RADIOLOGY AND ONCOLOGY

June 1999
Vol. 33 No. 2
Ljubljana

ISSN 1318-2099

preprosto neboleče

Napolnjene injekcijske brizge za samoaplikacijo

volumen (ml)	jakost (I.E.)
1	EPOMAX™ 4000
0,5	EPOMAX™ 2000
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RADIOLOGY AND ONCOLOGY

Editorial office
Radiology and Oncology
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June 1999
Vol. 33 No. 2
Pages 87-178
ISSN 1318-2099
UDC 616-006
CODEN: RONCEM

Aims and scope

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

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

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

Reader for English
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Design
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Printed by
Imprint d.o.o., Ljubljana, Slovenia

Published quarterly in 750 copies

Bank account number 50101 679 901608
Foreign currency account number
50100-620-133-27620-5130/6
NLB - Ljubljanska banka d.d. - Ljubljana

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

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

According to the opinion of the Government of the Republic of Slovenia, Public Relation and Media Office. The journal Radiology and Oncology is a publication of informative value, and as such subject to taxation by 5 % sales tax.

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

This journal is printed on acid- free paper

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

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ISSN 1318-2099
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CONTENTS

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

Radiol Oncol 1999; 33(2): 87-94.

Evaluation of intrarenal arterial Doppler spectra in healthy children

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Background. *The aim of the study was to determine the features of intrarenal arterial Doppler spectra in healthy children by measuring acceleration time (AT) and resistance index (RI).*

Subjects and methods. *RI and AT values were compared between different age-groups of children and with normal RI values in adult population. Color duplex Doppler sonography of the intrarenal arteries, using Acuson 128XP10 scanner, was performed in 150 children (300 kidneys), with no clinical or laboratory pathological changes of the urinary tract. All children were classified into three age groups: (1) 52 children between 2 to 6 years of age; (2) 48 children from > 6 to 11 years of age; (3) 50 children from >11 to 16 years of age.*

Results. *The mean RI +/- 1.S.D. value in the group I was 0.70 +/- 0.03, in the group II 0.625 +/- 0.025 and in the group III 0.585 +/- 0.03. AT ranged from 0.04-0.09 seconds and the mean value of 0.07 +/- 0.01 seconds was the same for all three groups.*

Conclusions. *RI in early childhood is considerably higher as compared to older children and the adult population; after the age of six, RIs become equal to those in the adults. The utilization of RI=0.70, as a threshold value for the increased renal vascular resistance in adults, can be also applied to children over 6 years. The detection of renal artery stenosis on the basis of the analysis of acceleration time is the same in children and in adults.*

Key words: renal artery-ultrasonography; colour Doppler imaging; child

Introduction

Duplex Doppler ultrasonography has been extensively used in the last few years in the assessment of various kidney diseases in adults and children. In pediatric nephrology, Doppler was first used to evaluate vascular changes in renal transplants, especially to detect renal artery stenosis and trans-

Received 22 September 1998
Accepted 28 December 1998

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plant rejection.[1-6] In the native kidneys, Doppler was used for the detection and follow up of hemolytic-uraemic syndrome, diagnosis of renal artery stenosis and renal vein thrombosis, detection of focal ischaemic areas in acute inflammation or infarction, and for the assessment of perfusion in renal insufficiency and several parenchymal diseases.[7-14]

Renal Doppler enables a non-invasive evaluation of renal vascular resistance, by measuring resistance index (RI) and other Doppler sonographic indices in the intrarenal arteries. It is especially important for the evaluation of blood flow in renal parenchymal diseases. Several studies in children and adults showed that an increased RI can be found in various parenchymal kidney diseases (hemolytic-uremic syndrome, interstitial nephritis, diabetic nephropathy, autosomal dominant polycystic disease, etc.), while in glomerulonephritis, the RI values are normal.[14-17] The detection of renal artery stenosis is based on the rise of flow velocity in the main renal artery and changes in the morphology of Doppler spectra in the intrarenal arteries, distally of the site of stenosis, particularly the changes of the acceleration time.[18-21] In order to interpret the changes of the Doppler spectra in all these pathologic conditions in pediatric nephrology, the normal morphologic features of intrarenal arterial Doppler spectra in healthy children have to be determined.

In this study, we analyzed the features of intrarenal arterial Doppler spectra in healthy children and measured acceleration time and resistance indices. The children were divided into several age groups in order to evaluate age-dependence of RI since it is known that the RI values in adult population are age-dependent.[22] The RI values in children were also compared with the normal RI values in adult population to determine the age at which RI in children reaches the values of the adults.

Materials and methods

Between December, 1996 and March, 1998, color duplex sonography was performed in 150 children (300 kidneys) with no clinical or laboratory pathological changes of the urinary tract. The criteria for the inclusion into the study were: normal urinalysis findings prior to US examination, normal B-mod US finding of both kidneys, absence of history of kidney diseases, hypertension, congenital or acquired heart disease, any chronic or metabolic disease. All children were classified arbitrarily into three age groups: (1) 52 children between 2 to 6 years; (2) 48 children > 6 to 11 years; (3) 50 children > 11 to 16 years. Of these, 74 were boys and 76 girls. They were recruited from a group of children undergoing ultrasound examinations for unrelated organ systems, without significant abnormality found in the area of original interest. Sixteen children were volunteers. Informed consent was obtained from the parents of all children.

Real time and color duplex US examinations were performed with a color Doppler scanner Acuson 128 XP10, with a curved-array 5-MHz transducer. All children underwent examination with conventional US; the length of the kidney and the thickness of the renal parenchyma were measured. During the examination, the children were in supine or lateral decubitus position. Color Doppler US studies of the interlobar and arcuate arteries were performed in both kidneys in each child. The Color identification of the intrarenal arteries considerably facilitated the positioning of the Doppler sample volume and examinations were performed at the lowest possible angle between the ultrasonic beam and the insonated vessel. Recordings were obtained in at least three different vessels from the upper, middle and lower third of the kidney. From each recording RI and acceleration time (AT) were measured only when at least three consecutive waveforms with simi-

lar appearance were noted. RI was measured with the following formula: (peak systolic frequency shift - minimum diastolic frequency shift / peak systolic frequency shift). AT was measured by positioning the caliper to the point of the beginning of the cycle and to the point of the maximum systolic velocity. The distance between these points represented the AT value. The wall filter was set at the lowest value of 50 Hz, and the Doppler sample volume was set at 1-3 mm. The minimal pulse repetition frequencies that did not produce alliasing were used. Only optimal spectral waveforms for a particular vessel were used for measurement. Measurements were obtained with the existing software capabilities of the scanner. Mean RI and AT were calculated from all measurements in each kidney. The RI and AT value differences between the right and left kidneys in the same child were also analyzed. For the comparison of the RI values in children with normal adults, the results from Brkljačić *et al.*[22] were used, where the age dependence and RI values in the intrarenal arteries in healthy adults were analyzed. The average duration of the examination per child was 30 minutes. Adequate spectral waveforms were obtained in all children. All examinations were performed by the first two authors. The mean values of measured parameters were used for the statistical analysis of differences between the groups of examinees. The statistical analysis was carried out with "SPSS/PC+". The statistical significance of observed differences was calculated with the nonparametric Mann-Whitney U-test. Standard descriptive statistic parameters were also used for the presentation of our results.

Results

Doppler sonography was successful in all the 300 kidneys of 150 children (74 boys and 76 girls) examined. All children complied with the prior described criteria for the inclusion into the study.

The distribution of RI +/- 1.S.D. by the age-groups is shown in the Figure 1. In order to compare the RI values in children with those in healthy adults, we added to the figure also the RI values in adults from the study of Brkljačić *et al.*[22]

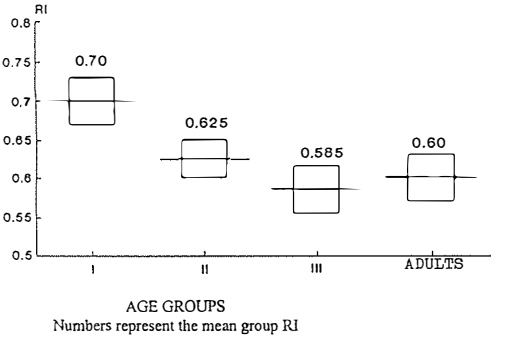

Figure 1. The distribution of RI by age groups.

The mean RI +/- 1.S.D. value in the group I was 0.70 +/- 0.03 (range 0.64-0.75), which is statistically significantly higher as compared to the group II (RI 0.625 +/- 0.025; range 0.575-0.655) and the group III (RI 0.585 +/- 0.03; range 0.55-0.65).

The statistical significance of the RI differences between age groups was tested. P values and the presence or absence of significance of the differences is presented in Table 1.

Table 1. Statistical significance of RI differences between age groups

Age-groups	p	Statist. significance
I vs. II	< 0,01	+
I vs. III	< 0,01	+
II vs. III	< 0,03	+
I vs. adults	< 0,01	+
II vs. adults	< 0,03	+
III vs. adults	> 0,10	-

In the group of the youngest children (group I, up to six years), the RI values were

significantly higher in comparison with the other groups of children and the adult group. In the group II (>6-11 years) the RI values were just slightly higher than in the adult group, with the statistical significance of differences in the level of only 95%. In the Figure 1, an overlap of many values can also be noted between this group (II) and the adult group, while there is practically no overlap in the RI values between the adults and the youngest children group (I). In the third group of children (> 11 years of age), no difference was observed in comparison with the adults.

To establish whether a threshold value of 0.70 of the adults can be used in children as well for the evaluation of higher renal vascular resistance, the ranges of RIs in children age groups were analyzed in intervals of 0.05, and the results are presented in Table 2.

Table 2. Ranges of RI values in age groups of children

Range of RI	Group I (N=52)	Group II (N=48)	Group III (N=50)
0,55-0,59	0	4 (8,3%)	6 (12%)
0,60-0,64	7 (13,5%)	32 (66,7%)	35 (70%)
0,65-0,69	8 (15,4%)	12 (25%)	9 (18%)
0,70-0,74	29 (55,8%)	0	0
>= 0,75	8 (15,4%)	0	0

These results indicate that RI is below 0.70 in all children over 6 years in the groups II and III. However, in younger children (group I), the RI>= 0.70 was observed in as much as 67.3% (35 of 52) children. Therefore, one can conclude that the threshold RI value of 0.70 in this age group is not an acceptable indicator of higher renal vascular resistance.

The measured acceleration time (AT) ranged from 0.04-0.09 seconds with very small variability, and the mean value was the same for all three groups: it was 0.07+/ 0.01 sec.

Discussion

Duplex Doppler ultrasonography of the intrarenal arteries enables non-invasive evaluation of renal vascular resistance and measurement of velocities in the renal and intrarenal arteries. Doppler findings have a considerable significance in adults and children in the diagnosis of renal artery stenosis, renal vein thrombosis, complications after kidney biopsy, evaluation of blood flow in acute and chronic kidney inflammation, distinction of obstructive and non-obstructive collecting system dilatation and in evaluation of renal vascular resistance in various parenchymal renal diseases (e.g. diabetic nephropathy, lupus, autosomal dominant polycystic kidney disease (ADPKD), hepatorenal syndrome, hemolytic-uremic syndrome, interstitial nephritis).[23-26] The longitudinal measurements of RI can be useful in predicting the normalization of renal function or progression of renal functional impairment. Doppler can be useful in the evaluation of vascularization in benign and malignant renal masses.[27] Power Doppler has been shown to be particularly useful for the detection of focal inflammatory changes in the kidneys and focal renal ischaemic areas.[27,28]

Several studies have been conducted in the adult population for the assessment of Doppler in various kidney diseases. One of the most important findings was the observation by Platt *et al.*[26] that the RI value below 0.70 can be used as an indicator of normal

renal vascular resistance in adults. The age-dependence of the RI values in the adults was observed, with RI rising in older age; this is manifested as a loss of the functioning nephrons and increase in renal vascular resistance, which is not detected from the changes of serum creatinine values. The RI values greater than 0.70 can be interpreted as a sign of elevated renal vascular resistance and can be found in several parenchymal renal diseases and some other conditions. However, in several reports, the RI values higher than 0.70 were reported in the intrarenal arteries of healthy children.[29-35] This is the reason for a limited and only lately growing use of Doppler techniques in children. In order to compare the RI values in children with those in healthy adults, we have compared our results with the results of the study published by Brkljačić *et al.*[22] They examined a group of 121 healthy adult examinees with normal kidney function (51 men and 70 women, age-range 19-83 years, mean age 44.1 +/- 14.8 years), in which the Doppler analysis of the intrarenal arteries was performed in the same way as in this paper. In that study on healthy examinees, the mean RI of 0.60 +/- 0.035 (range 0.535-0.685) was determined. The normal RI values in the literature ranged from 0.58 to 0.64; they were obtained with different examination techniques and number of examinees. We consider the given values in the adults to be adequate for the comparison with the results in this study because the examination was performed under identical technical conditions, using the same type of color Doppler scanner and with the same number of measurements in the kidneys.

The normal range of renal Doppler findings in children has not been extensively studied yet. Earlier studies found no age dependence of renal RIs in children,[36] but later ones reported that the renal vascular resistance changed with age. The recognition of abnormal renal RIs in children and interpretation of measured RI values, therefore, require the knowledge of normal RIs in children. Our data indicate that renal RIs in children under six years are above the level of the adult values. In older children, the RI values do not differ significantly from those in adults; in healthy children older than six years, RI is always under 0.70. Therefore, the RI values in children over 6 years can be interpreted in the same fashion as in adults while, in younger children. RIs should be interpreted according to the normal values of the particular age groups.

Increased RIs in young children are due to higher renal vascular resistance in the maturing human kidney. Renal functional parameters, like glomerular filtration rate, tubular excretory capacity and blood rate are decreased in newborns and mature by the first year. The higher activity of the renin-angiotensin system in infancy and childhood in comparison with the values in adults has been studied in detail. Plasma renin activity (PRA) in full-term infants is about 10 ng/ml/hour; it decreases during the first year of life to 5 ng/ml/hour, and the value of 1 ng/ml/hour, normal in adults, is not reached until about the age of six.[37,38] Higher renal vascular resistance in children under 6 years could be due to higher values of PRA in this age group; however, additional research should be conducted to prove eventual relation between PRA and Doppler resistance index.

Doppler ultrasonography plays an important role in the non-invasive diagnosis of renal artery stenosis.[18-20] Two important signs detectable by Doppler are: (1) high velocity at the site of stenosis in the main renal artery and (2) in high-degree stenosis "parvus and tardus" spectra in the intrarenal arteries, mainly characterized by increased acceleration time (AT). The latter sign is especially important in children in whom renal artery stenosis due to the fibromuscular dysplasia is much more common than in adults in whom renal artery stenosis (RAS) is most commonly

caused by the ostial atherosclerotic disease. The significance of the AT measurement in the intrarenal arteries and detection of "parvus and tardus" spectra in the diagnosis of renal artery stenosis was first described by Stavros in 1992[21] and, only since, this parameter has been measured in adults. No investigation of AT in healthy children could be found in the literature. We have therefore studied the acceleration time values in healthy children. AT was not measured in adult population in a larger group of examinees. But it was observed that the values are very low, and some authors consider the values below 0.10 sec. as normal AT values in adults. Apparently, there is no difference between children and adults in the systolic portion of intrarenal arterial spectra and, consequently, also in AT values. So, the AT values in children and in adults can be interpreted in the same way.

ATs were very low, with low variability of measured values, and no differences observed between age groups of children, as well as in comparison to adults. Thus, renal artery stenosis in childhood can be diagnosed on the basis of the same criteria as in adults.

Doppler studies are very dependent on the experience of the examiner, technical quality of the US scanner and optimal examination technique (arteries in which RIs are measured, number of measurements, automatic or manual calculation). It is very important to insonate as many intrarenal arteries as possible, to adjust the optimal pulse repetition frequency and wall filter and to have enough time on disposal to perform the study optimally. All these requirements are even more important in children who are often not cooperative enough during the examination.

There are only four prior reports of normal renal RI values with larger number of children, published between 1992 and 1997.[31,34] If data from these reports are converted to the mean renal RI of the similar age groups like in this study, the values of resistance index in the youngest age group (2-6 years) are significantly higher in our study (RI=0,70) compared to the studies of Lin *et al.* (mean RI=0,65)[32] and Bude *et al.* (mean RI=0,64),[33] but very similar to results of Scholbach *et al.* (RI=0,71)[31] and Vade *et al.* (RI=0,69).[34] In the age group II (>6-11 years), RI is similar in all studies (RI=0,62), except in Scholbach's[31] who reported higher values (RI=0,70). In the third age group (>11-16 years), the values of RI are equal to those reported by Vade *et al.* (RI=0,58) (34), similar to Bude's (RI=0,59)[33] and Lin's (RI=0,62),[32] and cannot be compared to the results of Scholbach *et al.*[31] where children over 6 years are not divided into further age subgroups.

