Metode: V raziskavo smo vključili 17 bolnikov z zamašenimi arterijami na spodnjih okončinah. Zdravili smo jih s selektivno pulzne razpršilno trombolizo. Pri vseh smo upoštevali standardni protokol zdravljenja s trombolizo. S pulzno razpršilno brizgalko Angiodynamics® smo v žilo vbrizgavali streptokinazo - Kabikinase® (5.000-20.000 IU/h), urokinazo - Ukidan® (20.000-40.000 IU/h) in heparin v fiziološki raztopini (500-1.000 IU/h), dokler se trombus ni popolnoma razpustil.
Rezultati: Od 1995 do 1997 smo uspešno pozdravili 17 bolnikov. Povprečni čas zdravljenja je bil 12 ur. V tem času ni bilo hujših zdravstvenih zapletov. Kirurški poseg je bil v prvih šestih mesecih po zdravljenju potreben le pri dveh bolnikih in sicer zaradi ponovitve kliničnih simptomov.
Zaključek: Farmakološko-mehanska selektivna tromboliza s pomočjo pulzno-razpršilne tehnike je zanesljiva, sorazmerno hitrejša in tudi varnejša metoda za zagotavljanje prepustnosti zamašenih arterij.

Radiol Oncol 1999; 33(1): 9-14.

Odkrivanje preobremenitve z železom in jeterne ciroze z analizo podatkov o spektralnih značilnostih CT-ja jeter z visoko ločljivostjo

Kurbel S, Kristek B, Kovačić D, Glavina K, Kurbel B

Izhodišča. Jeterno cirozo in preobremenitev z železom smo odkrivali z analizo podatkov, dobljenih iz skupka slikanj jeter s CT-jem z visoko ločljivostjo. Podatke smo obdelali s statističnimi metodami in Fourierjevo analizo.
Bolniki in metode. Obravnavali smo 11 bolnikov z jeterno cirozo, 7 hemodializiranih bolnikov s preobremenjenostjo z železom in 51 posameznikov za primerjavo. Uporabili smo CT Siemens Somatom DHR (kernel 2; 0,2 mm široki, 2 mm debeli piksli). Področja vzorčenja so bila kvadratne velikosti, velika 50 pikslov. Vsakemo bolniku smo določili tri področja posameznikom za primerjavo pa dva. Področja smo statistično obdelali in razgradili v 100 linearnih fragmentov (50 linij in 50 stolpcev s 50 piksli) za Fourierjevo analizo. Za vsak vzorec smo izračunali povprečje in standardno devijacijo.
Rezultati. CT visoke ločljivosti se je pri bolnikih z obremenitvijo z železom razlikoval od kontrolnih vrednosti po povišani povprečni gostoti (>=79 HU) in zmanjšani moči mnogih harmonij ($p<0.01$). CT visoke ločljivosti pri bolnikih s cirozo pa je pokazal zmanjšano gostoto (<50 HU) in povečano moč harmonij od 0.1 do 0.9 ciklov/mm (valovne dolžine od 10 do 1.1 mm) ($p< 0.01$).
Zaključki. S pomočjo podatkov, dobljenih s CT-jem jeter z visoko ločljivostjo, lahko razlikujemo med normalnimi, cirotičnimi ali z železom preobremenjenimi jetri.

Radiol Oncol 1999; 33(1): 77-82.

Radiol Oncol 1999; 33(1): 15-21.

Standardizirana slikovna dokumentacija v nuklearni medicini

Bohuslavizki KH, Buchert R, Mester J, Clausen M

V vsakdanji medicinski praksi ni splošno sprejetih standardov o slikovni dokumentaciji. Tako nastanejo težave pri ponovnem ocenjevanju slikovnih zapisov na rentgenskih filmih ali papirju, ki jih dobimo iz drugih nuklearnomedicinskih ustanov ali pa je težavna primerjava različnih slikovnih zapisov pri istem bolniku. Da bi olajšali ponovno ocenjevanje slikovnih zapisov, smo izdelali predloge za potrebno dokumentacijo. Opisani so primeri slikovnega dokumentiranja pri najbolj pogostih nuklearnomedicinskih raziskavah, kot so scintigrafija pljuč, ščitnice, kosti - s planarno ali SPECT kamero, pa tudi funkcionalna ledvična scintigrafija, perfuzijska miokardna scintigrafija in pozitronska emisijska scintigrafija. Ti primeri imajo namen vzpodbuditi razpravo v nuleranomedicinski stroki, kakšni naj bodo slikovni dokumentacijski zapisi pri nuklearnomedicinskih preiskavah.

