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Is quantitative salivary gland scintigraphy a mandatory examination prior to and after radioiodine therapy?

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The aim of this study was to evaluate possible deterioration of salivary gland function due to low dose radioiodine therapy using quantitative salivary gland scintigraphy (qSGS). In addition, the prevalence of salivary gland impairment (SGI) was estimated in thyroid patients. Prior to routine thyroid scintigraphy, qSGS was performed after i.v. injection of 36–126 MBq Tc-99m-pertechnetate, and the uptake was calculated as a measure of parenchymal function. 144 patients underwent qSGS prior to and 3 months after radioiodine therapy. The prevalence of SGI was estimated from qSGS in another 674 patients submitted to thyroid scintigraphy. Despite salivary gland stimulation with ascorbic acid during radioiodine therapy a significant dose related parenchymal impairment of 15–90 % could be measured after the application of 0.4–24 GBq of I-131. The prevalence of SGI was 77/674 = 11.4 % and 52/674 = 7.7 % in one/two and three/four glands, respectively. Thus, qSGS should be applied in all patients prior to and after radioiodine therapy to quantify and to document both the preexisting and the treatment induced SGI even by low dose I-131. With respect to forensic reasons qSGS might even be applied mandatory.

Key words: salivary glands – radionuclide imaging – iodine radioisotopes – adverse effects

Introduction

Radioiodine therapy using I-131 has been known to be effective for almost 50 years both to reduce hyperthyroidism or to treat differentiated thyroid carcinoma and its iodine trapping metastases. Despite an almost selective uptake of iodine in thyroid cells it can be mistaken for chlorid due to its similar atomic diameter and its comparable electrical charge. This leads to an undesired accumulation of I-131 via an energy consuming $Na^+/K^+/2Cl^-$-co-transport[1-4] in acinar cells of salivary glands as well as in gastric parietal cells. Therefore, a parenchymal impairment of salivary glands is a well-known undesired side effect of high dose radioiodine therapy as used in thyroid cancer with cumulative activities up to 40 GBq I-131.[5-7] Consequently, radioiodine therapy is performed under salivary gland stimulation using sialogoga, e.g. chewing gum or vitamin C drops in order to minimize the intraglandular transit time of I-131 and, thus, to minimize salivary gland impairment.[6-11]

However, there are only rare data on parenchymal damage in salivary glands after low dose radioiodine therapy as used for the treatment of benign thyroid diesease. This is mainly due to the lack of an easy to perform method which yields quantitative data on parenchymal function of all major salivary glands. However, recently a normal data base for quantitative salivary gland scintigraphy has been established[12, 13] on a large number of healthy subjects, and its value for the detection of mild parenchymal impairment has been demonstrated successfully in Sjögren's syndrome.[14-17]

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The aim of this study was therefore first to enlarge this normal data base in order to reduce the standard deviation of these reference values, second to quantify possible parenchymal damage in salivary glands due to low dose radioiodine therapy performed under salivary gland stimulation and third to estimate the prevalence of salivary gland impairment in patients submitted to thyroid scintigraphy.

Materials and methods

Patients

In a total of 1.130 patients quantitative salivary gland scintigraphy (qSGS) was performed prior to thyroid scintigraphy. Therefore, an additional radiation burden was omitted, and all patients gave their informed written consent. Details of patient populations investigated are given in Table 1.

In order to enlarge our nomal data base published previously[12, 13, 16] we included 312 patients without any salivary gland diseases. Inclusion and exclusion criteria were described in detail elsewhere.[12]

144 patients underwent quantitative salivary gland scintigraphy prior to and 3 months after radioiodine therapy with different cumulative activities up to 24 GBq I-131. 68 patients received radioiodine therapy for hyperthyroidism and 11 for thyroid cancer. Details of these patients are given in Table 2. Radioiodine therapy was performed under salivary stimulating conditions by peroral application of 200 mg ascorbic acid (Cebion®) three times a day during their stay in hospital.[6–11]

The prevalence of salivary gland impairment was estimated from results of quantitative salivary gland scintigrapy obtained from another 674 patients submitted for thyroid scintigraphy. Prevalence was given both for single parenchymal damage covering one or two salivary glands and for global parenchymal damage covering three or four salivary glands.

Table 2. Applied cumulative activity of I-131 in GBq and details of patient characteristics of the radioiodine therapy group.

I–131	Age			
[GBq]	range	mean ± SD	n	f/m
0.4–0.6	25–74	44.2 ± 7.4	44	34/10
0.7–1.1	36–83	56.1 ± 9.3	41	30/11
1.4–1.5	28–76	46.9±8.7	25	19/6
3.0	20–81	42.5 ± 11.3	19	14/5
6.0	19–65	43.7 ± 9.4	9	5/4
24.0	29–76	58.4 ± 10.2	6	4/2

Quantitative salivary gland scintigraphy

Quantitative salivary gland scintigraphy was performed in a standardized method as described previously[12, 16] after intravenous injection of 36–138 MBq Tc-99m-pertechnetate. For quantification one rectangular ROI over the brain, which served as a common background ROI, and four irregular ROIs over both parotid and submandibular glands were used. ROIs were copied from the study performed prior to radioiodine treatment to the study obtained after radioiodine treatment.

As a measure for parenchymal function of major salivary glands the uptake of Tc-99m-pertechnetate was calculated in percentage of the injected activity.[18–26] For compensation of noise and, thus for stabilization of data, the uptake was averaged from 12–14 min p.i. ($U_{12\text{-}14}$). Saliva excretion was stimulated by 3 ml diluted lemon juice 15 min p.i., and excretion fraction (EF) was calcultated from mean uptake at 17–19 min p.i. ($U_{17\text{-}19}$) expressed in percent of the uptake ($U_{12\text{-}14}$) measured according to equation 1.

$$EF\,[\%] = \frac{U_{12\text{-}14} - U_{17\text{-}19}}{U_{12\text{-}14}} \cdot 100 \qquad \text{(Eq. 1)}$$

Table 1. Patient characteristics of different populations investigated.

	Age			
Group	range	mean ± SD	n	f/m
Normal date base	18–82	44.2 ± 7.4	312	216/96
Radioiodine therapy	19–83	55.1 ± 12.3	144	106/38
Thyroid scintigraphy	19–83	48.7 ± 9.4	674	453/221

In order to test the reproducibility of salivary gland scintigraphy the same scintigram of a patient from the normal data base was reevaluated 10 times by 13 differently experienced technicians. Intraobserver reproducibility was expressed as variation coefficient and relative variation coefficient. To estimate the interobserver reproducibility the mean of both uptake and excretion fraction derived of all technicians was first calculated. Interobserver reproducibility was then expressed as percent deviation of each individual technician from this mean.

Statistics

Results are given as mean ± one standard deviation. Two-tailed students t-test for unpaired data were used to evaluate statistical differences, with $p < 0.05$ considered to be statistical significant.[27] Individual results of patients below the 2-sigma range of the normal data base were taken as pathological.

Results

Normal data base

The original scintigramm of a patient from the normal data base is shown in Figure 1. The mean ± one standard deviation of Tc-99m-pertechnetate uptake of all patients is given in Figure 2 for the major salivary glands. The corresponding normal values of Tc-99m-pertechnetate uptake and excretion fraction are summerized in Table 3. Tc-99m-pertechnetate uptake amounted to 0.45 ± 0.14 % and 0.39 ± 0.12 % for parotid and submandibular glands, respectively. The corresponding values for excretion fraction were 49.5 ± 10.6 % and 39.1 ± 9.2 %.

Excellent values were found for intraobserver reproducibility indicated by variation coefficients of 0.3 and relative variation coefficients of below 2 %. The deviation of the individual mean from the mean of all technicians of 1.1–5.2 % indicated an excellent interobserver reproducibility as well.

Parenchymal impairment after radioiodine

The uptake of Tc-99m-pertechnetate of parotid and submandibular glands prior to and after radioiodine therapy as well as the decrease of Tc-99m-pertechnetate uptake in percent of pretreatment values are given in Table 4 and Figure 3. A significant activity related decrease in parenchymal function could be shown in all subgroups even after as less as 0.4-0.6 GBq I-131. Parenchymal impairment increased from 0.15 % after 0.4-0.6 GBq I-131 to about 90 % after 24 GBq I-131. A patient receiving 6 GBq I-131 is shown in Figure 4.

Prevalence of parenchymal impairment

Significant parenchymal impairment was seen in 129/674 patients. Of these, one or two salivary glands were affected in 77/674 = 11.4 %, and three or four salivary glands were affected in 52/674 = 7,7 % of these patients.

In 69 of these 129 patients the etiology of parenchymal impairment could be evaluated successfully. In single salivary gland damage chronic sialolithiasis and external radiation therapy could be detected in 32 and 7 patients, respectively. In patients with global salivary gland impairment rheumatic diseases and drugs with anticholinergic side effects, i.e. neuroleptic and antidepressant drugs, were causal in 10 and 20 patients, respectively. However, in 60/129 =

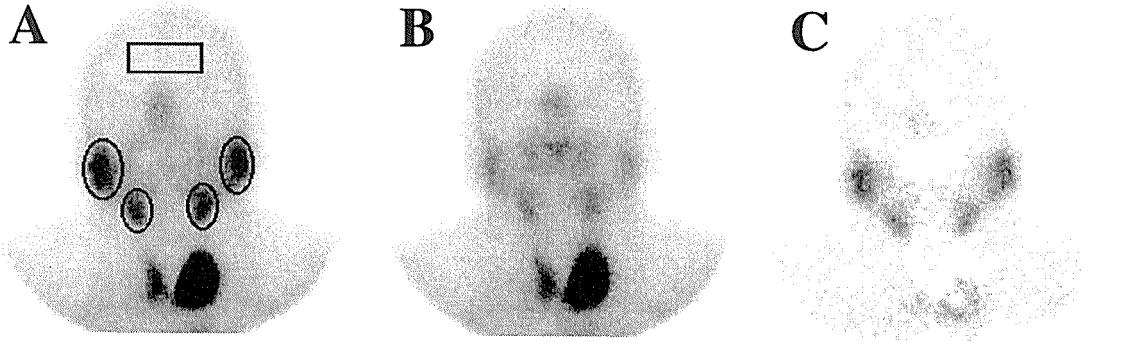

Figure 1. Sialoscintigramm of a normal patient at 13 min after injection of Tc-99m-pertechnetate prior to (A) and at 18 min p.i. after 3 ml of lemon juice p.o. as a sialogogum (B), and parametric image (C) for visualization of saliva excreted from salivary glands. ROIs used for quantification are shown in A.

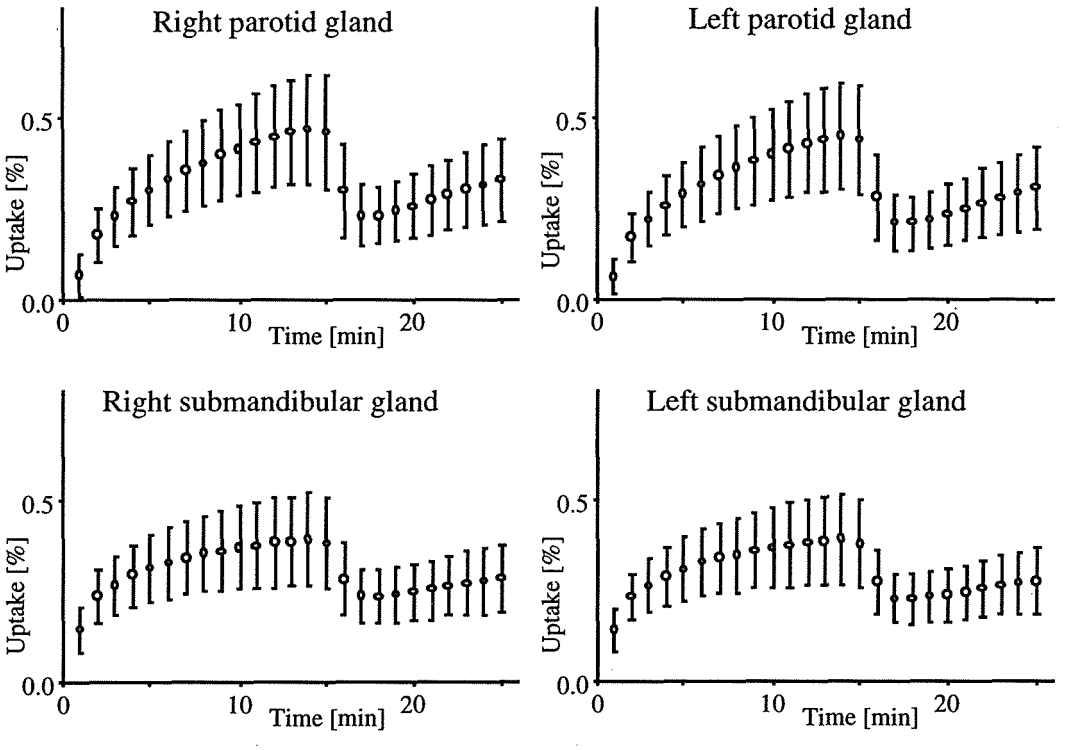

Figure 2. Time-activity-curves of Tc-99m-pertechnetate uptake in major salivary glands. Mean ± one standard deviation of 312 patients.

Table 3. Normal values of Tc-99m-pertechnetate uptake in percent of injected activity, and excretion fraction (EF) in percent of uptake. Numbers represent mean ± one standard deviation (n = 312). RPG, LPG: right, left parotid gland. RSG, LSG: right, left submandibular gland.

	RPG	LPG	RSG	LSG
Uptake [%]	0.46 ± 0.15	0.44 ± 0.14	0.39 ± 0.12	0.39 ± 0.12
EF [%]	48.2 ± 10.5	50.9 ± 10.5	38.2 ± 9.1	39.9 ± 9.2

Table 4. Mean ± one standard deviation of pertechnetate uptake in percent of injected activity in salivary glands prior to and after radioiodine therapy with different cumulative activities of I-131 in GBq, and decrease of parenchymal function in percent of pretreatment values (Δ Uptake). Note, that left and right parotid and submandibular glands were lumped together, respectively. a: decrease of parenchymal function was calculated versus normal data base since no pretreatment values were available.

Cumulative ativity I–131	n	Parotid glands			Submandibular glands		
[GBq]		prior to I–131	after I–131	Δ Uptake	prior to I–131	after I–131	Δ Uptake
0.4–0.6	44	0.42 ± 0.14	0.36 ± 0.13	14.3	0.36 ± 0.13	0.31 ± 0.11	13.9
0.7–1.1	41	0.40 ± 0.19	0.33 ± 0.13	17.5	0.35 ± 0.16	0.28 ± 0.10	20.0
1.4–1.5	25	0.38 ± 0.12	0.30 ± 0.14	21.1	0.32 ± 0.15	0.23 ± 0.14	28.1
3.0	19	0.44 ± 0.17	0.32 ± 0.16	27.3	0.37 ± 0.11	0.25 ± 0.09	32.4
6.0	9	0.46 ± 0.10	0.29 ± 0.12	34.8	0.42 ± 0.21	0.29 ± 0.19	30.9
24.0	6		0.04 ± 0.03	90.9[a]		0.05 ± 0.02	86.8[a]
Normal data base	312	0.45 ± 0.14			0.39 ± 0.12		

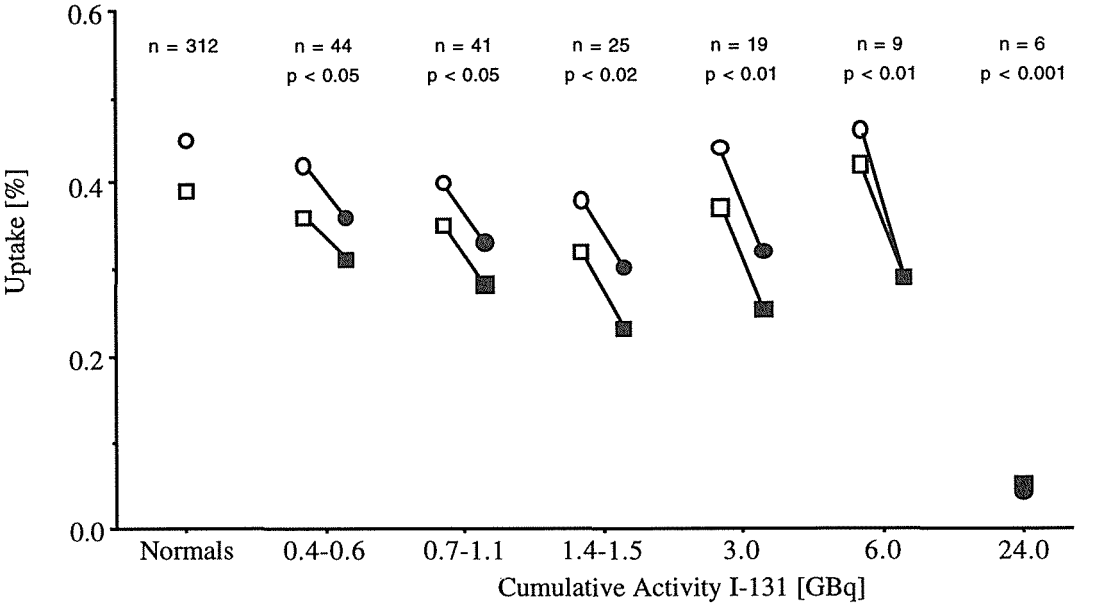

Figure 3. Mean uptake of pertechnetate in parotid (circles) and submandibular (squares) glands in normals and patients prior to (open symbols) and after (filled symbols) radioiodine treatment with increasing cumulative activities of I-131. Note, that left and right parotid and submandibular glands were lumped together, respectively. For standard deviation see table 4.

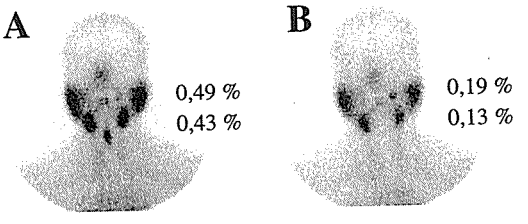

Figure 4. Sialoscintigramms 13 min after injection of Tc-99m-pertechnetate of a patient prior to (A) and and 3 months after (B) radioiodine therapy with 6 GBq-I-131 due to follicular thyroid carcinoma. Numbers denote mean uptake of Tc-99m-pertechnetate in percent of injected activity in parotid (upper) and submandibular (lower) glands, respectively. Observe the small ectopic thyroid remnant within the thyroglossal duct prior to therapy.

46.5 % of the patients with parenchymal impairment there was no reason detectable for their deminished uptake of Tc-99m-pertechnetate.

Discussion

Normal data base

A valid quantification of salivary gland function is mandatory to detect even mild or beginning parenchymal impairment.[17] Beside various semiquantitative and not routinely practical methods (for an overview see[12]) the calculation of Tc-99m-pertechnetate uptake in percent of the activity applied has been suggested[18–26] in analogy to well established state-of-the-art quantitative thyroid scintigraphy.[28] However, variable study protocols, small patient numbers and a lack of inclusion and exclusion criteria may be the main reasons for clearly different reference values and markedly enhanced standard deviations.

On the other hand, a reduction of standard deviation is desirable in order to ease the differentiation of normal and pathological parenchymal functions. This can be achieved both by enhancing the number of patients and by thoroughly selected inclusion and exclusion criteria. In our study Tc-99m-pertechnetate uptake was 0.45 ± 0.14% and 0.39 ± 0.12% for parotid and submandibular glands, and excretion fraction was 49.5 ± 10.6% and 39.1 ± 9.2%, respectively. The validity of our reference values is supported first by the similar shape of our time-activity-curves (see Fig. 2) and those reported as N(ormal)-type curves derived from qualitative salivary gland scintigraphy[29, 30] and second by successful demonstration of mild parenchymal impairment in patients suffering from ongoing Sjögren's syndrome.[17]

The excellent values measured for both intra- and interobserver reproducibility indicate a high reproducibility of salivary gland scintigraphy. Therefore, variability of Tc-99m-pertechnetate uptake of the reference values probably reflect physiological variability of parenchymal function in salivary glands.

Parenchymal impairment after radioiodine

Parenchymal impairment of salivary glands as an undesired side effect of high dose radioiodine therapy as used in thyroid cancer with cumulative activities up to 40 GBq I-131 could be shown in up to 80 % of the patients.[5–7] Consequently, radioiodine therapy is performed under salivary gland stimulation using sialogoga, e.g. chewing gum or vitamin C drops in order to minimize the intraglandular transit time of I-131 and, thus, to minimize undesired parenchymal impairment.[6–11] However, there are only limited data on parenchymal damage in salivary glands after low dose radioiodine therapy as used for treatment of benign thyroid disease.

Radioiodine therapy was performed under salivary stimulating conditions using ascorbic acid perorally three times during the patients stay in our therapeutic ward.[6–11] However, despite this commonly accepted procedure a dose related decrease of parenchymal function could be shown even after as less as 0.4-0.6 GBq I-131. This mild impairment of parenchymal function measured after radioiodine treatment did not cause any hyposialia which is in agreement to common clinical experience due to several reasons. First, a loss of function of some 15 to 33% could be demonstrated after low dose radioiodine therapy. Second, it is known from various diseases of both endocrine and exocrine glands that a loss of up to 90% of parenchyma is necessary to result in clinical symptoms, e.g. diabetes mellitus, diabetes insipidus, Sjögren's syndrome, chronic pancreatitis with exocrine insufficiency, hypopituitarism.[31] Third, patients with high dose radioiodine treatment with 24 GBq complained about hyposialia/asialia. The latter is in good agreement both with our observation that parenchymal function was impaired to about 90% and with data reported in the literature.[5, 16]

Impairment of parenchymal function was measured 3 months after radioiodine therapy. It is unclear up to now whether repair mechanisms may lead to a (partial) restoration of parenchymal function. This is suggested by a long-term follow-up in a few of our patients (unpublished data).

Quantification of parenchymal impairment with respect to forensic reasons

As to our knowledge there are no valid data in the literature concerning the prevalence of parenchymal impairment in salivary glands. Most probably this might be due to a lack of an easy to perform examination which yields valid quantitative data on salivary gland function. Using quantitative salivary gland scintigraphy we found a pathologically ecreased parenchymal function in 19.1% of 674 patients investigated. Single and global parenchymal dysfunction contributed approximately to equal amounts. Beside common reasons of global parenchymal impairment, e.g. rheumatic diseases, drugs with anticholinergic effect, i.e. neuroleptic and antidepressant drugs should be kept in mind.[32] However, we could not evaluate any reason in as much as 60/129 = 46% of these patients even though the patients history was obtained very carefully.

However, both the knowledge and extent of any kind of preexisting parenchymal damage is essential since salivary glands may be damaged even after low dose radioiodine. Consequently, clinical significant functional impairment may occur, and forensic problems may arise. Therefore, it is essential not only to inform patients with preexisting parenchymal impairment about possible occurence of hyposialia but to increase salivary gland stimulation during radioiodine therapy. Thus, quantitative salivary gland scintigraphy should be performed in all patients prior to and after radioiodine therapy in order to exclude or to quantify radioiodine induced parenchymal impairment in salivary glands.

Conclusions

Quantitative salivary gland scintigraphy is an easy to perform method with excellent intra and interobserver reproducibility which can be performed prior to thyroid scintigraphy without any additional radiation burden. It should be performed prior to radioiodine therapy in order to document salivary gland function, and it should be repeated 3 months after radioiodine therapy in order to either exclude or quantify possible radioiodine induced parenchymal impairment of salivary glands. With respect to forensic reasons quantitative salivary gland scintigraphy might even be applied mandatory.

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Relative DNA concentration in thyrocytes from scintigraphically hot nodi

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The aim *of our study was to study the cytological appearance and the relative DNA content of scintigraphically hot thyroid nodi.*
Methods: *Sixty-seven patients with hyperthyroidism due to hot nodi were treated. The relative DNA content of thyrocytes in hot nodi was determined by single cell cytophotometry and compared to results of cytology, and scintigraphy. T4, T3, TSH and thyroglobulin were measured in sera of the patients as well.*
Results: *The modal value of the relative DNA concentration in thyrocytes was in 16 hot nodi diploid (Type 1), in 21 hyperdiploid (Type 2). The 12 nodi with diploid (Type 3) as well as 18 with hyperdiploid (Type 4) modal value of the relative DNA concentration had signs of increased proliferation. The thyrocytes of 4 normal controls were diploid. Cytomorphological signs of atypia and degenerative changes of thyrocytes were significantly more frequent in Types 3 and 4 than in Types 1 and 2.*
Conclusion: *Dominant scintigraphically hot thyroid nodi are diploid or hyperdiploid. Some of them are in the state of proliferation. DNA cytophotometry can be useful as an additional diagnostic method in cases with thyroid (hot) nodi of uncertain cytology, especially when therapy with low dose of radioiodine is planned.*

Key words: hyperthyroidism – radionuclide imaging; DNA; cytophotometry

Introduction

Autonomous nodi are present in about 40% of patients with endemic goiter.[1] Scintigraphy with technetium (^{99m}Tc) or iodine (^{131}J) regularly shows an autonomous nodule as a hot spot with a more or less suppressed paranodal thyroid gland. A hot spot on the scan of the thyroid can present a true adenoma, a hyperplastic clone of hyperactive autonomous thyrocytes or a highly differentiated follicular carcinoma. It is therefore desirable to differentiate among these conditions before the therapy is given, although in general the incidence of thyroid carcinoma is low.[2,3] The aim of our study was to determine a pattern of DNA distribution in hyperactive thyroid nodi.