The comparison of our results to the studies of Scholbach *et al.*,[31] Bude *et al.*[33] and Lin *et al.*[32] is not precise due to older, technically inferior types of Doppler US scanners used in those studies. This is especially applicable to the study of Bude[33], who calculated manually from scans using micrometer on the old type of US scanner. Scholbach measured RI values only once, in main renal arteries where the values are known to be higher compared to intrarenal arteries.[31] He divided the children under 6 years into 3 age subgroups and found equal values of RI in all of these subgroups, significantly higher compared to older children. The values of RI in this study are about 5% higher then ours. Lin measured RI in segmental arteries without specifying the number of measurements.[32] The studies in adult population showed that the resistance index at the level of the interlobar-arcuate arteries is the most constant Doppler parameter and should be preferred in clinical applications.[39] Our data for children 2-16 years old are in agreement with Vade's, published in 1993; which comprised 95 children from 0 to 18 years and conducted measurement on the same state-of-art Acuson 128 XP scanner.[34] The measurements of RI were obtained also from interlobar arteries and calculated as an average of three values from different parts of

the kidney as in our study. The number of children in the same age range in this study is 150, and in Vade's report 56. Vade did not measure AT.

In conclusion, we were able to obtain an adequate Doppler sonogram and measure RI and AT in intrarenal arteries by color duplex Doppler ultrasonography in all examined children. It has been demonstrated that the Doppler index of resistance in early childhood is considerably higher as compared to the adult population; however, after the 6 years, all RI values are below 0,70. Therefore, the utilization of the 0.70 RI as a threshold value for the increased vascular resistance in adults can be also applied to children over 6 years. In younger children, the RI values should be interpreted on the basis of normal values in a particular age group. The acceleration time values in children are not different as compared to adults. The detection of renal artery stenosis on the basis of analysis of intrarenal Doppler arterial systolic spectral morphology and measurement of AT are the same in children and in adults.

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Ultrasound diagnosis of gallstone ileus - a case report

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Background. *The diagnostic method of choice for ileus was a plain abdominal film. However, in the recent years, x-ray has been increasingly replaced by ultrasonography which, in experienced hands, can reveal ileus at an earlier stage and also identify the cause and site of obstruction.*

Case report. *We reported about a male patient who was admitted to the Department of Gastroenterologic Surgery with severe abdominal pain and vomiting. A plain abdominal film showed an obstructive ileus and pneumobilia. Ultrasound examination of the abdomen confirmed the presence of ileus of the small intestine and demonstrated a gallstone impacted in the distal ileum. It also disclosed a distorted gallbladder adhering to the duodenum. The patient was treated surgically and had an uneventful postoperative course.*

Conclusions. *Ultrasonography, in addition to diagnosing the ileus caused by an ectopic gallstone, is also able to identify the aetiology and site of obstruction.*

Key words: cholelithiasis, intestinal obstruction-ultrasound, gallstone ileus

Introduction

Gallstone ileus (GI) is a form of obstructive ileus in which the obstruction is caused by gallstones that have passed into the bowel through a fistula between the gallbladder and the duodenum.[1] GI accounts for less than 3% of all cases of obstructive ileus, yet this figure is significantly higher in patients over 65 years of age, in whom gallstones are responsible for as many as 25% of cases of bowel obstruction.[1,2] The most frequent site of obstruction is the terminal ileum (60%), followed by other parts of the small intestine, whereas the colon, most frequently the sigmoid, is involved in less than 5% of cases.[3] The condition is more common in women than in men, the ratio ranging from 4:1 to 16:1 in various series.[2] Advanced GI requires immediate surgical treatment; the mortality is five times higher than in bowel obstruction from other causes. Until recently, the diagnostic method of choice was the plain abdominal film. However, plain radiography has several limitations: it fails to show bowel obstruction when the bowel loops are filled with fluid (without gas); it usually cannot detect gallstones as most are not sufficiently calcified; and it does not show minor pneumobilia. Therefore, in the recent years, x-ray has been increasingly replaced by ultrasonography which, in experienced hands, can

Received: 12 November 1998
Accepted 30 December 1998

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reveal ileus at an earlier stage and also delineate gallstones, thereby identifying the cause and site of obstruction. We describe a patient in whom GI was correctly diagnosed by ultrasound and surgical treatment was successful.

Case report

A 47-year-old man was admitted with a 2-day history of upper abdominal pain and vomiting. He was not jaundiced and had a normal temperature. He was constipated for the past few days. Three years before, he was treated in hospital for obstructive jaundice associated with fever. At that time, ultrasound examination demonstrated acute calculous cholecystitis and dilatation of intra- and extra-hepatic bile ducts due to a gallstone lodged in the distal common bile duct. Endoscopic retrograde cholangiopancreatography (ERCP) and papillotomy (EPT) were performed, and a 7 mm stone was removed from the common bile duct. The cholecystitis responded to antibiotic treatment. By the time of discharge, the fever and jaundice had subsided. A cholecystectomy that would be expected to follow the primary operation was not undertaken for unknown reasons. On the present admission, a plain abdominal film showed signs of obstructive ileus and pneumobilia (Figure 1). Ultrasonography performed a few hours later provided evidence of advanced ileus; it showed dilated fluid-filled loops of the small bowel with thickened walls, swollen mucosal folds and absence of peristalsis (Figure 2). Several solid echogenic structures with distal acoustic shadowing suggestive of calculi were visible in the distal ileum (Figures 3a,b). The gallbladder was small and shrunken, it had a thickened wall and adhered firmly to the duodenum (Figure 4). The bile ducts contained air (Figure 5). The liver displayed structural changes compatible with a diffuse parenchymal lesion. There was no free fluid in the peritoneal cavity. These findings spoke in favour

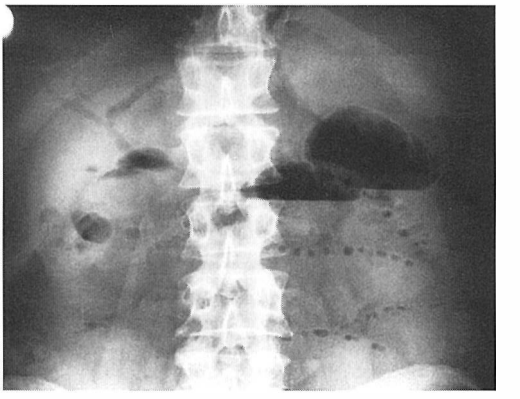


Figure 1. A plain abdominal film on the admission showing obstructive ileus and pneumobilia.

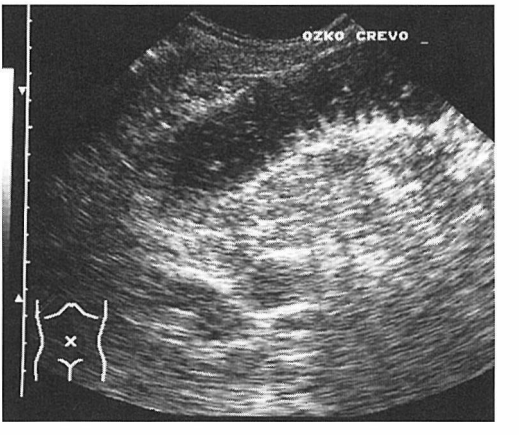
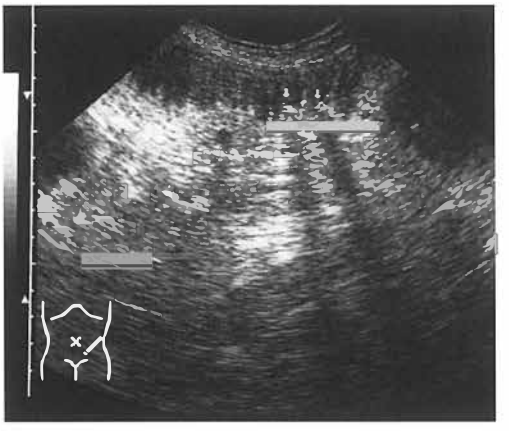


Figure 2. Ultrasonogram showing dilated fluid-filled loop of the small bowel with thickened wall and swollen mucosal folds.

of GI with occlusion in the distal ileum. The patient was immediately prepared for surgery. At operation, the bowel was found to be severely distended to a level about 40 cm proximal to Bauhin's valve, where a hard mass was palpated within its lumen. On enterotomy this proved to be a large gallstone, measuring 2.5 by 2.5 by 5 cm (Figure 6), and two smaller ones. The operation also disclosed adhesions to the liver, a shrunken gallbladder and a fistula, about 2 cm in diameter, between the gallbladder and the duodenum. The gallstones were removed from the ileum, a cholecystectomy was performed, and the



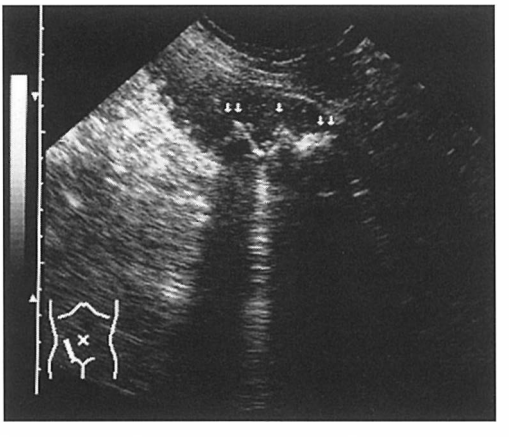


Figure 3a,b. Ultrasonogram showing calculi in the distal ileum.

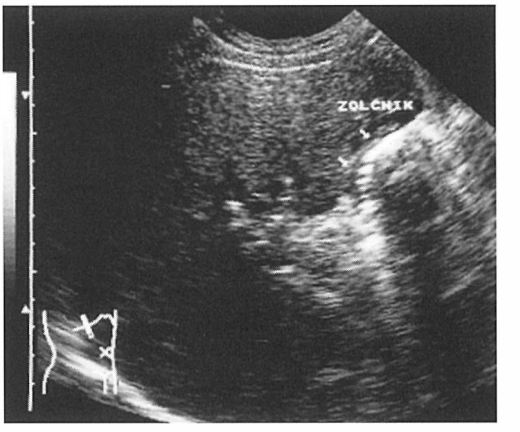


Figure 4. Ultrasonogram showing small, shrunken gallbladder with thickened wall and adhered firmly to the duodenum.

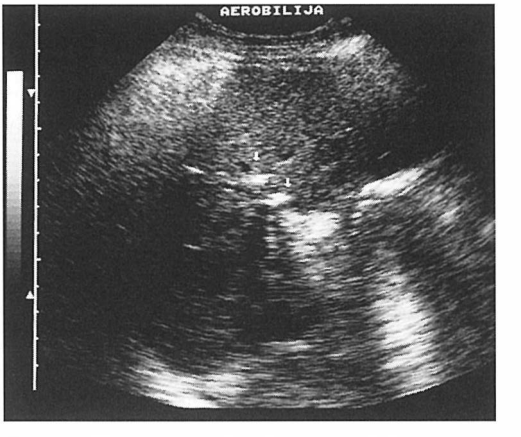


Figure 5. Ultrasonogram showing pneumobilia.

defect in the duodenum was closed. Postoperatively the patient remained in the intensive care unit for 5 days. His condition was good. On the 7th day, the sutures were removed and he was discharged from the hospital.

Discussion

Gallstone ileus is a form of obstructive ileus that develops as a result of the impaction of gallstones in the gut, mostly in the distal ileum.[3] A special form of GI is the so-called Bouveret's syndrome, caused by calculi lodged in the duodenum and obstructing the gastric outlet, which accounts for less than 10% of cases.[4] The calculus enters the intestine through a fistula connecting the gallbladder mostly with the duodenum[1] or, exceptionally, with the colon.[5] In our patient, the obstruction was located in the distal ileum, and a 2 cm wide fistula was found between the gallbladder and the duodenum.Gallstone ileus typically occurs in elderly women.[2] So our patient, a 47-year-old man, represents an exception which could be misleading and therefore underscores the need for proper diagnostic evaluation. Many patients are unaware of harbouring gallstones and experi-

Figure 6. A large gallstone, removed from the ileum.

ence no symptoms prior to the development of GI.[1,2] However, our patient was treated three years previously for acute calculous cholecystitis and obstructive jaundice due to a gallstone in the common bile duct. The cholecystitis was cured and the stone was removed by EPT during ERCP, but there are no data to explain why a cholecystectomy was not performed subsequently. Until recently the diagnosis of GI was based on plain abdominal films demonstrating Riegler's triad of signs: gas in the gallbladder or bile ducts or both, an ectopic gallstone, and partial or complete obstruction of the bowel.[2,4] However, radiography is sufficiently sensitive in only about a third of cases[1] as (a) it does not show distended bowel loops if they are filled with fluid (unlike ultrasound, which provides excellent visualisation of fluid-filled structures), (b) it demonstrates only about 10% of gallstones, which are adequately calcified, and (c) it does not detect small amounts of air within the bile ducts. In our patient, radiography demonstrated small bowel ileus. Also pneumobilia was clearly visible on the film, undoubtedly as a result of the papillotomy performed three years before, but the stone that caused the obstruction was not apparent. Ultrasound has opened new possibilities in the diagnosis of GI, as in many other areas. Ultrasonography provides reliable detection of (a) dilated fluid-filled bowel loops, (b) all gallstones regardless of their composition, (c) small amounts of gas within the bile ducts, and (d) a distorted gallbladder displaying even more pronounced changes in gallstone ileus than in cholecystitis or carcinoma.[2] All these features were present in our patient. Thus, ultrasound examination in GI can diagnose ileus at an early stage and can also identify the cause and site of obstruction, thereby enabling the surgeon to minimise the surgical trauma. It can detect any additional stones which must be removed promptly in order to prevent recurrent obstruction of the bowel. In the literature, a pitfall in ultrasound diagnosis is illustrated by a patient in whom ileus was caused by adhesions, while the presumed stone visible in the right lower quadrant was in fact a calcified mesenteric lymph node.[1] In our opinion, such errors are unlikely since an experienced examiner will readily locate a gallstone within a dilated bowel loop. The appraisal of a severely altered, emptied gallbladder, firmly adhering to the duodenum, may present some more difficulties since the presence of air within the connecting fistula precludes a clear view. However, the main shortcoming of ultrasonography is that its diagnostic yield continues to depend to a large extent on the examiner's skill. Contrast studies, computed tomography (CT) and endoscopy play a minor role in the diagnosis of GI. Attempts at endoscopic extraction of the stone may be successful mainly in Bouveret's syndrome.[1,2,4-7]

The treatment of GI is almost exclusively surgical. Spontaneous excretion of the stone is extremely rare[2,4] but successful removal by endoscopic short wave lithotripsy has been reported.[6] At operation the ileus must be released. If the patient's condition permits, cholecystectomy is also performed and the fistula is repaired at the primary operation, as was done also in our patient. In this way, recurrent ileus caused by additional stones present in the gallbladder, as well as recurrent cholecystitis, cholangitis and malignant transformation of a persistent fistula can be avoided.[2] Since GI mostly occurs in elderly

patients, the mortality is high, which is attributed mainly to long delays before admission and before the diagnosis is established, dehydration due to vomiting, and a deterioration of coexisting diseases that are common in the elderly.[2] Our patient was relatively young and his general condition was good; he experienced no complications during the operation, which comprised also a cholecystectomy and fistula repair, and he had an uneventful postoperative course.

Conclusions

Ultrasound examination affords rapid and reliable diagnosis of gallstone ileus and identifies the site of intestinal obstruction. This shortens the time to surgery, reduces the operative risk and contributes to a favourable outcome of this serious condition.

Our patient was relatively young and his general condition was good; he experienced no complications during operation, which comprised also a cholecystectomy and fistula repair, and he had an uneventful postoperative course.

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Quantitative and qualitative assessment of contrast-enhanced magnetic resonance imaging of the parotid gland in Sjögren's syndrome

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Background. *The aim of this study was to evaluate the potential of unenhanced and contrast-enhanced MR imaging for providing diagnostic information of the parotid gland in patients with Sjögren's syndrome.*
Subjects and methods. *In 27 patients with Sjögren's syndrome, unenhanced and GdDTPA-enhanced spin-echo MR imaging was performed. The morphologic MR findings were compared to the signal intensity (SI) measurements of unenhanced T1-weighted, T2-weighted and contrast-enhanced T1-weighted MR imaging. Quantitative and qualitative data were compared with those of normal subjects (n=12).*
Results. *T1 and T2 values on the unenhanced MR images of the patients with Sjögren's syndrome were significantly lower than those of normal subjects (T1: 62+/-4%, T2: 71+/-2% of baseline). Quantitative analysis of contrast-enhanced MR imaging showed a significant SI increase in all patients with Sjögren's syndrome. Even in 4 patients with no morphologic findings on MR imaging, the increase of SI on enhanced MR imaging was significantly higher than in normal subjects (34+/-3% versus 17+/-3%; p<.05. The extent of morphologic changes correlated with quantitative data. The sensitivity and specificity for MR imaging was 85% and 100%, respectively.*
Conclusions. *The use of paramagnetic contrast media provides additional diagnostic information, particularly in the patients without apparent changes in morphology as indicated by a significantly greater tissue enhancement compared to normal subjects. The extent of morphologic alterations correlates with the quantitative data of Gd-DTPA-enhanced MR imaging but not with those of unenhanced MR imaging.*

Key words: Sjögren's syndrome, parotid diseases; magnetic resonance (MR) imaging, image enhancement, paramagnetic contrast media, morphology

Introduction

In 1933, Sjögren first described a syndrome characterised by the triad of dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia), with the evidence of a systemic autoimmune disease.[1-3] Sicca syndrome or

Received 20 July 1998
Accepted 29 November 1998

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the primary form of Sjögren's syndrome refers to the patients without underlying systemic autoimmune disease, whereas the secondary is associated with the connective tissue disease.[2-5] The secondary form is more frequent than the primary, with rheumatoid arthritis being the most common associated autoimmune disease (35%).[2-5] However, no therapeutic cure is currently available for this disorder. Hence, the care of the patients is confined to the alleviation of symptoms and prevention of tissue damage. The efficacy of the therapy of the patients with Sjögren's syndrome relies ultimately on early findings.