Radiol Oncol 1999; 33(1): 23-6.

Odkrivanje metastaz osteogenega sarkoma v limfnih bezgavkah z 99mTc-MDP scintigrafijo. Prikaz primera

Ilić D, Zafirovski G, Simova N, Zisovski N, Glamocanin S, Janevska V, Tolevska C, Vaskova O, Samardziski M

Osteogeni sarkom se po telesu običajno širi hematogeno. Tako nastanejo pljučne in skeletne metastaze. Redkeje pa se širi limfogeno. Prikazujemo primer bolnika z osteogenim sarkomom, pri katerem smo z radioizotopno scintigrafijo kosti odkrili metastaze v limfnih bezgavkah.

Radiol Oncol 1999; 33(1): 77-82.

Radiol Oncol 1999; 33(1): 27-33.

Korelacija med NK citolitičnim in BLT esteraznim testom pri določevanju aktivnosti NK celis stimuliranih s tumorskimi celicami

Ihan A

Z meritvami encimske aktivnosti N-benzyloxycarboxy-L-lizinske esteraze smo preiskovali eksocitozo celic NK. Celice NK smo osamili iz levkocitnega koncentrata (Buffy coat) oz. venske krvi. Eksocitozo smo sprožili s kombinacijo PMA/ionomcin ali s tumorskimi celicami K542. Ob stimulaciji celic NK s tumorskimi celicami (K542) smo opazovali značilno soodvisnost (Cor=0.84) med rezultati citolitične aktivnosti celic NK in med izmerjenimi encimskimi aktivnosti N-benzyloxycarboxy-L-lizinske esteraze (BLT test). Ugotavljamo, da BLT test omogoča nadrobnejši študij dogodkov, ki se odvijajo med citotoksično aktivnostjo celic NK.

Radiol Oncol 1999; 33(1): 35-41.

Mikro jedra v limfocitih z zavrto citokinezo pri bolnikih po radioterapiji z I-131

Kašuba V, Kusić Z, Garaj-Vrhovac V

Pri 10 bolnikih s hipertireozo in različnimi vrstami karcinoma ščitnice smo raziskovali mikro jedra (MN) v limfocitih periferne krvi, v katerih citohalazin-B zavira citokinezo. Bolniki so bili zdravljeni z I-131 natrijevim jodidom, ki so ga dobili peroralno, v odmerkih 259-5180 MBq. Frekvenco v mikro jedrih smo izmerili pred zdravljenjem z I-131 in po njem. Rezultate merjenja pred zdravljenjem smo uporabili kot kontrolo. Rezultati naše raziskave so bili glede na starost in trenutno dejavnost precej variabilni. Iz števila mikro jeder in dvojedrnih celic z mikro jedri (BNMN) ter s pomočjo Poissonove regresije, prirerejene autokorelaciji za vsak osebek posebej, je bilo mogoče ugotavljati pretirano disperzijo. Samo pri začetni dozi nismo ugotovili značilne pomembnosti. Pri medsebojnem vplivanju časa in odmerka, še zlasti pri višjih odmerkih, pa smo ugotovili značilno pomebnost, medtem ko so spremembe v jedrih potekale počasneje. Za najnižji odmerek (259 MBq) smo izračunali relativni čas tveganja. Če smo odmerek podvojili, se je število mikro jeder in dvojedrnih celic z mikro jedri zmanjšalo povprečno za 5% na dan (relativno tveganje MN = 0.955; relativno tveganje $_{BNMN}$ = 0.954).

Radiol Oncol 1999; 33(1): 43-53.

Napovedni pomen urokinaznega aktivatorja plazminogena in njegovih inhibitorjev pri bolnicah z rakom dojk