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Materials and methods

Subjects

Sixty-seven hyperthyrotic patients with autonomous goiter, sent to our department for routine investigations before the therapy with radioiodine, were in-

cluded in the study. The inclusion criterion was the presence of a single hot node or utmost 3 hot nodi with a discernible dominant hot node. There were 57 females and 10 males, aged 43 to 89 years (mean 61.5 years).

The normal *control group* consisted of 4 females, aged 33 to 37 years, sent to our department for suspected autoimmune thyroiditis. In each of them thyroid disease was excluded by hormonal tests, negative thyroid autoantibodies and by cytological examination of the thyroid.

Methods

The final clinical diagnosis of thyroid disease was based on the disease history, the physical examination of the patients, the results of thyroid cytology, ultrasonography, scintigraphy, serum T4, T3, TSH and thyroglobulin concentration. The results of cytophotometry were compared with cytomorphology (cytology) and final diagnosis.

Scintigraphy and ultrasonography

The planar scintigraphy of the thyroid was performed with Siemens Basicam gamma camera, 20 minutes after intravenous application of 80 MBq of ^{99m}Tc-pertechnetate. Scintigram with 2 MBq of ^{131}J was accomplished 24 hours after the oral application of radiotracer. Ultrasonography (US) of the thyroid was performed by using high resolution transducer (10 MHz, Diasonics DRF 300). The diameter of dominant hot nodi was measured with US. Scintigraphically hot nodi were identified on the ultrasound scan with the help of scintigrams. Fine needle biopsy of a dominant hot node, guided by US, was done after scintigraphy. Smears for cytology and the single cell cytophotometry were prepared from each sample obtained with fine needle aspiration biopsy.

Cytomorphology

Smears were stained by the May-Gruenewald-Giemsa method. The morphological changes in thyroid cells were grouped into 6 classes:

1 normal thyrocytes
2 hyperactive thyrocytes
3 thyrocytes with degenerative changes
4 proliferation or atypical thyrocytes
5 malignancy suspected
6 definite malignancy

Cytochemical DNA assessment

The single cell cytophotometry was performed after the Feulgen staining procedure, including acid hydrolysis in 4 N HCl at 28°C for 60 min. DNA measurements were carried out on microspectrophotometer Opton USPM 30/50 at wave length of 580 nm and diaphragm 0.63. The objective's magnification was 25x or 40x. Processing was done by the computer. Hundred and fifty to 200 thyrocytes and 25-100 leucocytes were evaluated in each smear. The modal relative DNA concentration of leucocytes in the same thyroid biopsy smears served as a reference value for the normal diploid DNA concentration, the so called "L" value.[4,5] The thyrocytes with the modal relative DNA concentration were considered to be in G1 (gap1) phase of cell division cycle, G2 (gap 2) phase was double modal value. The cells with the intermediate DNA concentration were considered to be in S (synthesis) phase. According to their relative DNA content, thyrocytes from hot nodi were classified as diploid ($0.75<G1<1.25$ L) and hyperdiploid (1.25 L $<$ G1). The results were expressed as percentage of thyrocytes in each class. DNA frequency distribution histograms of thyrocytes in hot nodi were compared to the histograms of leucocytes and of normal thyrocytes in control patients.

Biochemical analyses

The analysis of serum total triiodothyronine – T3 (normal 1.09-3.12 nmol/l) was done with the RIA method (SPAC T3 – Byk-Sangtec, Dietzenbach), total thyroxine – T4 (normal 53-182 nmol/l) with SPAC T4 – Byk-Sangtec, Dietzenbach, thyrotropin – TSH (normal 0.17-4.05 mE/l) was measured with the immunoradiometric method, IRMA (Imunotech, Marseille) and thyroglobulin – Tg (normal 0-34 µg/l) with the RIA method (Henning, Berlin).

Statistical analysis

Mean, standard deviation, median and modal values and, when appropriate, Chi square test were calculated.

Results

Scintigraphy was performed in 67 patients:
37 pts had a solitary hot node,
18 pts had 2 hot nodi (with 1 dominant node),
12 pts had 3 hot nodi (with 1 dominant node),

The thyroid scintigrams performed with ^{99m}Tc-pertechnetate showed the same distribution pattern of radioactivity as the scintigrams with ^{131}J in all patients.

Results of cytology, ultrasound investigation, hormonal analyses and Tg of 67 patients with hot nodi are given in Tables 1 and 2.

Table 1. The size and cytological class of 67 scintigraphically hot nodi.

	units	mean ± sd	median	mode
radius of nodi	cm	2.7 ± 0.7	3	3
cytology	class	2.07 ± 1.1	2	1

Table 2. TSH, T4, T3 and Tg in plasma of 67 patients with scintigraphically hot nodi.

	units	mean ± sd	median
TSH	mE/l	0.08 ± 0.13	0.04
T 4	nmol/l	151.4 ± 45.5	139
T 3	nmol/l	3.47 ± 0.94	3.39
Tg	ug/l	58.7 ± 46.9	46

DNA measurements

According to the cellular DNA content in hot nodi we noticed four different types of DNA frequency distribution histograms (See also Table 3.):

Type 1- modal class was diploid (mean relative DNA concentration was 1.24 ± 0,05L),

Type 2 – modal class was hyperdiploid (mean relative DNA concentration was 1.57± 0,13 L),

Type 3 – modal class was diploid but some polyploid cells were noticed.

Type 4 – modal class was hyperdiploid and some polyploid cells were noticed.

There was no significant difference in percentage of cells in S phase between different types.

The results of cytomorphology were compared with individual types of DNA frequency distribution histograms in Table 4.

The *cytomorphology* in group of patients with DNA frequency distribution histograms of the types 1 and 2 differed significantly from the types 3 and 4 ($p<0.001$). There was no statistically significant difference in the results of cytomorphological examination between types 1 and 2, or 3 and 4.

Final diagnosis

By the clinical investigation and the hormone analysis subclinical hyperthyroidism was proved in two patients with antonomous nodi wheres overt moderate hyperthyroidism due to toxic nodular goiter was proved in 65 patients. One patient had histologically verified follicular carcinoma within the solitary hot nodule (cytologic class was 4, DNA frequency distribution histogram was type 4). One patient had oncocytic tumor within 3 hot nodi (cytological class 5, DNA frequency distribution histogram was type 3).

In the control euthyrotic group, all the results were within the normal limits.

Discussion

Two types of hormone producing scintigraphic hot nodi were found considering the modal value of the relative DNA concentration in thyrocytes: diploid and hyperdiploid. The proliferation was apparent in some of these nodi. Atypia as noted with the

Table 3. Types of DNA frequency distribution in 67 scintigraphically hot nodi compared to normal thyroid (controls) and leucocytes.

Histogram	Patients (N)	G1(%)	S (%)	G2 (%)	>G2 (%)
Type 1	16	94 ± 6	5 ± 6	0.25 ±0.5	1 ± 1.7
Type 2	21	94 ± 5	4 ± 4	0.7 ± 1.4	0.1 ± 0.4
Type 3	12	68 ± 19	5 ± 3	23.9 ± 17	2.7 ± 2.8
Type 4	18	78 ± 13	8 ± 8	14 ± 12	1.7 ± 2.9
Controls	4	96 ± 3.6	1-8	0	0
Leucocytes	67	100	0	0	0

Legend:
G1, G2, S (%) – percentage of thyrocytes in individual phases of cell division cycle
>G2 – percentage of thyrocytes with more than tetraploid DNA concentration

Table 4. The comparison of cytomorphologic results in different types of DNA frequency distribution histograms in 67 hot nodi.

Histogram	Patients (N)	Active thyrocytes (% pts)	Aniso-nucleosis (% pts)	Oncocytes (% pts)	Degenerated thyrocytes (% pts)	Microfollicles (% pts)
Type 1	16	100	0	0	0	10
Type 2	21	80	5	0	10	15
Type 3	12	83	42	8	33	25
Type 4	18	94	17	6	39	11

cytomorphology was more frequent in nodi with the signs of proliferation in the DNA frequency distribution histogram.

In a recent study the somatic mutation of TSH receptor gene was shown in the major part of autonomous nodi. The proof of this mutation might have an implication on the prognosis of thyroid adenoma.[6] According to standards somatic mutation is present in 58 % of autonomous nodi in our study. It is apparently more frequent among solitary hot nodi where the average relative DNA concentration was slightly higher than in groups with 2 or 3 hot nodi.

Several authors assume the aneuploidy characteristics of true adenomas.[7-10] Also Lukasz found predominant hyperdiploidy in thyroid adenomas.[11] According to these authors it is conceivable that among hot nodi in our patients those with the DNA frequency distribution histograms of Types 2 and 4 are true adenomas. In favour of this it is also the cytomorphology, which showed higher grades of atypia in these nodes.

Among 67 hot nodi in our patients 2 (2.8%) were malignant. Citomorphologically one patient had follicular carcinoma (solitary hot node) and the other one (3 hot nodi) oncocytoma. In both cases the single cell cytophotometry showed the DNA distribution histograms reflecting proliferation and hyperdiploidy. Since similar changes were found in some benign follicular nodi as well, such changes can not be considered a proof of malignancy. Contrary to our experience, Bengtsson et al.[12] considers the DNA cytophotometry convenient for the differentiation of malignant from benign lesions in the thyroid. Most other authors believe that DNA cytophotometry cannot reliably differentiate between thyroid cancer and benign thyroid adenoma,[7-9, 13-14]

The percentage of malignant hot nodi in our patients was small but significant. After our opinion cytomorphology is therefore mandatory in all dominant, especially solitary, hot nodi before the therapy, especially if the radioiodine therapy is considered. When cytomorphology is inconclusive, the DNA cytophotometry can be of a help in the final decision about the therapeutic plan.

Conclusion

Two types of DNA frequency distribution histograms are found in scintigraphically hot nodi of the thyroid: one with diploid and the other with the hyperdiploid modal DNA value. Increased proliferation was noted in some hot nodi. Both, benign and malignant hot nodi in the thyroid could be diploid or hyperdiploid.

The DNA cytophophotometry can be useful as an additional diagnostic tool in the assessment of hot nodi before the therapy, especially when the results of cytology are ambiguous.

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Diagnostic value of currently available tumor markers in thyroid cancers

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In the follow-up management of cancer patients, tumor markers are a powerful and essential tool for both early detection of tumor progress, i.e. relapse or metastases, and monitoring therapy response. In thyroid cancer well-established tumor markers are available for the most common tumor types such as papillary and follicular carcinomas of the thyroid epithelium and for medullary thyroid carcinoma, which arises from the parafollicular C-cells of the thyroid gland. The tumor markers thyroglobulin, calcitonin, and carcinoembryonic antigen and their employment in routine clinical work-up in patients with thyroid cancer are presented. Furthermore, tissue polypeptide antigen and neuron-specific enolase as potential tumor markers in selected cases of thyroid cancer are discussed.

Key words: thyroid neoplasms – diagnosis; tumor markers, biological; thyroglobulin – calcitonin – carcinoembryonic antigen – tissue polypeptide antigen – neuron-specific enolase

Introduction

Carcinomas are the most common tumor type of thyroid malignancies. They may be classified into two varieties depending on whether the tumor arises in thyroid follicular epithelium or from the parafollicular C-cells. The general histologic types of the former one are the well-differentiated papillary and follicular carcinoma including the oxyphilic cell subtype, and, least common, the histologically undifferentiated anaplastic carcinoma. The tumor type arising from the C-cells is the so-called medullary thyroid carcinoma (MTC), which occurs familial at least in 10%. It usually appears as a component of multiple endocrine neoplasia (MEN) tpye IIa or IIb. The thyroid may also be the site of other rare tumors such as squamous cell carcinomas, various kinds of sarcomas and lymphoproliferative diseases, and metastases from primary tumors elsewhere.

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Tumor markers in thyroid cancer

The term tumor marker in connection with thyroid cancer implicates a somewhat different and unique quality showing that the most important markers – i.e. thyroglobulin and calcitonin – are not specific antigens of tumor tissue but common specific components of normal thyroid tissue. Their use is based on a unique option in thyroid cancer treatment, namely on the possibility of completely removing any thyroid tissue by combined surgical and radioiodine therapy. Thus, the appearance of any thyroid-specific substrate in the patients plasma after total thyroid ablation is highly indicative of recurrence and/or metastases.

Next to the mostly used tumor markers thyroglobulin and calcitonin applied in the sense mentioned above, "classical" tumor markers such as carcinoembryonic antigen, tissue polypeptide antigen, and neuron-specific enolase, which are mainly used

in others than tumors of the thyroid gland, may be useful for follow-up examinations in selected cases. In the following short review, the tumor markers and their indications in thyroid cancers are described.

Thyroglobulin (TG)

TG is an iodinated glycoprotein peculiar to the thyroid, which is essential for synthesis and storage of thyroid hormones.[1] Under physiological conditions usually small amounts of TG can be found in the blood with normal serum levels of less than 50 ng/ml. Since TG is exclusively produced by thyroid epithelium cells, serum concentrations should be less than 2 ng/ml in athyrotic state. For follow-up examinations the total absence of thyroid tissue is required as achieved by thyroidectomy and subsequent ablative radioiodine therapy.[2] Since TG release is stimulated by TSH, TG measurements should be performed under TSH elevation, i.e. 2–4 weeks after stopping exogenous thyroxin hormone substitution.[3] Under TSH supression there may be low TG serum concentrations and, thus, false negative results in 10–20% of the patients with even extended thyroid cancer including metastases or local recurrences.

Since false negative results may also be caused by plasma antibodies directed against TG, the determination of TG antibody concentrations and recovery measurements after adding a defined amount of TG are essential for reliable TG measurements.[4] Taking these prerequisites into account, any post-therapeutic TG elevation indicates either remnant thyroid tissue requiring further ablative treatment or it is indicative of metastases or local recurrences. TG plasma concentrations correlate well with tumor mass thereby indicating successful therapy. Thus, TG is a useful tumor marker for both therapy monitoring and follow-up examinations in patients with differentiated thyroid carcinoma, i.e. follicular, papillary, and oxyphilic cell carcinomas.[3] Since TG is usually produced only by differentiated thyroid carcinomas, TG is neither of use in nearly all cases of anaplastic carcinomas nor in MTC.

Tissue Polypeptide Antigen (TPA)

TPA is a cytokeratin-related non-specific proliferation marker for nearly all kinds of carcinomas. Its sensitivity for thyroid cancer including papillary, follicular, and medullary thyroid carcinoma, however, is only about 40–60% showing a good correlation to tumor progression or therapeutic response with a high positive predictive value of 90%.[4] In combination with more specific tumor markers such as TG or calcitonin both the sensitivity and the specificity for thyroid cancer can be increased. Additionally, TPA may be used as a substitute for standard thyroid tumor markers in patients with non-reliable tumor marker values, e.g. in patients with high levels of anti-thyroglobulin antibodies.

Calcitonin (CT)

CT, a peptide hormon for regulating calcium metabolism, is produced in the C-cells of the thyroid gland, and, to some extent, in the central nervous system. CT release is stimulated by increasing calcium levels as well as by pentagastrin and other hormones of the gastrointestinal tract. In thyroid cancer CT is a highly specific and sensitive tumor marker for diagnosis and follow-up of MTC. In patients with clinically manifested MTC the serum levels of CT are elevated in about 90% of the cases so it can be used for differential diagnosis in suspected thyroid cancer.[4] Furthermore, CT measurement in side-specific jugular venous blood sampling may be helpful for the localization of occult MTC.[3,4] After tumor removement normal serum concentrations indicate successful therapy. However, for follow-up examinations as well as for screening tests in patients with suspected MEN IIa/IIb or their relatives a pentagastrin stimulation test is mandatory for efficient diagnosis.[4] After intravenous injection of 0.5 μg pentagastrin per kg body weight only normal serum levels with no significant increase up to 5 min p.i. as compared to basic CT levels sufficiently exclude MTC or any relapse while a more than 2-fold increase of CT is evident for MTC.

Carcinoembryonic Antigen (CEA)

CEA is an unspecific tumor marker with a good sensitivity for colorectal cancer, breast cancer, and MTC. Since up to 10% of extended MTC have no increase of CT serum concentrations even after stimulation with pentagastrin a combination of CT and CEA is recommended for the follow-up of pa-

tients with MTC. The diagnostic sensitivity of CEA ranges from 10 to 80 % for MTC depending on the stage of disease. However, there is only a weak correlation of CEA serum concentration and tumor relapse with a positive predictive value of 70 %.[4] Therefore, CEA provides essential information only in those subjects with no CT release and, thus, false negative CT tests. Furthermore, CEA may serve as a tumor marker in anaplastic thyroid carcinomas in which TG serum levels are in the normal range in most cases. However, increased CEA serum concentrations are often found in extended disease only.

Neuron-specific Enolase (NSE)

Another marker for neuro-endocrine tumors including MTC is NSE. While this enzyme of the glycolysis, the γ-enolase of neuro-endocrine cells, is highly sensitive for small cell lung cancer and neuroblastomas, only a sensitivity of about 15% is reported for MTC. Furthermore, the positive predictive value for a relapse in NSE positive patients with MTC averages only 70% and there is no strong correlation of NSE serum levels and tumor spread as compared to CT.[4] NSE therefore, may be of clinical use only in those patients with non-reliable CT values and it cannot be recommended for routine clinical follow-up examinations.

Conclusion

In agreement with the recommendations of the German Society of Endocrinology the following regimen of routine tumor marker measurements in patients with cancers of the thyroid gland is suggested:[3]

Thyroglobulin: for the follow-up in patients with papillary, follicular, or oxyphilic carcinoma

Calcitonin: for diagnosis and follow-up in patients with medullary thyroid carcinoma as well as for screening of the relatives of patients with multiple endocrine neoplasia type II

CEA: for the follow-up in patients with medullary thyroid carcinoma

In contrast, there is no general recommendation for the use of TPA and NSE. These tumor markers, however, may be useful for the follow-up in selected patients with thyroid cancer.

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Value of scintigraphic imaging in the detection of pancreatic tumors – the role of FDG-PET

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One of the main challenges in diagnostic radiology is the early and the accurate detection of pancreatic carcinoma, which is indeed difficult by morphologically orientated methods, i.e. US, CT, MRI. Therefore, functional imaging using nuclear medicine procedures may be useful. In patients with suspected pancreatic cancer, several imaging procedures have been investigated i.e. immunoscintigraphy, receptor scintigraphy, unspecific perfusion scintigraphy. However, none of them convinced in routine patient management. Best diagnostic results with an overal accuracy of about 80% were obtained using F-18-fluorodeoxyglucose – positron emmission tomography (F-18-FDG-PET). Due to inherent technical limitations PET probably cannot depict lesions smaller than 10–15 mm in diameter even when using a high resolution PET scanner. Thus, it is probably not suitable for early detection of pancreatic carcinoma. It is not yet clear, whether the performance of FDG-PET is high enough to reduce the current number of diagnostic laparotomies. Prospectively performed comparative studies with CT, US, ERCP and MRI using state of the art equipment are still needed to establish an optimal diagnostic strategy. Beside lesion detection, FDG-PET may offer valid data on both the prognosis of pancreatic masses, and the effectiveness of therapeutic procedures. However, further effort is still necessary to define the exact position of nuclear medicine in the management of pancreatic cancer.

Key words: pancreatic – neoplasms – radionuclide imaging; tomography, emission – computed – methods, fenorine radioisotopes; FDG-PET – diagnostic value

The clinical problem

One of the current challenges in diagnostic radiology is the early and the accurate detection of pancreatic carcinoma and its differentiation from mass-forming pancreatitis using noninvasive imaging methods.[1, 2] The diagnostic accuracy of morphologically oriented imaging techniques is presently suboptimal. Ultrasonography is hampered by the dorsal position of the pancreas in the abdomen and the bowel located in front of it. CT and MRI have an excellent geometric resolution, but the differentiation between malignant and benign lesions remains difficult even with this leading edge technology. On the other hand, functionally oriented nuclear medicine procedures offer the possibility of imaging organ metabolism when using appropriate radiolabelled tracers. At the beginning, the aim of radioisotope studies was merely the visualization of the pancreatic tissue. For this purpose Selenium-75-selenmethionine and (I-125)-N,N,N'-trimethyl-N'(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine (I-123-HIPDM) were tested. Both tracers are accumulated in the normal pancreatic tissue, but they can not differentiate between malignant tu-

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mors and benign lesions.[3,4] Thus, in the era of high resolution radiological methods they are of a more historical value. Recently, the introduction of several new types of tracers opened exciting perspectives. Therefore, the possibilities of these radiopharmaceuticals will be discussed.

Immunoscintigraphy

Since the mid eighties big expectations have been connected to the introduction of radiolabelled monoclonal antibodies against tumor associated antigenes in oncologic nuclear medicine. The characteristic feature of these antibodies is their extremely high specificity. Using chemical procedures, they can be broken either into Fc and F(ab')2 fragments or into Fc and Fab fragments. The F(ab')2 of Fab fragments are responsible for the antigen specificity of the antibodies. These antibodies can be labelled with I-123, I-131, In-111 or, recently with Tc-99m.

Different types of monoclonal antibodies have been tested for the detection of pancreatic cancer. A coctail of I-131 labelled F(ab')2 fragments of antibodies directed against the tumor associated antigenes CA 19-9 and CEA was used by Montz.[5] Despite the detection of some large tumors without elevated level of tumor markers in the peripheral blood, this tracer is considered of only limited diagnostic value due to its relatively poor sensitivity. Using In-111 for labelling and SPECT acquisition at least 3 days after the tracer injection some increase in diagnostic accuracy, due to elimination of unspecific early tracer accumulation, was reported by Bares in various gastrointestinal carcinomas.[6] As a further attempt, preliminary results have been reported with the I-131 labelled murine monoclonal antibody AR-3-IgG1 directed against the mucin-like antigen CAR-3.[7]

Investigations with the In-111 or I-131 labelled F(ab')2 fragments of the monoclonal antibody BW 494/32, the corresponding antigene which is often expressed by pancreatic carcinomas, failed as well in presenting a break through in immunoscintigraphy.[8] Nevertheless, in a study with only 3 patients, Abdel Nabi and coworkers presented favourable images of primary tumors as well as of their metastases using In-111 labelled monoclonal anti CEA antibody ZCE 025.[9]

In conclusion, at present there is no radiolabelled monoclonal antibody available with clearly documented high clinical performance necessary for routine patient management. Despite of this, immunoscintigraphy can be considered as a possible investigation in diagnostically problematic cases.

Receptor scintigraphy

New possibilities in tumor imaging have been offered recently by the introduction of small receptor analogue molecules. A subgroup of these compounds, the radiolabelled somatostatine analogues can be used for imaging of endocrine tumors i.e. of those derived from the so called APUD cells. Somatostatine analogues have been primarily used for imaging of carcinoids and islet cell tumors of the pancreas. Bakker and coworkers reported the successful localization of pancreatic tumors in rats with I-123-Tyr-3-octreotide.[10] However, the main disadvantage of this radiopharmaceutical is its predominantly hepatic clearance and therefore its high liver accumulation, which may mask pancreatic tracer uptake.

When labelling octreotide with In-111-DTPA, the longer halflife of In-111 can be combined with the facilitated renal clearance of the DTPA-containing compound. These features offer the advantage of 24 hours imaging when interferring background activity is already minimized by renal clearance. Bakker and coworkers reported successful investigations of pancreatic tumors in a rat model using (In-111-DTPA-D-Phe1)-octreotide.[11] They documented an increasing tracer uptake with time within the tumor tissue, which could be clearly visualized by gamma camera scintigraphy in somatostatin receptor positive rat pancreatic carcinoma.[11] As an attempt for supporting surgical interventions, Ohrvall and coworkers introduced a non imaging method for the intraoperative detection of tumors and its metastases by using a hand-held gamma probe. However, the feasibility of this interesting method is limited by the relatively high background activity.[12]

Unspecific perfusion tracers

Thallium-201 uptake is considered to reflect the regional perfusion as well as the viability of tumor cells.[13] The theoretical background of this feature of TI-201 is the correlation of the growth of malignant transformed cells and the activity of the Na/K-ATPase.[14]

In 1993 Suga and coworkers demonstrated the possibility of monitoring the efficacy of antineopla-

stic treatment using quantified TI-201 uptake in pancreatic cancer. In three patients, the TI-201 uptake by the tumor correlated well with the serum level of the tumor marker CA 19-9.[15] In a subsequent publication of this group results with subtraction scintigraphy were presented.[16] When the boundary between abnormal TI-201 uptake and adjacent liver activity was unclear on the TI-201 SPECT image a SPECT image of the liver using Tc-99m-phytate was acquired and subtracted from the TI-201 image in order to separate hepatic and pancreatic TI-201 uptake. Using this technique a favorable sensitivity of 91% could be demonstrated. However, in the same study, four of sixteen patients with benign pancreatic disorders exhibited abnormal increased TI-201 uptake. Based on these data, TI-201 scintigraphy is a nuclear medicine method with relatively low cost and acceptable clinical performance for detecting pancreatic cancer and can be recommended, especially when PET facility is not available.