Magnetic resonance (MR) imaging is well suited to imaging the patients with inflammatory disease because of its potential to visualise inflamed tissue.[6-9] Former data suggest that the difference in signal intensity between normal and pathologic tissues can be increased by administering MR contrast medium;[6-9] it alters both T1 and T2 relaxation times in tissue by creating local magnetic fields that fluctuate with appropriate frequency components. For instance, Gadolinium-chelates produce significant enhancement on the T1-weighted images in the areas with blood supply, oedema, and necrosis.[10-13] This class of agents has been previously used for the delineation and characterisation of inflamed tissue in rheumatoid arthritis.[6,8,9,14]

However, there is no information available regarding the characterisation and staging of Sjögren's syndrome using contrast-enhanced MR-imaging. Accordingly, this study was designed (1) to evaluate the potential of MR imaging in the assessment of morphologic changes, and (2) to evaluate the potential of unenhanced and contrast-enhanced MR imaging for additional diagnostic information of the parotid gland in the patients with Sjögren's syndrome

Patients and methods

Study population

The study protocol comprised 12 normal subjects (7 female, 5 male, average age 48 years) without evidence of parotid disease and 27 patients with Sjögren's syndrome (25 female, 2 male, age 29-79, mean 51). All patients had signs and symptoms that met the criteria for the diagnosis of Sjögren's syndrome as defined by Sjögren's Disease Research Committee of Europe.[15] The diagnosis was pendent if the patients met 4 of the following 6 criteria; (1) xerophtalmia, (2) xerostomia, (3) positive Schirmer test results (<10 mm of wetting in 5 minutes), (4) positive lip biopsy, (5) positive scintigraphy of the salivary glands, and (6) evidence of auto antibodies (anti-Ro/SS-A and anti-La/SS-B). All patients had xerophtalmia and xerostomia. In 21 patients, the Schirmer test was positive. Scinitgraphy of the salivary glands was obtained in 13 patients (positive n=12). Fourteen patients had the biopsy of the salivary glands performed (positive n=12, not specific n=2) The diagnosis of Sjögren's syndrome was made after the identification of a periductal lymphocytic infiltration. The evidence of anti-Ro/SS-A antibodies and anti-La/SS-B (n=19) was positive in 14 patients. The patients with an associated pre-existing lymphoma, a HIV-related infection, a Sarcoid or a graft versus host reaction were excluded from the study. Twenty-two patients (81.5%) had a primary Sjögren's syndrome and 5 (18.5%) had a secondary form. The duration of the disease was from 1 to 7 years (mean 3). None of the studied patients underwent surgery of the parotid gland before. Sialography was not performed in the present population because of the reported evidence of serious exacerbation of the inflammatory process.[16-18]

MR Imaging

MR imaging was performed with 1.0 T unit (Siemens-Impact; Erlangen, Germany) using a commercially available head coil. The T2-weighted turbospin-echo(TSE) sequence followed by the T1-weighted SE sequence before and after the contrast medium application in coronal and axial scan planes were obtained. Gadopentetate dimeglumine (0.1 mmol/kg) (Gd-DTPA) (Magnevist; Schering, Berlin, Germany) was administered intravenously. For a direct comparison of the T1- and T2-weighted images, the same slices in the same location spacing were used. Imaging parameters for the T1- and T2-weighted (T)SE sequences were the following; repetition time (TR)/echo time (TE):600/15 msec and TR/TE:2000/15-90 msec, respectively. The acquisition matrix was 256x256, slice thickness 4 mm, intersection gap 0.4 mm, and field of view 23 cm. All images were displayed on the monitor with the same gray-scale level and window.

The qualitative analysis of the parotid gland on the unenhanced MR images entailed the following criteria; (1) enlargement present-absent, (2) parenchymal structure: homogeneous-inhomogeneous, and (3) presence of pseudotumors (parenchyma alterations with a size > 5 mm). The findings of the inhomogeneous gland parenchyma were further divided into two groups; mottled pattern (lesions < 2 mm in diameter) and honeycomb-like pattern (lesions 2-5 mm in diameter), known as "salt and pepper" appearance.[1]

On the unenhanced T1-weighted images, the signal intensity (SI) of the masseter muscle was defined as isointense. A circular region of interest (ROI) (4 mm^2; pixel size 0.14 cm^2) was used in order to quantify regional signal intensity (SI). For each image before and after the administration of contrast medium, ROIs were placed at the identical location bilaterally in the centre of the parotid gland. The co-ordinates of each region of interest were fixed in the first image and remained constant for the analysis of the subsequent post-contrast media injection images. The signal-intensity-time curves were generated with an implemented evaluation program. SI was calculated with the following equation; $SI = (SI_{max} - SI_0)/SI_0 \times 100$, ($SI_{max}$: maximal signal intensity following the administration of the contrast agent; SI_0: signal intensity before the injection of the contrast agent).

Data analysis

For morphological findings, MR images were interpreted independently by three radiologists. The final diagnosis was made following the agreement of at least two readers. All values were expressed as mean +/-standard error of mean value (SEM). The paired two-tailed Student's t-test was used for comparison of SI within the groups before and after the administration of the contrast agent. The unpaired Student's t-test was used for the comparisons between the groups to determine the differences of SI increase. A probability level of less than 0.05 was considered as significant.

Results

Normal subjects

Morphologic findings and signal intensity analysis

On MR imaging, the parotid gland showed homogeneous low level signal and intermediate signal on the T1- and T2-weighted MR images, respectively. Following the administration of contrast agent the increase in signal intensity was 17±3%.

Patients with Sjögren's syndrome

Morphologic findings

Morphologic findings on MR imaging are summarised in Table 1. In 23/27 patients

Table 1. MR imaging findings of the parotid gland in 27 patients with Sjögren's syndrome

Morfologic findings	MRI
Normal size	6
Enlargement	21
Homogeneity	8
Mottled pattern	8
Salt and pepper pattern	11

(85%), alterations in regard to the size or parenchymal homogeneity of the parotid gland were evident on the unenhanced T1- and T2-weighted MR images. In 4/23 patients, MR imaging showed only an enlargement of the gland. Unenhanced MR imaging was normal in 4 patients (15%). Post-contrast scans did not improve the delineation of parenchymal inhomogeneities.

Signal intensity analysis

Signal intensity of all alterations of the gland compared to normal gland tissue was hypointense on the unenhanced T1- and T2-weighted images in all patients (Figures 1-3). Figure 4 shows a significant difference in SI values on the unenhanced T1- and T2- weighted images between normal subjects and the patients with Sjögren's syndrome ($p<0.05$). No significant difference in SI values on the

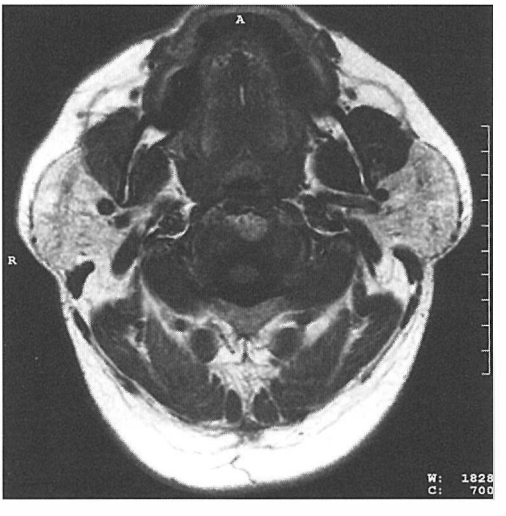

Figure 1a. Sjögren's syndrome – mottled appearance. Transverse (trans.) T1-W SE image (600/15 msec, TR/TE) precontrast: The gland appears enlarged with a granular internal structure.

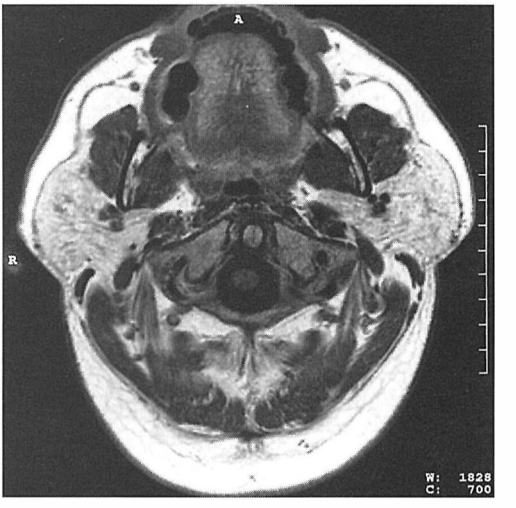

Figure 1b. Sjögren's syndrome – mottled appearance. Gadolinium-enhanced trans. T1-W SE image: The same pattern is seen on the enhanced image; no additional morphological information concerning the gland structure can be obtained using contrast medium in comparison to T2-weighted image.

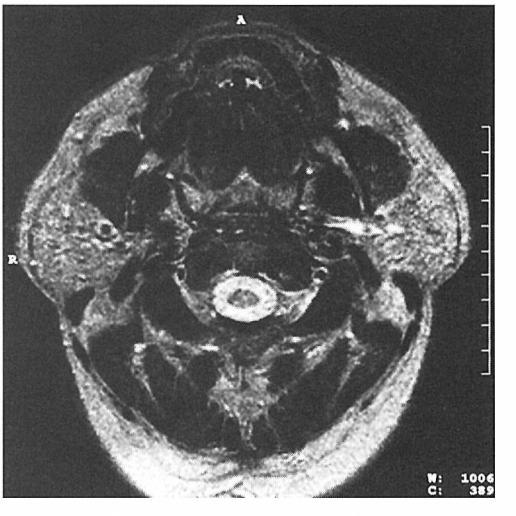

Figure 1c. Sjögren's syndrome – mottled appearance. Trans. T2-W SE image (2500/90 msec, TR/TE): The inhomogeneous granular internal structure is best seen on the T2-weighted image.

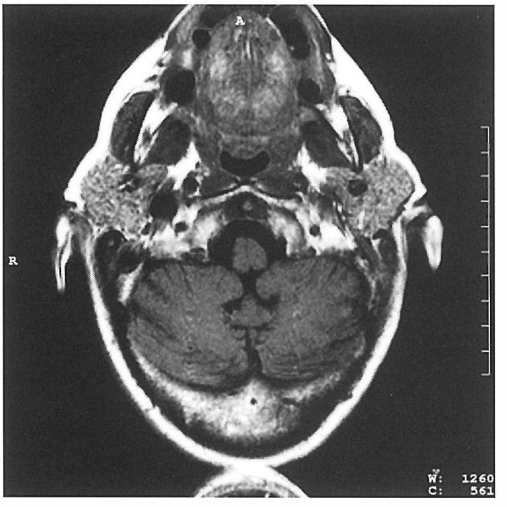

Figure 2a. Sjögren's syndrome – honeycomb-like appearance. Transverse (trans.) T1-W SE image (600/15 msec, TR/TE) precontrast: Significant enlargement of the gland with coarse nodular internal structure.

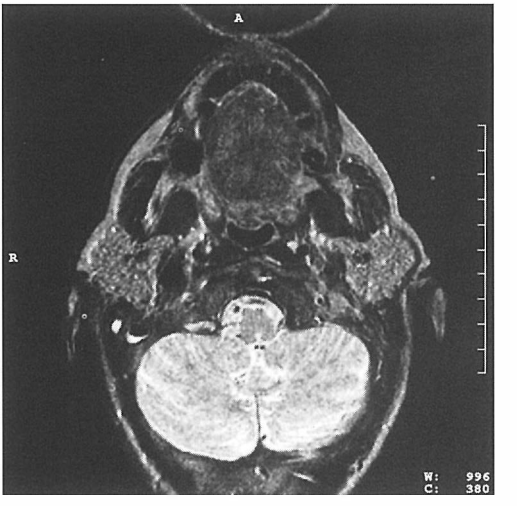

Figure 2c. Sjögren's syndrome – honeycomb-like appearance. Trans. T2-W SE image (2500/90 msec, TR/TE): The T2-weighted image represents best the typical salt and pepper appearance of the parotid gland in Sjögren's syndrome.

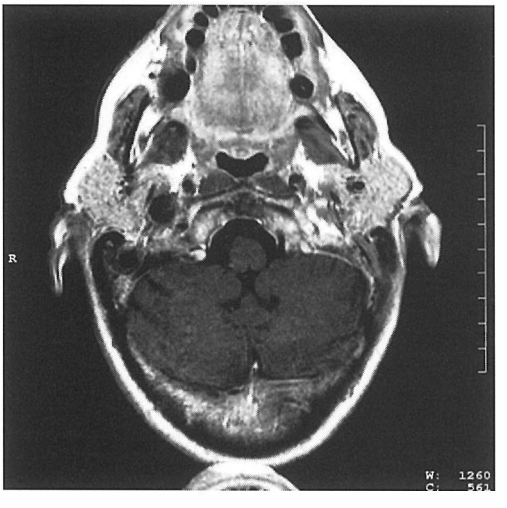

Figure 2b. Sjögren's syndrome – honeycomb-like appearance. Gadolinium-enhanced trans. T1-W SE image: Contrast medium outlines the coarse nodular internal structure but with no additional morphological information in comparison to the T2-weighted image.

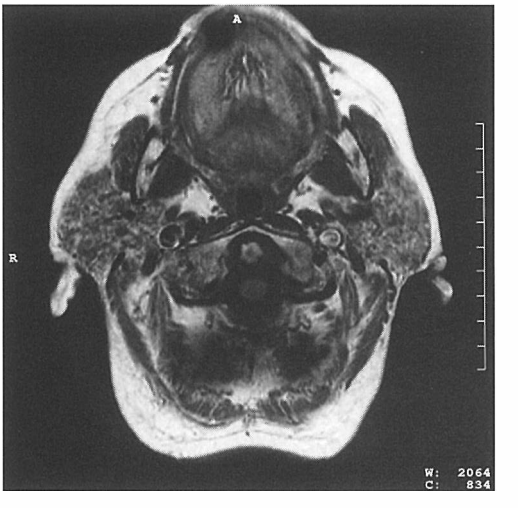

Figure 3a. Sjögren's syndrome – patient with honeycomb-like appearance on MRI. Transverse (trans.) T1-W SE image (600/15 msec, TR/TE) precontrast: Enlarged gland with coarse nodular internal structure.

unenhanced T1- and T2- weighted images was found between the patients with mottled and nodular honeycomb-like patterns.

Following the administration of the contrast medium, a significant increase of signal intensity in all patients with Sjögren's syndrome (46+/-19% versus 17+/-3% increase of the baseline value, $p<0.05$) was noted in com-

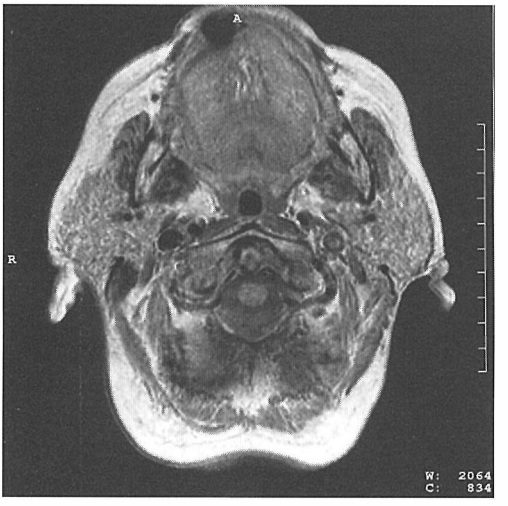

Figure 3b. Sjögren's syndrome – patient with honeycomb-like appearance on MRI. Gadolinium-enhanced trans. T1-W SE image: Honeycomb-like appearance is clearly shown on enhances T1-weighted image.