Borštnar S, Čufer T, Vrhovec I

Urokinazni aktivator plazminogena (u-PA), in njegova inhibitorja (PAI- 1 in PAI-2) igrajo pomembno vlogo pri razgradnji medceličnega tkiva in s tem pri prodoru tumorskih celic v okolico ter metastaziranju. Namen naše raziskave je bil ugotoviti morebitno napovedno vrednost uPA, PAI-1 in PAI-2 v retrospektivni seriji 87 bolnic z rakom dojke stadijev I-III, njihove citosole hranimo na Onkološkem inštitutu v Ljubljani. Srednja opazovalna doba je bila 35 mesecev. Napovedni pomen uveljavljenih napovednih dejavnikov ter uPA, PAI-1 in PAI-2 smo ocenjevali z univariatno statistično analizo in delnimi multivariatnimi modeli. Vrednost uPA so bile zelo nizke in niso korelirale s preživetjem brez znamenj bolezni, PAI-1 in PAI-2 pa sta značilno vplivala na čas prve ponovitve bolezni. Bolnice, ki so imele vrednost PAI-1 večjo od 5ng/mg proteinov, so imele statistično značilno slabše preživetje brez znamenj bolezni kot bolnice z manjšimi vrednostmi (58% vs 85% , p = 0.0046). PAI-2 je pokazal nasprotno sliko, bolnice z vrednostmi PAI-2 večjimi od 6.4 ng/mg proteinov so imele statistično značilno boljše triletno preživetje brez znamenj bolezni kot bolnice z manjšimi vrednostmi (90% vs 48%, p = 0.0178). PAI-1 in PAI-2 sta ohranila svojo neodvisno napovedno vrednost ob dodajanju uveljavljenih napovednih dejavnikov v delne multivariatne modele in le lokalna razširjenost bolezni je pokazala večjo napovedno moč od PAI-1 in PAI-2.

Radiol Oncol 1999; 33(1): 55-8.

Radioterapija neovaskularizirane žilnice pri starostni makularni degeneraciji

Wagner W, Beeke E, Barsnick P

Starostna makularna degeneracija zaradi neovaskularizacije žilnice predstavlja v oftalmologiji vse večji problem. Zdravljenje doslej ni bilo preveč uspešno in obenem povezano s številnimi stranskimi učinki. Pred kratkim pa smo zasledili ugodne rezultate zdravljenja z obsevanje z nizkimi dozami. Zato smo ponovno proučili podatke o bolnikih, vključenih v pilotsko študijo, da bi lahko potrdili v literaturi objavljene podatke. Raziskava je zajemala 43 bolnikov, ki smo jih obsevali z lineranim akceleratorjem (6 MV) pri skupni dozi 16Gy. Šest mesecev po zaključenem zdravljenju se je pri 69% bolnikov ostrina vida ohranila ali celo izboljšala. Pri proučevanju stranskih učinkov pa v času spremljanja bolezni v povprečju 12 mesecev nismo zasledili niti akutnih niti kasnih posledic zdravljenja.

Radiol Oncol 1999; 33(1): 77-82.

Radiol Oncol 1999; 33(1): 59-61.

Sekundarni malignomi po zdravljenju seminomov z radioterapijo

Fuchshuber PR, Lee RJ, McGrath B, Gibbs JF, Kraybill WG, Proulx GM

Opisujemo primer bolnika, ki je bil pred 19-imi leti adjuvantno zdravljen z radioterapijo zaradi seminoma, stadij I, nato pa je zbolel zaradi malignega perifernega tumorja živčnih ovojnic, ki je nastal v obsevalnem polju.
Razpravljamo o novejših podatkih, ki govore o morebitnem tveganju bolnikov s seminomom, da bi zboleli za sekundarnim malignomom glede na ostalo populacijo.
Tudi opisani primer bolnika kaže na potrebo skrbne evaluacije dosedanje radioterapije pri bolnikih z zgodnjo obliko seminoma.

Radiol Oncol 1999; 33(1): 63-8.

Sarkom mehurja: prikaz primera s pregledom literature

Proulx GM, Gibbs JF, Lee RJ, Velagapudi S, Huben R

Sarkomi mehurja so redki tumorji, zato temeljita prognoza bolnika in odločitev o vrsti zdravljenja na redkih poročilih posamičnih ustanov. Tako tudi nimamo standardne razvrstitve bolnikov po stadijih bolezni, niti ne standardnega zdravljenja.
Na Roswell Park inštitutu v Buffalu smo naredili retrospektivni pregled bolnikov, ki so se od januarja 1960 do decembra 1997 zdravili zaradi sarkoma mehurja. Te izkušnje in pregled literature nam omogočajo boljše razumevanje naravnega poteka bolezni in napovedi bolnikovega preživetja glede na vrste zdravljenja. V članku prikazujemo primer bolnika, ki smo ga radikalno zdravili z namenom ozdravitve in podajamo izkušnje drugih ustanov.
Če je bolezen omejena na mehur, lahko bolnika zdravimo s popolno ali delno kirurško odstranitvijo mehurja ali pa se odločimo za kombinirano zdravljenje z delno odstranitvijo mehurja, obsevanjem in kemoterapijo, saj s tem zmanjšamo nevarnost sistemskega obolenja in in ima bolnik dobre možnosti, da ga pozdravimo.

Radiol Oncol 1999; 33(1): 69-75.