PET investigations using FDG

The rational of using radiolabelled glucose analogues for tumor imaging is based on the increased metabolic activity of tumor tissue. The accelerated rate of glykolysis in aggressive malignant transformed tumors was published first by Warburg in 1956.[17]

Fluorodeoxyglucose (FDG), a glucose analogue, is supposed to enter the cells by the same transport mechanisms as used by native glucose. After phosphorylation, however, FDG-phosphate is trapped intracellulary due to its extremely slow dephosporylation as compared to native glucose. This feature enables imaging of FDG distribution by positron emission tomography (PET). Experimental and human studies have demonstrated an increase in FDG uptake in various malignant tumors.[18–20] All of these studies have confirmed that an increased FDG uptake is a reliable indicator of the presence of viable malignant tumor tissue. In contrast, benign processes or metabolically less active neoplasms generally have lower or even normal levels of glucose uptake.[21] The mechanism of FDG accumulation in tumors is probably multifactorial. First, it is induced by activation of glucose transporter proteins and elevated glucose consumption, which both are considered to be early and prominent features of an oncogene-mediated malignant transformation in cell culture systems.[22] Second, tumor associated tissue inflammation produces an increased FDG uptake as well.[23]

The value of F18-FDG for the detection of malignant pancreatic processes was first documented in patients by Zanzi and coworkers in 1990.[24] Based on the results of F18-FDG-PET, Klever and coworkers reported clear differentiation of pancreatic carcinoma and chronic pancreatitis.[25] Bares and coworkers[26] found focally increased FDG accumulation in 12 out of 13 patients with histologically proven pancreatic adenocarcinoma. Eight of nine known lymph nodes and four of five known liver metastases were detected in their study. In contrast, in two patients with chronic pancreatitis no FDG uptake was documented. One patient suffering from an adenocarcinoma and lacking of FDG uptake had diabetes, probably because the fasting state could not be established sufficiently prior to the study. In a second study with 40 patients investigated by the same group,[27] PET helped to correctly classify 25 of 27 malignant pancreatic tumors and 11 of 13 benign disorders of the pancreas. False negative findings were obtained again in 2 patients with insulin-dependent diabetes. False-positive findings were associated either to retroperitoneal fibrosis or, in one patient, to pancreas divisum with chronic pancreatitits. However, FDG-PET was shown to be superior to both CT and ultrasound in the detection of lymph node metastases. These results have been confirmed by Friess and coworkers.[28] Fourty one of fourty two patients with pancreatic cancer and four of six patients with a periampullary carcinoma presented a focally increased FDG uptake. In contrast, in 28 of 32 patients with chronic pancreatitis no FDG accumulation occured.

In a comparative study of 46 patients suspected of having a pancreatic cancer the diagnostic performance of FDG-PET was superior to both CT and transabdominal and endoscopic ultrasound.[29] This superiority of FDG-PET over CT was also demonstrated by Stollfuss and coworkers.[30] Based on the result of a recent comparative study with FDG-PET and TI-201 SPECT in patients with histologically proven pancreatic cancer it can be concluded that, if PET facility is available, FDG-PET is at present the nuclear medicine method of choice.[31]

An exciting and widely discussed possibility to increase the performance of PET studies is the use of quantitative methods to obtain numerical values of the phosphorylation rate of deoxyglucose. For this purpose, several methods have been suggested.

Most of them are based on the three-compartment model introduced by Sokoloff.[32] However, there are some practical and theoretical difficulties connected to the application of the three compartment model of FDG metabolism, especially to the determination of the velocity constants of the biochemical reactions and to that of the lumped constant.[33–35] Therefore, most investigators are simply using ratios of FDG uptake in tumors as compared to that in normal tissue. A further possibility of quantification is the estimation of the net tumor uptake of FDG in the tumor tissue normalized to the body surface or body weight.

The most widely used quantitative method of estimation of FDG uptake in tissue is the determination of standardized uptake values (SUV). The main advantage of the SUV method is its methodologic simplicity. The main disadvantage is the fact, that the SUV itself is time-dependent and, therefore, potentially subject to error if images are not obtained at the same time interval after tracer injection.[36, 37]

The impact of quantification of FDG uptake by SUV could be shown in patients with pancreatic cancer. The tumor region exhibited significantly higher values compared to pancreatic regions in patients with pancreatitis.[28] Using ROC analysis it could be demonstrated that an SUV value of about 1.5 optimally separates malignant and benign pancreatic processes.[30]

However, very recent investigations in clinical patients report a lower accuracy as found in the early works.[38, 39] Dohmen and coworkers found an accuracy of about 80% regarding the differentiation of pancreatic cancer from chronic pancreatitis. Furthermore, they could not demonstrate any advantage of using quantitative parameters as compared to the simple visual analysis.[38] The limited performance of the SUV was also reported by Vomocil and coworkers.[39]

PET tracers for endocrine pancreatic tumors

Results with C-11-labelled L-dihydroxyphenylalanine (L-DOPA) and hydroxytryptophane (HTP) have been recently reported in pancreatic endocrine tumors with promising results particularly regarding glucagonomas.[40] These findings, based on the investigation of 22 patients, suggest further possibilities in metabolic characterization of tumors. However, the role of FDG in this type of tumors has not yet been explored.

Multimodality imaging

The combination of morphologic and functional information is a very new and exciting trend in diagnostic imaging. It is especially helpful in the exact anatomic localization of functional disorders. Detailed morphologic information presented by the excellent geometric resolution of CT or MRI can be combined with the visualization of metabolic parameters and presented in one single image. However, for the coregistration of tomographic images from different modalities sophisticated external or anatomical markers are needed. The first successful fusion of F-18-FDG-PET and MR images using a system of surface markers in patients with pancreatic adenocarcinoma was demonstrated by Benyounes and coworkers.[41] The combined images were impressive for exact delineation of the tumor on the high quality slices of the MR study. Thus, multimodality imaging might be of increasing interest in future studies.

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Nuclear medicine, IBM PC PIP-GAMMA-PF computer system

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Our own development of nuclear medicine computer system was mainly due to very high prices of commercial systems and was based on our knowledge and experiences in the development of some other nuclear medicine equipment and processing software.[1,2] The project was started 5 years ago on the low cost IBM PC and after two prototypes a highly professional acquisition board was built along with the acquisition and data analysis software. The acquisition board is characterised by stable functioning and simple installation. After testing different modes of processor input of scintigraphic signals (DMA, PORT) the latest one was chosen because of its simpler programming code, good stability and the possibility of using it in DOS and WINDOWS. The acquisition software consists of a predefined set of acquisition protocols for standard nuclear medicine examinations, simulation of persistence scope with a possibility of changing the image orientation, zoom size, automatic adjustment of the image size, offset and pixel size, analysis of clinical image data and archiving the patient population and scintigraphic data by using departments network. To the database management and system processing routines - PIP by A. Todd-Pokropek (London University) we added some clinical processing modules for the most common examinations in nuclear medicine. The system was successfully applied in daily routine work at the clinic for nuclear medicine in Ljubljana and in some other places.

Key words: nuclear medicine; radionuclide imaging; image processing, computer assisted; software

Acquisition board

The Gamma camera is functionally connected to the computer via an analogue-digital converter and control logic circuit (Diagram 1.1.).

1. Position signals

The position signals X and Y (Diagram 1.2.) are defined by the following characteristics:

– the displacement of scintigraphic image from the middle of matrix defined as X or Y offset

– their variation range (scan height and width) corresponding to X and Y gain

– analogue signals in V (volts)

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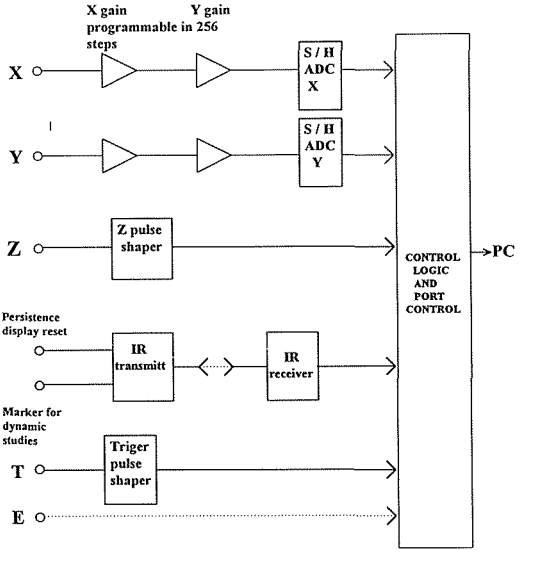


Diagram 1.1. Functional diagram of interfacing board.

– generally non-symmetric signals in relation to 0 V

– signal variation means the X - Y range (Siemens BASICAM : ± 2V, GE 300 A : ± 1.5 V, Nuclear Chicago Searle : ± 2 V)

– commonly constant values of X - Y signals until they are changed by Z sampling signal

– coaxial cabling from gamma camera console to computer interfacing card with 70 Ω impedance of BNC connector

– amplifier needed for more than 10 m long X - Y cables

– IC designed for 0 V in the middle of the matrix

– non-symmetric variation of X - Y signals related to 0 V means the image offset

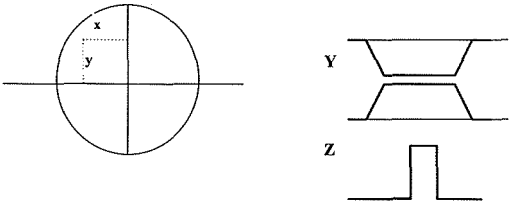


Diagram 1.2. Gamma camera signals: definition of X and Y offset and gain

2. Logical energy signal

The logical signal Z, which represents the correct gamma ray energy, starts the X and Y conversion and sampling (Diagram 2.1.).

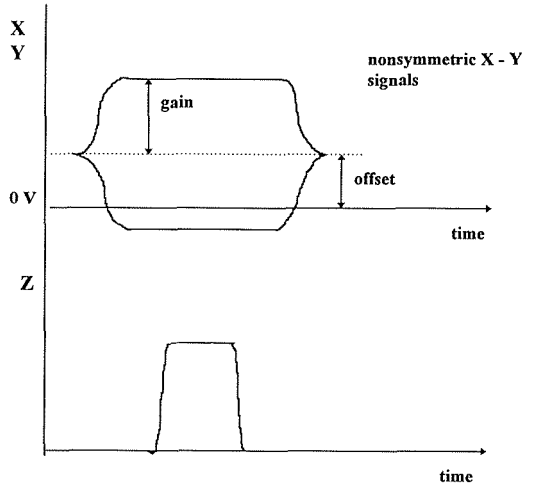


Diagram 2.1. Shape of Z signal and offset and gain of X and Y signals

3. Analogue-digital conversion of X and Y signals

The digitalisation of X and Y signals is characterised by the following features:

– the accuracy improvement of signal conversion: the AD converter with more bits is used for more reliable higher bits (pp. 12 bits for 1 byte magnitude conversion; after conversion the 4 less significant bits are rejected), dynamic linearity is improved

– in high count scans (> 2 million counts) the correction for dynamic non-linearity of ADC should generally be applied

– each AD converter has its own characteristic linearity curve

4. Autoadjustment of interfacing board's gain and offset for input gamma camera signals

The position signals X and Y from the gamma camera to the interfacing card must fit the computer matrix.

This can be done basically by two methods either by manual adjustment of the gamma camera position signals on the interfacing card or by the feedback intervention "interfacing card - computer" (Diagram 4.1.).

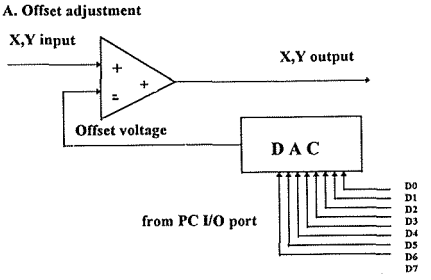
The last method considerably improves the effectiveness and simplicity of the whole acquisition system. We developed the logica circuit for a continuous change the gain of input X and Y signals from the gamma camera prior the analogue-to-digital conversion.

The mean value of the gain setting 128 (range 0-255) corresponds to the range of x,y ± 1.5 V for the whole matrix size. If the input signal's range varies up to ± 3 V, then at this gain setting half of the image will be cut in diameter (diagram 4.2.).

With such a range, which can be manually changed for really extremely non-standard gamma camera signals, all the available gamma cameras can be used.

When the input signals come from the gamma camera with a range of ± 0.75 V and the chosen gain is 128 then the image in the matrix will have half of the diameter's value of the gamma camera with signals in the range of ± 1.5 V. To set up the interfacing gain we shall start with the highest possible value of gain parameter (255). In this case we shall normally get a minimized image for most gamma cameras. Our task is now to increase

A. Offset adjustment



B. Gain adjustment

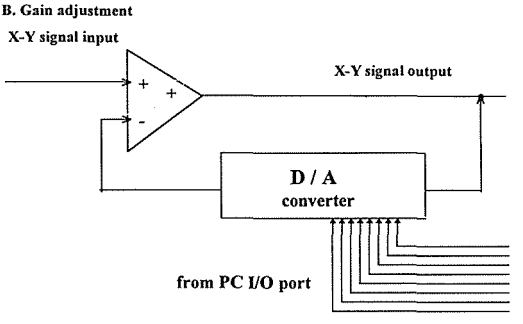


Diagram 4.1. Digital gain and offset control of X and Y.

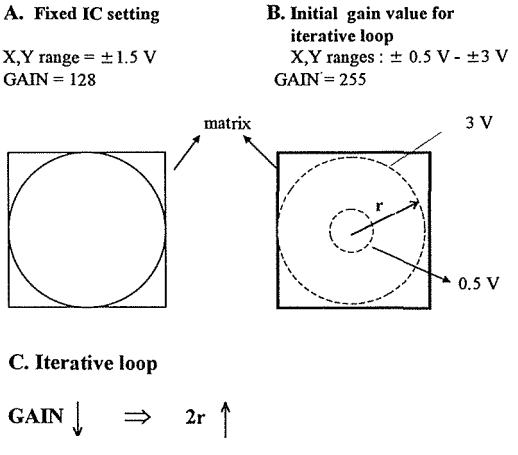


Diagram 4.2. Adjusting the interfacing card's gain and offset values

the image size step by step by decreasing the interfacing gain value. The problem of non-central positioning of the image in the matrix can be solved simply by moving the image centre to the matrix centre. To start the image position in the matrix centre we set the offset to 128. If the image is not centred (non-symmetrical X,Y range), then we change the offset to a new value which is equal to the displacement of the image centre from the matrix centre.

Instrumental needs

To start the gain and offset set-up, the uniform radioisotope source (Co57 plate on collimator or point source 2 m from gamma camera without collimator) is used.

Algorithm for autoadjustment of interfacing board's gain and offset

Initial values

The following starting values are chosen: For iterative loop gain = 255, offset in both directions = 128

Main loop

The scan of 50000 counts in the matrix 256x256 is acquired. The activity profile in X and Y direction with thickness of 3 lines is formed, the maximum number of counts per profile element is searched for and the coordinates of this maximum are used to find the scan edges by applying threshold criteria. Displacement of the scan centre from the matrix centre (Diagram 4.3.) is computed on the basis of the following formulas:

$$Xds = (Xl + Xr)/2 - Xm \qquad Yds = (Yu + Yl)/2 - Ym$$

where Xl, Xr are the x co-ordinates of the left and the right scan edge

Yu, Yl are the co-ordinates of the upper and lower scan edge

Xm, Ym are co-ordinates of the matrix centre

Xds, Yds are displacements of the scan from the matrix centre

These values are subtracted from the current offsets. Similarly, the deviations from the scan and matrix size in both directions are computed using the following formulas:

$$Dx = (Md - (Xr-Xl))/2 \qquad Dy = (Md - (Yl-Yu))/2$$

where Md is one dimensional matrix size

Dx, Dy are halves of the difference between the matrix size and the scan size

These values should be subtracted from the current gain values if the edge coordinates are computed with high precision. But the smaller the starting image's diameter the less exact computation for edge coordi-nates is possible. The subtracted gain

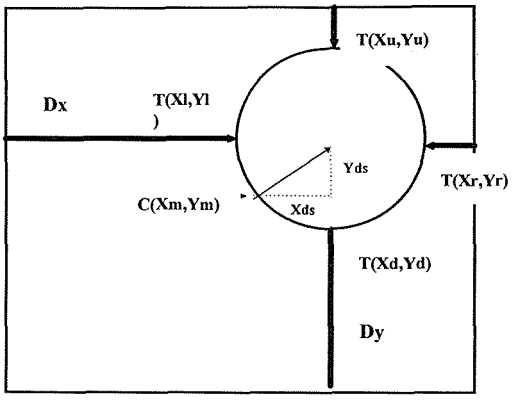


Diagram 4.3 Definition of iterative loop variables.

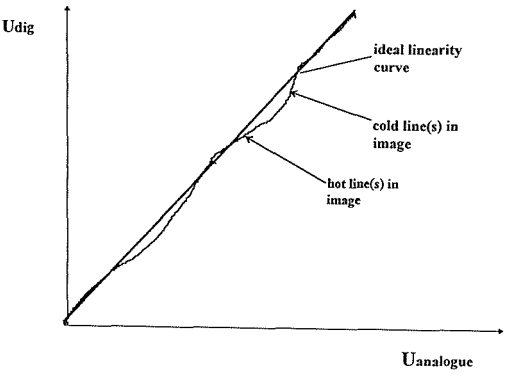


Diagram 5. Dynamic non-linearity curve.

values can lead to the image size greater than the matrix size and therefore it is difficult to find the optimal gain setting using the software technique described above. Because of this we prefer to subtract only half of the Dx and Dy and the image size is slowly approaching the matrix size and the corresponding gain values the optimal settings.

Stopping condition

If Dx and Dy are smaller than 2, the procedure for gain/offset-up is stopped and the corresponding values for gain and offset stored in the calibration file and set to the interfacing card..

To visually check the algorithm's success, all intermediate scans are displayed during software adjustment of the gain settings until the scan size equals to the matrix size.

Problems

The following problems arise most frequently durign the autoadjustment of gain and offset:

– insuficient radioactivity in flood or point source,

– nonadequately calibrated gamma camera with "holes" or "hot spots" in the scan (the edge co-ordinates can be miscomputed).

5. Correction of dynamic non-linearity

At high count images (over 2 million counts) this correction is necessary to remove the stripes which appear at some horizontal and vertical matrix lines. The stripes in the image appear at all horizontal and vertical lines where the bit content of the co-ordinate is changing from total "1" to "0" in most bit positions (e.g. from X(Y) =11111 to X(Y) =100000) because of the so called bit flipping.The effect of "bit flipping" is not the same for all co-ordinates in question. Higher content of the "1" bits leads to more frequent flipping (diagram 5.). To correct this ADC "malfunction", the uniform distribution of input signals in X and Y direction is applied and the extent of bit flipping is measured in each position.

6. Acquisition software

It consists of four basic units:

– entering the patient population, instrumental and acquisition data (part of the PIP system). For clinical work we built a set of predefined studies which can be called up to avoid the time consuming input of instrumental and acquisition parameters for each patient. A new study protocol can be easily added to the existing set..

– functioning as persistence scope

for checking the patient positioning, the adjustment of the zoom and the image orientation and, if necessary (in the installation phase) the adjustment of the image size, offset and pixel size.

– starting the predefined acquisition

During the acquisition we have a possibility of finishing the complete study (static and dynamic studies) of finishing the current scan (in static mode acquisition). Each scan is currently displayed if the acquisition time is exceeds 10 seconds for static acquisition or 2 seconds for dynamic acquisition, otherwise only the frame and group numbers, the time, the countrate and the total time are displayed (the reason for this is to prevent the count loss in fast framing and scan displaying).

– storing the sequence of images from virtual memory to hard disk and returning to the main PIP menu (part of PIP system). This phase can be upgraded if required in such a way that the data can be stored on some other unit as well (e.g. on archive disk).

– hardware and acquisition software installation

The interfacing card called GAMMA-PF and relevant acquisition software were constructed in such a way that the hardware and software can easily be installed (see installation manual). The interfacing card is installed in an empty computer slot like any other computer card without any additional intervention in the computer switches. The same is also true for the acquisition software installation which is done by running the INSTALL command from the installation disk. For the optimal image size and shape the software is included which recursively adjusts them from the uniform radiation flux on the gamma camera (uniform source on the gamma camera detector or point source two meters from the detector). No hardware adjustment of the image gain and offset is needed. The functioning of the interfacing card is very stable and no intervention is needed except in the case of electrical or physical damage to the card.

– maintenance

The best way of maintaining the interfacing card would be either sending back the malfunctioning interfacing card or which is also acceptable because of the low card price of the card, to have an additional card at hand, which can immediately be installed and the damaged one sent back to the supplier for repair (highly recommended to prevent any interruption of clinical investigations).

7. Networking

The basic function of the nuclear medicine network system should be providing the records of the patient population and a scintigraphic data acquisition on large scale storage media, communication between the acquisition, processing and reporting stations, printing the reports on network or local printer and access to external network.[3] The solution of this task can be achieved by applying low cost network hardware and software (eg NOVEL, MS WORKS for WINDOWS, etc). When planning the network system for the department we should take into account the department's organisation and economy. The network system can considerably lower the overall department's expence per patient's examination study, by increassing the effectiveness of the department in using the digital storage and displaying system as patient study retrieval system and low cost high resolution printer for reporting. All these demands can be fullfiled by using the low cost IBM PC hardware and software technology.

The following basic functions of PIP-GAMMA-PF network (Diagram 7) are available:

– storing patient study files (IMG, XAC) on the network server's archive disk (hard disk with a capacity more than 2 GB)

– storing patient's coded images (the image with sequential scans, curves, ROIs, patient's population data and quantitative estimates of organ's function) on the server's archive disk

– archiving patient document files (the physician's report, the image of results, patient population data) on the archive disk

– printing the patient's documents on the network printer (laser or inkjet printer with resolution at least 600x600 dots/inch)

– distributing the application software from the server's disk to acquisition and processing IBM PC stations

– access to hospital, regional and international network

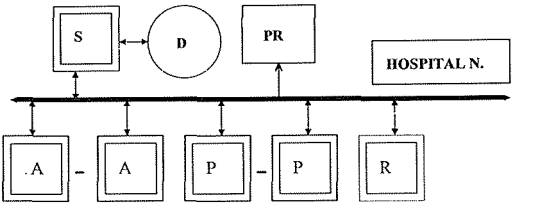


Diagram 7. Functional schema of PIP-GAMMA-PF network.

where A is PIP-GAMMA-PF acquisition system
P is PIP-GAMMA-PF processing system
R is PC reporting system (MS WORD)
S is network server
D is server's archive disk
PR is network printer

The acquisition system needs the following settings in PIP.LGO file:

acqndir = C:/PIP/DATA
datadir = C:/PIP/DATA
archive = H:/PIP/DATA

where C is the logical label for the system disk in the acquisition system and H is the logical label for the network server's archive disk

The processing system should be set at the following values in PIP.LGO: datadir = H:/PIP/DATA and the output coded result images (PCX format) should be stored in an appropriate subdirectory on H:/GAMMA/ (e.g. H:/GAMMA/LUNGS/), which is for the time being programmed in GAMMA-PF user program.

Results

The following results were achieved in the project:

– one full-size AT bus professional interfacing card (ISA bus, four layers, PORT input), installation disk and manual

– simple installation of the interfacing card in an empty slot of IBM PC

compatible computer and installation of software with the "install" command from the installation disk

– a simplified and shorter acquisition programming code with PORT input of data

– a set of predefined studies for clinical examinations

– the acquisition can be stopped either to finish the current image or to finish the study

– the sequence of images is displayed continuously

– the continuous displaying the frame and group numbers, time and count rate

– acceptable count loss for clinical examinations[4]

– a high functioning stability observed over a period of two years

– network system with acquisition, processing, reporting, archiving, printing and communicating on local or extended level[3]

– good results with MAG3 kidney studies.[5]

Conclusions

An upgraded four layer professional interfacing card was built for the ISA bus, together with the installation disk and a manual. The whole installation procedure of inserting the interfacing card in an empty slot of on IBM PC compatible computer and the installation of the acquisition software are simple and can be performed by a nuclear medicine technologist. Entering the scintigraphic data has been simplified by replacing the absolute addressing (DMA) with PORT input/output access. The image orientation, the zoom size, image size and the shape are controlled by software. The ADC dynamic non-linearity correction is applied to high count images. As cardiac gated acquisition with two low-memory buffers has high count loss (over 20%), there is a need for 32-bit operational system and 32-bit C programming language.

To improve the speed of the acquisition (first pass studies), upgrading the interfacing card by using the new PCI bus is necessary in the next project activities.