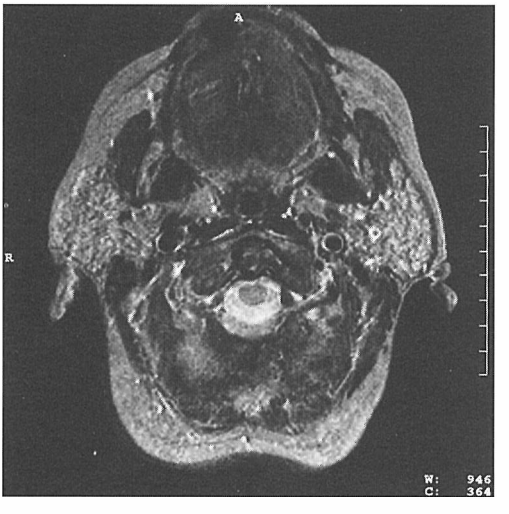

Figure 3c. Sjögren's syndrome – patient with honeycomblike appearance on MRI. Trans. T2-W SE image (2500/90 msec, TR/TE): Honeycomb-like appearance is represented best on enhances T2-weighted image.

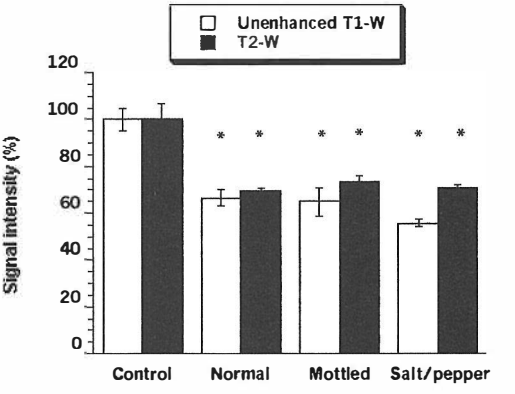

Figure 4. Difference in mean signal intensity between control group, patients with Sjögren's syndrome and no morphological findings, mottled pattern, and nodular honeycomb-like pattern of parotid gland parenchyma at T1-weighted unenhanced and T2-weighted image. Data are expressed as mean +/-SEM. (*significant compared with control group).

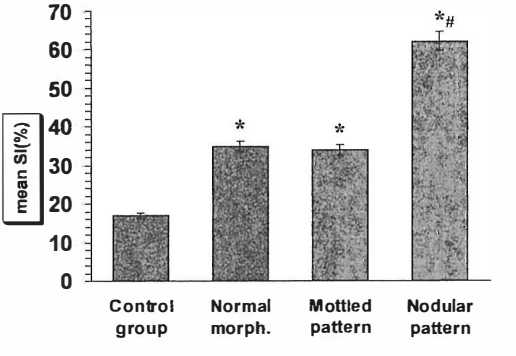

Figure 5. Comparison of differences in mean signal intensity (SI) on contrast-enhanced MR imaging between control group and MR-morphology of patients with Sjögren's syndrome with no morphological findings, mottled pattern, and nodular pattern of parotid gland parenchyma. Data are expressed as mean +/-SEM. (*significant compared with control group, # significant compared with patients with either no morphological findings or mottled pattern).

parison to that in normal subjects. Moreover, 8 patients (4 patients with the enlargement and 4 patients with no enlargement of the gland), in whom MR morphology was negative, showed a significantly higher SI_{max} (34+/-3% of baseline) than normal subjects. In the patients with mottled pattern of the parotid gland, SI_{max}, compared to normal, increased significantly (37+/-4% versus 17+/-3% of baseline, $p<0.05$). However, the magnitude of enhancement was similar to that in the

patients with no morphological findings. SI_{max} on MR imaging was significantly higher in the patients with nodular honeycomb-like pattern than in normal subjects and all other patients (59+/-6% of baseline, $p<0.05$). Figures 4 and 5 summarise the data of contrast-enhanced MR scans in the patients with or without Sjögren's syndrome.

Discussion

The major findings of the current study are as follows; the T2 and T1 signal intensity values on the unenhanced MR images were significantly lower than in normal subjects. The decrease of the T2 and T1 signal intensity values on the unenhanced MR imaging was not concordant to pathologic MR-morphology in the patients with Sjögren's syndrome. The signal intensity measurements following the administration of contrast media showed a significant increase of the signal intensity in patients compared to normal subjects. Even in the patients with no pathologic gland morphology, the signal intensity increase was significantly higher than in the subjects of the control group. The contrast-enhanced MR imaging is superior to the unenhanced MR imaging as indicated by a significantly different increase of the signal intensity in the patients with nodular pattern compared to the patients with either no morphologic findings or mottled pattern. The signal intensity increase did correlate with the extent of morphologic findings on MR imaging.

Sjögren's syndrome is believed to be a chronic inflammatory disease of autoimmune origin affecting usually postmenopausal women (90%).[5] The criteria for Sjögren's syndrome have not been universally established because of the lack of clarity in the diagnostic evaluation.[15] While some authors call for sialography, others see the salivary gland biopsy as an indispensable diagnostic tool.[19-24] Recent trials to stage Sjögren's syndrome on the basis of MR imaging are also not too satisfying because they are missing a reasonable pathological grading system.[25] Moreover, the underlying pathogenesis of Sjögren's syndrome is still not known. The antibodies to SS-A and SS-B are often detected both in the patients with sicca syndrome (70% and 48%, respectively) and in the patients with rheumatoid arthritis and Sjögren's syndrome (9% and 3%, respectively);[2] the antibodies to SS-B are considered highly specific for Sjögren's syndrome.[5] The clinical features in our patients were compatible with those reported in the literature.

MR imaging showed a high accuracy in revealing the mottled and/or honeycomb-like patterns ("salt and pepper"). Both pattern of inhomogeneity, defined also as granular for mottled and speckled for honeycomb-like pattern,[25] have been demonstrated with equal image quality on the unenhanced T1- and T2-weighted MR images as hypointense areas of varying size, which is in accordance with previous reports.[25,26] Microscopically, a periductal mononuclear cell infiltrate consists, initially, of small lymphocytes and, during the course of disease, of large lymphocytes and reticular cells. Vascular disease seems to be responsible for extravasation of lymphocytes. Mitchell and Sundaram *et al.* suggested that aggregates of lymphocytes might be responsible for this specific pattern in Sjögren's syndrome.[27,28] Our findings are consistent with those of previous studies which demonstrated a "salt and pepper" appearance in plain MR imaging.[25,26] In contrast to the inhomogeneity of the gland parenchyma, the enlargement of the parotid gland is an unreliable sign because the term 'enlargement of the gland' still lacks exactness in definition.

The significant decrease in signal intensity on the unenhanced T1-weighted images can be explained by interstitial oedema. However, this finding is not specific to Sjögren's syndrome as reported by others.[1,25,27] In contrast to previous studies, the T2-weighted MR

images demonstrated a significant decrease of signal intensity in patients compared to normals.[1,25] Furthermore, the calculations of signal intensity on the T2-weighted images did not allow to differentiate between the patients with mottled and nodular honeycomb-like pattern of the parotid gland. In agreement with previous reports, the data of the present study suggest that the amount of lymphocytic infiltration and fibrotic tissue might be responsible for an absolute decrease in signal intensity. This observation is also supported by a significant increase in signal intensity following the administration of contrast medium compared to normal subjects. Moreover, even the patients with no morphologic findings demonstrated a significant increase in signal intensity after the contrast medium administration. This marked tissue enhancement can be related to vasculitis which causes lymphocytic extravasation due to capillary permeability. This leads to an increased leaking of small GdDTPA molecules through the capillary endothelium in the surrounding tissue causing an increase in SI_{max}. This is in contrast to previous MR imaging studies which hypothesised that hyperperfusion may account for this phenomenon.[30]

In conclusion, MR imaging is a suitable non-invasive method to detect morphologic changes in Sjögren's syndrome. The mandatory use of paramagnetic contrast media to rule out lymphoma in Sjögren's syndrome provides additional diagnostic information, particularly in the patients without apparent morphologic changes. Future work may prove contrast-enhanced MR imaging an important diagnostic method for monitoring the response to therapeutic interventions in the patients with Sjögren's syndrome.

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Positron emission tomography (PET) in clinical routine

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Background. *Positron emission tomography (PET) is a non-invasive imaging technique that utilizes positron emitting isotopes of biological elements in order to assess metabolism in vivo. The most commonly used tracer is the F-18-labelled glucose analogue called fluorine-18-fluorodeoxyglucose (F-18-FDG). Due to increased restrictions in the national health system and marked expenses for PET studies indications have to be defined thoroughly. Therefore, in the last years several round table expert meetings have been held in order to clearly define the clinical impact of PET in various diseases, e.g. neurological, oncological, and cardiovascular disorders resulting in well-defined indication lists.*

Conclusions. *The indication lists for PET may help both the referring clinician as well as the nuclear medicine physician to optimize cost- effectiveness. Moreover, national health services increasingly utilize these indication lists to decide on reimbursement regarding PET studies in the individual patient.*

Key words: tomography, emission-computed; positron emission tomography, F-18-FDG; utilization, cardiology, neurology, oncology; economics, cost-effectiveness

Introduction

Positron emission tomography is a non-invasive imaging technique that utilizes positron emitting isotopes of biological elements. Thus, PET allows both an assessment and quantification of metabolism *in vivo*. During the last two decades PET has become a part of clinical routine due to both technical advances and an increasing availability of positron emitting isotopes.

Received 21 September 1998
Accepted 15 November 1998

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The most commonly used tracer is a F-18-labelled glucose analogue called fluorine-18-fluorodeoxyglucose (F-18-FDG). FDG is taken up by cells and converted to F-18-FDG-6-phosphate in the same manner as conventional glucose. However, in contrast to physiological glucose-6-phosphate, F-18-FDG-6-phosphate can not be utilized and therefore, accumulates within the cell. Thus, distribution of F-18-FDG reflects glucose metabolism *in vivo*. This was first used in the assessment of neurological and cardiovascular disorders. However, since cancer cells have an increased rate of anaerobe glycolysis as compared to non-malignant transformed cells, PET allows a selective visualization of vital tumor tissue. Due to the intracellular accumulation of F-18-

FDG-6-phosphate the contrast between malignant and non-malignant transformed cells is increased, thereby providing the high sensitivity of PET in the detection of vital tumor tissue. Thus, PET plays a more and more important role in diagnosis of oncological disorders.

The high costs for a PET study as well as the growing public focus on health care expenses are the main reasons for the necessity of clearly defined indications for PET imaging. Therefore, in the last years several round table expert meetings have been held [1-4] in order to clearly define the clinical impact of PET in various diseases, e.g. neurological, oncological and cardiovascular disorders resulting in well-defined indication lists. These indication lists may help both the referring clinician as well as the nuclear medicine physician to optimize cost-effectiveness. Moreover, national health services increasingly utilize these indication lists to decide on reimbursement regarding PET studies in the individual patient.

Table 1. Classification of indications for PET studies

Indications PET is...	
Ia	usually appropriate and considered useful
Ib	acceptable but usefulness less well-established
IIa	helpful
IIb	evaluation not possible at that time
III	generally not appropriate

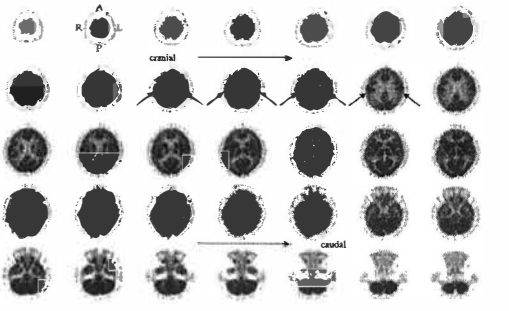

Figure 1. 60-year-old patient with progressive loss of memory and concentration. PET was appropriate (Ia) in order to establish differential diagnosis of dementia. Transversal slices show a global decreased tracer accumulation of the cortex when compared to the basal ganglia. Note, physiological glucose metabolism in area close to the central sulcus as well as in the area of the visual cortex. Typical findings of a progressive Alzheimer's disease.

Evaluation of PET

In several round table meetings[1-4] the value of PET studies has been defined in various diseases, and indications for PET studies were assigned according to three classes as described in detail in Tables 1 and 2 based on the results of clinical research and published studies. If a PET study was shown to be "usually appropriate and considered useful" or "acceptable but usefulness was less well established" PET was assigned to classes Ia and Ib, respectively. If a PET study was shown to be "helpful" or due to lack of experience there is "no evaluation possible at that time" the indications for PET studies were assigned to classes IIa or IIb, respectively. If a PET study is "generally not appropriate" it was assigned to class III. However, one should be aware that a class Ia indication does not imply that a PET study has to be performed necessarily. On the other hand, a class III indication does not automatically exclude a PET study from clinical patient work-up. In general, the indication for a PET study has to be thoroughly checked in the individual patient.

PET in Neurology

For early diagnosis of Huntington's disease, which is based on an atrophia of the caudate and the lentiforme nucleus, PET was classified appropriate (Ia). Moreover, PET was shown to be of high clinical impact in the localization of an epileptic focus. For detection of temporal lobe epilepsy PET was classified appropriate (Ia), whereas for detection of an epileptic focus located extratemporally

Table 2. Summary of indications for PET in clinical routine

Neurology			
Huntington's disease	early diagnosis		Ia
Dementia	early diagnosis		Ia
	differential diagnosis		Ib
Depression	cognitive alteration	differentiation from dementia	Ib
Epilepsy	temporal lobe epilepsy	preoperative localization of focus	Ia
	epilepsy located extratemporally	preoperative localization of focus	Ib
Cardiology			
Diagnosis of viable myocardium			Ia
Oncology			
Glioma	high-grade	diagnosis of relapse	Ia
	known relapse	suspected malignant de-differentiation	Ia
	suspected glioma	localization of biopsy site	Ia
	known glioma	determination of biological aggressiveness	Ib
Head and neck	search for primary tumor	histology positive, conventional imaging negative	Ia
	primary tumor	lymph node staging if primary tumor is resectable	Ib
	local relapse	more than 3 months after therapy	IIa
Differentiated thyroid cancer	I-131-scan negative	relapse of tumor or metastases suspected	Ia
	I-131-scan positive	search for further tumor manifestations if therapeutic regimen will be altered	Ib
Non-small-cell lung cancer	peripheral pulmonary nodule	high-risk patient	Ia
	lymph node staging		Ia
	suspected local relapse		Ia
	therapy monitoring		IIa
Breast cancer	primary tumor suspected		IIa
	local relapse suspected		IIa
	lymph node staging		IIa
	diagnosis of distant metastases	high-risk patient	IIa
	therapy monitoring		IIa
Pancreatic cancer	primary tumor suspected	differential diagnosis tumor tissue versus inflammatory tissue	Ia
	local relapse suspected	therapeutic option is considered	Ib
Colorectal cancer	relapse suspected	elevation of tumor markers and unclear findings of conventional imaging	Ia
	after chemotherapy	therapy monitoring	Ib
Cancer of the bladder	lymph node staging		IIa
Ovarian cancer	relapse		IIa
	re-staging		IIa
Germ cell tumor	non-seminomateous	therapy monitoring (not for differentiated teratoma)	Ib
		lymph node staging	IIa
		re-staging	IIa
Malignant lymphoma	primary staging		Ib
	after therapy	diagnosis of tumor remnants	Ib
	re-staging		IIa
	diagnosis of tumor relapse		IIa
Malignant melanoma	grade II and III	lymph node staging	Ia
		assessment of distant metastases	Ia

PET was defined acceptable (Ib). In case of early diagnosis of dementia PET was determined to be appropriate (Ia). Moreover, in differential diagnosis of dementia PET was classified acceptable (Ib) as shown in Figure 1.

PET in Cardiology

A PET-study has definitely been shown appropriate (Ia) in the diagnosis of myocardial viability (Figure 2). In combination with a

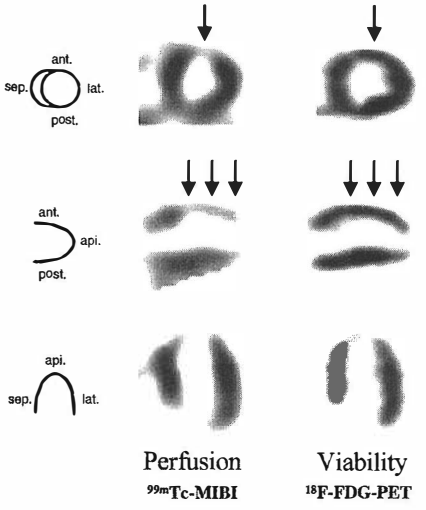

Figure 2. 74-year-old patient with a known stenosis of the left anterior descending artery. PET was appropriate (Ia) in order to establish viability prior to surgical revascularization. Myocardial perfusion scintigraphy (left) exhibited a deficiency of perfusion in the anterior wall and the apex corresponding to rest ischemia. PET study (right) shows a focal lack of glucose uptake in the apex (so called match) indicating scar tissue. In contrast, PET revealed significant FDG-uptake in the anterior wall (so called mis-match) indicating hibernating myocardium. Thus, recanalization probably may improve contractility of the anterior wall in this patient.

rest myocardial perfusion study using Tc-99m-MIBI PET allows a visualization of hibernating myocardium, i.e. myocardium which shows markedly diminished perfusion but is still vital. Thus, in assessing myocardial viability PET helps the clinician to choose the appropriate therapeutic approach in the individual patient.