Epitermični izvor nevtronov za terapijo raka z zajetjem nevtronov v boru na reaktorju TRIGA Instituta Jožef Stefan – modeliranje in eksperimentalna preveritev

Maučec M, Glumac B, Rant J, Krištof E

Zaradi specifičnih lastnosti obratovanja spadajo reaktorji TRIGA med najvarnejše raziskovalne reaktorje. V svetu se jih vedno več uporablja kot izvor termičnih oz. epitermičnih nevtronov v namene terapije raka z zajetjem nevtronov v boru (angl. Boron Neutron Capture Therapy). Prispevek prikazuje rezultate Monte Carlo modeliranja, optimizacije ter eksperimentalne verifikacije obsevalne naprave z epitermičnimi nevtroni razvite v radialnem kanalu reaktorja TRIGA na Institutu Jožef Stefan v Ljubljani. Rezultati potrjujejo široko uporabnost razvitega tri-dimenzionalnega modela reaktorja, predvsem sredice in obsevalnih kanalov. Zaradi visoke atenuacije nevtronskega fluksa ($\Phi_{epi} = 4.1\times10^6$ n/cm^{2}s, kar je dobra dva reda velikosti pod priporočeno terapevtsko mejo) obsevalna naprava v obstoječi izvedbi ni primerna za klinična BNCT obsevanja. Prispevek zračne reže, ki obdaja sistem filtrov dodatno prispeva k visoki specifični dozi gama žarkov in hitrih nevtronov na obsevalnem mestu, ki sta skoraj 60-krat višji od priporočenih vrednosti. Vendar pa bi z minimalno predelavo obsevalne naprave ter uporabo zaščitnega ohišja s Pb (ali Bi) ter LiF (ali Li_2CO_3) lahko občutno izboljšali kvaliteto obsevalnega curka kot orodja za "in-vitro" študije transporta bora v malignih celičnih kulturah ali laboratorijskih živalih.

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Contact European Lung Cancer Working Party c/o Prof. J-P. Sculier, Institute Jules Bordet, 1, rue Héger-Bordet, B1000, Brussel, Belgium, or call +32 2 539 04 96; or fax +32 2 534 37 56; or e-mail 101473.1044@compuserve.com

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March 19-21,1999

The ESO training course will take place in Zakopane, Poland.

Contact ESO office for Central and Eastern Europe, Ms. Dagmar Just, Ärztekammer fü Wien, Fortbildungsreferat, Weihburggasse 10-12, 4th floor, 1010 Vienna, Austria; or call +43 1 51501262; or fax +43 1 51501200; or e-mail just@aekwien.or.at

Breast cancer

March 22-25, 1999

First Acta Oncologica Symposium on Management of the axilla in Breast cancer. Implications for Diagnosis, Prognosis, Treatment and Morbidity will take place in Geilo, Norway.

Contact Jens Overgaard, Experimental Clinical Oncology Department, Aarhus University Hospital, Norrebrogade, 44, Building 5, 8000 Aarhus C, Denmark, or call +45 89 49 26 29, or fax: +45 86 19 71 09

Radiation treatment planing

March 24-27, 1999

Principles and Practice of 3-D Radiation Treatment Planning will be held in Munich, Germany.

Contact DR. HJ Feldmann, Radiooncology Department, Technische Universität München, Klinikum rechts der Isar, or call +4 49 8941404512/11, or fax +49 8941404587

Radiophysics

April 6-11, 1999

5th Biennial ESTRO Meeting on Physics for Clinical Radiotherapy will be held in Göttingen, Germany.

Contact ESTRO office, Av. E. Mounierlaan, 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

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New drugs in cancer treatment

April 12-14,1999

The ESO training course will take place in Smolenice Castle, Slovakia.

Contact ESO office for Central and Eastern Europe, Ms. Dagmar Just, Ärztekammer fü Wien, Fortbildungsreferat, Weihburggasse 10-12, 4th floor, 1010 Vienna, Austria; or call +43 1 51501262; or fax +43 1 51501200; or e-mail just@aekwien.or.at

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Radiotherapy

April 12-15, 1999

Hadron Beam Therapy, Physics and Biology, will take place in Cape Town, South Africa.

Contact Dr Dan Jones, National Accelerator Centre, P O. Box 72, Faure, 7131, South Africa, or call +27 21 834 3820, or fax: +27 21 834 3382

Clinical research

April 18-22, 1999

ESTRO course on Methodology of Clinical Research, will be held in Como, Italy.