Acknowledgement

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Study of blood perfusion with Patent Blue staining method in LPB fibrosarcoma tumors in immuno-competent and immuno-deficient mice after electrotherapy by direct current

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Electrotherapy of tumors by low-level direct current can be used effectively to reduce tumor mass in experimental and clinical tumor models. The effects of such treatment on blood perfusion of tumors were studied on solid subcutaneous LPB fibrosarcoma model in immuno-competent C57Bl/6 mice and in immuno-deficient Swiss nude mice. Tumors were treated for one hour with electric current of amplitudes 0.6 mA and 1.0 mA delivered by Pt/Ir needle electrodes inserted subcutaneously on two opposite sides of the tumor. Study of tumor growth response to single-shot electrotherapy yielded highly significant growth retardation in C57Bl/6 mice but only insignificant effect on tumor growth in nude mice. Effect of electrotherapy on blood perfusion of tumors as one of the proposed mechanisms of antitumor action was evaluated by tissue staining method using Patent Blue Violet dye. Perfusion was estimated immediately after electrotherapy and 24 hours after the treatment. Perfusion of tumors in C57Bl/6 mice was only moderately decreased due to electrotherapy, whereas in nude mice there was practically no effect observed. The difference in growth response of the two models indicates that vascular occlusion which occurs due to the products of electrolysis at the site of insertion of the electrodes may not be the major cause for the observed tumor retardation in immuno-competent mice.

Key words: fibrosarcoma electric stimulation therapy; regional blood flow; direct current electrotherapy, tumor growth retardation, blood perfusion

Introduction

It has been demonstrated in several studies that electrotherapy by low-level direct current can be applied successfully as a local treatment for solid malignancies with minimum side effects for the host. The antitumor effectiveness has been achieved in various experimental animal tumor models[1-6] and in clinical trials.[7,8] In addition, electrotherapy can be used as an adjuvant treatment to other conventional therapies for it has been shown that it potentiates the effectiveness of certain biological response modifiers and anticancer drugs, e.g. tumor necrosis factor, interleukin-2 and bleomycin.[9-11] Regardless of the way of its application, either as a single treatment or in combination with other therapies, it

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is of great importance to understand the mechanisms of its antitumor action in order to be able to optimise existing and develop new treatment strategies.

Many attempts have been made to explain antitumor effectiveness of electrotherapy. It can hardly be expected that one single mechanism could be responsible for it since the parameters of treatment vary considerably from one author to the other. The differences appear in the type of tumor model, in electrical parameters (e.g. amplitude and shape of the signal), in electrode number and in material and the way of application.[12]

Several possible mechanisms that could be responsible for antitumor effectiveness of direct current electrotherapy have been proposed. When one or more of the electrodes are inserted into the tumor then the extreme pH changes measured in vicinity of the electrodes are believed to be responsible for cell killing. It has also been shown that with an appropriate configuration of multiple electrodes in the tumor it is possible to effectively eliminate most of the tumor mass[13]. Surprisingly enough it was found that when electrodes are inserted subcutaneously on two opposite sides outside the tumor so that the tumor is brought into so-called "field" configuration, then similar growth retardation is produced as in the case with one of the electrodes in the tumor. For the "field" configuration antitumor effectiveness of electrotherapy cannot be ascribed to pH changes and also not to temperature rise which were both not found in the tumor.[12] There was also no correlation found between tumor growth retardation and deposition of the electrode material[14].

One of the possible mechanisms of antitumor action of sub-thermal low-level direct current electrotherapy for electrode configurations where tumor is not penetrated by stimulation electrodes is vascular occlusion at the site of insertion of the electrodes.[15] It was suggested that altering the blood supply to tumor tissue might lead to eradication of tumor mass. In one of our previous studies we have found that significant growth retardation of one particular tumor model was indeed accompanied by and correlated to large decrease of tumor blood perfusion as assessed by a tissue staining method.[16] In the present study an attempt was made to investigate this effect in LPB murine fibrosarcoma tumor in both immuno-competent and immuno-deficient animals.

Materials and methods

Animals and tumors

Female and male animals of C57Bl/6 strain were purchased from two sources, namely Rudjer Bošković Institute, Zagreb, Croatia and C.E.R.J animal facilities Laval, France. Nu/nu Swiss nude mice were bred at the animal facilities of the Institute Gustave-Roussy, Villejuif, France. Animals were housed in plastic cages in convenient colonies and kept at constant temperature of 24°C. They were watered and fed ad libitum. Additional standard measures were taken in order to prevent infection in nude mice. Animals in good condition and free of any infection, aged 8-12 weeks, were used in experiments. LPB fibrosarcoma cells syngeneic to C57Bl/6 mice were cultured in vitro. Solid subcutaneous tumors were initiated dorsolaterally in the right flank of mice by injection of $0.8 \cdot 10^6$ and $1.6 \cdot 10^6$ viable LPB cells in C57Bl/6 and nude mice respectively. Tumors of approximately 7 mm in diameter were obtained about 10 days latter, when animals were randomly assigned to different experimental groups with 7-9 animals per group in each single experiment.

Electrotherapy

Single-shot electrotherapy consisted of constant direct current which was applied for 60 minutes. Current amplitudes used were 0.6 mA and 1.0 mA. Selection of current level and duration was based on our previous studies.[12] Current was generated by a multichannel current source thus enabling simultaneous treatment of up to eight animals. Needle electrodes 1 mm in diameter and 20 mm in length made of Pt/Ir alloy (90/10%) with rounded tips were used to deliver the current. Electrodes were inserted subcutaneously through small punctures in the skin made by a sharp needle. Electrodes were inserted in parallel on two opposite sides of the tumor with the cathode on caudal side of the tumor. Distance between the electrodes was 20-22 mm and each electrode was placed 5-8 mm away from the tumor edge. During electrotherapy mice were anesthetised by intraperitoneal injection of 100 mg/kg of ketamine (Ketanast, Parke-Davis, Germany), 10 mg/kg of xylazine (Rompun, Bayer, Germany), and 0.1 mg/kg of atropine. Animals in control groups were treated in the same way except that no current was applied.

Assessment of tumor growth

Tumor sizes were estimated before electrotherapy (day 0) and on the days following electrotherapy. Two largest perpendicular diameters were measured with vernier calliper and tumor volume was calculated using formula $V=\pi ab^2/6$ (a and b being measured diameters). For each individual tumor its doubling time (DT) was calculated as a time needed for the tumor to double its initial size. Mean average tumor volumes and their standard errors were calculated and presented as growth curves. Mean average doubling times were also calculated for all experimental groups. Experiments were repeated three times in case of nude mice and twice for C57Bl/6 mice. Statistical significance of differences between experimental groups were evaluated using Student t-test and values of p less than 0.05 were considered as an indication of statistical significance.

Assessment of tumor perfusion

Perfusion study was performed on separate animals from those used in tumor growth study. Saline solution (0.1 ml, 1.25%) of biological dye Patent Blue Violet (Byk Gulden, Switzerland) was injected in retroorbital sinus of animals. After the dye has been left to distribute evenly through tissues for approximately 3 minutes, animals were euthanised and tumors were carefully removed. Tumors were cut along their largest diameter and the percentage of stained versus non-stained cross-section area was visually estimated independently by two persons. The mean of both estimations was used as a relative measure of tumor perfusion. Solidly stained parts of the tissue were considered to be well perfused, absence of the dye was an indication of poor perfusion. Perfusion was assessed immediately after electrotherapy and 24 hours following the treatment. For each experimental group the mean value and standard error of the mean were calculated and presented. Student t-test was used to evaluate statistical significance of differences between experimental groups.

In our study Patent Blue Violet dye was used instead of a more widely used Evans Blue dye. The former yielded a much better color contrast between stained and non-stained parts of tissue than the latter. Furthermore, a study performed on fibrosarcoma SA-1 tumors in A/J mice has shown that both dyes produce essentially the same results.[19] This was confirmed in control tumors as well as in tumors treated with electrotherapy. Therefore in our opinion the use of Patent blue instead of Evans Blue is entirely justifiable in perfusion studies such as ours.[17,18]

Results

Tumor growth

Application of the single-shot electrotherapy induced statistically significant tumor growth delay in immuno-competent C57Bl/6 mice. Typical growth of tumors in one of the experiments is given in Figure 1, where growth delay of treated tumors with respect to control tumors is clearly shown. It was also evident that larger dose (higher current level) yielded better antitumor effects. In Table 1 tumor doubling times (DT/days) are given for all experimental groups in two separate experiments. Tumors were arrested in their growth and their size was temporarily reduced. When regrowth occurred after several days, the growth rate was similar to that observed in control tumors. Apart from the observed fact that control LPB tumors in immunodeficient mice grew faster than in C57Bl/6 mice it was also demonstrated that electrotherapy was much less effective in the case of nude mice, as shown in Figure 1 and presented in Table 1. Doubling time of tumours treated with 0.6 mA electrotherapy was not significantly different from doubling time of control tumors. Application of 1.0 mA however yielded significant growth delay but it was still much smaller than in immuno-competent mice.

Tumor perfusion changes

In Table 2 perfusion of tumors as assessed by means of Patent Blue staining method is presented. Data of several experiments were pooled together. All control tumors in both strains of mice were practically completely stained, which indicates that tumors at that stage of development were well perfused. No reduction in staining of tumor tissue was observed in nude mice immediately after electrotherapy regardless of the current level applied. In C57Bl/6 mice reduced staining was observed immediately after treatment and this effect was maintained for at least 24 hours. As in the tumor growth study the effect was more pronounced for higher dose. There was also a large variability in staining among individual tumors treated with electrotherapy. On the other hand staining of control tumors was very uniform in both strains of mice.

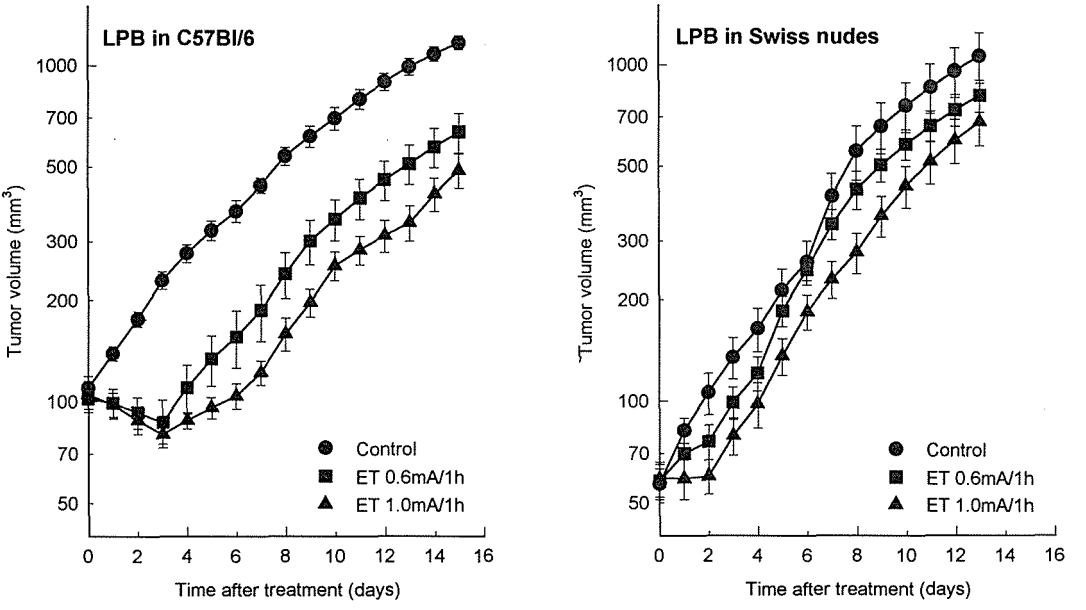

Figure 1. Growth of LPB tumors in immuno-competent C57Bl/6 mice and in immuno-deficient Swiss nude mice following a single-shot one-hour electrotherapy with 0.6 mA and 1.0 mA. Data points represent the mean values for 7-8 animals with standard error bars.

Table 1. Effect of one hour electrotherapy (ET) with 0.6 mA or 1.0 mA on growth of LPB fibrosarcoma tumors in C57Bl/6 and Swiss nude mice. Tumor doubling times (DT) are given separately for each experiment and statistical significance of difference of DT in treatment groups with respect to corresponding control group is indicated by value of p as assessed by Student t-test.

		C57B1/6			Swiss nude		
Exp.	Treatment	n	DT(days) mean±SD	p	n	DT(days) mean±SD	p
	control	8	4.0±2.2		8	2.8±1.5	
1	0.6mA/1h	8	6.3±2.9	0.096	8	3.4±1.0	0.362
	1.0mA/1h	8	11.7±5.4	0.002	8	4.5±1.2	0.025
	control	8	2.8±0.7		7	1.8±0.8	
2	0.6mA/1h	8	7.5±2.0	<0.001	7	2.5±1.0	0.174
	1.0mA/1h	8	10.4±3.1	<0.001	7	2.7±0.4	0.021
	control				7	1.9±0.8	
3	0.6mA/1h				6	2.3±0.8	0.388
	1.0mA/1h				7	3.2±1.2	0.034

Table 2. Percentage of stained tumor cross-section area as an indicator of quality of tumor perfusion for LPB fibrosarcoma in immuno-competent C57Bl/6 and immuno-deficient Swiss nude mice. Perfusion was estimated immediately after one hour electrotherapy (ET) for two current intensities (0.6 mA and 1.0 mA) and 24 hours after 0.6 mA ET. Value of p indicates statistical significance of difference between each particular treatment group and corresponding control group, as assessed by Student t-test.

	C57B1/6			Swiss nude		
Experimental group	n	% stained (mean±SD)	p	n	% stained (mean±SD)	p
control	19	98±5		11	100±0	
0.6mA/1h (0h after ET)	27	80±30	0.013	7	100±0	1.000
0.6mA/1h (24h after ET)	8	81±35	0.044			
1.0mA/1h (0h after ET)	30	70±35	0.001	14	98±5	0.200

Discussion

In this study it was demonstrated that low-level direct current electrotherapy induces significant retardation of LPB fibrosarcoma tumors growing in immuno-competent C57Bl/6 mice and that this effect is also dose dependent. This is in agreement with our previous work on this and other tumor models.[12,14,20] When LPB tumors were treated in immuno-deficient animals, electrotherapy appeared to be much less effective. In another tumor model of SA-1 fibrosarcoma growing in immuno-competent A/J mice the electrotherapy of the same type as in this study produced similar growth retardation as observed in C57Bl/6 mice. An extensive study on A/J mice by means of Patent Blue staining showed rapid decrease in perfusion during electrotherapy (data not shown).[16,19] Staining was decreased to the mean value of approximately 20% in treated tumors and even three days after treatment tumors were only partially reperfused (approximately 50% of stained tumor cross-section), which is very different from perfusion of 80% found in LPB tumors for the same treatment (0.6 mA for 60 minutes). That means that vascular occlusion due to electrotherapy in SA-1 tumors was much more expressed than in LPB tumors. Since the dynamics of deperfusion and reperfusion of SA-1 tumors was in good correlation with dynamics of growth retardation of that particular tumor model it was suggested that vascular occlusion occurring at the site of insertion of the electrodes might be the main factor of antitumor effectiveness. The presented study has raised some doubt to this hypothesis because the same extent of LPB tumor growth retardation was accompanied by a much less expressed occlusive effect. Furthermore, LPB tumors in immuno-deficient animals were significantly less affected by electrotherapy than the same type of tumors in immuno-competent animals, which indicates that host's immune response might play an important role in effectiveness of electrotherapy.[20] In our opinion, occlusion of supplying blood vessels outside the tumor inevitably occurs at the site of electrode insertion due to extreme pH changes that were measured in immediate vicinity of electrodes.[12] The extent of this occlusion is probably significantly tumor model-dependent, as indicated by the difference in Patent Blue staining between the two fibrosarcoma models. Some other factors beside interrupted blood supply are probably responsible for the observed tumor retardation and immune system of the host organism is one of them.

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Superficial thermoradiotherapy: Clinical result favor immediate irradiation prior to hyperthermia

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Purpose. *The aim of the present paper is to report the analysis of some relevant tumor and/or therapeutic parameters and to compare different treatment strategies used in our patients treated by local thermoradiotherapy (TRT).*

Methods and materials. *In the period 1989-1995, fifty-two patients with locally advanced tumors accessible to local TRT were treated at the Institute of Oncology in Ljubljana, Slovenia. A majority of 39 (75%) patients failed to respond to previous radiotherapy, while in 13 patients TRT was used as primary treatment. Interstitial TRT (ITRT) as primary treatment was used in 13 (25%) patients, interstitial hyperthermia combined with simultaneous external irradiation (STRT) in 7 (14%), and external TRT (ETRT) was applied in 32 (61%) patients.*

Results. *In all 52 patients a complete response (CR) rate of 60% was achieved, while 2-year recurrence-free and disease-specific survivals were 51% and 45%, respectively. Among tumoral and therapeutic parameters tested, CR rate was found to be significantly influenced by histology other than squamous cell carcinoma* (p=*0.045), tumor volume < 55 ccm* (p=*0.02), minimum intratumoral temperature* (T_{min}) $\geq$ *42.5C* (p=*0.015), total tumor dose (TTD) of radiotherapy* $\geq$ *45 Gy* (p=*0.048), fraction size of irradiation used concurrently to hyperthermia > 3 Gy* (p=*0.03), and by those TRT treatments where irradiation preceded hyperthermia* (p=*0.026). Repeating of hyperthermia (HT) treatments did not improve the CR rate. The use of RT immediately prior to HT resulted in a 2-year recurrence-free survival (RFS) of 66% compared to 38% for patients in whom HT treatment was followed by irradiation* (p=*0.07). For the subgroup of 20 patients in whom fraction size of >3 Gy was delivered immediately before HT treatment, an even better RFS of 85% was achieved* (p=*0.03) . The enhancement ratio of 1.7 was found between the dose response curves for 29 patients receiving RT prior to HT and 23 patients in whom HT was used before RT. Acute and late toxicity of grade 3 and/or 4 were recorded in 28% and 23% of treated patients, respectively. TRT-related acute toxicity was more pronounced in patients with maximum temperature measurements inside the heated volume* (T_{max}) *45C* (p=*0.006), while a higher grade of late toxicity correlated with a tumor volume* $\geq$ *55 ccm (p=0.02) and TD of RT > 45 Gy (p=0.04). There was no significant correlation found between a higher toxicity grade and CR rate.*

Conclusions. *Our clinical results are in favor of an application of somewhat higher fraction size of RT than conventional, employed immediately before heating, when combined with HT.*

Key words: neoplasms-radiotherapy; hyperthermice, induced; local thermoradiotherapy; advanced tumors; clinical results; prognostic parameters

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Introduction

When introduced to clinical practice, local thermoradiotherapy (TRT) proved useful first of all in the treatment of advanced and recurrent/residual tu-

mors, either applied with invasive technique,[1-7] non-invasive technique,[8-12] or in combination of both.[13, 14] The advantage of TRT over radiotherapy (RT) alone was proved in some non-randomized,[8, 9, 14-16] as well as in a few randomized clinical trials.[17-20] Believing in biological reasons for combining RT and hyperthermia (HT) treatment,[21-25] at the Institute of Oncology in Ljubljana, efforts were made to develop technical equipment and our own treatment strategies for the application of TRT prevalently in order to overcome radioresistance of locally advanced tumors[26] and tumor lesions which failed to respond to previous RT. The aim of the present paper is to report the analysis of some relevant tumor and/or therapeutic parameters in our patients treated by local TRT.

Materials and methods

The patients

Altogether 52 patients (42 male and 10 female) were treated by TRT between 1989-1995 at the Institute of Oncology in Ljubljana, Slovenia; 39 of these were with recurrent and/or residual tumors after previous standard RT and 13 with primary advanced malignancy. Tumor sites were as follows: head and neck region in 46 patients, breast and/or thoracic wall in 5, and inguinal lymphnodes in 1 patient. Histologically, 38 (73%) tumors were squamous cell carcinoma, 8 (15%) adenocarcinoma, 5 (10%) malignant melanoma, and 1 (2%) Mb Hodgkin. By the time of combined HT and RT treatment, patients were free of distant metastases and were not receiving any other concurrent cancer therapy. Only patients with Karnofsky performance score $\geq$ 70% were eligible for TRT. Tumor volume ranged from 10 - 180 ccm (median 54 ccm).

Hyperthermia devices

Interstitial heating was performed by means of interstitial water hyperthemia system. Prior to clinical utilization our device had been tested on experimental animals. The results of animal experiments were published elsewhere.[27, 28] First clinical experience using interstitial water hyperthermia system showed acceptable homogeneity of temperature distribution inside the heated volume.[29, 30] Intratumoral insertion of plastic or metal tubes for application of interstitial water hyperthermia technique was done under a general anesthesia in 20 patients. Percutaneous heating was performed in 32 patients by non-invasive 432 MHz microwave unit using two different antennae to cover adequately the total tumor surface within safety margins. In the majority of cases no water bolus was used. Extensive local anesthesia using 2% Xylocain was utilized with percutaneous application of thermotherapy.

Radiotherapy

In 13 patients brachytherapy was applied in combination with interstitial HT. Ir-192 wires were inserted through the same plastic tube implant as used for HT. In all implants, X-ray and/or ultrasound verification was used to assure that the implant encompassed the whole tumor volume. A dose-rate of 0.5 - 0.7 Gy/h was delivered to the tumor periphery. Total tumor dose (TTD) in patients treated by interstitial TRT (ITRT) ranged from 20 - 70 Gy (median 60 Gy). In 39 patients percutaneous RT was applied using a fraction size of 1.8 - 3 Gy. The fraction size of brachyradiotherapy was estimated from dose-rates at the tumor periphery given within 4 hours after HT treatment.

In 7 patients interstitial heating using metal tubes was performed combined with simultaneous irradiation (STRT) by teleradiotherapy using electron beam. In one patients STRT using single fraction of 5 Gy was the only therapy, while in 6 patients TTD of RT ranged from 25 - 60 Gy (median 55 Gy). For the rest of 32 patients percutaneous HT combined with external RT using either electron beam or Co-60 was employed with TTD of 20 - 70 Gy (median 40 Gy).

TTD of RT depended on the time interval from previous RT and TTD of previous RT. Total cumulative dose of radiotherapy did not exceed 100 Gy. Thirteen patients without previous irradiation received 45 - 70 Gy (median 60 Gy), while TTD for 39 previously irradiated patients ranged from 5 - 66 Gy (median 40 Gy). Fraction size of RT used concurrently with HT differed from 1.8 - 8 Gy (median 3.5 Gy).

Thermoradiotherapy and thermometry

Thirteen patients receiving interstitial hyperthermia were treated under general anesthesia once only. Hyperthermia session started after steady state temperature distribution inside the tumor volume had been reached and lasted 60 minutes. Placement of Ir-192 wires followed immediately after the heating session in 9 cases, while in 4 patients brachythera-

py had to be postponed for more than one hour due to a substantial swelling of the heated region.

Seven patients were treated with simultaneous interstitial hyperthermia and external irradiation. This specific therapeutic approach has been presented previously.[31] On the day before HT treatment, metallic tubes were implanted through the tumor volume under a general anesthesia. The next day, the patient was placed in a room close to the linear accelerator, and the implant was connected to a water HT unit. Approximately 30 minutes after the beginning of HT session, the patient and the HT device were moved together to the linear accelerator unit and irradiation using electron beam was performed while uninterrupted heating continued. After completed RT session, the patient was moved again to the nearby location and heating proceeded until total HT treatment time of 60 minutes elapsed. In all 7 patients treated simultaneously TRT was not repeated.

Combined percutaneous HT and RT was performed in 32 patients. After a TD of 10-20 Gy had been reached, the first HT treatment was performed. A single session of HT during the RT course was performed in 13 patients whereas 19 patients received 2-3 HT treatments. Repeated HT was applied once weekly. The total heating time depended on maximum and minimum temperature measured inside the heated volume and lasted 45 - 60 minutes for each HT session. Owing to technical problems, in 5/32 patients the time interval between the application of both modalities exceeded one hour. In 3 patients HT was performed immediately before irradiation while in the remaining 29 patients HT followed RT.

Altogether, there were 23 patients treated with HT preceding RT, and 29 patients in whom HT followed RT treatment.

Invasive thermometry was performed in 20 patients treated by interstitial HT using five-point manganin-constantan thermocouple probes which were moved stepwise through 2-3 (depending on tumor volume) plastic tubes inserted perpendicularly to the implant. It was considered that HT treatment started when intratumoral temperature of >=42.5°C was obtained at the tumor periphery. Temperatures were registered every five minutes during the heating session; minimum and maximum temperatures were recorded.

In 32 patients heated with an external HT device, 2-3 (depending on tumor volume) plastic tubes were inserted through the heated volume, and temperature measurements performed by means of a one-point non-conducting temperature probe. The same protocol for temperature monitoring as in invasive HT treatments was used. with the exception of an extra thermal probe on the skin surface.

Tmin i.e., mean minimum temperature measured in 3-5 measurement points at the tumor periphery during entire heating session was taken as a reference for estimation of HT treatment quality. In patients with multiple HT treatments, the highest Tmin observed was recorded. The mean value of maximum temperatures measured intratumorally and/or on skin surface was expressed as Tmax and was used for treatment toxicity estimation.