PET in Oncology

During the last years several studies have been published evaluating the impact of PET in oncological diseases. These studies were focused on following main topics:

1. Differentiation of primary tumor tissue or relapse of tumor versus soft tissue or scar.
2. Lymph node staging and identification of distant metastases.
3. Therapy monitoring.

In diagnosis of *malignant melanoma* (Grade II and III) lymph node staging and detection of distant metastases by PET were shown to be appropriate (Ia).

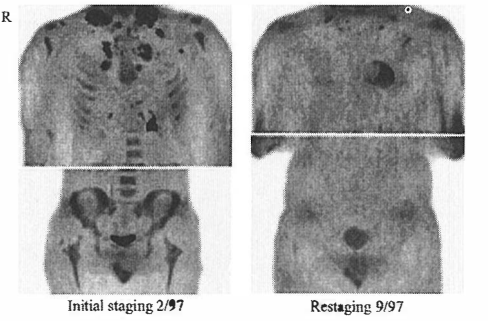

Figure 3. 24-year-old patient with Hodgkin's disease. PET was acceptable (Ib) for primary staging (left) as well as e-s restaging (right). Images are given as maximum intensity projections. Primary staging showed multiple focal accumulations of FDG indicating vital tumor tissue located at both sides of the neck, the mediastinum, the left axillary region, left and right supraclavicular region, behind the sternum, and around the coeliac truncus. Note, bone marrow infiltration as documented by homogenous tracer accumulation of the bone marrow. Initially, according to PET findings Hodgkin's disease was classified as grade IV. Re-staging seven months later showed no evidence of vital tumor tissue documenting complete remission.

In *malignant lymphoma* primary staging (Figure 3, left) and diagnosis of tumor remnants after therapy (Figure 3, right) were assigned as acceptable (Ib). Moreover, PET was classified as "acceptable" (Ib) in re-staging and in detection of relapse of malignant lymphoma.

In *non-seminomateous germ cell tumors* therapy monitoring of non-differentiated teratoma was defined as acceptable (Ib).

Moreover, PET was shown to be helpful (IIa) both in re-staging as well as in lymph node staging of non-seminomateous germ cell tumors.

PET was shown to be helpful (IIa) in several clinical settings in gynecology concerning *ovarian and breast cancer.* In ovarian cancer these included diagnosis of relapse and re-staging. In breast cancer PET was assigned as helpful (IIa) in diagnosis of the primary tumor, exclusion of local relapse, in lymph node staging, in therapy monitoring, and in diagnosis of distant metastases.

In lymph node staging of patients with *cancer of the bladder* a PET study was determined as helpful (IIa). In *pancreatic cancer* functional imaging using PET was assigned as "appropriate" (Ia) in differentiation between tumor tissue and inflammatory tissue. Moreover, PET was defined as acceptable (Ib) for an exclusion of local relapse of pancreatic cancer if these findings provided a therapeutic option.

In *colorectal cancer* PET was defined as appropriate (Ia) for re-staging, i.e. assessment of local relapse, staging of lymph nodes, and detection of distant metastases, in patients who present with unresolved elevated tumor markers or in patients in whom conventional imaging revealed suspicious findings. For therapy monitoring after chemotherapy and after radiotherapy PET was defined acceptable (Ib) and helpful (IIa), respectively.

In *non-small-cell lung cancer* PET was shown appropriate (Ia) in differentiating benign from malignant disease in solitary pulmonary nodules. Moreover, PET was assigned appropriate (Ia) in lymph node staging (Figure 4) as well as in exclusion of a local relapse in these patients. In order to assess treatment response PET was defined as helpful (IIa).

PET was described to be valuable in therapy monitoring of patients with *differentiated thyroid cancer.* In these patients presenting with elevated thyroglobulin levels but negative whole-body I-131 scintigraphy PET was

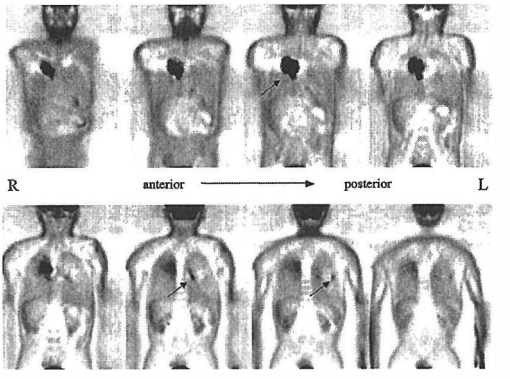

Figure 4. 61-year-old patient with suspected lung cancer of the right superior lobe. PET was appropriate (Ia) in order to determine dignity of the pulmonary mass. Coronal slices show massive tracer accumulation in the right superior lobe indicating vital tumor tissue. In addition, PET showed two pulmonary foci contralaterally (arrows). Thus, curative surgery was abandonned in this patient.

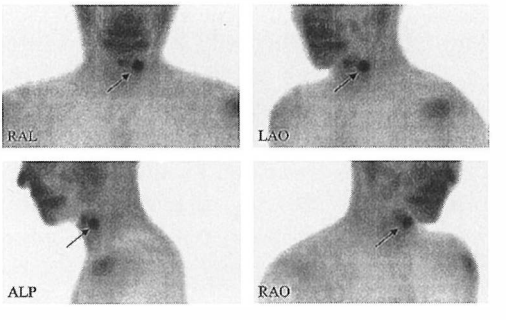

Figure 5. 39-year-old patient with differentiated thyroid cancer after total thyreoidectomy and radioiodine treatment presented with elevated thyroglobulin levels. Since whole-body I-131 scintigraphy was normal PET was appropriate (Ia) in order to detect metastases. Focal FDG uptake in the left paramedian neck corresponding to vital tumor tissue yielded to subsequent surgery.

shown to be appropriate (Ia) for identification of tumor relapse or distant metastases (Figure 5). Moreover, PET was assigned as acceptable (Ib) in patients presenting with positive I-131 whole-body scintigraphy in order to identify additional sites of metastases which may significantly alter treatment regimen.

In patients with *cancer of unknown origin in the head and neck region* PET was defined as appropriate (Ia) if a positive histology of

lymph node metastasis is not accompanied by tumor localization using conventional imaging technique. Moreover, in patients with a potentially resectable primary tumor lymph node staging by PET was assigned as acceptable (Ib).

Potential impact of PET was assigned appropriate (Ia) in patients with *brain tumors* in several clinical settings, i.e. for diagnosis of tumor relapse in patients with high-grade glioma, for the detection of a malignant dedifferentiation, and for localization of the biopsy site in suspected glioma. Furthermore, PET was assigned to be acceptable (Ib) for determination of the biological aggressiveness of a known glioma.

Cost-effectiveness

Most studies dealing with cost-effectiveness of PET studies are based on analysis of the American health system. PET has been shown helpful in cost reduction in certain indications as compared to other diagnostic procedures.[5-21] PET was shown to be both a more sensitive and more cost-effective strategy for differential diagnosis of pulmonary nodules when compared to fine-needle biopsy.[22] Thus, especially in differentiation benign from malignant masses PET helps to avoid more expensive strategies like biopsy or surgery.

Conclusion

PET provides additional information to conventional morphologically orientated imaging in numerous clinical settings. Whereas PET was first used in assessment of neurological and cardiovascular disorders, today, PET plays an increasing role in diagnosis and therapy monitoring in oncology. Due to growing restriction in the reimbursement policy of the national health system clearly defined indication lists have been established in several round table expert meetings. Thus, both performance of PET studies and cost-effectiveness may be improved.

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Assessment of renal function from creatinine clearance measurement and ^{131}I-hippuran renography in cancer patients before chemotherapy

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Background. *Serum creatinine and endogenous creatinine clearance (CrCl) are widely used measures of renal function before prescribing nephrotoxic chemotherapy. This study compares the precision and bias in glomerular filtration rate (GFR) estimation without the need to collect urine by using Cockcroft-Gault formula on a single serum creatinine concentration (CrCo) and ^{131}I-hippuran clearance (HC) determined from the renographic curves.*

Patients and methods. *Fourty-seven patients aged between 27 and 73 years were studied. In all patients, we determined serum creatinine concentration, CrCl, CrCo and HC simultaneously before treatment by combined chemotherapy with cisplatin (CDDP) and in 31 patients, before the third cycle. Serum and urine creatinine concentrations were determined with a Hitachi 911, an automated biochemical analyser. CrCl was calculated from the urine flow, from the ratio between the serum and urine creatinine concentrations and was standardized for the body surface area. Serum creatinine was used to estimate CrCo using a Cockcroft and Gault formula. HC was determined from 131I-hippuran uptake by both kidneys, results were compared to our Nuclear Medicine Department normal values with regard to the age of each patient. For the evaluation of results, Pearson's correlation coefficient and t-test with 95% confidence interval were used.*

Results. *The sensitivity of serum creatinine, CrCo and HC to predict CrCl < 78 mL/min/1.73m^2 was 41%, 68% and 46% and specificity was 95%, 71% and 76% respectively. Value of CoCr for prediction of reduced CrCl (sensitivity) was statistically significantly better than the HC (p=0.03). Value of CoCr for prediction of normal CrCl (specificity) was as good as HC (p=0.3).*

Conclusions. *CrCl for the GFR estimation in the patients treated with nephrotoxic chemotherapy cannot be changed by CrCo and/or HC.*

Key words: glomerular filtration rate, creatinine clearance, renal function; radioisotope renography, iodohippuric acid, ^{131}I-hippuran renography; chemotherapy, cisplatin

Received 18 February 1999
Accepted 3 March 1999

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Introduction

Many chemotherapeutic agents can induce renal failure or a specific renal lesion, e.g. in the glomeruli or tubules. Moreover, alternation in the renal function may lead to

impaired metabolism and accumulation of chemotherapeutic agents and their metabolites and can enhance systemic toxicity and renal failure.[1] Cisplatin (CDDP) plays a central role in the treatment of many solid malignant tumors. In addition to many other side effects, an acute and chronic type of renal injury is a result of proximal and probably distal tubule cell necrosis.[2] The nephrotoxicity of CDDP is dose-related and cumulative; it depends on the level of diuresis and pre-existing alternation in renal function. Therefore there is a necessity for early recognition of renal injury for safe and effective usage of this agent.

The glomerular filtration rate (GFR) provides the best overall measure of renal function. The urinary clearance of exogenous substances, such as ^{51}Cr-EDTA and inulin, are accepted as gold standards for the estimation of GFR. However, because of cost and convenience, serum creatinine and endogenous creatinine clearance (CrCl) are the most widely used measures of renal function.[3,4]

Serum creatinine concentration (CrCo) is of limited value in early detection of renal insufficiency because it is well established that it may be seen within normal limits despite more than 50% reduction in GFR. It does not significantly change until CrCl is less than 70 mL/min/1.73m^2 or the inulin clearance is less than 50 mL/min/1.73 m^2.[4,5] CrCl overestimates true glomerular filtration rate (GFR) because creatinine is not only filtrated by the glomeruli but is also secreted by the tubuli.[5] The contribution of tubular secretion to the total CrCl varies widely over time and is increased in those with glomerular disorders.[6]

The measurement of CrCl is easy. It involves collecting a 24 or 48 hour urine, measuring its volume and creatinine concentration in urine and serum. However, collecting the urine for at least 24 hours often cannot be entirely controlled by trained technicians, it is inconvenient for the patients and frequently results in errors.[3,4] Several publications have demonstrated that due to its susceptibility to error, the 24-hour creatinine clearance correlated worse with GFR than the estimates based on serum creatinine.[3,7-9]

The estimation of GFR from the plasma creatinine, using Cockcroft and Gault formula, avoids the need to collect urine.[10] It provides a better estimate of GFR than the plasma creatinine alone because age, gender and bodyweight as determining factors of muscle mass are taken into account.[11,12] The assesment of renal function by Cockcroft and Gault equation was safely used by many oncologists before administering a low weekly dose of cisplatin.[13]

Renography with radioactive ^{131}I-hippuran provides us with renography curves, which show isotope uptake by the kidneys.[14] The technique is short, simple, fairly harmless for the patient and provides a comparison of the function of the two kidneys. Hippuran is excreted exclusively by the kidneys; 20% is filtrated by the glomeruli and 80% by the renal tubules. It is not reabsorbed into the blood. After the intravenous injection of hippuran marked with radioactive iodine, the g-ray detectors are counting the course of hippuran clearance (HC) by curve-drawing; this record is called renogram. From the shape of the curves, data on excretion disorders, tubule impairment and renal circulation are obtained. Good correlations were noted between different parameters of the averaged renogram curves and kidney function parameters (creatinine clearance,[14] inulin and PAH clearance[15,16]), especially after values are standardised for age of the patient. With comparing calculated vs. age-standard value of hippuran clearance, quantitative value of renal function is available.

In the present study, we investigated how accurately and precisely GFR can be approached using plasma creatinine, Cockcroft formula or renography hippuran clearance estimation in comparison with CrCl. The study group was confined to the

patients with normal and mild to moderate impairment of renal function because accurate information on GFR is particularly important in treatment decision-making in nephrotoxic chemotherapy.

Patients and methods

Patients

Fourty-seven patients with malignant melanoma, gastric cancer and ovarian cancer (16 males and 31 females), aged between 27 and 73 years (mean 55) were studied. Half of the patients had metastatic disease and the other half was without evidence of disease after radical surgical treatment. All except 5 patients with impaired renal function were treated by combined chemotherapy with CDDP as adjuvant or palliative setting. Before treatment, all patients were without clinical evidence of serious internal disease and they didn't receive any diuretics or drugs known to interfere with creatinine secretion. No change in nutrition (meat ingestion), hidration rate or daily physical activity was observed. Their body weight ranged from 46 to 114 kg (mean 73) and their body surface from 1.4 to 2.3 m^2 (mean 1.8).

Study design

In all patients serum creatinine, CrCl and HC before treatment and, in 31 patients, before the third cycle were determined. All together, we obtained 78 measurements. A twenty-four hour urine collection on an inpatient basis was carefully controlled by trained technicians. The patients were instructed to begin the 24-hour urine collection in the morning, discard their first voided urine and then collect all their urine for the next 24-hours. In the morning of the second day when urine collection was finished, blood samples for serum creatinine were taken before breakfast. CrCl and CrCo were calculated using that morning creatinine. On the basis of their CrCl values, normalized for body surface, the measurements were divided into two groups: group A (n=41) with CrCl ≥78 mL/min/1.73m^2, and group B (n=37) with CrCl < 78mL/min/1.73m^2. Renography was performed 2 to 6 hours after the urine collection was completed.

Laboratory methods

Serum and urine creatinine concentrations were determined enzymatically by spectrometric method (reagents Boehringer Mannheim, Germany) with a Hitachi 911, an automated biochemical analyser. CrCl was calculated from the urine flow, from the ratio between the serum and urine creatinine concentrations and was standardized for the body surface area:

$$\text{CrCl} = \frac{[\text{urine creatinine concentration (mmol/L)}] \times [\text{urine flow (ml/min)} \times 1.73\ (\text{m}^2)]}{[\text{serum creatinine concentration (mmol/L)}] \times [\text{body surface area (m}^2)].}$$

In addition, the serum creatinine was used to calculate a Cockcroft and Gault estimate of the CrCl using the formula:

$$\text{CrCo (ml/min)} = \frac{[140 - \text{age (years)}] \times [\text{body weight (kg)}] \times [0.85 \text{ for women}]}{[49] \times [\text{serum creatinine concentration } (\mu\text{mol/L})]}$$

Because of different relative amounts of fat and muscles in women, a 15% reduction of a Cockcroft and Gault is recommended in women.[10]

Radiograpy was done without special preparation of the patient (only good oral hydration), using three detectors (both kidneys and the heart). After bolus injection of 100 mCi of sodium o-iodohippurate-^{131}I a continuous tracing was recorded for 15 minutes. For clearance determination, combined figures for parameters from 5 to 15 minutes were obtained by automatically averaging values from right and left renograms by computer. Renograms were also interpreted by visual

inspection of the images to qualitatively assess renal function and technical adequacy of the test.

Statistical analysis and reference values

In the evaluation of results Pearson correlation coefficient and t-tests with 95% confidence interval were used. The statistical analysis was performed using the program Statistica for Windows, version 4.3, StatSoft Inc., 1993.

Reference values were set according to Slovenian National Board for Clinical Chemistry and Clinical Biochemistry Guidelines. The normal serum concentrations irrespective of sex and age are: serum creatinine 44 do 97 µmol/L, CrCl 1.3 do 2.0 ml/s (78-120 ml/min).