Contact ESTRO office, Av. E. Mounierlaan, 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

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Colorectal cancer

May, 1999

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

Good clinical practice in oncology

May 6-7, 1999

The ESO training course Good Clinical Practice – Scientific Aspects: What Kind of Clinical Trials Do Need in Oncology will take place in Vienna, Austria.

Contact ESO office for Central and Eastern Europe, Ms. Dagmar Just, Ärztekammer fü Wien, Fortbildungsreferat, Weihburggasse 10-12, 4th floor, 1010 Vienna, Austria; or call +43 1 51501262; or fax +43 1 51501200; or e-mail just@aekwien.or.at

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Gynecological oncology

May 8-13, 1999

The 11th International Meeting of Gynecological Oncology will take place in Budapest, Hungary.

Contact Prof. Dr. Péter Bõsze, 1301 Budapest, PO. Box 46, Hungary; or fax + 36 1 275 2172

Brachitherapy

May 10-12, 1999

Annual Brachytherapy Meeting GEC-ESTRO, will take place in Utrecht, Netherlands.

Contact ESTRO office, Av. E. Mounierlaan, 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

Breast pathology - oncology

May 11-15, 1999

The ESO training course 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

Radiology

May 17-19, 1999

The UK's Premier Radiological Meeting will be held in Birmingham, United Kingdom.

Contact Radiology 1999 Secretariat, 36 Portland Place, London, WIN 4AT, UK; or call + 44 (0) 171 307 1410; or fax + 44 (0) 171 307 1414

Paediatric oncology

May 17-19, 1999

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

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

Euthanasia in oncology

May 21-23, 1999

The ESO training course 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

Paediatric oncology

May 24-29, 1999

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

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

Epidemiology in cancer

May 27-29, 1999

The ESO training course will take place in Olomouc, Czech Republic.

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Lymphoma

May 28 - June 1, 1999

The Postgraduate ESMO/ESO course will take place in Monte Verita, Ascona, Switzerland.

Contact the ESMO Head office, Via Soldino 22, 6900 Lugano, Switzerland; or call +41 91 950 07 86; or fax + 41 91 959 07 87; or e-mail esmosecr@dial.eunet.ch

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Molecular oncology

May 30 - June 3, 1999

Course on Molecular Oncology for Radiotherapy will be held in Izmir, Turkey.

Contact ESTRO office, Av. E. Mounierlaan, 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

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Paediatric and medical oncology

May 31 – June 3, 1999

The ESO training course "Basic Science and Treatment in Paediatric and Medical Oncology" will take place in Yerevan.

Contact ESO Office for Russia and Community of Independent States, M. Vukelic, CSC Ltd, Heiligenstädter Strasse, 395b, 1190 Vienna, Austria; or call +43 1 3690444; or fax +43 1 369044420

Lymphoma

June 2-5, 1999

The VII. International Conference on Malignant Lymphoma will be held in Lugano, Switzerland.

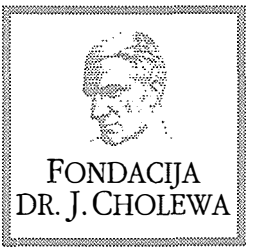
Contact the ESMO Secretariat, Via Soldino 22, 6900 Lugano, Switzerland; or call +41 91 967 54 11; or fax +41 91 967 57 44

High dose chemotherapy and autologous stem cell transplantation

June 3-5,1999

The ESO training course High Dose Chemotherapy and Autologous Stem Cell Transplantation in Cancer: Indications and Unresolved Questions will take place in Brijuni Island.

Contact ESO office for Central and Eastern Europe, Ms. Dagmar Just, Ärztekammer fü Wien, Fortbildungsreferat, Weihburggasse 10-12, 4th floor, 1010 Vienna, Austria; or call +43 1 51501262; or fax +43 1 51501200; or e-mail just@aekwien.or.at



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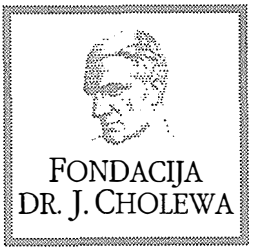

Activity of "Dr. J. Cholewa" foundation for cancer research and education - report for the first quarter of 1999

In the first quarter of 1999 the "Dr. J. Cholewa" foundation for cancer research and education continued with its activities, as already outlined in previous reports. The activity of the Foundation is slowly gathering momentum, and it is thus necessary to acknowledge the efforts of the majority of its members and supporters. As the activity of the Foundation needs considerable financial support, additional efforts shall be required to guarantee its continuation at the present level. Worldwide financial crisis left its impact on the willingness of the donors to contribute to the Foundation, which nevertheless managed to collect enough support not to compromise its previously stated goals.