Estimation of response to treatment and toxicity

Only complete clinical disappearance of the treated tumors (CR-complete response) 2 - 3 months after completion of TRT was estimated as a therapeutic success. All other responses were considered as treatment failures. The results were analyzed using Biomedical Statistical Software Package (BMDP);[32] the survival was calculated from the end of treatment using Kaplan-Meier's method.[33] A log-rank, χ^2-test, and Fisher exact tests were used to analyze the difference between groups. Sigma-plot computer program was used for the dose-response curves drawing. In order to estimate treatment related toxicity RTOG/EORTC system was used,[34] introducing HT related formation of blisters as grade 3 early toxicity. Only the most severe grade of toxicity for each patient was recorded.

Results

In all 52 patients treated with TRT at the Institute of Oncology, Ljubljana, Slovenia, a CR rate of 60% and a 2-year recurrence-free survival of 43% were achieved. The observation time ranged from 3 - 42 months (median 11 months). The prognostic significance of some tumoral characteristics is evident from Table 1 while the prognostic importance of the observed therapeutic parameters is apparent from Table 2. Next to tumor histology, tumor volume was found to be the most prominent prognostic factor among tumoral parameters. The only significant parameter among hyperthermic factors was T_{min} while neither the type of HT used nor the number of HT treatments showed any prognostic significance. Among radiotherapeutic treatment parameters, except for the type of RT used, all three

Table 1. Prognostic importance of patients' and/or tumor characteristics.

Tested parameter		N° of pts.	CR (%)	*p*-value
Sex	Men	42	23 (55%)	
	Women	10	8 (80%)	0.11
Tumor site	Head & neck	34	19 (56%)	
	Other	18	12 (67%)	0.65
Histology	SCC	38	19 (50%)	
	Other	14	12 (86%)	0.045
Tumor volume	< 55 ccm	31	23 (74%)	
	≥ 55 ccm	21	8 (38%)	0.02
Previous RT	Yes	39	24 (62%)	
	No	13	7 (54%)	0.8
	≤ 50 Gy	22	15 (68%)	
	> 50 Gy	17	9 (53%)	0.5

CR – complete response, SCC – squamous cell carcinoma, RT – radiotherapy

Table 2. Prognostic significance of therapeutic parameters

Tested parameter		N° of pts.	CR (%)	*p*-value
Type of HT	Interstitial	20	10 (50%)	
	Percutaneous	32	21 (66%)	0.4
Type of RT	Interstitial	13	6 (46%)	
	Percutaneous	39	25 (64%)	0.1
N° of HT	1	33	23 (70%)	
	> 1	19	8 (42%)	0.1
T_{min}	≥ 42.5°C	43	29 (67%)	
	< 42.5°C	9	2 (22%)	0.015
TTD of RT	≥ 45 Gy	30	21 (70%)	
	< 45 Gy	22	10 (45%)	0.048
Fraction size/HT	> 3 Gy	28	21 (75 %)	
	≤ 3 Gy	24	10 (42%)	0.03
Sequence	RT + HT	29	21 (72%)	
	HT + RT	23	10 (43%)	0.026

CR – complete response, RT – radiotherapy, HT-hyperthermia, T_{min} – minimum intratumoral temperature, TTD – total tumor dose, Fraction size/HT – fraction size of RT concurrent to HT

remaining tested parameters, i.e. total dose of RT, fraction size of immediate irradiation, and sequence of RT showed a significant influence on local treatment outcome. While, all patients in RT + HT group were treated by external TRT (ETRT) only, CR rates in HT + RT group differed regarding the type of TRT applied. There were 6/13 (46%) CR recorded in patients using ITRT, 4/7 (57%) using STRT, while no CR was obtained in 3 patients treated by ETRT. Figure 1 presents a 2-year recurrence-free survival of 66% for the patients in whom RT was used immediately before HT, vs. 38% achieved in patients where RT followed HT (p=0.07). For 20 patients in whom an immediate fraction size of >3 Gy preceded HT, a recurrence-free survival of 84% was achieved (p=0.03). In Table 3 the two groups of patients treated with different sequence of the two modalities are compared regarding some prognostic parameters. It is shown that both treatment groups are acceptably comparable. Figure 2 presents dose response curves for the two groups of patients in whom different sequencing of both modalities was used. An enhancement ratio of 1.7 was found for the group of 29 patients in whom RT was used immediately prior to HT when plotted against those 23 patients in whom RT followed HT.

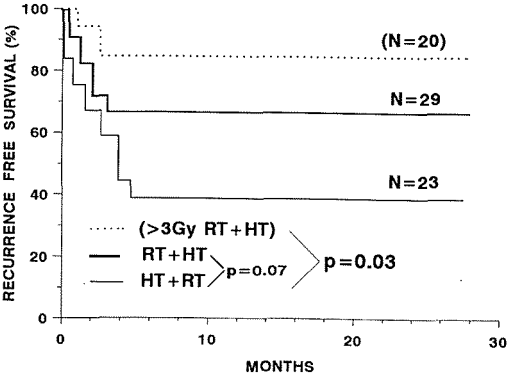

Figure 1. A 2-year recurrence-free survival (RFS) of 38% achived in 23 patients treated by TRT using HT prior to an immediate irradiation is compared to 66% for 29 patients in whom HT followed RT (p=0.07). Among those 29 patients in the latter group, there were 20 patients, refered in brackets, receiving a fraction size of > 3 Gy immediately before the heating. A significantly better RFS of 84% achieved in this subgroup of patients is presented by a dotted line (p=0.03).

The majority of our patients tolerated TRT treatment well, and there was no reason to terminate the therapeutic session before 45-60 min of HT treatment was reached. In 39 interstitial and/or percutaneous HT treatments, where no general anesthesia was used, generous infiltration of the heated volume with 2% Xylocain was employed. In a few patients pressure over the heated area was used in order to diminish the cooling effect of enhanced blood flow. Using RTOG/EORTC system, there were 29% of acute and 23% of late toxicities grade 3-4 recorded in our patients. Tables 4 and 5 present the prevalence of tested tumor and/or therapeutic

Table 3. The prevalence of prognostic parameters within the two groups of patients treated by different sequencing of the two treatment modalities.

Sequence of RT & HT	T_{min} ≥42.5°C (%)	Volume < 55 ccm (%)	TTD of RT ≥ 40 Gy (%)	Fr.size/HT > 3 Gy (%)	N° of HT > 1 (%)	Histology no SCC (%)
RT + HT	24/29 (83%)	19/29 (66%)	16/29 (55%)	19/29 (66%)	13/29 (45%)	8/29 (28%)
HT + RT	19/23 (83%)	12/23 (52%)	18/23 (78%)	9/23 (39%)	6/23 (26%)	6/23 (26%)
p-value	0.9	0.5	0.2	0.12	0.3	0.8

RT – radiotherapy, HT – hyperthermia, T_{min} – minimum intratumoral temperature, TTD of RT – total tumor dose, N° of HT – number of HT treatments, SCC – squamous cell carcinoma

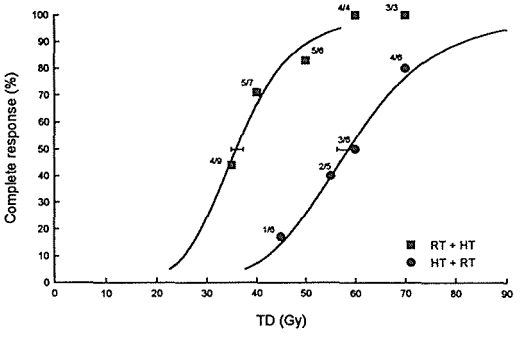

Figure 2. An enhancement ratio of 1.7 was found when the dose-response curve calculated for 29 patients receiving an immediate fraction of radiotherapy prior to the heat treatment had been compared to the dose-response curve for 23 patients in whom irradiation followed hyperthermia immediately.

Table 4. The influence of tumor and/or therapeutic parameters on acute toxicity grade.

Tested parameter		N° of pts.	Grade 3-4	*p*-value
Tumor volume	≥ 55 ccm	24	10	
	< 55 ccm	28	5	0.04
T_{max}	≥ 45°C	30	13	
	< 45°C	22	2	0.006
N° of HT	> 1	19	7	
	1	33	8	0.2
TTD of RT	> 45 Gy	30	11	
	≤ 45 Gy	22	4	0.1
Response	CR	31	9	
	no CR	21	6	0.2

T_{max} – maximum temperature measured in heated volume, HT – hyperthermia, TTD – total tumor dose, RT – radiotherapy, CR – complete response

Table 5. The influence of tumor and /or therapeutic parameters on late toxicity grade.

Tested parameter		N° of pts.*	Grade 3-4	*p*-value
Tumor volume	≥ 55 ccm	23	9	
	< 55 ccm	26	3	0.02
T_{max}	≥ 45°C	28	8	
	< 45°C	21	4	0.2
TTD of RT	> 45 Gy	27	7	
	≤ 45 Gy	22	5	0.3
Cumulative TTD	≥ 85 Gy	29	10	
	< 85 Gy	20	2	0.04

* In 3 patients evaluation of late toxicity was not possible because they died shortly after 3 months following TRT. T_{max} – maximum temperature measured inside the heated volume, TTD-total tumor dose, RT – radiotherapy

parameters at risk for the expression of higher grade toxicity. Tumor volume of 55 ccm was found equally significant for the appearance of both acute and late toxicity, T_{max} showed an important influence on acute toxicity rate, while higher cumulative TTD of RT was found to be responsible for a significant expression of high-grade late toxicity. There was no relation found in our patients between CR rate and the expression of either acute or late higher toxicity grade.

Discussion

Although, different treatment schedules of TRT were used at the Institute of Oncology, Ljubljana, it was possible to detect some important prognostic parameters with significant influence on local response rate. Next to tumor volume, TTD of RT, and T_{min} which has been previously recognized as prognostically important[16, 19, 35, 36] in our analysis also tumor histology, fraction size of RT concurrent to HT, and sequence of RT and HT were found of prognostic significance regarding CR rate (Tables 1

and 2). On the other side, tumor site, previous RT, type of RT and/or HT used, and more than one HT treatments showed no significant influence on response rate.

On basis of preclinical[21] and clinical data[37, 38] referring to the effects of HT treatment when combined with RT, it is possible to conclude that only appropriate heating should result in improvement of therapeutic effect when compared to RT alone. For multiple heatings used in clinical trials it was shown that CR rate, although strongly dependent of TTD of RT, is directly proportional to the cumulative equivalent time (CEM) at 43°C.[39, 40] Unfortunately, it is impossible to predict the sufficiency of heat treatment in clinical HT. In recently published randomized studies[18, 19] the planned intratumoral temperatures were reached only in a minority of treated patients. Although both trials failed regarding the planned threshold of minimum intratumoral temperatures, a significant influence of TRT over RT alone on CR rate (62% vs. 35%) was shown in a multicentric melanoma trial using ETRT,[19] while no benefit (57% vs. 54%) of combined treatment was recorded in RTOG study using ITRT.[18] From these data, regardless the differences in tumor histology, tumor volume, and TTD of RT for the patients treated in both series, two conclusions can be derived as follows: First, in external TRT where RT using a higher fraction size had been followed by an immediate heating, even "mild HT" significantly enhanced the treatment results. Second, "mild HT" followed by an interstitial low dose-rate brachyradiotherapy did not enhance already acceptable results achieved by RT alone. In meta analysis of clinical ETRT trials[15] it was found that the thermal enhancement ratios (TER) for different tumor types were similar regardless their response to RT alone. In the majority of published non-randomized series of head & neck tumors treated by TRT, the mean CR rate of 64% for ETRT[15] and 55% for ITRT[41] achieved were similar to the results of the two above mentioned randomized studies. In summary, although the heating in the majority of clinical trials has been equally inadequate, a constant superiority of ETRT over ITRT was recorded. It is hard to believe that thermal enhancement was more pronounced in ETRT trials, simply due to inferior results obtained by fractionated irradiation alone when compared to continual low-dose irradiation. It is more likely, that some other (presumably radiotheraputic) treatment parameters are responsible for the loss of thermal enhancement in ITRT.

In our treatments T_{min} of 42.5°C, measured at the tumor periphery throughout the 45 - 60 minutes of HT treatment course has been chosen as a therapeutic goal.[12] With respect to our HT strategy as much as 83% of patients (43/52) were adequately heated. Surprisingly, our overall CR rate of 60% was not found any better when compared to previously published TRT treatment series.[15, 42] However, when two groups of equally heated patients treated with different sequencing (i.e., RT + HT vs. HT + RT) (Table 3) were tested, a CR rates of 72% vs. 43% were estimated, respectively (p=0.025). RFS was significantly better for those 20 patients in whom >3 Gy of RT was used immediately before the heating (p=0.03) (Figure 1). When the dose response curves for the same two groups of equally heated patients has been drawn (Figure 2), the shape of the curve obtained in 23 patients in whom RT followed HT strongly resembled the curves for RT alone published in previous clinical trials.[15] According to the published data, similar effect are to be expected when HT is employed either before or after RT,[43] however in our patients an enhancement ratio of 1.7 was calculated in favor of TRT using RT immediately prior to HT. In one published animal study using ITRT,[44] the opposite findings were recorded, however, neither hyperthermic nor radiotherapeutic conditions used in aforementioned experiment are clinically obtainable.

The prediction that mild HT combined with low-dose rate RT would yield the best response[45] clinically failed.[18] On the other side, the possibility of tumor reoxygenation during mild HT[46] most likely does not affect tumors bigger than only few ccm.[26] If it would, the number of HT treatments should , by the process of reoxygenation, significantly improve the therapeutic results, not only in small experimental animal tumors,[47] but also in substantially larger human tumors.[48, 49] Though, in most of our patients cytotoxic level of intratumoral temperatures has been reached, still, practically no benefit of HT was recorded in patients in whom RT followed HT. The same observation was reported from animal experiments, when HT preceded RT.[50] It should be stressed that in a majority of our sufficiently heated patients the signs of circulatory collapse have been observed 30 minutes after T_{min} of 42.5°C at the tumor periphery has been reached. It is impossible to expect an enhancement of radiation damage in such a hypoxic situation. This is also a probable explanation for the achievement of only average treatment results (57% CR rate) achieved

in 7 patients in whom a really simultaneous TRT was employed, introducing the irradiation 30 minutes after HT treatment has been started. The fact, that in the majority of clinical trials an enhancement of TRT over RT alone was proved, although intratumoral temperatures achieved were far below from those proposed by results achieved from *in vitro* and *in vivo* experiments, is leading to a reasonable conclusion that in clinical circumstances tumors are more sensitive to HT. However, according to CEM,[40] it is not understandable that even more than 30 minutes of an "adequate heating" (i.e., $T_{min} > 42.5°C$), achieved in the majority of our patients, the therapeutic effect, obtained by HT + RT sequence, yielded the treatment results comparable only to those achieved by RT alone. The possible explanation for these findings is that the mechanism of thermal efficiency in clinical TRT most probably acts prevalently by inhibiting the ability of repair system for radiation induced damage. Because of somewhat greater sensibility of human tumors, in clinical conditions, to the heat, even non-cytotoxic HT could enhance an immediately preexisting radiation damage, however, sufficient heating probably results in better enhancement. Therefore, it looks like, it is not HT itself to be blamed for the disappointing treatment results obtained by ITRT, but more likely the placement and the amount of immediate irradiation used concurrently with HT. Our CR rate achieved in patients, where RT + HT sequencing has been used (i.e., CR rate of 72%), were even slightly better when compared to other randomized trials with similar sequencing employed.[15] Except for malignant melanoma, the positive influence of a higher fraction size of immediate RT, which in our case resulted in CR rate of 84%, in former clinical trials had not been found relevant. If radiosensitization induced by HT is a consequence of disturbances in DNA repair,[51] then only irradiation applied immediately before HT could result in the potentiation of radiation damage. A larger fraction size of RT should, therefore, result in more expressed potentiation of induced radiation damage. It is likely that higher intratumoral temperatures, rather than mild, interfere with the ability of repairing processes more effectively. Presumably, the recorded influence of somewhat higher fraction size on CR rate in our report is also related to the substantially higher level of intratumoral temperatures achieved in our HT treatments. Taking into account that in our report fairly good treatment results were achieved when RT was used immediately prior to somewhat more intense HT, even our higher rate of toxicity reported (Tables 4 and 5) could still be acceptable, considering the fact that the treatment by TRT was the only chance of prolonged survival due to a local cure in our patients.

It is true that a variety of tumor types and different treatment schedules used in our report can bias the final results. However, it is also true that by using strict protocols the differences would probably never show up.

It is our conclusion, that in the treatment of superficial human tumors by HT, either "mild" or "sufficient" heating, could be an important additive to RT, when appropriately employed. In our patients a significant influence of reasonably higher fraction size of RT on treatment results, when applied immediately before the heating session, was detected. Most likely, the low-dose irradiation, by having no ability to provide a productive amount of instant radiation damage to be increased by heat, rather than insufficient heating, was responsible for a poor expression of thermal enhancement in ITRT clinical trials.

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Radiotherapy in nephroblastoma. Pre- and postoperative combination treatment. Radiotherapy in localized (stage II, III, IV) and metastatic disease. Acute and long-term side effects.*

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Nephroblastoma, an embryonic type of malignant tumor in the kidney found in infancy and early childhood is sometimes associated with congenital anomalies. In recent years the research in genetics of this tumor has been extremely active. For decades radiation has generally been accepted as a valuable supplement to surgery in the treatment of nephroblastoma. Unfortunately it may produce undesirable late effects. With the survival rates steadily improving from about 25% before up to 80% and more, the problem of late sequelae is becoming the most important one. With the aim to diminish late sequelae with adjustment of treatment to known variables several clinical trials have been conducted in Europe, USA and Great Britain. The results of these are presented together with adverse effects and second malignancies. Further prospects are discussed. The success of therapy for children with nephroblastoma has resulted in growth to adulthood of a large population of former patients. Radiation therapy is considered responsible for a great deal of early and late toxicities. However, the number of children at risk for this has certainly diminished.

Key words: nephroblastoma-radiotherapy

General aspects

Nephroblastoma is a term used for an embryonic type of malignant tumor in the kidney, found in infancy and early childhood, seldom seen beyond the age of 10 and very rarely in adult life.

Wilms suggested in 1899 that the tumor arises from undifferentiated mesoderm. This was accepted and his name has been associated with the tumor ever since. Nephroblastoma comprises 10% of malignant diseases in children and afflicts one in 10.000 children.

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Nephroblastoma is sometimes associated with congenital anomalies. Children with aniridia, genito-urinary malformations and mental retardation (WAGR), Denys-Drash syndroma or Beckwith-Wiederman syndroma are considered to run a higher risk for nephroblastoma.[1]

In Wilms' tumor associated with specifical congenital syndromes, WT 1 germ-line mutations are frequently detected, while in sporadic Wilms' tumor WT 1 - DNA mutations are present only in 6% of the cases. Possible molecular markers for prognosis, using DNA techniques, are being developed. The loss of heterozygosity for chromosomes in Wilms' was seen for chromosomes 11 p, 16 q and 1 p in 33,17 and 12% respectively. Patients with loss of heterozygosity for chromosome 16 q had mortality rates 12 times higher than those without it. However, the correlation of chromosome 16 q and poor prognosis seems related to tumor progression rather than initiation, as there is no constitutional deletion

of the chromosome in Wilms' tumor. Whether IGF binding protein -2- levels will be a useful serum marker of Wilms' tumor activity is no yet established.[2,3]

Radiation treatment - development

The first cure of a child with nephroblastoma was reported in 1894, the report on the first child treated for nephroblastoma by radiation therapy (RT) alone in 1916 and in 1945 the first cures of children with nephroblastoma treated with RT were reported. It has since then been generally agreed upon that RT is a valuable supplement to surgery for nephroblastoma.

A beneficial effect of preoperative radiation has been first shown in 1947 and later by many others. Opponents, however, warned of the delayed surgery and thereby risk for metastases. They also denied that preoperative shrinkage of the tumor was of importance for the surgeon. The discussion on whether radiation should be used preoperatively or postoperatively has been continued until 2 decades ago, when more systematical studies provided evidence of the value of preoperative radiation and of the value of preoperative treatment of nephroblastoma in general. This experience is still not generally used outside Europe, preoperative chemotherapy (ChT) is at present widely accepted as a mode of treatment for the great majority of children with malignant solid tumors.

The optimal dose of radiation for nephroblastoma was also a matter of discussion for decades. It was shown more than 20 years ago that 20 Gy to the tumor bed postoperatively was sufficient for tumor control. The decision, that in the great majority of cases not more than that is necessary, was made only after the introduction of Actinomycin D. A significant influence of preoperative RT in locally advanced tumors on the cure rate was shown in a retrospective multivariate analysis in 1973 and was the basis for the first study to determine whether preoperative radiation improves survival as compared to postoperative radiation, in a prospective randomized trial.[4] Postoperative radiation was still much more in use.

The technique of RT as regard the design of the treatment volume, fractionation schemes, daily dose and planning has not changed much during the decades. Two opposing fields are used to cover the tumor bed. After recognizing the risk for scoliosis, when irradiating only one half of the vertebral body, the 2 opposing fields were enlarged to cover the whole vertebral bodies. Fields for treatment of the whole abdomen or the whole lung in disseminated tumors have also remained essentially the same, although the dose had to be reduced because of additional ChT. Nephroblastoma being a rare condition; studies have been conducted in cooperative groups: The National Wilms' Tumor Study (NWTS) [5] started in 1969 in the USA by the Children Cancer Study Group (CCSG). The Nephroblastoma SIOP 1 in Europe in 1971[6] and in 1979 the United Kingdom Children's' Cancer Study Group Wilms' trial (UKW) in Great Britain and Norway.[7]

The stage of the tumor and its histological type have been recognized as the most important factors for the prognosis in nephroblastoma. Consequently, the criteria applied for randomization were mainly the same in the three trials, the questions asked, however, varied, as they were based on previous experiences and traditions at the 3 different regions. Recognizing the importance of lymph node involvement for prognosis[8], the staging has been slightly modified, from that which was first accepted by the NWTS 1, and in the SIOP 1 Study trial.

The significant difference in the outcome of tumors with favourable histology (FH) and unfavourable histology (UH) (10% of all cases), has been confirmed in the trials.[9] For further randomization, only localized stages with FH were included; infants and stage IV, Stage V and all tumors with UH were dealt with in other ways.[10] In the course of these studies several histological subtypes have been recognized e.g. the Stockholm histological classification of nephroblastoma (Table 1).

Table 1. The Stockholm working classification of renal tumors of childhood (1994).

I. LOW RISK TUMOURS ("FAVOURABLE")
– Cystic partially differentiated nephroblastoma
– Nephroblastoma with fibroadenomatous-like structures
– Nephroblastoma of highly differentiated epithelial type
– Nephroblastoma - completely necrotic (after preoperative chemotherapy)
– *Mesoblastic nephroma*
II. INTERMEDIATE RISK TUMOURS ("STANDARD")
- Non-anaplastic nephroblastoma with its variants
– Nephroblastoma - necrotic but some features left (< 10%)
III. HIGH RISK TUMOURS ("UNFAVOURABLE")
– Nephroblastoma with anaplasia
– *Clear cell sarcoma of the kidney*
– *Rhabdoid tumour of the kidney*

Groups of patients with very good prognosis have been identified; e.g. children younger than 2 years with tumors of FH, not greater than 550 ccm (NWTS) or stage I tumors[11] in which after preoperative chemotherapy no vital tumor cells were found (SIOP). In such cases postoperative treatment could possibly be omitted. Also, groups with very poor prognosis have been identified; those with advanced tumors and UH. For those, more aggressive treatment schemes were designed.[12]

By increasing the rate of Stage I tumors with preoperative chemotherapy in the ongoing SIOP trial, postoperative radiation is given only to 20% of children as compared to 80% in the first SIOP study.[13] The number of children receiving postoperative RT has diminished in the UKW when the study confirmed that omittance of postoperative RT for Stage I tumor is safe and RT was given to Stage II and Stage III tumors only if at the second look operation no residual tumor was found. Fewer children receive RT in the NWTS as well and also the doses in general are lower.

After the most important questions were resolved: on the value of preoperative treatment in the SIOP, on the effect of chemotherapy combinations VCR, VCR - AMD, VCR + AMD + Doxo in the NWTS, on the safe omittance of postoperative RT for Stage I tumors in the UKW, the main goal, that is, to decrease the number of children receiving postoperative radiation seemed to be achieved.

The experience of the different trials was constantly exchanged and incorporated into treatment schemes. Preoperative treatment is used in the ongoing SIOP trial for all cases, in the USA it is given to children with "unresectable" tumors and its beneficial effect is reported in selected groups of patients with intracaval and intraatrial tumor extension.[14] Also, in the UKW study, preoperative treatment is given to individually considered unresectable tumors. The main drawback, according to the opponents of preoperative chemotherapy, is the difficulty of histological classification. They accept, however, a 100% tumor response to preoperative chemotherapy as a good prognostic sign. There is still no known effective treatment for some of the tumors of UH, especially if disseminated. For these children with poor prognosis, RT is still used to reduce the risk of abdominal recurrence, while new chemotherapeutic agents or new combinations are being introduced to reduce the risk for metastases.