Table 1. Patients characteristics

Characteristic	Mean	Range
Age (years)	55	27-73
Serum creatinine (µmol/L)		
Men (n=16)	91	68-193
Women (n=31)	96	62-367
Body surface area (m^2)	1.8	1.4-2.3
Weight (kg)	73	46-114
Hight (cm)	164	152-194
Endogenous creatinine clearance (ml/min)		
A (≥ 78 (n=41))	96	78-145
B (< 78 (n=37))	53	15-76
Hippuran clearance estimation (ml/min)	498	170-1170
Estimation of endogenous creatinine clearance by Cockcroft and Gault formula (ml/min)	80	21-153

Results

In 41 samples in group A (creatinin clearence ≥78 mL/min/1.73m2), the serum creatinine ranged from 62 to 110 µmol/L (mean 82 µmol/L). In 37 samples in group B (creatinin clearence < 78 mL/min/1.73m2), the serum creatinine ranged from 62 to 367 µmol/L (mean 107 µmol/L). Table 1 shows the characteristics of the 47 study participants.

The comparison of CrCl with the serum concentration of creatinine, the CrCo estimation of CrCl and HC estimation of renal function is shown in Table 2. The serum creatinine was elevated above reference values in 17 samples and CrCo and HC were reduced below reference values in 37 and 27 measurements, respectively. In group B the serum creatinine concentration was within normal range in 22 out of 37 cases. The sensitivity of serum creatinine concentration to predict CrCl < 78 mL/min/1.73m2 was 41% (95% confidence interval (CI) 31-50). The specificity was 95% (95%CI 74-100). The correlation coefficient between CrCl and the serum creatinine concentration was 0.48 (p=0.1).

In group A CrCo was reduced in 12 of 41 measurements and in group B was normal in 12 of 37 measurements. The sensitivity of CrCo to predict CrCl < 78 mL/min/1.73m2 was 68% (95%-CI 52-83) and specificity 71% (95%CI 57-85).

The comparisson of CrCl and HC showed that HC was reduced in 10 of 41 cases in group A and it was within normal range in 20 of 37 cases in group B. The sensitivity of HC for prediction of CrCl < 78 mL/min/1.73m2 was 46% (95%CI 30-62) and specificity 76% (95%CI 62-89).

As shown in Figures 1 and 2, the relationship between the CrCl and CrCo or HC was analyzed according to the expectation of an ideal case where CrCl is equal to GFR. By plotting values of CrCo or HC (y) versus GFR(x) as determined by CrCl, linear regression showed rather weak correlation between CrCl and GFR estimation by CrCo or HC. The correlation between CrCl and CrCo was slightly better (r=0.6) than the correlation between CrCl and HC (r=0.55), but the difference was not significant.

The prediction of CrCl < 78 mL/min/1.73m2

Table 2. Comparison of endogenous creatinine clearance with serum concentration of creatinine, estimation of endogenus creatinine clearance using Cockcroft and Gault formula and estimation of glomerular filtration rate by hippuran clearance in 78 concomitant measurements

	S-creatinine		CrCo		HC	
	normal	elevated	normal	reduced	normal	reduced
No of measurements	61	17	41	37	51	27
CrCl						
A (≥ 78 ml/min)	39	2	29	12	31	10
B (< 78 ml/min)	22	15	12	25	20	17

CrCl - endogenous creatinine clearance, S-creatinine - serum concentration of creatinine, CrCo - estimation of endogenous creatinine clearance by Cockcroft and Gaut formula, HC - ^{131}I-hippuran clearance estimation

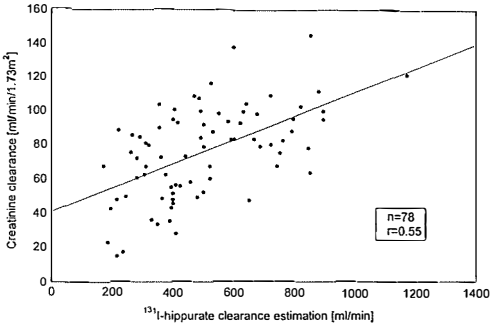

Figure 1. Correlation between creatinine clearance and estimation of ^{131}I-hippurate clearance in 47 patients and 78 simultaneous determinations.

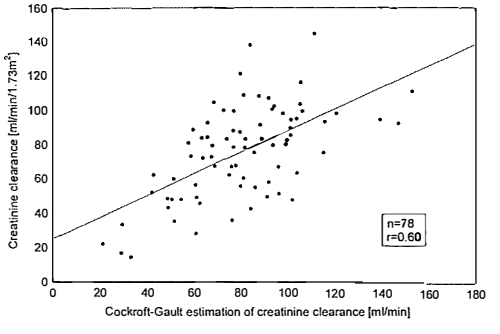

Figure 2. Correlation between creatinine clearance and Cockroft-Gault estimation of creatinine clearance in 47 patients and 78 simultaneous determinations.

(sensitivity) by CrCo was statistically significantly better than HC (p= 0.03) and serum creatinine concentration. There were no differences in the prediction of CrCl ≥78 ml/min/m^2 (specificity) between CrCo and HC (p=0.3).

Discussion

The present study, comprising 47 cancer patients in whom 78 simultaneous measurements of serum creatinine concentration, CrCl, HC and estimation of CrCl using Cockroft and Gault formula, indicates that the serum creatinine concentration, HC and CrCo cannot be used for early detection of renal insufficiency instead of CrCl.

The sensitivity of serum creatinine concentration to estimate CrCl < 78 mL/min /1.73m^2 was 41%. The poor sensitivity of creatinine in detecting CrCl < 78 mL/min /1.73m^2 may be due to a variety of renal and non-renal influences on the creatinine concentration. It is well established that the serum creatinine concentration is influenced by protein intake, the muscle mass, age, gender, race and drugs like cimetidine interfere with tubular secretion of creatinine.[4,9] The coefficient of day-to-day variations in creatinine excretion ranges between 3% to 14%, and may be as high as 70% when 24-hour urine collection errors are not eliminated.[8,9] Part of this variation arises from daily variation of GFR, which is variously reported to

have a coefficient of variation of 11% or even 17%.[8,9]

To account for differences between individuals in creatinine production, creatinine clearance can be obtained. However, estimation of GFR by CrCl is usualy overestimated for 10 to 40% due to errors during the 24-hour urine collection and to the tubular secretion of creatinine.[5] The ratio of CrCl to GFR was almost always greater and increased with decreasing GFR to a maximum of approximately 1.7 at a GFR of approximately 20 ml/min.[9,17] However, several reports have also shown that, in conditions where GFR is moderately impaired or normal and urine collection errors are reduced substantially by technicians who are trained to measure the urine volume and times of voiding, the CrCl is just about equal to GFR with a ratio of CrCl and GFR of approximately 1.15.[7,17,18] In this study, GFR was estimated by CrCl at a high accuracy rate because a majority of patients had mild to moderate impairment in renal function and, in order to minimize error in 24-hour urine collection, all patients were hospitalized and carefully monitored by trained technicians.

The estimation of GFR from the serum creatinine concentration, using Cockcroft and Gault formula usually overestimates GFR.[10] In our study, CrCo overstimated CrCl in 32% cases. This overestimation may be partly explained by day-to-day variability of creatinine metabolism and overweight of majority of study participants. Their mean body weight was 73 kg and mean body mass index was higher than 26 kg/m^2. Among obese patients, serious errors arise in the Cockcroft and Gault equation. It has been therefore suggested that among obese patients the Cockcroft and Gault equation should take account of lean body weight.[19,20] So, the standarization of the equation for body size is important for an appropriate comparison with a measure of GFR that is standarized similarly.[11] Due to the overestimation of GFR and great variability using of Cockcroft and Gault equation was not recomanded in the patients with advanced renal failure. Toto et al[10] found less variability in predicting GFR from the serum concentration of creatinine alone than from Cockcroft and Gault equatation in the subjects with creatinine values ranging from the upper limit of normal to 400 μmol/L. Several studies have shown that, in the patients with normal or a mild to moderate decrease in renal function, despite substantial errors in 24-hour urine collection and variability of creatinine metabolism and GFR, the estimation of CrCl using Cockcroft and Gault formula is less precise for GFR than CrCl.[9,11,17] The sensitivity of CrCo to predict reduced CrCl was 68%. However, the prediction of CrCl < 78mL/min/m^2 by CrCo was significantly better than prediction by serum creatinine concentration.

We found out that the estimation of GFR by HC overestimated CrCl in 54% of cases.

The sensitivity of HC in the estimation of reduced GFR was 46% only and was hardly any better than that of creatinine serum concentration (41%), whereas the specificity of serum creatinine concentration was considerably better. GFR depends upon the renal plasma flow and filtration fraction. In normal conditions, 20% of the blood plasma entering the kidneys is filtrated within the kidney.[20] As HC is an important measure of renal circulation, the GFR estimation can hardly be reliable with regard to plasma flow irrespective to the variability of filtration fractions. Our results are in accordance with the results of Chachati and coworkers,[16] who found that despite fair correlation between uptake of ^{131}I-hippuran clearance estimation and PAH clearance, the variability of HC was too large.

Renography is by far the best method for the early recognition of renal tubular disorders. In patients with early detected intrarenal and extrarenal secretion disorders by radioisotope renography and normal GFR dose modification of CDDP is not required. It

is generally known that mild secretion disorders, though impairing GFR, do not considerably increase the CDDP toxicity. Therefore, this method is as harmless in the patiens with one kidney only as in the patients with both kidneys because CDDP does not induce any serious renal failure at a normal level of CrCl.[1]

To prevent acute and chronic renal injury by CDDP, despite aggressive and careful hydration, the dose of CDDP must be modified according to GFR which provides the best overall measure of renal function. In general, the dose of CDDP in GFR 30 to 60 ml/min has to be lowered by half, whereas at a GFR lower than 10 or 30 ml/min the treatment with CDDP should to be discontinued.[1,2] Our results indicate that the serum creatinine concentration, estimation of CrCl by CrCo and estimation HC cannot equivalently replace CrCl as the best estimation of GFR before chemotherapy.

Conclusions

In patients with normal or moderately reduced renal function, the CrCl is more informative than serum creatinine concentration, CrCo and/or HC. Low sensitivity and specificity of this methods cannot provide a good screening test for early renal failure and cannot substitute the CrCl in the estimation of GFR. The estimation of renal function before chemotherapy by CrCo and HC is therefore unnecessary and inconvenient; moreover, serious damage to patients can be induced by considering their normal values before chemotherapy.

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Radiol Oncol 1999; 33(2): 127-36.

Predictive assays of tumor response to chemo and radiotherapy

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Currently used predictive assays of treatment outcome of tumors treated by chemo- or radiotherapy and radiation damage of normal tissues are presented. This review is focused on the assays where tumor cells derived from the human tumors need to be cultured in in vitro conditions to perform the test. In addition, an overview of the clinical studies dealing with the correlation between predictive assays and treatment outcome or radiation damage to the normal tissues is given.

Key words: neoplasms-drug therapy-radiotherapy; radiation injuries; treatment outcome

Introduction

The best treatment for a particular patient is based on a variety of factors predictive of the outcome of the therapy. In radiotherapy, these factors include tumour- and host- related factors, technical aspects of treatment and knowledge of the dose response relationship for tumour control and normal tissue injury. At present, the treatment plan is usually based on parameters such as tumour site, histology, stage, size, morphology, patterns of invasion of anatomical structures, location with regard to vulnerable normal tissues, and patient's performance status. Within these categories, some tumours show greater response to radiotherapy than others. If these were identified before treatment, alternative therapies offering a better chance of cure than the standard conventional therapy might be selected. Therefore, there is a need for additional (other, more specific) predictive assays, which will provide the information that can be useful in the selection of an optimal treatment protocol for each patient.[1-3]

An ideal predictive assay should,

(a) correlate specifically with local tumour control, independently of other prognostic parameters,
(b) be measurable precisely,
(c) be relatively insensitive to sampling error,
(d) be measurable quickly with regard to the initiation of treatment,
(e) have low probability of falsely predicting resistance to conventional treatment,
(f) be relatively harmless.

The research in the field of predictive assays started almost 30 years ago with the investigation of the relationship between clinical and tissue culture response to chemotherapeutic agents of human cancer.[4] After this first report, a lot of researchers started to

Received 4 January 1999
Accepted 15 February 1999

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The paper was presented at: Seminar on cell cultures. Handling and trends. November 25, 1998, Ljubljana, Slovenia.

work on the development of tests that would predict the response of tumors to a particular treatment. Current research in predictive assays can be divided into three categories;

(a) Studies of intrinsic cellular radio- and chemo- sensitivity;
(b) Detection and quantification of hypoxic cells in human tumours;
(c) Tumour cell proliferation kinetics and ploidy (repopulation).

Intrinsic radio- and chemo-sensitivity

The research in predicting the outcome of treatment started with the development of methods which enable to grow *in vitro* human tumor cells.[3] The described assays can be used for predicting the treatment outcome either after radiotherapy or after treatment with chemotherapuetic drugs. The differences in responses of particular tumors to treatment with drugs are usually larger than to treatment with radiation. Therefore, the use of predictive assays in chemotherapy would be highly beneficial for a particular patient. If the patients with a resistant disease could be identified before the initiation of treatment, the toxicity of ineffective treatment would be spared to them.

Intrinsic radio- and chemo-sensitivity can be measured by survival, growth of cells, DNA damage and chromosome damage after treatment. Only colony forming assay which measures out the cell kill after a particular treatment, is a direct assay. All others tests, which measure either growth, DNA or chromosome damage, are indirect and measure the parameters which should correlate with cell kill.

Survival

The survival of cells is measured by colony forming, i.e. clonogenic assay. This assay is the gold standard for determination of treatment efficiency, since, with this test, the ability of tumor cells to proliferate is measured directly. From the theoretical point of view, the principle of this test is very simple. The cells have to be removed from the tumors, prepared as single cell suspension, placed into appropriate growth environment, and exposed to radiation or drugs. After certain period of time, depending on the growth rate of the tumor cells, the formed colonies are fixed, stained and counted. By comparing the number of colonies in the treated group with the number in the control group, the surviving fraction can be calculated. As a predictor of treatment outcome after radiotherapy, a surviving fraction at 2 Gy is commonly used, as this is a usual daily dose in clinical radiotherapy.[5]

Growth

Since there are several practical problems associated with clonogenic assay, such as that not all human tumors can be grown *in vitro* and long duration of this test, alternative tests that measure the growth of the cells have been developed. The growth of the cells can be measured simply by counting the cells after certain period of time by means of dye-exclusion technique or by means of automated colorimetric assays. One of the examples of these assays is methyl tetrazolium test (MTT test), which estimates cell survival based upon the capacity of living cells to reduce a tetrazolium compound to a formazan crystals, a colored product that can be measured spectrophotometrically.[6,7] The principle of this test is very similar to that of clonogenic assay. Cells are plated in microtiter plates and subjected to treatment. The difference between these tests is at the end of growth period, which is usually shorter in MTT assay than in the clonogenic assay. In the case of MTT assay, a substrate, methyl tetrazolium compound, is added to the cells which are further incubated for approx. 3-4 hours.

The formed formazan crystals are then dissolved in dimethyl sulfoxid and absorbance measured using microplate reader. The results of these assays show very good correlation with the clonogenic assay, therefore these assays represent a promising alternative to the clonogenic assay (Figure 1).[6,7]

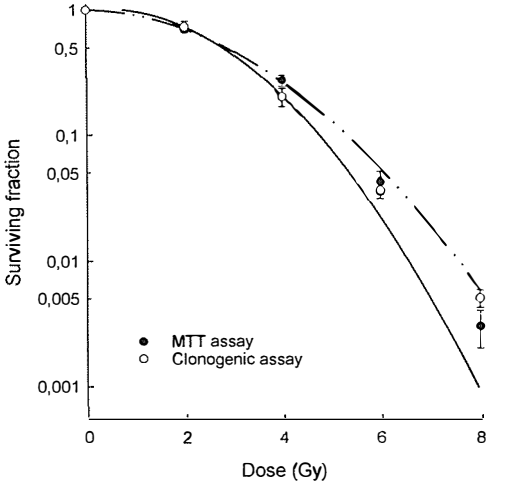

Figure 1. Survival curves for mouse fibrosarcoma cells SA-1 using MTT and clonogenic assay as a function of irradiation dose. Cells were irradiated in Petri dishes using Darpac 230 kV irradiation machine at a dose rate 2 Gy/min. For MTT test cells were transfer to microtiter plates and incubated for 7 days. After that period MTT test was performed. In clonogenic assay, colonies were fixed and stained 10 days after the irradiation (Čemažar unpublished data).