Despite the problems mentioned in the previous paragraph, the Foundation continues to support regular publication of "Radiology and Oncology" international scientific journal, and the regular publication of the "Challenge ESO Newsletter", the newsletter of the European School of Oncology from Milan, Italy. Both medical journals mentioned are edited and published in Ljubljana, Slovenia. It is also worth mentioningthe educational grants awarded to three oncologists for their training in foreign countries, as well as the support the Foundation granted to the Organizing Committee of the 1st. Slovenian Congress of Surgery, held in the city of Maribor. All of this in addition to the grants and support mentioned before in the previous reports in "Radiology and Oncology" journal.

For 1999 the Foundation plans to continue to help with the provision of grants for the various European School of Oncology courses, and to support publishing and editorial activity from the various fields of oncology. Additional administrative help will thus be needed to ensure proper and smoothe execution of the Foundation's goals, with the necessary steps already taken in this direction.

" Dr. J. Cholewa" Foundation for Cancer Research and Education continues to pursue its goals, as defined by its statute and meetings of the Board of directors in the past year. It continues to cooperate with similar institutions in Slovenia and abroad, especially with the "Mali Vitez" Foundation from Ljubljana, Slovenia. In the rest of 1999 additional steps will have to be taken to further consolidate the Foundation financially.

Tomaž Benulič, MD
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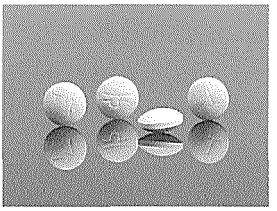

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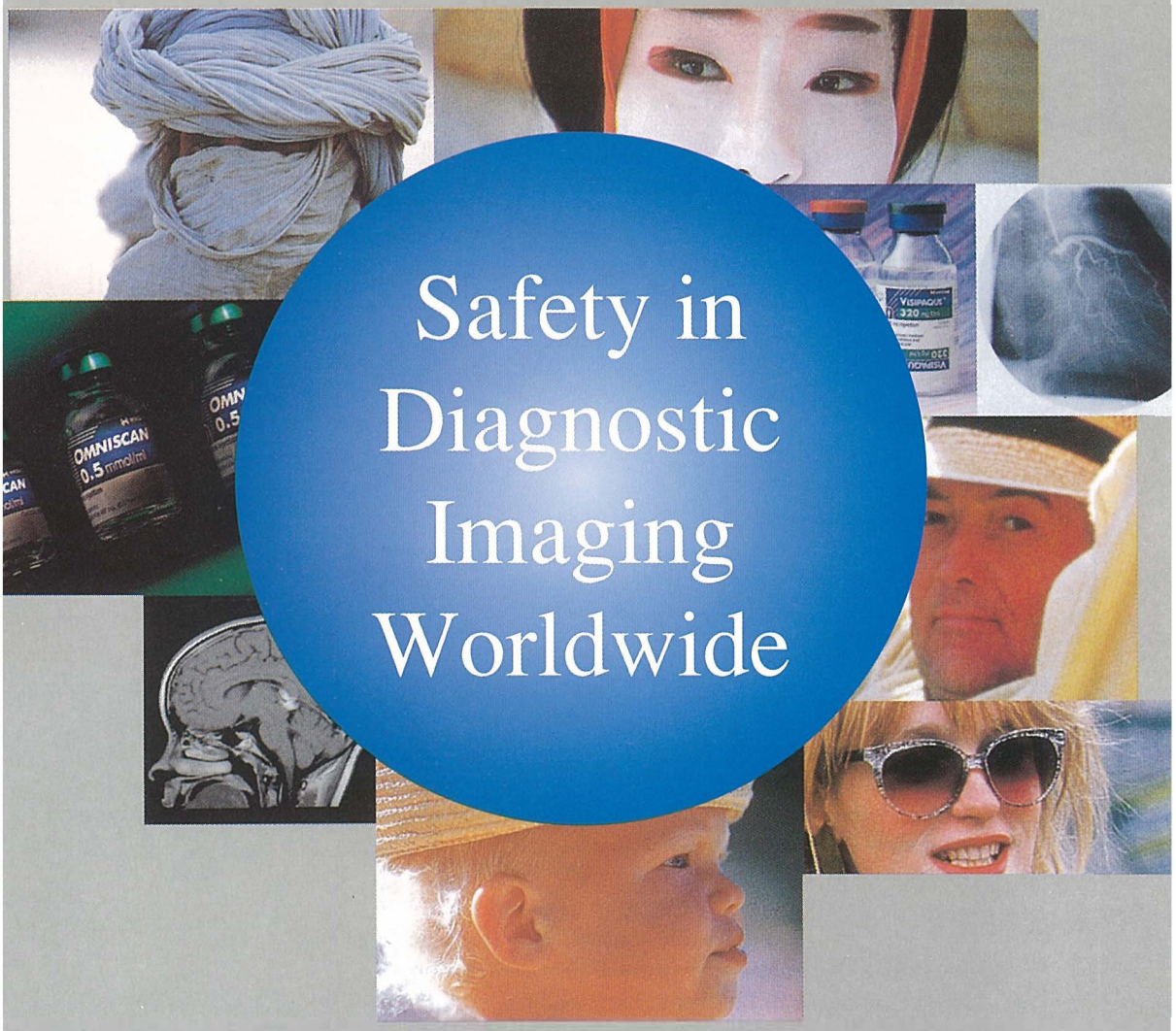