In the majority of cases, radiotherapy has been replaced by chemotherapy. For advanced tumors and those with UH, Doxorubicin is usually added. It has also been a part of treatment for UH and recurrent tumors, often in combination with RT. Fatal late effects of cardiotoxicity have been observed after treatment with Doxorubicin. The answer to the question how best to balance the merits and demerits of chemotherapy vis-a-vis those of RT has yet to be found.

Adverse effects of treatment

With the survival rates of Wilms' tumor steadily improving from about 25% before the introduction of chemotherapy, with the amounting knowledge and treatment tailored to age, stage, histology type, the survival rates are now up to 80% and more in some good risk groups; the problems of late sequelae are becoming the most important ones.

The tolerance for RT of different organs has been agreed upon after decades of experience and observation, the late effects of chemotherapy are still being recognized and the effects of combination of chemotherapy and RT are a rather new chapter of radiobiology.

Radiation nephrotoxicity

In general, the tolerance to radiation of the kidney in the child is similar to that in the adult. It is about 20.0 Gy when radiation has been delivered to both kidneys in 3-5 weeks, using reduced daily fractions, when delivered without chemotherapy. The duration of follow-up is critical, signs and symptoms of late radiation damage to the kidney may not develop for years. The underlying process of late radiation damage is progressive nephrosclerosis.

It has been suggested that renal function compatible with clinical health in nephrectomised patients can be preserved as long as the remaining kidney has received less the 12.0 Gy. It has been reported that one child out of four, who received 14-15 Gy, experienced transient nephropathy, and one child in the NWTS has developed fatal nephropathy. Thus, the dose of 14 Gy appears relatively safe even on a long-term basis. An excess of diastolic hypertension has been reported in the long-term survivors (5 years from diagnosis) of the NWTS, notably among younger children. The results of this retrospective study have to be taken with caution for several reasons.[15]

It has been suggested that moderate doses of radiation (14.5 - 20.0) may reduce or eliminate the

ability of a remaining kidney to hypertrophy. The enhancing effect of AMD on radiation nephropathy is controversial, but in animal experiments BCNU, cisplatinum and Doxorubicin showed this effect. Iphosphamide may produce nephropathy, but it is not clear if it enhances the effects of RT.[16]

Radiation hepatotoxicity

Hepatotoxicity is one of the major acute reactions to combined postoperative treatment in nephroblastoma patients. 30.0 Gy is considered a safe RT dose to the whole liver. The tolerance of the liver varies a great deal depending on the volume irradiated and on additional chemotherapy. Hepatotoxicity identical in presentation and course to radiation hepatitis occurred in children who received postoperative chemotherapy only, in about 10%. In the SIOP group of patients who received postoperative radiation and AMD it occurred in 11 out of 58 children (19%) who had major parts of the liver or whole liver within the irradiation fields. Four of them had veno-occlusive disease (VOD). The dose range was 12.0 - 22.5 Gy with a few who had a boost up to 30 Gy. All four patients who had VOD had received doses > 20 Gy and AMD on 5 consecutive days (15 y/kg). None of the patients who received a single dose of AMD (0/13) developed liver toxicity but 11/24 with AMD on 5 consecutive days did. All children recovered from toxicity with conservative treatment and dose reduction of AMD.[17, 18]

Radiation pneumonitis

Radiation induced interstitial pneumonitis and pulmonary fibrosis are common complications of treatment for lung metastases in Wilms' tumor patients. A safe dose to the whole lung is considered to be 20 Gy when given in conventional fractionation and without chemotherapy; 1400 Gy in 10 fraction in combination with ChT have resulted in no complications. In the NWTS, however, with 14 Gy to the whole lung plus AMD and VCR, pneumopathy was found in 10 - 13%.

Whether Doxorubicin addition increased the rate of late pulmonary dysfunction is not clear. Radiation pneumonitis has been seen as a "recall effect" of AMD after RT.[19]

Cardiac toxicity

Congestive heart failure is a known complication of therapy with antracyclines. Its frequency is directly proportional to the cumulative dose of doxorubicin, with a reported incidence of congestive heart failure of about 5% in patients who received a cumulative dose of 400-500 mg/m2 or more. Although the initial reports of cardiac failure were limited to the first year after completion of therapy, reports of heart failure and dysarrhytmias, leading in some cases to sudden death, are now appearing in patients treated 4-20 years earlier. In patients from the NWTS 1, 2 and 3, 8 cases (1.7%) were found of congestive heart failure after doxorubicin treatment. Additional risk factors included whole lung irradiation and concurrent therapy with cyclophosphamide. Only further follow-up will define the magnitude of the risk for different combinations of chemotherapy and RT.[20]

Reproductive system

Damage to the male and female reproductive system caused by cancer therapy may result in infertility or hormonal dysfunction. Up to 12% of female survivors of childhood cancer who received abdominal radiation have ovarian failure. In males, gonadal radiation may result in temporary azoospermia and elevated levels of follicle stimulating and luteinizing hormones. A study of pubertal development in children treated for Wilms' tumor found that 3 out of 10 girls and 1 out of 6 boys had delayed development associated with elevated hormone levels. The relative fertility of the 20 Wilms' tumor survivors was 1.49%. Serious adverse pregnancy outcomes have been observed in women who had received abdominal radiation for Wilms' tumor. Perinatal mortality has been estimated to be eight times higher than among USA white women in general and the rates of low births weight 4 times higher. Similar observations regarding the effect of abdominal radiation on low birth weight have been made in babies born to other childhood cancer survivors. The risk for children born to Wilms' tumor patients who had chemotherapy, but no RT, is not known.

Second malignancies

Children treated for cancer are at increased risk for both malignant and benign second tumors. Cyclophosphamide, doxorubicin and cisplatin currently used to treat high risk Wilms' tumor patients, or those who had recurrences, have been implicated in leukemogenesis and carcinogenesis. Radiation and chemotherapy in combination may be more onco-

genic than either agent alone. Studies of second malignant neoplasms in Wilms' tumor survivors have documented a 1% cumulative incidence at 10 years from diagnosis and rising thereafter. All but 2 of the 26 SMN identified in 2 studies occurred in irradiated patients, most within the radiation field.

In the NWTS, among 5415 patients treated, 46 developed SMNs, 34 of these had RT and 12 had no RT. Radiation, doxorubicin and recurrence, alone or in combination appear to account for 86% of the SMN cases recorded.[21] These 46 cases represent a 8.5 times higher rate than excepted. One possible cause of SMN might also be related to genetic predisposition; there are some observation that suggest such correlation: patients with congenital anomalies have an increased risk of SMN, the patients who experienced SMN were relatively young (5 out of 7 less the 2 years old and in one study of 36 patients with SMN after Wilms', eight had first degree relatives with Wilms' tumor or congenital anomalies associated with Wilms' tumor.[22]

How much the risk for SMNs has decreased by decreasing the use of RT only time will show. Much work is still needed to define the risks depending on treatment modality, doses of RT and particular chemotherapy agents.

The success of therapy for children with Wilms' tumor has resulted in growth to adulthood of a large population of former patients. The quality of their lives and the lives of their offspring must be our first concern after saving their lives.

RT is considered responsible for a great deal of early and late toxicities and the most serious late sequelae - second malignant tumors. The number of children at risk for this has certainly diminished.

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Three-layer template for low-dose-rate remote afterload transperineal interstitial brachytherapy

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Our experience with a low-dose-rate (LDR) remote afterload device (RAD) for transperineal interstitial brachytherapy with ^{192}Ir wires has shown that in commercially available templates the smallest distance between individual needle guides is 10 mm due to the fixation technique used; the fixation of the guides also turned out inadequate since these tended to move uncontrollably when attached to the LDR RAD. Therefore, a special three-layer template was developed at our Institute. The two outer plates of the device are joined to form a rigid unit; there is a third, longitudinally movable plate placed in between the other two. The distribution of holes for guides insertion is identical in all three plates, and can be adjusted as required. By tightening two screws mounted on the outer plates, the medium plate is moved longitudinally, thus simultaneously fixing all the guides inserted. We believe that this device proves very useful, thanks to the simplicity of technical solution, more suitable distribution of holes for guides as well as firm and simultaneous fixation of all guides.

Key words: brachytherapy-methods; interstitial brachytherapy, transperineal template

Introduction

According to the rules of Paris system for dose calculation,[1,2] a template for needle guides fixation in the interstitial brachytherapy using low-dose-rate (LDR) remote afterloading device (RAD) with ^{192}Ir wires should provide a geometrically correct distribution of needle guides in the treated volume, and also ensure strong fixation of the guides during implantation.

Evaluating our previous work,[3] we found commercially available templates imperfect as a result of the guide fixation method used.[4-6] The distribution of holes for guides in these templates permitted for a minimum distance of 10 mm between guides. Taking into account the irregular shape of tumors implanted, this would not always enable an optimum distribution of guides in the implanted area. Besides, the fixation of guides in these templates was insufficient as they would move uncontrollably while attached to RAD during implantation.

Therefore, at the Department of Brachytherapy of the Institute of Oncology in Ljubljana, we have made a template which is devoid of the previously mentioned drawbacks.

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Design

We have developed and constructed a three-layer template presented in Figure 1. The two thinner, 3 mm thick outer plates are joined in four corners with screws and distance washers to form a rigid unit. The third, median 5 mm thick plate is movable longitudinally with respect to the outer plates. All three plates have identical perforations, the hole diameter being the same as the outer diameter of

the guides. The guides are inserted through the three plates and fixed by tightening of the two screws mounted on the outer plates. At doing this, the median plate slides longitudinally; the movement is gradual which allows precise exertion of pressure on the guides and thus simultaneously fixing all the guides inserted. This is necessary to prevent guide distortion and at the same time ensure sufficient shear forces required for stable fixation of the guides inserted. Generally, we use a template with regular 5-mm square hole distribution pattern which ensures good coverage of the treated volume. Its low weight, simple construction and solid fixation of conveniently distributed guides solve the problems associated with massiveness, elaborate construction, and inadequate distribution of holes which results in inappropriate distribution of guides in the existing commercially available templates.

Conclusion

Our experience with the presented template indicates that this meets the requirements for LDR remote afterload interstitial brachytherapy. It enables greater flexibility as to the selection of distance between guides, and their distribution with respect to the shape and size of the volume treated. Also, the fixation of the guides inserted is simultaneous, which prevents the guides from being distorted. Accordingly, when attached to RAD tubes, no uncontrollable movements may occur during implantation.

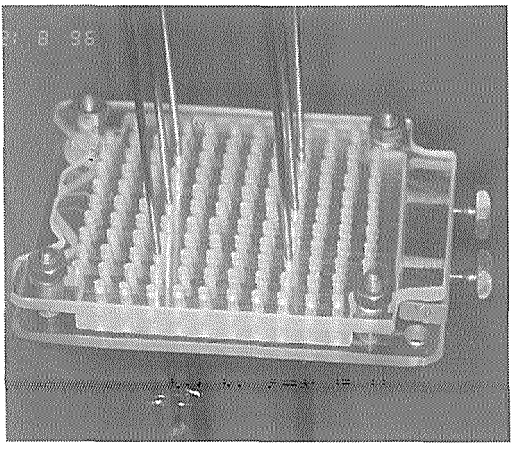

Figure 1A. Template assembly for LDR RAD interstitial brachytherapy with ^{192}Ir wires designed and worked out in the Institute of Oncology, Ljubljana, Slovenia.

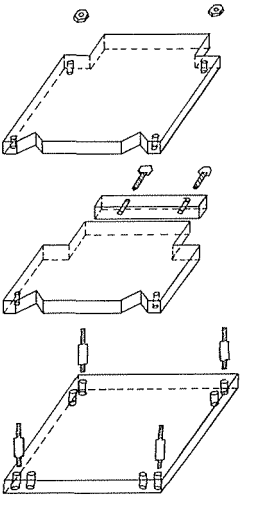

Figure 1B. Schematic description of template.

We believe that the template designed and worked out in the Institute promotes the quality of work with LDR RAD in our transperineal interstitial brachytherapy.

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ESTRO teaching course in Basic Clinical Radiobiology

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This year ESTRO Teaching course in Basic Clinical Radiobiology was organized in co-operation with the Dukuz Eylul University of Ismir and The Turkish Radiation Oncology Society. Almost 150 medical doctors, physicists and biologists from different European countries and also from Japan, the United States of America and Australia attended this very well prepared teaching course. The course provided an introduction to radiation biology as applied to radiotherapy.

The first day of the course was dedicated to the basic lectures introducing radiobiology. Prof. Dr. A. van der Kogel from Institute for Radiotherapy, the Netherlands described the concept of clonogenic cells and cell survival. The clonogenic cells could be ascribed as the cells that form colonies exceeding 50 cells (5-6 divisions) within a defined growth environment. He gave an overview of different assays of clonogenic tumor and normal tissue cells. By these assays one can detect the stem cells since they have the ability to form a colony. Models of radiation cell killing were mathematically explained by Dr. M. C. Joiner from the CRC Gray laboratory, United Kingdom. Three models were explained as follows:

a) Single target single hit model; the idea of this model is that just one hit by radiation on a single sensitive target leads to cell death. The survival curve is exponential and the probability of survival can be calculated by means of Poisson statistics.

b) Multi-target single-hit model. This model assumes that there is more than one sensitive target and again a Poison statistics can be applied to determine the probability of cell survival.

c) Linear-quadratic model gives a better description of radiation response in the low-dose region (0-3 Gy). The shape of the curve is determined by the α/β ratio; the dose at which the linear contribution to the damage equals the quadratic contribution. Linear component of the equation might be due to a single track event while the quadratic component might arise from two-track events. A high α/β ratio is characteristic of response of cells to densely-ionizing radiations such as neutrons and α-particles, while a low α/β ratio is characteristic of X-rays.

Dr. F. A. Stewart from the Netherlands Cancer Institute presented the lecture entitled "Cell Proliferation in Normal Tissues". Normal tissues can be divided into two categories: hierarchical tissues with a clear separation between the stem cell compartment, an amplification compartment and post-mitotic compartment of mature functional cells (bone marrow, epidermis, intestine...) and flexible tissues, where all cells are capable of cell division (lung, kidney, spinal cord...). The hierarchical tissues are rapidly responding tissues where the amount of damage and the time to repair the damage caused by radiation are dose dependent while the time to response is not. In contrast, in the flexible tissues it takes weeks to see the damage, and the rate of damage expression is strongly dose dependent.

Dr. M. Baumann from Carl Gustav Carus University Clinic, Germany listed in his lecture on clinical radiobiology the radiosensitive and radioresistant tumors. He also stressed the importance of dose per fraction and overall treatment time for tumor response and normal tissue damage. A more accurate description of how changing the dose per fraction influences the shape of isoeffective curves for radiation damage to normal tissues was given by Dr. S. M. Bentzen from Cancer Research Institute, Denmark. The sensitivity to large fraction sizes is much higher for late responding than for early responding normal tissues. He also explained shortcomings of Ellis NSD formula and strongly opposed its further use. Growth kinetics of tumors were explained by Prof. Dr. G. G. Steel. He presented the method of measuring tumor cell proliferation by using flow cytometry and described the parameters: growth fraction (the proportion of cells that are proliferating), population doubling time (time required for a cell population to double its number), potential doubling time (the predicted cell population doubling time in the assumed absence of cell loss), cell cycle time (time between two mitoses) and cell loss factor (the rate of cell loss from

a tumor, as a proportion of the rate at which cells are being added to the tumor by mitosis).

The second day of the curse dealt with radiobiology of normal tissues, re-treatment tolerance of normal tissues, the linear-quadratic approach to fractionation and hyperfractionation, and accelerated therapy. Prof. Dr. A. van der Kogel held a lecture about radiobiology of normal tissues, focused on radiation effects on the central nervous system. In the central nervous system where the turnover of the cells is very long, the early radiation damage (white matter necrosis) is expressed after 4-6 months, while late vascular damage is detectable only after 7 months. These results were obtained from the studies of radiation effects on rat spinal cord. In general, a high, α/β ratio is characteristic for early radiation effects, and a low one for late effects. This year, a new lecture on re-treatment tolerance of normal tissues was given by Dr. F. A. Stewart. All prospective studies on this topic were done on animal models. Rapidly renewing tissues such as the skin or intestine have full reirradiation tolerance within 2-3 months, since they show almost complete recovery within 1-2 months after the first treatment. In slowly renewing tissues (central nervous system, lung...) recovery is much slower and depends on the size of the initial radiation dose.

The lecture on use of linear-quadratic model in fractionation was presented by Dr. M. C. Joiner. The linear-quadratic model describes the relationship between a total isoeffective dose and dose per fraction more satisfactory than Ellis NSD formula. For late responding normal tissues a low α/β ratio (0.5-6.0 Gy) is characteristic indicating that for the same effect you must increase the total dose if you decrease dose per fraction. A higher α/β ratio (7-20 Gy) is characteristic for early responding tissues and tumors, which indicates a less rapid increase in total dose with decreasing dose per fraction. The benefits and disadvantage of hyperfractionation (use of a reduced dose per fraction over a conventional overall treatment time using multiple fractions per day) and accelerated radiotherapy (use of a conventional dose per fraction over a reduced overall treatment time using multiple fractions per day) were explained by Dr. M. Baumann. In hyperfractionation the gain is that late reactions are reduced, while keeping the early radiation damage to normal tissues and the effect on tumor in same range as in conventional radiotherapy. The benefit of accelerated radiotherapy is that the tumor control curve in dose response plot is shifted to the left as a consequence of reducing the protective effect of tumor cell repopulation during radiotherapy. The radiation effects on normal tissue damage based on the data of three clinical trials are constant or even increased. The problems associated with the dose response curves for tumor response were explained in detail by Dr. S. M. Bentzen. His lecture was focused primarily on the problems of estimating the dose response relationship and on the prescriptions bias which could be very small but could lead to enormous change in tumor response to radiation therapy.

The first lecture on the third day was devoted to radiobiology of tumors. Prof. Dr. G.G. Steel explained the advantages and disadvantages of different experimental systems for studying the radiation effects on tumors from *in vitro* tumor cell lines and multi spheroids to *in vivo* transplanted and primary tumors of animal and also human tumor xenografts. The basics, and when to use the different end points e.g. growth delay, tumor control and cell survival for measuring radiation effects on tumors were also explained. Tumor growth delay is applied when the treatment performed is not a curative one. Tumor growth delay is defined as the time in the treated group minus the time in control group to reach the given size. The parameter most often used in tumor control assay is tumor control dose (TCD_{50}); i.e. the radiation dose that controls 50% of tumors. The advantage of cell survival assay is that by removing of cells into growth environment that is uniform and unaffected by the treatment there is less artefact due to effects of treatment to the host animal.

The antitumor effectiveness of radiotherapy is strongly dependent upon oxygen. The enhancement of radiation damage by oxygen is dose modifying and is constant at all levels of cell survival curve. Cells in tumors could be either chronically or acutely hypoxic. Chronically hypoxic cells are the cells that are situated on the edge of the viable tumor cells that surround blood vessels. Acute hypoxia occurs when blood vessels in the tumor are transiently occluded. The mechanisms for this type of perfusion-limited hypoxia are not yet known, but could be due to the plugging of vessels by blood cells or by circulating tumor cells; collapse of the vessels in the regions of high interstitial pressure; or by spontaneous vasomotion in incorporated host arterioles affecting blood flow in tumors (presented by Dr. M. C. Joiner). Since hypoxic cell are resistant to radiation several attempts were made to reduce hypoxic cells in the tumors, including:

a) Chemical radiosensitizing of hypoxic cells using drugs such as misonidazole or nimorazol;

b) Preferential killing of hypoxic cells using bioreductive drugs that are activated under hypoxic conditions;

c) Increasing oxygen availability by using hyperbaric oxygen (in this case you need a lower dose to control the tumors, but the studies on patients were stopped because this method was very inconvenient);

d) By introducing perfluorochemical emulsions into vessels to increase the oxygen-carrying capacity;

e) By using drugs that increase tumor blood perfusion;

f) By using carbogen (95% O_2 + 5% CO_2) under the normobaring conditions (Dr. M. Baumann).

The lecture on different classifications of normal tissue damage used in clinical practice was held by Dr. S. M. Bentzen. It is essential to score the normal tissue damage and treatment-related mortality in any study of cancer treatment. He also presented basic features and limitations of RTOG/EORTC, WHO, French/Italian and European systems for reporting and grading of treatment-related morbidity after radiotherapy.

The fourth day of the course was dedicated to clinical implications of volume effect, brachytherapy (dose-rate effect) and combination of radiotherapy and chemotherapy. The volume effect is described as dependence of the radiation damage to normal tissues on the volume of tissue irradiated. The radiosensitivity of the tissue depends largely on its organization into separate functional units and on the potential of the surviving stem cells to migrate and repopulate in irradiated part of the tissue. Prof. Dr. A. van der Kogel presented the volume effect on the spinal cord, lung, kidney and skin. He also pointed out that Ellis NSD formula is not suitable for the evaluation of volume effect. Mechanisms underlying the dose-rate effect are repopulation, cell-cycle progression (reassortment) and repair. Repair is the fastest (half time is approx. 1 hour) of these processes and modifies the radiation effects over the dose range in between 1 Gy/min and 1 cGy/min. Reassortment is a slower process and modifies the radiation effects over an intermediate range of dose rates. Repopulation is the slowest process; doubling times of tumors and normal tissues range from few days to several weeks or even months, therefore the repopulation affects cellular response at very low dose rates (below 2 cGy/min) depending on cell proliferation.

The combination of radiotherapy and chemotherapy was the subject of lecture given by Dr. F. A. Stewart. Aims of combined modality treatments are improvement of local control, eradication of distant metastases and avoiding mutilating surgery. Interaction between radio and chemotherapy could be either short-term interaction (sensitization), medium-term interaction or long-term modification. Short-term interaction comprises alterations in the shape of drug/radiation survival curve. The mechanism underlying the short term interactions is repair inhibition. Medium-term interactions are alterations of repopulation kinetics, oxygenation and pH status of the tumors and cell cycle distribution. In long-term interactions residual damage to tumors is a consequence of one modality that influences the response of the other.

Chemotherapeutic drugs could be given either concomitantly, before (neoadjuvant chemotherapy) or after radiotherapy (adjuvant chemotherapy). A disadvantage of concomitant radiotherapy is that normal tissue toxicity is increased. The rationale for neoadjuvant chemotherapy is the use of chemotherapy to debulk the primary tumor and eradicate distant metastases, however, a disadvantage of this therapy is that radiotherapy (which is more effective than chemotherapy) started with delay. A rationale for adjuvant chemotherapy is an additive local tumor effect and eradication of distant metastases. Alternating use of chemo/radiotherapy was also discussed. There are several rationales for this therapy, including early administration of both treatment approaches, reduced risk for developing resistance, and temporal separation of both modalities, which results in increased normal tissue tolerance. Clinical trials for non-Hodgkin lymphomas, small cell lung carcinomas, breast cancers and several others conducted in the Institute Gustave Roussy in Villejuif demonstrate excellent response. In view of radiotherapy, the future perspectives of chemotherapy are in the search for new, more effective drugs, development of methods for drug targeting, and prediction/identification of patients likely to benefit from this treatment modality.

The use of predictive assays for tumor response to radiotherapy was presented by Dr. S.M. Bentzen. One of the assays is the measurement of potential doubling time using flow cytometry, which is probably not a very good predictor, because of intratumoral cell variability, cell loss and problems asso-

ciated with differentiation between normal and tumor cells. Another assay is the measurement of survival at 2 Gy. The disadvantage of this assay is its long duration and a lot of experimental noise due to the presence of fibroblasts. The measurement of pO_2 shows in general a positive correlation with outcome, but is suitable only for accessible (and large) tumors only. Several new methods, including the use of PET, NMR, and p53 mutations, are foreseen as predictive assays for tumor response to radiotherapy.

On the last day of the course Dr. M. C. Joiner held a lecture on linear energy transfer (LET) and relative biological effectiveness (RBE). LET is a term used to describe the density of ionization in particle tracks. LET is defined as the average energy (in keV) released by a charged particle traversing a distance of 1 μm. X and γ-rays have a low LET while some particle radiations (neutrons, α-particles) have a high LET. High-LET radiations are biologically more effective than low-LET radiations. Biological effectiveness is measured by RBE, which is defined as the ratio of dose of the reference low-LET radiation (usually 250 kV X-rays) and the dose of tested radiation that produce equal effect. RBE is not constant, but depends on the level of biological damage (dose level). RBE dependence on radiation dose is different in different tissues.

Stages in process of damage produced by radiation was presented by Prof. G.G. Steel in his lecture on "Molecular Aspects of Radiation Biology". These processes can be divided into induction (ionization and formation of free radicals), processing (initial DNA damage and fixation/misrepair on these damages) and manifestation (chromosomal aberrations, fragment loss, micronuclei and cell death).