DNA damage

Another method used to determine intrinsic cellular sensitivity is based on measuring DNA damage. To assess DNA damage several test can be employed such as sucrose velocity sedimentation, filter elution, alkaline gel electrophoresis, membrane filtration, DNA precipitation, nucleoid sedimentation, alkaline unwinding, Halo, end tailing, viscoelastic and high performance liquid chromatography (HPLC), pulsed-field gel electrophoresis and single cell electrophoresis. The last two listed tests are the most promising test to be employed as a predictive one.[8-13] However, the predictive value of these two assays has to be confirmed, since the results of several studies are controversial, some showing very strong correlation with the colony forming assay, and others the lack of it.[11-13] The advantage of pulsed field gel electrophoresis is high sensitivity and specificity for measurement of DNA double strand breaks. The principle of pulsed field gel electrophoresis is as follows: the cells that have been taken from human tumor and treated are either radiolabelled before lysis and electrophoresis or stained with ethidium bromide after the electrophoresis. The advantage of pulsed field electrophoresis over the conventional one is that, by alternation of electric field the separation of DNA fragments is improved. This technique is therefore especially suitable for separation of large DNA fragments up to 12Mbp. If the DNA molecule of a known molecular weight is used as calibration, the separation of DNA from irradiated cells can be subsequently translated into a measure of strand breaks.[9-13]

Single cell electrophoresis (comet assay) is also widely studied for potential use as a predictive assay. The advantage of this assay is that we can monitor the response of a single cell to treatment and thus the problem of tumor or normal cell specificity may be overcome. The basis of this test is first to embed the cells into low-density agarose gel on a microscope slide. Then, the cells are lysed and subjected to electrophoresis. The broken DNA molecules migrate away from the general mass of DNA towards to anode and produce a typical feature which is called "comet". Variations in lysis conditions allow us to detect single and double DNA strand breaks, cross links and base damage.[8,12,13]

Chromosome damage

One of the most obvious effects of radiation is chromosome damage. It has been demonstrated that certain chromosome changes

such as deletion of substantial part of chromosome lead to cell death. Therefore, the measurements of chromosome damage are another possible approach to measure intrinsic cellular sensitivity. The conventional technique to asses chromosome damage is the preparation of the karyotype of cells that have been exposed to radiation in *ex vivo* conditions and count the aberrations.[14] Chromosomes are conventionally examined during metaphase. When chromosomal samples (karyotype) are prepared the colhicine or related agents that disrupt the formation of mitotic spindle fibres are added to arrest the cells in metaphase. The cells are then further exposed to hypotonic solution, fixed, placed on microscope slide and stained.

Another test used for the measurement of chromosome damage is micronucleus assay.[15-19] Micronuclei arise from acentric chromatide or chromosome fragments induced by drugs or irradiation. In diploid cells, the presence of micronuclei signals cell death. The basis of this test is, first, to culture the cells after their exposure to drugs or radiation in the presence of cytochalasine B, the drug which in appropriate concentration allow karyokinesis, but inhibits cytokinesis. After that, the cells are fixed on the microscope slide and stained. Micronuclei can be counted by means of microscope (Figure 2). Some studies have shown very good correlation of the micronucleus assay with the cell kill measured by colony forming assay, and some have not.[15,20] Therefore, the use of this assay as a possible predictor of tumor response have to be validated in further *in vitro* studies and also correlated with treatment outcome in clinical studies.

The newer techniques employed for measuring chromosome damage are premature chromosome condensation and fluorescence in situ hybridization (FISH technique).[14, 21-23] When the interphase cell is fused with a cell in mitosis, it undergoes a process of premature chromosome condensation in which chromosomes become visible. The mitotic cell can be of different type and its chromatin can be labelled with BrUdR so that, in binucleated fusion product, it is possible to identify the chromosome of target cell. The advantage of this technique is that it is very quick. It enables the scoring of breaks in chromatin within 10-15 minutes after irradiation and also the speed of their rejoining.[21, 22]

The analysis of chromosome damage has been greatly facilitated by the development of specific probes (chromosome-specific lengths of DNA) that can be used in FISH. In this technique the chromosomes of target cell are fixed on microscope slide after exposure of cells to irradiation and heated to the level that much of their DNA becomes single stranded and incubated in the presence of labelled probes. The probes bind to the regions of chromosome DNA with which they are homologous. The bound probe is then detected with a fluorescent ligand which binds to the probe and which can be seen under fluorescence microscope.[14,21-23] FISH technique has the following advantages over other techniques of measuring chromosomal damage: it is highly sensitive and requires small samples of tissue.

Gene expression after drug or radiation treatment

Cell death after therapy occurs by at least three mechanisms: apoptosis, necrosis and reproductive cell death. There are numerous genes that are associated with the cell response to agents and radiation. The development of techniques in molecular biology, which enables rapid assessment of gene expression and mutation, have stimulated an increasing number of reports dealing with correlation of molecular parameters with treatment outcome and prognosis.[24-26] The screening of mutations in genes that are involved in radio and chemo resistance, cell

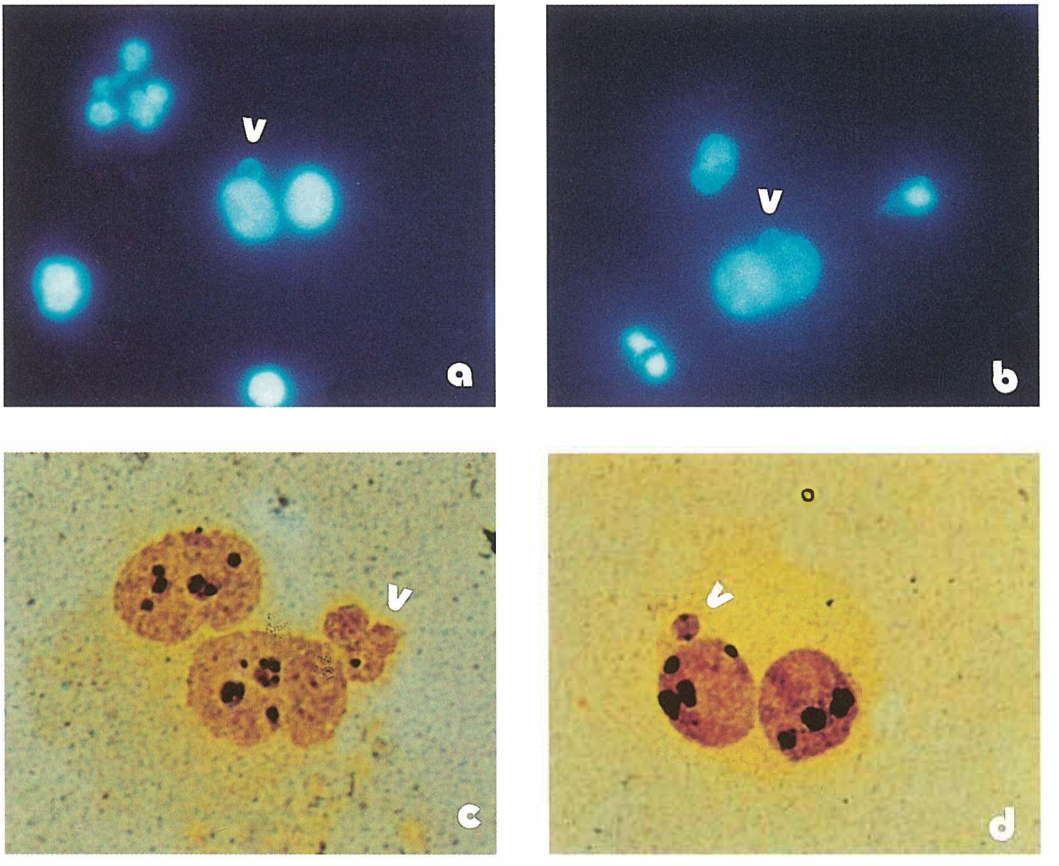

Figure 2. Presence of micronuclei in binucleated lymphocytes taken from nurses, occupationally exposed to chemotherapeutic drugs.(a) signal-positive micronucleus stained with DAPI, (b) signal-negative micronucleus stained with DAPI, (c) cell with two Ag-NOR- and one Ag-NOR+ micronuclei after silver staining, (d) Ag-NOR+ micronucleus with two signals after silver staining. From Garaj-Vrhovac *et al.* (1998) with permission.[19]

proliferation and repopulation, inflammatory response (growth factors), vascular damage, together with the cell cycle checkpoint and apoptosis genes could be, in future, one of the possibility to help predicting the sensitivity of human tumours to treatment with drugs or radiation therapy and also for predicting the radiation induced damage to normal tissues.[24] At present, at least five genes influencing radiation sensitivity were cloned, *viz. Ku70, Ku80, SCID, XRCC4 and ATM* gene.[27] In the area of chemotherapy, expression of multidrug resistance mdr genes can be measured and correlated with treatment outcome.[28]

Hypoxia

The evidence that tumor hypoxia can reduce the efficiency of radiotherapy was provided a long time ago.[29] The identification of hypoxic tumors before the initiation of radiotherapy is now feasible with new quick and reliable techniques.[1,30-34] The methods to detect hypoxia in human tumours are the following;

- Polarographic measurements using oxygen electrodes;
- Autoradiographic detection of radiolabelled misonidazole or its analogues which selectively bind to hypoxic cells;
- The detection of fluorine-labelled misonidazole or its derivates using positron-emission tomography;
- The detection of ^{123}I-labelled nitroimidazoles by external scanning;
- Immunohistochemistry or detection by flow cytometry of nitroimidazole compounds;
- Non-invasive determination of oxygen distribution in tumours using magnetic resonance techniques;
- Invasive determination of oxygen using a flurophore-tipped optical fibre (0.2 mm diameter) where the change in fluorescence lifetime, resulting from quenching of the fluorescence by oxygen, is measured in real time.

Tumor cell kinetics

The third category in predictive assays, that is subjected to extensive research, is tumor cell repopulation. The evidence for the importance of repopulation during radiotherapy has been obtained by the analysis of clinical data and measurements of the kinetics of cell proliferation in human tumour biopsies. Tumor cell proliferation can be measured by the use of radioactive precursors of DNA such as tritiated thymidine (^{3}HTdR) and autoradiographic detection of radiolabelled ^{3}HTdR in cells or tissue sections, by measuring total DNA content and iodo- or bromodeoxyuridine (IUdR or BrUdR) uptake using flow cytometry, or by detection of BrUdR-labelled cells on frozen sections or slides.[35-37] In addition, proliferation status of tumors can be detected by staining tumor section with proliferation dependent antibodies, such as Ki67 (proliferation associated protein) and proliferating cell nuclear antigen (PCNA).[38,39] When the cell proliferation is measured by flow cytometry, labelling index (LI; proportion of cells within S phase) and potential doubling time (T_{pot}; doubling time of clonogenic cells in the assumed absence of cell loss) can be determined.[37]

Correlation with treatment outcome

In general, the results of predictive assays showed a good correlation with treatment outcomes in different types of tumors. In the case of intrinsic radiosensitivity, the survival at 2 Gy (SF_2) of carcinoma of the cervix correlated with both, local tumor control and survival of patients after treatment with radiotherapy.[40] However, for head and neck squamous cells carcinoma, this correlation was not demonstrated.[41-43] Correlation of *in vitro* drug sensitivity testing with response to chemotherapy showed that, in the case of small and non-small cell lung cancer, there was no correlation.[44] On the other hand, strong correlation of ^{3}H-uridine uptake assay and clinical response in patients with metastatic breast cancer was noted.[45] In a study of Klumper *et al.*, MTT assay was used to assess the chemosensitivity in childhood acute non-lymphoblastic leukemia. Among the drugs tested, the only failures of chemotherapy in these patients were found to be due to the resistance to cytosine arabinoside.[46] The role of micronucleus assay in predicting response to radiotherapy was demonstrated on 11 tumors of different origin. The tumors that produced more micronuclei after irradiation of cells *in vitro* showed better response to radiotherapy.[18]

In cervical cancer, it has been demonstrated by several authors that oxygenation of tumors can predict radiation response and survival of patients.[47,48] In a study of Fylers *et al.*, it was shown that pO_2 reading below 5 mmHg as well as tumor size are significant prognostic factor in an univariate analysis of disease-free survival of patients with cervical

cancer.[48] In head and neck tumors, the differences in pO_2 measurements were observed between tumors and, for the majority of 35 tumors included in the study, the values of pO_2 were lower than that of normal tissue. However, there was no correlation with the treatment outcome, probably due to the limited number of patients included in that study.[41]

The T_{pot} as a predictor of tumor response to therapy did not prove its usefulness. In a study of Begg *et al.* as well in the study of Eshwege *et al.*, it was demonstrated that T_{pot} did not predict the treatment outcome of patients with head and neck carcinoma. However, LI showed to be more promising as a predictor of tumor response in head and neck tumors.[41,49-52] Proliferation marker Ki67 showed to be associated with recurrent disease and PCNA with prediction of survival in patients with laryngeal cancer.[38]

Radiation induced damage to normal tissues

Besides measurement of intrinsic radiosensitivity of tumor cells, several studies were also dealing with the response of normal tissue to radiotherapy and its use as a predictor of normal tissue complications after radiotherapy. Currently, the doses used in a conventional treatment are determined primarily by the most sensitive patients. Therefore, if a predictive assay that would recognise sensitive patients prior to the treatment could be developed, the doses given to those patients could be reduced and, consequently, the risk of severe complications could also be reduced. On the other hand, the doses given to more resistant patients could be increased to achieve an improved tumor control. It has been already recognised that at identical treatment regimens, the reactions of the normal tissues to treatment are more severe in some patients than in others. This is not due only to the interpatient difference in tissue physiology and biology or genetically based difference in radiosensitivity, but also to the physical parameters, such as dosimetry (differences in the actual radiation dose delivered to the target cells of the normal tissue), treatment volume (irradiation volume of normal tissue vary with tumour size) and Poisson statistics (critical levels of "Tissue rescuing unit").[37,53]

To predict the susceptibility to radiation damage, the same predictive assays as for the treatment outcome can be applied. Most of the current studies involve the measurements of colony formation, chromosome damage, counting of micronuclei, measurement of differentiation and DNA damage in fibroblasts or lymphocytes. Some studies indicate that a significant therapeutic gain could be achieved for a subset of patients from the use of the predictive assay of normal tissue radiosensitivity.[15, 54-59] However, further validations of these results are needed on larger groups of patients.

Conclusion

Despite numerous predictive assays available at the time, their use has neither been widely accepted nor integrated into at least some aspects of the care of patients with cancer. There are several problems associated with predictive assays: not all patients' tumours can be grown *in vitro*, quality control, mimicking the in vivo pharmacokinetics of drugs in *in vitro* cell cultures, and long duration of clonogenic assay. Most of the problems have been solved with new *in vitro* predictive assays such as FISH assay for intrinsic cellular sensitivity or use of compounds that binds selectively to hypoxic cells, which will probably lead into clinical practice, hopefully in near future.

Acknowledgement

This work was supported by the Ministry of Science and Technology of the Republic of Slovenia

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Biochemical failure of surgical stage T3N0 prostate carcinoma with or without adjuvant radiotherapy

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Background. *Patients with extracapsular extension or seminal vesicle involvement of prostate adenocarcinoma are known to have a worse prognosis than patients without these adverse features. Multiple studies have assessed the impact of adjuvant postoperative radiotherapy on clinical outcome, but there are fewer studies examining the effect on biochemical (prostate specific antigen or PSA) failure.*

Methods. *This is a retrospective analysis of 100 patients found to have prostate adenocarcinoma extending through the prostatic capsule or involving the seminal vesicles (stage T3) after prostatectomy. Thirty-one patients received adjuvant radiotherapy to the prostatic bed and 69 patients did not receive radiotherapy. Prognostic factors were not evenly distrubuted between the two groups. Mean follow-up was 60 months.*

Results. *Actuarial freedom from PSA failure at 5 and 10 years was 64% and 31%, respectively, in the group that received radiotherapy. For the non-irradiated group, the results for the same endpoint were 55% and 30% at 5 and 10 years (p=.76). The only endpoint analyzed which was significantly improved with adjuvant radiotherapy was clinical local control, which was 95% at 10 years for the radiotherapy group and 65% at 10 years for the non-irradiated group (p=.03). Among patients who received radiotherapy, biochemical failure was similar when comparing patients with or without seminal vesicle involvement. Potency in patients undergoing nerve sparing prostatectomy was not affected by postoperative radiotherapy.*

Conclusions. *Adjuvant radiotherapy after prostatectomy in patients with stage T3 disease significantly reduced the clinical local failure rate, but did not improve the biochemical failure rate or overall survival. The benefit of adjuvant radiotherapy should be tested in clinical trials.*

Key words: prostatectomy; prostatic neoplasms- radiotherapy oncology; prostate-specific antigen; neoplasm staging

Received 3 August 1998
Accepted 8 January 1999

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Introduction

Following radical prostatectomy for prostate cancer, one-third to two-thirds of men are found to have disease extending through the prostatic capsule or into the seminal vesicles (stage T3).[1] Clinical failure rates are increased

in these patients compared to those with disease confined to the gland. Radiation therapy (RT) is frequently used as adjuvant therapy in this situation, but its efficacy is controversial. Multiple retrospective studies have examined the effects of adjuvant radiotherapy on clinical failure rates, with most showing improved local control.[2-6] However, most have shown no impact on overall survival. Due to the long natural history of prostatic carcinoma and the high incidence of death from intercurrent disease, it is often difficult to assess the efficacy of treatment. With the advent of prostate specific antigen (PSA) testing, the outcome of treatment modalities in prostate cancer can be evaluated with shorter follow-up and with less influence of intercurrent disease. We present a retrospective case-control analysis of the efficacy of adjuvant radiation therapy in pathologic stage T3 prostatic carcinoma, with special emphasis on biochemical (PSA) outcome.