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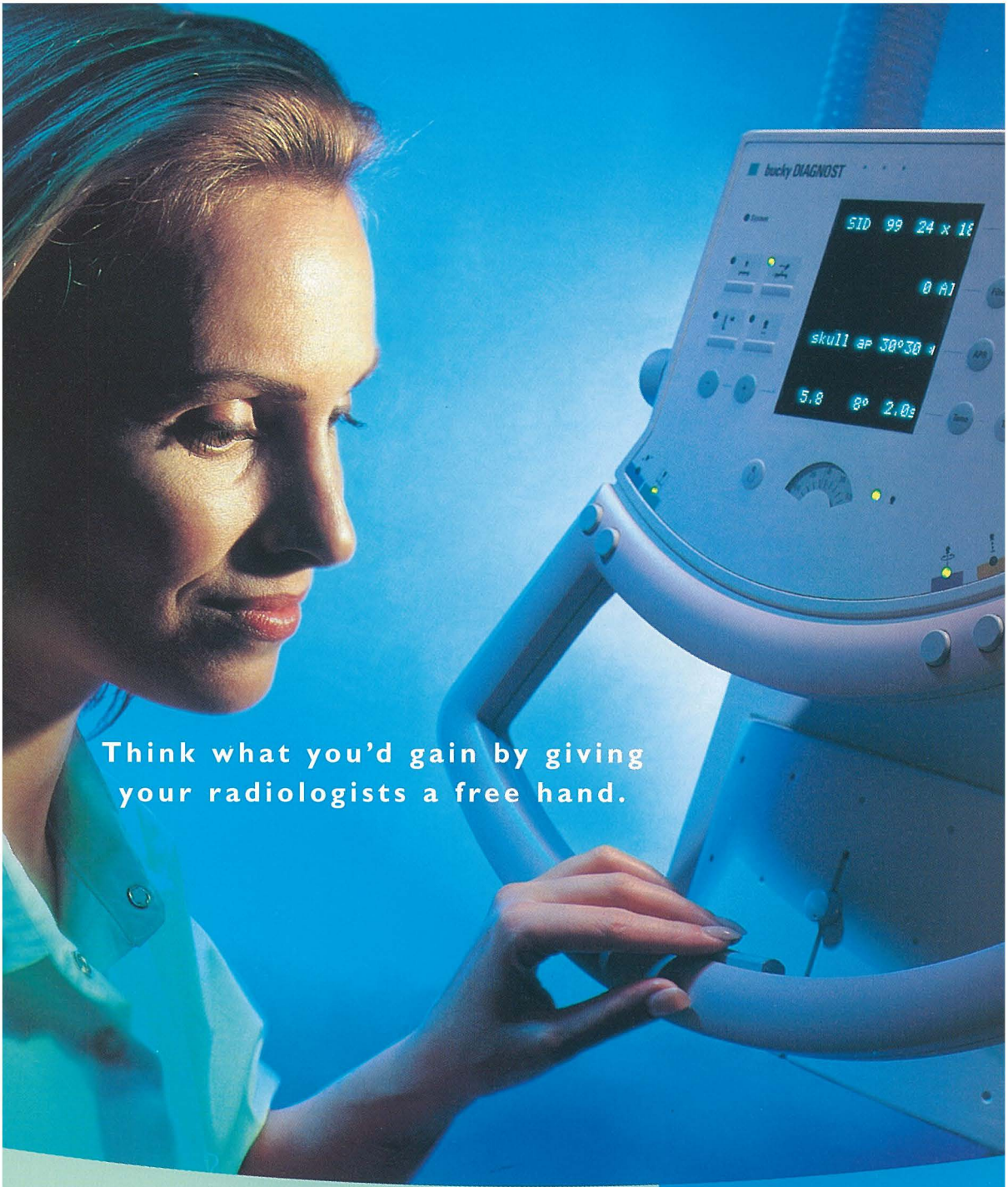

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kriptokokni meningitis	*prvi dan 400 mg,* *nato od 200 do 400 mg na dan*
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Manuscript written in English should be submitted to the Editorial Office in triplicate (the original and two copies), including 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).