During the course, some practical calculations based on the linear-quadratic model and clinical examples were discussed. A particular attention was paid to unplanned gaps that could occur during ongoing fractionated radiotherapy. It was stressed that an increased dose per fraction in order to deliver the same total dose, as well as adding an additional dose at the end of therapy, are not satisfactory solutions. The best way to overcome an unplanned gap is to accelerate radiotherapy by treating the patient twice per day till the planned schedule is obtained. It is important that the overall treatment time is not changed.

This very educational course ended with course evaluation, examination and announcement that a new edition of "Basic Clinical Radiobiology" with some new chapters and the latest developments in radiotherapy will be published next year (1997).

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Study tour in Cambridge: A report

With the help of Cambridge Colleges Hospitality Scheme for Central and Eastern European Scholars I spent a month at the Department of Radiology of the Cambridge University Teaching Hospital in July, 1996.

There are over 5000 people employed in the hospital, daily treating 900–1000 patients, which makes about 60 000 patients, and 290 000 outpatients per year. The hospital has approximately 30 departments and clinics, one of them is the Department of Radiology dealing with medical imaging. Within the hospital complex there are also Blood Transfusion Centre, Clinical School with Medical Library, Molecular Biological Laboratory and Ambulance Station.

The Department of Radiology has the following units: conventional radiology, angiography, US-, CT-, MR-unit and a department of Nuclear Medicine. Sixten consultants and twenty junior radiologists (registrar, senior registrar) work in the Department together with fifty radiographers (including ultrasonographers). The radiotherapeutical department works independently of the department of radiology. The Brain Imaging Centre which houses the PET Unit is a Neurosurgery collaborative unit.

At the Department of Radiology the work with the patients starts at 9 a.m., but the radiologists come one hour earlier to attend conferences, meet the consultants, demonstrate new techniques and tutor their junior colleagues.

I spent the majority of my time in CT-, MR-, US-units. In the US-unit there are 5 US-machines available, all suitable to perform color, Doppler investigation and facilities for intracavitary examinations. Comparable to the Hungarian US routine, this hospital did not perform "total abdominal" investigations, but smaller regions were investigated (e.g. liver + biliary tract, kidneys + bladder; thoracic fluid, child hip, neck). The US investigations were performed by three-four persons, some radiologists, some ultrasonographers. In the CT-unit there are two CT-scanners, a scanner for head and spine (General Electric High Speed Advantage GE 9800), and another body-scanner (Siemens Somatom plus4 spiral CT). In both units interventional radiological procedures were performed, e.g. biopsy, abscess and empyema drainage, etc. In the MR-unit there are 2 General Electric Signa machines (0.5, 1.5 T). The main indication fields were the nervous system, the abdomen, the joints. These machines are capable MR spectroscopy also. I had the opportunity to see for the first time MR investigation of the biliary and pancreatic ducts, MRCP and the 3D-box technique. Computers are used extensively being the part of the information system within the hospital. Each unit has a reception; part of the administrative work is made by the reception staff. The application of the computer is very useful in administrative work. Comparable to the Hungarian practice, this hospital has many more radiographers and nursed, therefore the physicians do not waste time with non-medical work.

Aboute the Cambridge Colleges Hospitality Scheme for Central and Eastern European Scholars: in the beginning of the 1980's a linquist of the University of Cambridge originating from Hungary decided to promote the study tour of scientists and teachers from universities of Central and Eastern Europe. Since 1984 there has been an opportunity to spend a month in Cambridge during summer for 7 countries from this region. The University and the Colleges provide free accommodation, food and tutoring. The applicant can apply for a travel grant at the Soros Foundation. Visitors get a bursary from the Soros Foundation and the British Council for professional purposes (xeroxing, books). In 1996 the number of the visitors from Central and Eastern Europe was approx. 20, and 4 or 5 of us from Hungary. The majority of the visitors deal with human sciences and arts, I was the only one spending my time in the Cambridge University Teaching Hospital. I was there as an observer, it was necessary to avoid the clinical contact with the patients, but nevertheless, I was able to increase my expertise.

I'm very grateful to professor Dixon and his colleagues for the kind welcome and permission to participate in the work of the Department of Radiology; to Sir John Meurig Thomas, The Master of Peterhouse for his invitation to live in his House and to Mrs. M. Ferguson-Smith for organizing my visit (the Cambridge Colleges Hospitality Scheme for Central and Eastern European Scholars).

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Ali je kvantitativna scintigrafija pri žlezah slinavkah primerna preiskava pred in po radiojodnem zdravljenju?

Bohuslavizki KH, Brenner W, Tinnemeyer S, Lassmann S, Kalina S, Mester J, Clausen M, Henze E

Namen pričujoče preiskave je bil s kvantitativno scintigrafijo ugotoviti zmanjšanje funkcije pri žlezah slinavkah, ki lahko nastane zaradi nizkodoznega radiojodnega zdravljenja. Prav tako smo ugotavljali pogostnost okvar žlez slinavk pri ščitničnih bolnikih. Kvantitativno scintigrafijo pri žlezah slinavkah smo delali pred rutinsko ščitnično scintigrafijo. Injicirali smo intravenozno 36-126 MBq Tc-99m-pertehnetata in s kopičenjem izotopa v slinavkah izračunali parenhimsko funkcijo. Preiskavo smo naredili pred radiojodnim zdravljenjem in 3 mesece po njem pri 144 bolnikih. Pri 674 drugih bolnikih, ki so bili napoteni na ščitnično scintigrafijo, pa smo s predhodno kvantitativno scintigrafijo ugotavljali pogostnost okvar žlez slinavk. Kljub spodbujanju delovanja žlez slinavk med radioiodnim zdravljenjem z askorbinsko kislino, smo izmerili 15-90% parenhimske okvare žlez slinavk pri bolnikih, ki so z radiojodnim zdravljenjem prejeli 0,4-24 GBq J-131. Pogostnost okvar žlez slinavk pa smo našli v 11,4% (77/674) v eni ali dveh žlezah in 7,7% (52/674) pri treh ali štirih žlezah. Menimo, da je kvantitativna scintigrafija pred in po radiojodnem zdravljenju primerna preiskava za kvantitativno ugotavljanje funkcijskih okvar žlez slinavk - tako predhodnih okvar kot okvar zaradi zdravljenja, kar je pomembno tudi pri uporabi nizkih doz J-131. Posebaj priporočamo kvantitativno scintigrafijo pri žlezah slinavkah zaradi forenžičnih razlogov.

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Relativna koncentracija DNK v tirocitih scintigrafsko vročih nodusov

Budihna NV, Zupanc M, Zorc-Pleskovič R, Porenta M, Vraspin-Porenta O

Cilj *naše raziskave je ugotoviti citološko sliko in izmeriti relativno koncentracijo DNK v tirocitih dominantno scintigrafsko vročih nodusov ščitnice.*
Metode: *Preiskali smo 67 bolnikov z hipertirozo zaradi lokaliziranega avtonomnega tkiva (vročih nodusov). Relativno koncentracijo DNK v vročih nodusih smo določili s pomočjo celične citofotometrije in jo primerjali z rezultati citologije, scintigrafije, TT4, TT3, TSH in tiroglobulinom v serumu bolnikov.*
Rezultati: *Modalna vrednost relativne koncentracije DNK v tirocitih je bila v 16 vročih nodusih diploidna (tip 1), v 21 hiperdiploidna (tip 2). 12 nodusov v diploidno (tip 3) in 18 z hiperdiploidno (tip 4) modalno vrednostjo relativne koncentracije DNK je imelo hkrati znake povečane proliferacije celic. Tirociti 4 normalnih ščitnic so bili diploidni. Citomorfološki znaki atipije in degenerativne spremembe so bili signifikantno bolj pogosti pri tipih DNK distribucije 3 in 4 kot pri tipih 1 in 2.*
Sklepi: *Dominantni scintigrafsko vroči nodusi v ščitnici so diploidni ali hiperdiploidni. Nekateri vroči nodusi so v fazi proliferacije. DNK citofotometrija je lahko koristna kot pomožna diagnostična metoda v primerih, kjer citološka slika (vročih) ščitničnih nodusov ni zanesljiva, zlasti kadar načrtujemo radiojodno zdravljenje hipertiroze z lokaliziranim avtonomnim tkivom.*

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Diagnostična vrednost razpoložljivih tumorskih označevalcev pri ščitničnem karcinomu

Brenner W, Bohuslavizki KH, Klutmann S, Henze E

Pri sledenju onkoloških bolnikov so tumorski označevalci pomembno sredstvo - tako za zgodnje ugotavljanje napredovanja ali ponovitve bolezni in za ugotavljanje oddaljenih metastaz, kot tudi za ugotavljanje učinkovitosti zdravljenja. Za najbolj pogoste vrste ščitničnih karcinomov so na razpolago preizkušeni tumorski označevalci. Uporabljamo jih pri papilarnem in folikularnem karcinomu, ki izhajata iz ščitničnega epitela ter pri medularnem karcinomu, ki nastane iz parafolikularnih C-celic žleze ščitnice. V članku so predstavljeni tumorski označevalci tireoglobulin, kalcitonin in karcinoembrionalni antigen ter njihova uporaba v običajni klinični praksi. Avtorji obravnavajo tudi tkivni polipeptidni antigen in neuron specifično enolazo kot možna tumorska označevalca pri ščitničnem karcinomu.

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Vrednost scintigrafije s FDG-PET pri odkrivanju tumorjev trebušne slinavke

Bohuslavizki KH, Mestner J, Brenner W, Buchert R, Klutmann S, Clausen M, Henze E

Zgodnje odkrivanje tumorjev trebušne slinavke predstavlja v diagnostični radiologiji velik izziv. Odkrivanje teh tumorjev z morfološkimi metodami, kot so ultrazvok, računalniška tomografija in slikovna magnetna resonanca, je še vedno težavno. Tumorje trebušne slinavke so poizkušali odkrivati tudi z imunoscintigrafijo, receptorsko scintigrafijo in nespecifično perfuzijsko scintigrafijo, vendar se nobena od teh diagnostičnih metod ni pokazala primerna za običajno klinično prakso. Najbolj uspešni so bili z uporabo F-18-fluorodeoksi-glukozno pozitronsko emisijsko tomografijo (F-18-FDG-PET), kjer so dosegli 80% natančnost. Zaradi tehničnih omejetiv pozitronske tomografije pa ne moremo odkriti manjših sprememb od 10 do 15 mm, tudi če uporabljamo visoko resolutivni PET čitalec. Tako se tudi ta metoda kaže kot manj primerna za zgodnje odkrivanje raka trebušne slinavke in ni jasno, ali lahko uporaba FDG-PET zmanjša pogostnost diagnostičnih laparotomij. Še vedno so potrebne prospektivne primerjalne raziskave z ultrazvokom, računalniško tomografijo, retrogradno kontrastno pankreatikografijo in magnetno resonanco, da bi določili najprimernejše zaporedje preiskav. FDG-PET ima posebno vrednost, ker nam poleg odkrivanja sprememb v trebušni slinavki daje tudi podatke o naravi tumorskih mas v trebušni slinavki in o učinkovitosti zdravljenja. Seveda so potrebni še nadaljnji napori, da bomo točno določili, kakšen pomen imajo nuklearno medicinske metode pri obravnavi raka trebušne slinavke.

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Nuklearno medicinski IBM PC PIP-GAMMA-PF računalniški sistem

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Lasten razvoj računalniškega sistema za zajemanje in obdelavo nuklearno medicinskih slik temelji na naših bogatih izkušnjah pri razvoju nuklearno medicinske strojne in programske opreme ter visokih cenah komercialnih sistemov. Projekt smo začeli pred petimi leti na cenenem IBM PC z razvojem lastnega zajemalnega modula (GAMMA-PF), ki je v končni profesionalni obliki izdelan v štiriplastni tehnologiji z avtomatiziranimi funkcijami kontrole kvalitete slike ter analize kliničnih preiskav. V sedanji fazi je programska oprema izdelana za DOS okolje v programskem jeziku C++. Sistem je zelo lahko vgraditi ter poganjati in ne potrebuje veliko specializiranega znanja. Poleg avtomatske prilagoditve ojačanja in odmika vhodnih pozicijskih signalov z gama kamere na računalniški matriki, velikosti posameznega matričnega elementa, izbire preddefiniranega kliničnega protokola in registriranja interventnih posegov med snemanjem omogoča sistem tudi analizo različnih kliničnih preiskav na avtomatski način z možnostmi korekcije. Prav tako omogoča arhiviranje in izpis rezultatov obdelave z računalniškem mrežnem sistemu na centralno arhivno in izpisno enoto. Pri izgradnji sistema sodelujemo z londonsko univerzo (razvoj PIP – portable image processing sistema), mednarodno agencijo za atomsko energijo in ministrstvom za znanost in tehnologijo RS. Do sedaj je bil sistem uspešno uveden na kliniki za nuklearno medicino v Ljubljani, v nuklearno medicinskem laboratoriju v Mariboru ter v tridesetih laboratorijih po svetu.

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Študij prekrvljenosti fibrosarkoma LPB po elektroterapiji z enosmernim tokom pri imuno-kompetentnih in golih miših z metodo barvanja tkiva s Patent modrim barvilom

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Z elektroterapijo s šibkim električnim tokom lahko učinkovito zmanjšamo velikost tumorjev tako v eksperimentalnih kot kliničnih tumorskih modelih. Proučevali smo učinke elektroterapije na prekrvljenost trdnih podkožnih tumorjev fibrosarkoma LPB pri imuno-kompetentnih miših C57Bl/6 in pri golih miših z zmanjšanim imunskim odzivom. Uporabili smo enourno terapijo s tokom jakosti 0.6 mA in 1.0 mA, ki smo ga dovajali preko igelnih elektrod iz zlitine platine in iridija. Elektrode so bile vstavljene podkožno na dveh nasprotnih straneh tumorjev. Upočasnitev rasti tumorjev pri miših C57Bl/6 je bila po enkratni terapiji statistično značilna. Učinek na rast tumorjev pri golih miših je bil neznačilen. Z metodo barvanja tkiva s Patent modrovijoličnim barvilom smo ocenjevali učinek elektroterapije na prekrvljenost tumorjev, ki je eden od možnih mehanizmov električnega protitumorskega delovanja. Prekrvljenost smo ocenjevali takoj po terapiji in 24 ur po terapiji. Prekrvljenost tumorjev pri miših C57Bl/6 se je po elektroterapiji nekoliko zmanjšala, medtem ko pri golih miših praktično nismo zaznali nobenega učinka. Različen odziv v rasti tumorjev na elektroterapijo pri obeh tumorskih modelih kaže na to, da poškodba napajalnega ožilja v neposredni bližini vstavljenih elektrod, do katere pride zaradi produktov elektrolize, verjetno ni glavni vzrok za zavoro v rasti tumorjev pri imuno-kompetentnih miših.

Radiol Oncol 1997; 31: 33-8.

Površinska termoradioterapija: klinične izkušnje kažejo na prednost obsevanja neposredno pred pregrevanjem

Lešničar H, Budihna M

V poglavju smo razčlenili nekatere pomembnejše tumorske in terapevtske dejavnike, ki so vplivali na uspeh terapije pri bolnikih zdravljenih z lokalno hipertermijo. V obdobju med 1989-1995 smo na Onkološkem inštitutu v Ljubljani s termoradioterapijo zdravili 52 bolnikov z napredovalimi malignimi tumorji. Pri 39 (75%) bolnikih smo se za tak način zdravljenja odločili, ker predhodno zdravljenje s standardno radioterapijo ni bilo uspešno, pri 13 (25%) bolnikih pa je termoradioterapija predstavljala primarno zdravljenje. Pri 32 (62%) bolnikih smo uporabili perkutani način, pri 20 (38%) bolnikih pa intersticialni način zdravljenja. Popoln odgovor na terapijo smo dosegli pri 60% bolnikov, 2-letno preživetje brez ponovitve bolezni pri 51%, 2-letno za bolezen specifično preživetje pa pri 45% bolnikov. Na končno uspešnost zdravljenja so značilno vplivali: vrsta histologije, tumorski volumen, minimalne intratumorske temperature, skupna tumorska doza obsevanja, neposredni odmerek obsevanja ob pregrevanju ter vrstni red obsevanja in pregrevanja. Ponavljanje pregrevanja ni vplivalo na izid zdravljenja. Značilno boljše 2-letno preživetje brez ponovitve bolezni (85%) smo v primerjavi z bolniki, ki smo jih najprej pregrevali in nato obsevali (38%), ugotovili pri bolnikih, kjer je obsevanju ob uporabi nekoliko višjega enkratnega odmerka neposredno sledilo pregrevanje (p=0.03). Krivulji dozne odvisnosti, izračunani za posamezni skupini bolnikov z različnim zaporedjem obsevanja in pregrevanja, sta dodatno pokazali, da je ob enakem učinku zdravljenja celokupna obsevalna doza pri uporabi obsevanja tik pred pregrevanjem lahko bistveno nižja. Izračunani količnik izboljšanja zdravljenja (enhancement ratio) med skupinama je znašal 1.7. Izrazitejše akutne in kronične posledice zdravljenja smo opažali pri 28% oziroma 23% bolnikov. Stranski učinki zdravljenja so bili značilnejši pri bolnikih z višjo maksimalno izmerjeno temperaturo v ogrevanem področju, pri bolnikih z večjim tumorskim volumnom in pri uporabi višje celokupne tumorske obsevalne doze. Naši klinični izsledki nakazujejo pomembno vrednost uporabe višjih odmerkov obsevanja neposredno pred pregrevanjem. Tak način kombiniranega zdravljenja lahko privede do zadovoljivih rezultatov že pri razmeroma nizki celokupni dozi obsevanja, zato je uporaba termoradioterapije smiselna tudi pri predhodno že obsevanih bolnikih.

Radiol Oncol 1997; 31: 39-47.

Radioterapija nefroblastoma. Pred- in pooperativno kombinirano zdravljenje. Radioterapija pri lokalizirani (stadij II, III, IV) in diseminirani bolezni. Akutne in kasne posledice.

Jereb B

Nefroblastom – embrionalni maligni tumor ledvice – se pojavlja najpogostejše v zgodnji otroški dobi, nekateri bolniki imajo tudi prirojene anomalije. V zadnjih letih so se močno razvile genetske raziskave tega tumorja.
Pri zdravljenju nefroblastoma se je obsevanje uveljavilo že pred desetletji kot učinkovit dodatek kirurgiji, žal pa povzroča nezaželene kasne posledice. Preživetje se je trajno izboljševalo od prvotnih 25% pa do 80% ali več. S tem je problem kasnih posledic postal še bolj pomemben.
V Evropi, ZDA in Veliki Britaniji so s številnimi randomiziranimi kliničnimi študijami poskušali predvsem zmanjšati kasne posledice na ta način, da so čim bolj prilagodili zdravljenje znanim prognostičnim dejavnikom. Rezultati teh randomiziranih kliničnih študij so opisani, pa tudi komplikacije zdravljenja in sekundarni tumorji. Zaradi uspešnosti zdravljenja otrok z nefroblastomom se je močno povečala populacija bivših bolnikov, ki so odrasli. Menijo, da je obsevanje najpogostejši vzrok zgodnjih in kasnih komplikacij zdravljenja. Vsekakor se je število ozdravljenih otrok s tveganjem za kasne posledice zadnja desetletja bistveno zmanjšalo.

Radiol Oncol 1997; 31: 48-53.

Trislojna šablona za transperinealno intersticijsko brahiterapijo pri obsevanju z nizko hitrostjo

Kuhelj J, Strojan P, Burger J

Pri obsevanju z nizko hitrostjo (low-dose-rate – LDR) in remote afterload aparatom (RAA) za transperinealno intersticijsko brahiterapijo z ^{192}Ir žicami smo ugotovili, da je zaradi načina fiksacije vodil pri komercialno dostopnih šablonah najmanjša medsebojna razdalja med vodili suma 10 mm; sama fiksacija vodil pa je neustrezna, saj je ob njihovi priključitvi na LDR RAA prihajalo do nekontroliranih premikov le-teh. Zato smo v naši ustanovi izdelali novo, troslojno šablono. Zunanji dve plošči sta med seboj spojeni in tvorita rigidno celoto; med njima je tretja, vzdolžno pomična plošča. Razpored odprtin za vnašanje vodil je v vseh treh ploščah enak in je lahko poljuben. S privijanjem dveh vijakov, pritrjenih na zunanjih ploščah, vzdolžno pomikamo srednjo, ki s strižno silo pritrdi naenkrat vsa vstavljena vodila. Menimo, da tehnična enostavnost rešitve, primernejša razporeditev odprtin za vodila ter čvrsta in hkratna pritrditev vodil povečuje uporabnost opisane šablone.

Radiol Oncol 1997; 31: 54-5.

Notices

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

Oncology

May 28-31, 1997.

The International Conference 1997 Comprehensive Cancer Care "Focus on Cancer Pain" will be offered in Limassol, Cyprus.

Contact Congress Secretariat, Options Eurocongress, P.O.Box 4723, Limassol, Cyprus; or call +357 5 399 722; or fax +357 5 399 494.

Brachytherapy

March 5-7, 1997.

Deutsche Brachytherapy Konferenz '97 will be held in Luebeck, Germany.

Contact Prof. Dr. E. Richter, Med. Univ. zu Luebeck, Klinik fuer Strahlentherapie, Ratzeburger Allee, 160, 23538 Luebeck, Germany; or call +49 451 500 6660; or fax +49 451 500 3324.

Familial cancer and cancer genetics

April, 1997.

The ESO training course "Familial Cancer and Cancer Genetics" will be offered in Haifa, Israel.

Contact ESO Balkans and Middle East Office, c/o Egnatia Epirus Foundation, 7A Tzavella St., 453 33 loannina, Greece; or call +30 651 72315/76992; or fax +30 651 36695.

Radiotherapy

May 11-12, 1997.

The two day teaching seminar "Sterotactic Radiotherapy / Radiosurgery Using Linear Accelerator" will be held in London, U.K.

Contact Mrs Chris Cassell, Neuro-oncology Unit, The Royal Marsden Hospital, Downs Road, Sutton, Surrey SM2 5PT, U.K.; or call +44 181 642 6011 Ext. 3272; fax +44 181 643 5468.

As a service to our readers, notices of meetings or courses will be inserted free of charge.
Please sent information to the Editorial office, Radiology and Oncology, Vrazov trg 4, SI-1105 Ljubljana, Slovenia.

Radiation oncology

May 9-10, 1997.

The "Second Annual Multidisciplinary Radiation Oncology Conference" will be offered in Washington, DC, USA.

Contact Tamara Gilmore, CMP; Every Last Detail, 14203 Woolen Oak Court, Suite 7, Silver Spring, MD 20906, USA; or call +1 800 592 8337; fax +1 800 994 8757; e-mail: Detail_erols.com

Clinical trials

June, 1997.

The EORTC course "Clinical Trials Statistics for non Statisticians" will be offered in Brussels, Belgium.

Contact Ms. A.Marinus, EORTC Education and Training Division, Av. E. Mounier 83, 1200 Brussels, Belgium; or call +32 2 772 4621; or fax +32 2 772 6233.

Prostate cancer

June, 1997.

The ESO training course will be held in Moscow, Russia.

Contact ESO Russia and Community of Independent States, c/o CSC Ltd., Mrs. Mira Vukelic, Heiligenstaedter Strasse 395b, 1190 Vienna, Austria; or call +43 1 3188 466; or fax +431 3188 466-20.

Radiotherapy

June 4-7, 1997.

The "International Congress of Radiation Oncology 1997 (ICRO'97)" will take place in Beijing, China.

Contact Marleen Stevens, ISRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 85; or fax +32 16 34 76 81.

Peripheral stem cell transplantation

June 5-7, 1997.

The ESO training course will be offered in Delphi, Greece.

Contact ESO Balkans and Middle East Office, c/o Egnatia Epirus Foundation, 7A Tzavella St., 453 33 loannina,

Greece; or call +30 651 72315/76992; or fax +30 651 36695.

Gastric and intestinal cancers

June 10-11, 1997.

The ESO training course will be held in Mexico DF, Mexico.

Contact ESO Latin America, Dr.G.Farante, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; or fax +39 2 57 307 143.

Laeukemias and lymohomas

June 10-11, 1997.

The ESO training course will be held in Bogota, Colombia.

Contact ESO Latin America, Dr.G.Farante, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; or fax +39 2 57 307 143.

Breast cancer

June 10-11, 1997.

The ESO training course will be held in Santiago, Chile.

Contact ESO Latin America, Dr.G.Farante, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; or fax +39 2 57 307 143.

Gynaecological oncology

June 10-11, 1997.

The ESO training course will be held in Buenos Aires, Argentina.

Contact ESO Latin America, Dr.A.Rancati, Av. Las Heras 1666, 1018 Buenos Aires, Argentina; or call +54 1 8141 129; or fax +54 1 8141 129.

Headache

June 10-14, 1997.

The "8th International Headache Congress" will be offered in Amsterdam, The Netherlands.

Contact Lidy Groot, Congress Events, PO Box 83005, 1080-AA Amsterdam, The Netherlands.

Pulmology

June 11-13, 1997.

The clinical seminar "Strategy on Mechanical Ventilation in Newborns and Infant" will take place in Bernried am Starnberger See (near Munich), Germany.

Contact IPOKRaTES International Head Office, Rosengartenplatz 2, D-68161 Mannheim, Germany; or call +49 621 4106 134; or fax +49 621 4106 202.

Digestive tract

June 12-14, 1997.

The ESO advanced course "Digestive Tract (part I): Gastroesophageal Tumours" will be offered in Milan, Italy.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; or fax +39 2 57 307 143.

II Latin America education convention

June 12-14, 1997.

The ESO convention will be held in Sao Paulo, Brazil.

Contact ESO Latin America, Dr.A.Frasson, Ave. Ipiranga 6690, Porto Alegre, Brazil; or call +55 51 3392 709; or fax +55 51 3392 709.

Utrasonography

June 23-25, 1997.

The clinical seminar "Neonatal & Pediatric Utrasonography" will take place in Bernried am Starnberger See (near Munich), Germany.

Contact IPOKRaTES International Head Office, Rosengartenplatz 2, D-68161 Mannheim, Germany; or call +49 621 4106 134; or fax +49 621 4106 202.

Magnetic Resonance

June 26-29, 1997.

The "Clinical Magnetic Resonance Society Annual Meeting" will be offered in Orlando, USA.

Contact Walt Disney World SWAN, Disney World/EPCOT Center, Orlando, Florida, USA; or call +1 800 823 2677 / +1 513 221 0070; fax +1 513 221 0825; e-mail: cmrs_one.net

Asthma

August 24-27, 1997.

The "2nd Annual Meeting of the Central and Eastern European Regional INTERASMA organisation" will be offered in Budapest, Hungary.

Contact Central and Eastern European Regional INTERASMA Conference, Dr. Endre Laszlo, MAV Gyermekgyogyhaz, Budapest, XII. ker., Manyet u. 11., H-1121, Hungary.

Radiotherapy

August 31 - September 4, 1997.

The ESTRO and ERTED teaching course "Radiation Physics for Clinical Radiotherapy" will be offered in Lueven, Belgium.

Contact the ESTRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 80; or fax +32 16 34 76 81.

Clinical trials

Autumn, 1997.

The EORTC course "Data Management in Cancer Clinical Trials" will be held in Leuven, Belgium.

Contact Ms. A.Marinus, EORTC Education and Training Division, Av. E. Mounier 83, 1200 Brussels, Belgium; or call +32 2 772 4621; or fax +32 2 772 6233.

Clinical trials

September, 1997.

The EORTC course "One Day Introduction to EORTC Trials" will be held in Brussels, Belgium.

Contact Ms. A.Marinus, EORTC Education and Training Division, Av. E. Mounier 83, 1200 Brussels, Belgium; or call +32 2 772 4621; or fax +32 2 772 6233.

Cytokines

September, 1997.

The ESO training course "Cytokines" will be offered in Hofburg Conference Center, Vienna, Austria.

Contact European School of Oncology, Office for Central and Eastern Europe, c/o Arztekammer fuer Wien, Fortbildungsreferat Weihburggasse 10-12, 1010 Vienna, Austria; or call +43 1 3188 466; or fax +43 1 3188 466 20.

Breast reconstructive surgery

September, 1997.

The ESO training course will be held in Caracas, Venezuela.

Contact ESO Latin America, Dr.G.Farante, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; or fax +39 2 57 307 143.

Neuropathology

September 7-12, 1997.

The 13th International Congress of Neuropathology will be offered in Perth, Australia.

Contact Ms Angela Schaefer, ICMS Conference Secretariat, PO Box 8120, Hindley St., PO, Adelaide, SA 5000, Australia; or call +61 8 210 6776; fax +61 8 212 5101; e-mail: icnp97_icms.com.au

Radiotherapy

September 12-13, 1997.

The ESTRO workshop on "3D Treatment Planning and Optimization for Clinical Radiotherapy" will take place in Nice, France. (It will be linked to the 4th Biennial ESTRO Physics Meeting).

Contact the ESTRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 80; or fax +32 16 34 76 81.

Oncology

September 14-19, 1997.

The ESTRO 16 / ECCO 9 Congress will be offered in Hamburg, Germany.

Contact the ESTRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 80; or fax +32 16 34 76 81.

Radiotherapy

September 14-19, 1997.

The ESTRO teaching course "Radiotherapy in Integrated Cancer care will be held in Izmir, Turkey.

Contact the ESTRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 80; or fax +32 16 34 76 81.

Radiobiology

September 14-19, 1997.

The ESTRO teaching course "Basic Clinical Radiobiology" will be offered in Como, Italy.

Contact the ESTRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 80; or fax +32 16 34 76 81.

Medical physics and engineering

September 15-19, 1997.

The "11th International Conference on Medical Physics" and "18th Internaional Conference on Medical and Biological Engineering" will be offered in Nice, France.

Contact Nice'97, SEE - 48, rue de la Procession, F-75724 Paris Cedex, France.

Pediatric neurology

September 18-20, 1997.

The clinical seminar "Pediatric Neurology" will be held in Krems, Austria.

Contact IPOKRaTES International Head Office, Rosengartenplatz 2, D-68161 Mannheim, Germany; or call +49 621 4106 134; or fax +49 621 4106 202.

Neonatology

September 22-25, 1997.

The clinical seminar "Neonatology" will take place in Krems, Austria.

Contact IPOKRaTES International Head Office, Rosengartenplatz 2, D-68161 Mannheim, Germany; or call +49 621 4106 134; or fax +49 621 4106 202.

Melanoma and skin cancer

September 24-25, 1997.

The ESO training course "Melanoma and Skin Cancer" will be offered in Hofburg Conference Center, Vienna, Austria.

Contact European School of Oncology, Office for Central and Eastern Europe, c/o Arztekammer fuer Wien, Fortbildungsreferat Weihburggasse 10-12, 1010 Vienna, Austria; or call +43 1 3188 466; or fax +43 1 3188 466 20.

Good clinical practice

September 24-25, 1997.

The ESO training course "Good Clinical Practice" will be offered in Hofburg Conference Center, Vienna, Austria.

Contact European School of Oncology, Office for Central and Eastern Europe, c/o Arztekammer fuer Wien, Fortbildungsreferat Weihburggasse 10-12, 1010 Vienna, Austria; or call +43 1 3188 466; or fax +43 1 3188 466 20.

Oncology

September 25-27, 1997.

The ESO training course "New Approaches in Diagnosis and Treatment of Cancer" will be held in Belgrade, Yugoslavia.

Contact ESO Balkans and Middle East Office, c/o Egnatia Epirus Foundation, 7A Tzavella St., 453 33 loannina, Greece; or call +30 651 72315/76992; or fax +30 651 36695.

Oncology

September 26-27, 1997.

The ESO training course "Modern Trends in Cancer Screening" will be held in Belgrade, Yugoslavia.

Contact ESO Balkans and Middle East Office, c/o Egnatia Epirus Foundation, 7A Tzavella St., 453 33 loannina, Greece; or call +30 651 72315/76992; or fax +30 651 36695.

Oncology

September 26-28, 1997.

The ESO training course "Myeloproliferative and Myelodysplastic disease" will take place in Patras, Greece.

Contact ESO Balkans and Middle East Office, c/o Egnatia Epirus Foundation, 7A Tzavella St., 453 33 loannina, Greece; or call +30 651 72315/76992; or fax +30 651 36695.

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Biophysics and Biology of Tumors

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Diagnostic and Prognostic Factors in Oncology
Molecular Oncology
Immune System and Biological Response Modifiers
MR Imaging
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Functional Electrical Stimulation
Physiology
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ORGANIZING COMMITTEE ADDRESS

Gregor Serša, Institute of Oncology, Dept. of Tumor Biology, Zaloška 2
SI-1105 Ljubljana, Slovenia
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Symposium on

ORGAN SPARING TREATMENT IN ONCOLOGY

Ljubljana, Slovenia
19–21 June 1997

Organized by:

Institute of Oncology, Ljubljana, Slovenia

Under the auspices of:

Alps Adriatic Working Community

Design and Content

The Symposium is designed for medical doctors and other specialists involved in cancer treatment who wish to exchange experiences with oncologists and to contribute to upgrading the knowledge in organ sparing treatment. It will cover the following topics:

- Breast Conserving Therapy
- Bladder Sparing Treatment in Muscle Invasive Bladder Carcinoma
- Organ Sparing Treatment in Head and Neck Carcinoma
- Organ Sparing Treatment in Soft Tissue Tumors
- Quality of Life after Organ Sparing Treatment

Mailing Address

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Institute of Oncology
Zaloška 2, 1105 Ljubljana
SLOVENIA
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Trajanje: 2-4 tedne** **za tineo pedis je morda potrebno 6 tedensko zdravljenje	**Trajanje:** Enkratni odmerek	**Trajanje:** 6-8 tednov	**Trajanje:** Odvisno od kliničnega odziva	**Trajanje:** 1-2 tedna** **pri okužbah sluznice je morda potrebno 30 dnevno zdravljenje
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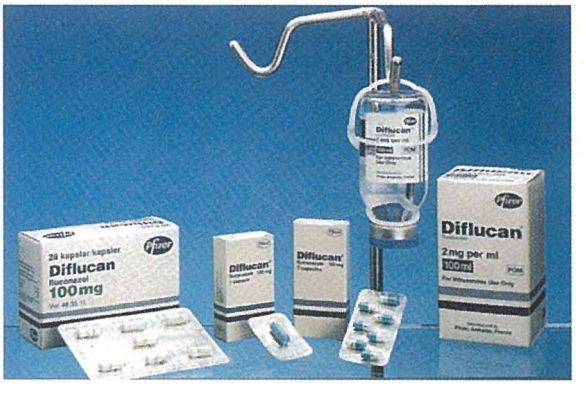

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Povzetek informacij za predpisovanje zdravila

Indikacije in odmerki: obrni. **Uporaba pri starejših:** kot zgoraj, razen pri ljudeh z ledvičnimi okvarami - glej popolne informacije za predpisovanje. Ne priporočamo uporabe pri otrocih mlajših od 16 let, razen kadar se zdi to lečečemu zdravniku nujno potrebno - glej popolne informacije za predpisovanje in priporočljive odmerke za otroke starejše od enega leta. Bolniki z ledvičnimi okvarami: morda so potrebni manjši odmerki - glej popolne informacije za predpisovanje. **Dajanje zdravila:** DIFLUCAN lahko dajemo oralno ali v obliki intravenske infuzije. Odmerki so pri oben načinih enaki. **Kontraindikacije:** Znana preobčutljivost na flukonazol ali sorodne triazole. **Opozorilo:** Pri bolnikih, pri katerih pride do pomembnega povečanja jetrnih encimov, moramo pred nadaljevanjem zdravljenja z DIFLUCANOM oceniti razmerje med tveganjem in koristnostjo. Poročajo tudi o anafilaktičnih reakcijah. **Varnostni ukrepi:** Ker so na voljo le omejeni podatki, odsvetujemo uporabo pri nosečnicah, razen kadar se zdi to lečečemu zdravniku nujno potrebno. **Dojenje:** ne priporočamo uporabe. **Interakcije z drugimi zdravili:** Potrebno je skrbno spremljanje bolnikov, ki istočasno jemljejo antikoagulanse, oralno sulfoniluree ali fenitoin, ciklosporin in teofilin. Pri bolnikih, ki istočasno jemljejo rifampicin, so morda potrebni večji odmerki DIFLUCANA. **Stranski učinki:** Najpogostejši stranski učinki so vezani na gastrointestinalni trakt: slabost, abdominalno nelagodje, diareja in vetrovi. Poročajo tudi o kožnem izpuščaju. Pri bolnikih s hudo osnovno boleznijo so med zdravljenjem z DIFLUCANOM in primernimi učinkovinami opazili spremembe v testnih rezultatih ledvičnih in hematoloških funkcij ter bolezenske spremembe jeter, vendar pa sta klinična pomembnost in povezava z zdravljenjem še nepotrjeni.

Na željo posredujemo dodatne informacije. Pred predpisovanjem DIFLUCANA se je potrebno seznaniti s celotnimi informacijami za predpisovanje zdravila.

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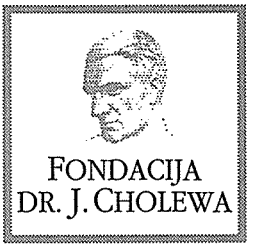

Activity of "Dr. J. Cholewa Foundation" for cancer research and education – report for the second half of 1996

"Dr. J. Cholewa Foundation" for cancer research and education continued its activity through the second half of 1996 as it was outlined at the meetings of the executive and scientific concils of the Foundation that took place at the end of 1995. One of the most important aspects of Foundation's activity is the support it lends to the publishing activity concerned with cancer research, education and treatment, that takes place in the Republic of Slovenia.

The Foundation continues to give part of the support needed for the regular publication of the "Radiology and Oncology" scientific journal. A source of relevant information is thus provided in this way for physicians and nurses involved in the treatment of cancer patients and in cancer research, and for those that are involved in various levels of cancer education. "Radiology and Oncology" is an international scientific journal and therefore offers the possibility to serve to all those interested as an important point of reference and scientific discussion.

Support for the regular publication of "Challenge" is another important activity of "Dr. J. Cholewa Foundation" for cancer research and education. "Challenge" is the newsletter of the European School of Oncology distributed free of charge to health professionals working in countries with limited resources to deal with the issue of cancer diagnosis and treatment in these parts of the world. The scarcity of resources is not limited to the developing countries alone and "Challenge" thus provides interesting information to all interested in this issue in the Republic of Slovenia and other countries as well. As one of the publications of European School of Oncology it is available on the Internet, together with the information concerning the activities and efforts of this important European scientific institution. In this way part of the activities of "Dr. J. Cholewa Foundation" appears regularly on the Internet.

The Foundation is proud to be one of the supporters of the International Conference on Epithelial Hyperplastic Lesions of the Larynx that took place on October 28–30, 1996, in Ljubljana, Slovenia. The fact that a large number of attendants of the Conference from Slovenia fulfills one of the important goals of "Dr. J. Cholewa Foundation" for cancer research and education.

The opening of "Dr. J. Cholewa Foundation" office is expected to take place in Ljubljana in the first quarter of 1997. This will enable the Foundation to further advance its activities in the future.

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

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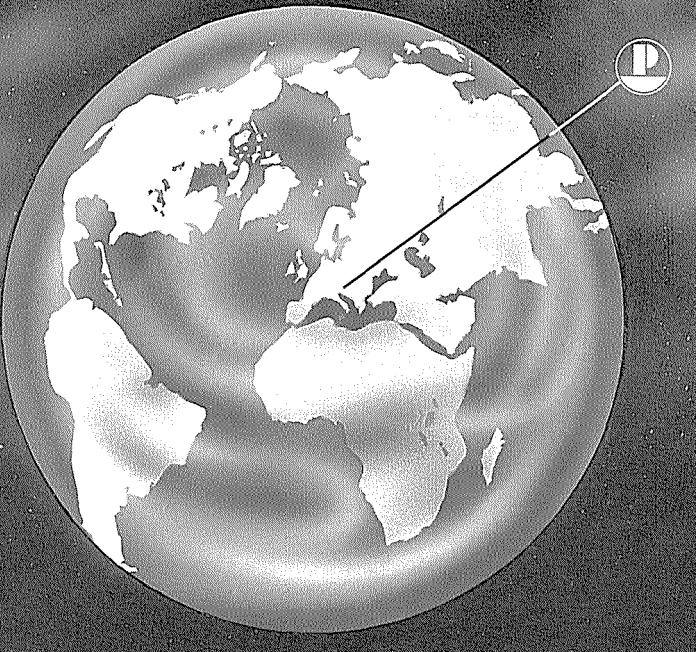

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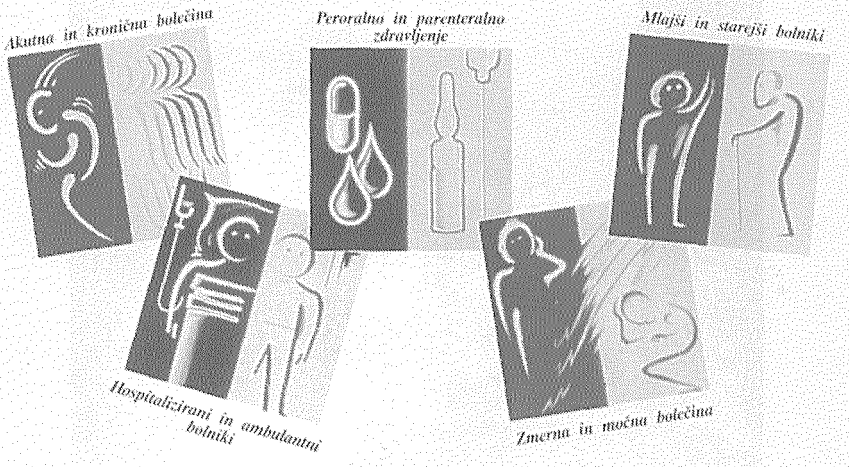

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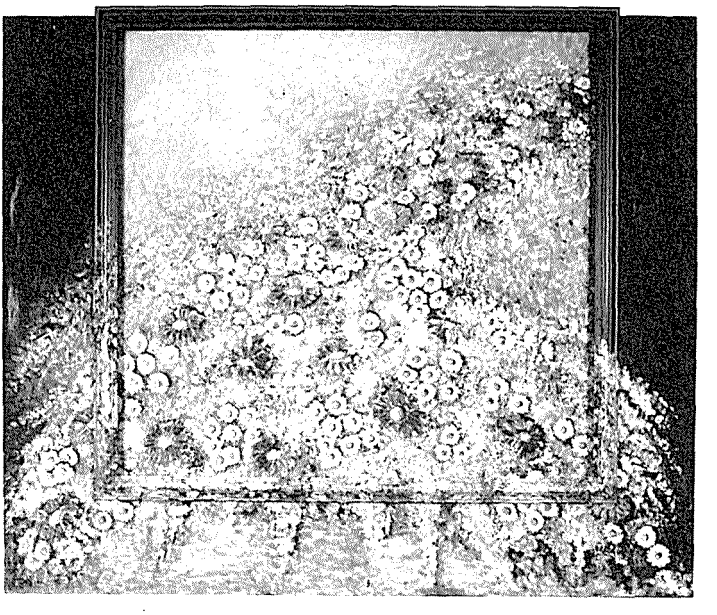

- centralno delujoči analgetik za lajšanje zmernih in hudih bolečin
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Kontraindikacije: Otroci do 1 leta starosti, akutna zastrupitev z alkoholom, uspavali, analgetiki in drugimi zdravili, ki vplivajo na CŽS, zdravljenje z inhibitorji MAO. **Interakcije:** Pri sočasni uporabi zdravil, ki delujejo na osrednje živčevje, je možno sinergistično delovanje v obliki sedacije pa tudi močnejšega analgetičnega delovanja. **Opozorila:** Pri predoziranju lahko pride do depresije dihanja. Previdnost je potrebna pri bolnikih, ki so preobčutljivi za opiate, in pri starejših osebah. Pri okvari jeter in ledvic je potrebno odmerek zmanjšati. Bolniki med zdravljenjem ne smejo upravljati strojev in motornih vozil. Med nosečnostjo in dojenjem predpišemo tramadol le pri nujni indikaciji. Bolnike s krči centralnega izvora skrbno nadzorujemo. **Doziranje:** *Odrasli in otroci, starejši od 14 let:* 50 do 100 mg 3- do 4-krat na dan. *Otrokom od 1 leta do 14 let* dajemo v odmerku 1 do 2 mg na kilogram telesne mase 3- do 4-krat na dan. **Stranski učinki:** Znojenje, vrtoglavica, slabost, bruhanje, suha usta in utrujenost. Redko lahko pride do palpitacij, ortostatične hipotenzije ali kardiovaskularnega kolapsa. Izjemoma se lahko pojavijo konvulzije. **Oprema:** 5 ampul po 1 ml (50 mg/ml), 5 ampul po 2 ml (100 mg/2 ml), 20 kapsul po 50 mg, 10 ml raztopine (100 mg/ml), 5 svečk po 100 mg.

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

Submission of manuscript: Manuscripts written in English should be submitted in triplicate (the original and two copies), including the illustrations to the Editorial Office: *Radiology and Oncology*, Institute of Oncology, Vrazov trg 4, SI-1105 Ljubljana, Slovenia; (Phone: + 386 61 1320068, Fax: +386 61 13 14 180, e-mail: oshrestha@mail.onko-i.si). Authors are asked to submit their manuscripts also on a 3.5" 1.44 Mb formatted diskette. The type of computer and word-processing package should be specified (Word for Windows is preferred).

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

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

Title page should include a concise and descriptive title for the paper; the full name(s) of the author(s); the institutional affiliation of each author; the name and address of the corresponding author (including telephone, fax and e-mail) and abbreviated title. This should be followed by the abstract page, summarising in less than 200 words the reasons for the study, experimental approach, the major findings (with specific data if possible), and the principal conclusions, and providing 3-6 key words for indexing purposes. The text of the report should then proceed as follows:

Introduction should describe the background of the investigation, citing only the essential references and stating the aim of the study.

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

Discussion should explain the results, and not simply repeat them, interpret their significance and draw conclusions. It should review the results of the study in the light of previously published work.

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

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

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

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

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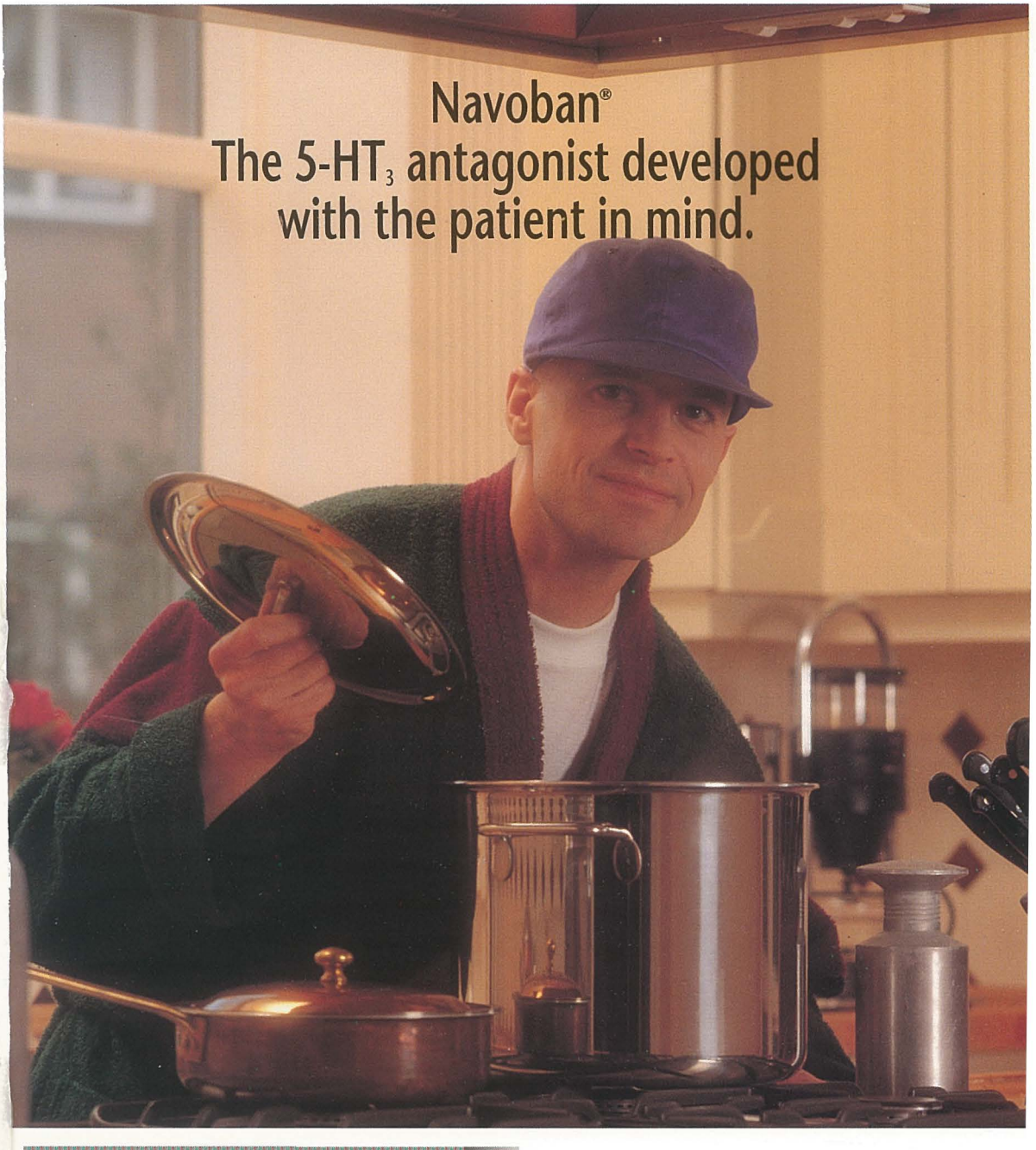

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