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- **vedno 1-krat na dan**
- **vedno 5 mg**

Skrajšano navodilo za uporabo: Navoban® kapsule, Navoban® raztopina za injiciranje 2 mg in 5 mg. Serotoninski antagonist. **Oblika in sestava:** 1 trda kapsula vsebuje 5 mg tropisetronovega hidroklorida. 1 ampula po 2 ml vsebuje 2 mg tropisetronovega hidroklorida. 1 ampula po 5 ml vsebuje 5 mg tropisetronovega hidroklorida. **Indikacije:** Preprečevanje slabosti in bruhanja, ki sta posledici zdravljenja s citostatiki. Zdravljenje pooperativne slabosti in bruhanja. Preprečevanje pooperativne slabosti in bruhanja pri bolnicah, pri katerih je načrtovana ginekološka operacija v trebušni votlini. **Odmerjanje in uporaba:** Preprečevanje slabosti in bruhanja, ki sta posledici zdravljenja s citostatiki. **Odmerjanje pri otrocih:** Otroci starejši od 2 let 0,2 mg/kg telesne mase na dan. Največji dnevni odmerek ne sme preseči 5 mg. Prvi dan kot intravenska infuzija ali kot počasna intravenska injekcija. Od 2. do 6. dne naj otrok jemlje zdravilo oralno (raztopino v ampuli razredčimo s pomarančnim sokom ali koka kolo). **Odmerjanje pri odraslih:** 6-dnevna kura po 5 mg na dan. Prvi dan kot intravenska infuzija ali počasna intravenska injekcija. Od 2. do 6. dne 1 kapsula na dan. **Zdravljenje in preprečevanje pooperativne slabosti in bruhanja: Odmerjanje pri odraslih:** 2 mg Navobana z intravensko infuzijo ali kot počasno injekcijo. Glej celotno navodilo! **Kontraindikacije:** Preobčutljivost za tropisetron, druge antagoniste receptorjev 5-HT3 ali katerokoli sestavino zdravila. Navobana ne smemo dajati nosečnicam; izjema je preprečevanje pooperativne slabosti in bruhanja pri kirurških posegih, katerih del je tudi terapevtska prekinitev nosečnosti. **Previdnostni ukrepi:** Bolniki z nenadzorovano hipertenzijo; bolniki s prevodnimi ali drugimi motnjami srčnega ritma; ženske, ki dojijo; bolniki, ki upravljajo s stroji ali vozili. **Medsebojno delovanje zdravil:** Rifampicin ali druga zdravila, ki inducirajo jetrne encime. Glej celotno navodilo! **Stranski učinki:** Glavobol, zaprtje, redkeje omotica, utrujenost in prebavne motnje (bolečine v trebuhu in driska), preobčutljivostne reakcije. Zelo redko kolaps, sinkopa ali zastoj srca, vendar vzročna zveza z Navobanom ni bila dokazana. **Način izdajanja:** *Kapsule:* uporaba samo v bolnišnicah, izjemoma se izdaja na zdravniški recept pri nadaljevanju zdravljenja na domu ob odpustu iz bolnišnice in nadaljnjem zdravljenju. *Ampule:* uporaba samo v bolnišnicah. **Oprema in odločba:** Zloženka s 5 kapsulami po 5 mg; številka odločbe 512/B-773/97 z dne 10. 11. 1997. Zloženka z 1 ampulo po 2 ml (2 mg/2 ml); številka odločbe 512/B-772/97 z dne 10. 11. 1997. Zloženka z 10 ampulami po 5 ml (5 mg/5 ml); številka odločbe 512/B-771/97 z dne 10. 11. 1997. **Izdelovalec:** NOVARTIS PHARMA AG, Basel, Švica. **Imetnik dovoljenja za promet z zdravilom: NOVARTIS PHARMA SERVICES INC., Podružnica v Sloveniji, Dunajska 22, 1511 Ljubljana, kjer so na voljo informacije in literatura.**
Preden predpišete Navoban, prosimo preberite celotno navodilo.

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