Materials and methods

Patients

Between January 1, 1974 and January 1 1993, a total of 659 patients underwent radical retropubic prostatectomy for clinically localized prostate cancer by the authors. Surgical techniques have been described previously.[7] Of these, 184 (28%) were pathologic stage T3. Thirty-four of these patients had involvement of pelvic lymph nodes upon pathologic review and are excluded from analysis. Also, fifty patients were excluded who had hormonal therapy before RT or who have less than one year follow-up. The remaining 100 patients with more than 12 months of follow-up and PSA testing available make up the patients included in this study. Sixty-nine patients received no adjuvant treatment. Thirty-one patients received adjuvant radiotherapy to the prostatic bed within 6 months of surgery. Patients were referred for RT according to physician preference. Mean dose was 5700 centigray (cGy) using a four field technique with a megavoltage linear accelerator (6-15 MV). Doses were given in 180-200 cGy fractions, five days a week. No patient received hormonal therapy before clinical failure. Mean follow-up was 60 months for the entire group, with a range of 12 to 168 months. See Table 1 for patient characteristics according to treatment group. Note that more patients in the radiotherapy group had positive margins or seminal vesicle involvement.

Table 1. Patients characteristics

	RT	No RT
Number of patients	#1	69
Mean age	66	65
+ seminal vesicles (%)	21(32)	10(15)
+ margin (%)	26(84)	25(36)
Gleason score >6(%)	11(35)	23(33)

Follow-up

Patients were followed every three months for the first year and then every six months for two more years, then annually. Serum PSA was obtained at every follow-up visit after it became available and was determined by the Hybritech assay. Clinical failure was defined as either local, with recurrent disease noted on rectal examination or computed tomography, and/or distal, with painful metastases confirmed by bone scan. Biochemical (PSA) failure was defined as a persistent PSA above 0.5 ng/ml at least 12 months after the completion of radiotherapy. Prostatic biopsies and bone scans were not routinely performed unless clinical signs of failure were present. Therefore, clinical failure rates could be underestimated. Patients provided subjective information regarding urinary continence and sexual potency, but no objective testing of these functions was performed.

Statistics

Data were analyzed by actuarial analysis using the product-limit method,[8] and subgroups were compared using the Cox-Mantel method.[9] All follow-up times were calculated from the date of surgery. Patients dying with no evidence of disease were censored from analysis of local control, PSA failure, and clinical failure, but not overall survival.

Results

Patients in the radiotherapy group had a significantly improved local control rate, 95% at 5 and 10 years, compared to the non-irradiated group, which had 76% and 65% local control at 5 and 10 years as seen in Figure 1 (p=.03). When analyzing local and distant clinical failure rates, the radiotherapy group had a better outcome (84% and 62% vs. 62% and 42% at 5 and 10 years), but the results were not statistically significant (p=.15). Overall survival was also not significantly different between the two groups, with the 10 year survival rates being 64% in the radiotherapy group and 47% in the non-irradiated group (p=.83). Freedom from biochemical (PSA) failure is shown in Figure 2. Again, no difference is noted between the radiotherapy patients, with PSA control of 64% and 31% at 5 and 10 years, and the non-irradiated patients, with 55% and 30% PSA control at 5 and 10 years (p=.76). See Table 2 for a summary of actuarial results.

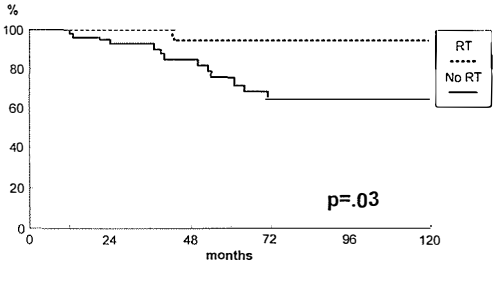

Figure 1. Local control in pathologic stage T3 prostate cancer with or without adjuvant radiation therapy (RT).

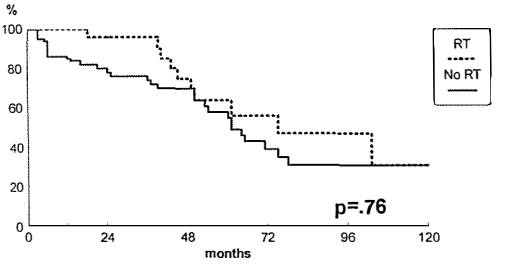

Figure 2. Freedom from biochemical (PSA) failure in patients with pathologic stage T3 prostate cancer with or without adjuvant radiation therapy (RT).

Seminal vesicle involvment did not predict for increased PSA failure in the radiotherapy group (Figure 3). In the non-irradiated group, there were only 10 patients with seminal vesicle involvement, but they did not have a significantly worse outcome, compared to those patients without seminal vesicle involvement. Margin status also was not a significant factor

Table 2. Actuarial results

	Local contol (%)*	Local +/- distal control (%)	Overall survival (%)	PSA control (%)
RT				
5 yrs	95	84	92	64
10 yrs	95	62	64	31
No RT				
5 yrs	76	62	97	55
10 yrs	65	42	47	30

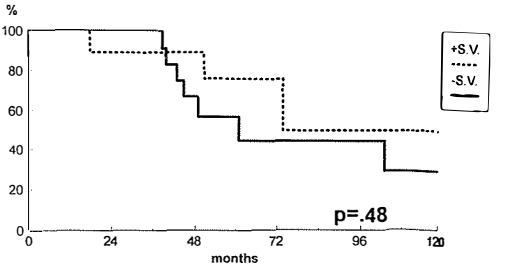

Figure 3. Freedom from biochemical (PSA) failure in patients with pathologic stage T3 prostate cancer receiving radiation therapy according to seminal vesicle (SV) involvement.

in the radiotherapy group, but the number of negative margin patients was small.

Complications were acceptable, with both groups having three patients with urethral stricture requiring dilatation. Significant long-term incontinence occurred in less than five percent of patients in both groups. Five of nine patients (55%) who had unilateral or bilateral nerve sparing prostatectomy in the radiotherapy group are able to obtain erections significant for intercourse. This is similar to the non-irradiated patietns, with 11 of 21 (52%) maintaining potency. There were no treatment related deaths. More detailed surgical complications have previously been reported.[7]

Discussion

Due to the inaccuracy of clinical staging techniques for prostate cancer, we are frequently faced with the dilemma of how to manage patients found to have extracapsular extension or seminal vesicle involvement after prostatectomy. Multiple retrospective studies have assessed the impact of adjuvant radiotherapy on the clinical outcome of these patients.[2-6] Most show improvement in local control rates with adjuvant radiotherapy to close to 100% compared to 75-85% without radiotherapy. However, these studies have shown no significant improval in survival. Due to the long natural history of prostate cancer and the high rate of death from intercurrent disease, it is difficult to assess the efficacy of treatment modalities with respect to survival. With the introduction of PSA testing, outcome of treatment can be determined with shorter follow-up and with less effect of intercurrent disease. This is one of the few reports analyzing the PSA failure rates after postprostatectomy radiotherapy for stage T3 disease. We found a statistically signifcant difference in local control in the two groups, but not in biochemical failure. Overall survival and clinical disease free survival had 17% and 20% differences, respectively, between the two groups, but these differences were not statistically significant due to small patient numbers and short follow-up. The increased percentage of patients with poor prognostic factors in the radiotherapy group, such as positive margins and positive seminal vesicles, could have also biased the results in favor of the no RT arm.

Stein *et al.*[10] at UCLA reported on twenty-four patients who received adjuvant radiotherapy for pathologic stage T3 disease found at prostatectomy and 91 patients who had no further treatment. This study found a statistically significant difference in freedom from PSA failure, with 75% in the radiotherapy group free of PSA failure at 5 years versus 43% without PSA failure in the non-irradiated patients ($p<.043$). Median follow-up was 43 months. In this study, the radiotherapy group had more patients with positive seminal vesicles, but fewer patients with capsular invasion or positive margins. In our study, all poor prognostic factors were higher in the radiotherapy group and this could have contributed to the fact that no improvement in freedom from PSA failure was seen.

Freeman *et al.*[11] found a freedom from PSA failure of 66% at 5 years in 95 patients with pathologic stage T3 disease after postoperative radiotherapy. Zietman *et al.*[12] had similar

results of 64% PSA control at 5 years in sixty-eight patients with similar surgical findings. Both of these studies found that failure rates were higher in patients with seminal vesicle involvement. Our study showed no difference in PSA failure based on seminal vesicle involvement, but a larger number of patients may be necessary to demonstrate this difference. Jacobson *et al.*[3] found that clinical local control improved from 70% to 100% if postoperative radiotherapy was added to patients with seminal vesicle involvement.

A few studies have examined the freedom from PSA failure in patients undergoing prostatectomy without adjuvant therapy. Robinow *et al.*[13] reported a freedom from PSA failure of only 37% at 3 years in forty-one patients with pathologic stage T3 disease. They noted an increased failure rate in patients with positive margins, whose PSA control was only 34%, compared to patients with only capsular invasion or seminal vesicle involvement. Frazier *et al.*[14] noted a 51% freedom from PSA failure in 124 patients with pathologic stage T3 disease, but the data were not analyzed actuarially, so the true PSA failure rate may be higher. Comparing these data with those of studies using adjuvant radiotherapy show a trend toward improved biochemical control with postoperative radiotherapy, at least at 5 years. However, follow-up is short in most of these studies and longer follow-up is needed to determine if this represents a true improvement in survival or just a delay in progression of disease. As seen in our study, the difference in biochemical failure seen at 5 years was no longer seen at 10 years. Nevertheless, deferring the morbidity of relapse seems worthwhile, especially since it has been shown that adjuvant radiotherapy is much more effective when there is only microscopic disease present, as opposed to after clinical recurrence.[15] A prospective intergroup study is currently open and randomizes patients with stage T3 disease to adjuvant radiotherapy or no further therapy.[16] This study should provide important information on this controversial topic.

Since some patients with pathologic stage T3 prostate cancer do not fail without adjuvant therapy, there has been interest in treating patients with radiotherapy only if the PSA is detectable postoperatively or increases after being undetectable postoperatively.[17,18] McCarthy *et al.*[18] reported that patients who were treated with adjuvant radiotherapy for delayed PSA elevation had a PSA control rate (68%) as high as those treated immediately postoperatively (67%). Patients with persistently detectable PSA levels after prostatectomy had a significantly lower biochemical control rate, with only 33% having a persistent undetectable PSA (p=.0008). These results suggest that adjuvant radiotherapy may be delayed until PSA levels rise in patients with undetectable PSA after prostatectomy, but longer follow-up is needed.

Early analysis of complications in our study shows no significant difference in the radiotherapy and non-irradiated groups. This has been shown in other studies.[2-6] Freeman *et al.*[11] analyzed morbidity extensively in their adjuvant radiotherapy study and compared the findings to the morbidity of patients they treated with prostatectomy alone. Eighty-eight percent of patients in both groups had urinary control or only mild stress incontinence. Urinary stricture rates were also similar in the two groups. However, in the postoperative radiotherapy group, only 18 percent of patients retained potency sufficient for intercourse after unilateral or bilateral nerve sparing prostatectomy, compared to a 46% potency rate for patients not receiving radiotherapy. In our study, postoperative radiotherapy did not decrease potency.

In conclusion, adjuvant radiation therapy for pathologic stage T3 prostate cancer significantly improves clinical local control rates in this and other studies. Early PSA follow-up demonstrates a trend toward improved biochemical control rates with adjuvant radio-

therapy, but this may not persist with longer follow-up. A prospective, randomized trial is currently investigating this clinical dilemma, and should provide valuable information. The optimal timing of radiation therapy has yet to be determined, as it may be possible to treat patients with equal success only when PSA rises after it has been undetectable postoperatively. Complications of adjuvant radiotherapy are acceptable.

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Cathepsin H in squamous cell carcinoma of the head and neck

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Purpose. *To estimate a prognostic value of cathepsin H (CH) in squamous cell carcinoma of the head and neck (SCCHN).*

Materials and methods. *CH concentration was measured using a quantitative immunosorbent assay (ELISA; KRKA d.d., Novo mesto, Slovenia) in serum samples from 35 patients, obtained at surgery (Serum no.1) and 7-407 days (median, 55 days) after therapy (Serum no.2). As control, CH concentration as measured in sera from 30 healthy volunteers was used from the study of Kos et al. (Clin Cancer Res, 1997). The prognostic significance of serum concentration of CH was compared with that of its tissue concentration from the study of Budihna et al. (Biol Chem Hoppe-Seyler, 1996), and for this purpose the follow-up of patients from the latter report was updated.*

Results. *A significantly elevated concentration of CH was measured in Serum no.1 as compared to Serum no.2. (8.9 vs. 8.0 ng/mls, P=0.04) or the sera from healthy volunteers (8.9 vs. 4.9 ng/mls, P<0.0001). The CH concentration in Serum no.1 appeared to be grade dependant (G_{1+2} vs. G_3, 9.1 vs. 5.0 ng/mls, P=0.06); no correlation was observed with other established prognostic factors or the presence of subsequent recurrence/dissemination of the disease. The time of Serum no.2 collection did not influence the CH concentration in these samples. There was a trend towards a better prognosis with increasing levels of CH in Serum no.1 in both the analysis of disease-free survival (DFS) and disease-specific survival (DSS). The maximal differences in survival rates between patients with low and high CH levels were calculated at cut-off concentration 10.7 ng/mls (DFS: 60 vs. 89%, P=0.13; DSS: 65 vs. 86%, P=0.25). The results of tissue concentration of CH were equivocal, with the maximal difference between low and high CH groups at cut-off concentration 720 ng/mgp (DFS: 14 vs. 48%, P=0.04; DSS: 23 vs. 60%, P=0.27).*

Conclusions. *Our results provide indirect evidence for the specific role of CH in the processes of invasion and metastasis in SCCHN. Besides, its serum and particularly tissue concentration might also be of prognostic value in this particular type of cancer.*

Key words: cathepsins, cathepsin H; head and neck neoplasm, carcinoma, squamous cell; prognosis

Received 11 February 1999
Accepted 12 March 1999

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Introduction

Cathepsin H (CH) is an ubiquitous lysosomal enzyme and belongs to the class of cysteine proteinases, such as cathepsins B (CB) , L (CL) and S. In the cell, it is involved in the processes of intracellular protein turnover and post-translation processing of some biologically important protein precursors.[1] The proteolytic activity of CH is regulated by the endogenous protein inhibitors of cystatin superfamily, i.e. stefins, cystatins and kininogens.[2]

Biochemically, CH is a glycoprotein. Mature enzyme molecule of human kidney consists of 220 amino acid residues,[3] and is found as a single chain form of 28 kDa or two chain form of 24 kDa (heavy chain) and 4 kDa (light chain).[4] Besides endopeptidase activity, it possesses also aminopeptidase activity, which is a unique feature when compared with other lysosomal cathepsins.[5] For optimal activity, CH requires acid pH, and is unstable at neutral or alkaline pH values.[1]

In normal cells, CH molecules are stored in lysosomes, but under certain conditions they can be secreted from the cell, mainly in the inactive precursor form. Their activation could result in uncontrolled extracellular proteolysis, which is implicated in pathogenesis of various diseases, also of cancer.[6] It has been demonstrated for CH to participate actively in the degradation of extracellular matrix components,[7] which is believed to be a crucial step in local invasion and metastatic spread of tumour cells.[6] Increased protein and/or activity levels of the enzyme have been determined in tumour tissue and sera of patients with breast cancer,[8] glioma[9] and malignant melanoma,[10,11] compared to adjacent normal tissue or sera of healthy controls. The opposite relation has been established in squamous cell carcinoma of the head and neck (SCCHN).[12,13] In a clinical study on melanoma, the serum concentration of CH was shown to be related with the survival probability,[11] whereas in the head and neck cancer, no statistically significant difference but only a trend was observed between groups with low and high concentration of the enzyme in tumour tissue.[13]

In present study, we examined the levels of CH in sera from patients with SCCHN (Group 1) using a quantitative immunosorbent assay (ELISA). In an attempt to ensure more reliable evaluation of the results, the follow-up of the patients from our previous study (Group 2),[13] in which the survival significance of enzyme concentration in tumour tissue had been investigated, was updated, and the results presented here.

Patients and methods

Patients from both groups were treated with curative intent at the University Department of Otorhinolaryngology and Cervicofacial Surgery, and at the Institute of Oncology, Ljubljana, Slovenia. The study protocol was approved by the Medical Ethics Committee at the Ministry of Health of the Republic of Slovenia, and all included patients gave their consent to voluntary participation in the study. The concentration of CH was measured at the Departments of Biochemistry of the Institute of Oncology (serum samples, Group 1) and of the Jožef Stefan Institute (tissue samples, Group 2), Ljubljana, Slovenia.

Patients

Group 1.

Between November 1995 and December 1996, 35 patients (two females, 33 males) with primary SCCHN entered the study. Median age of the patients was 58 years, range 37-71 years. They were primarily operated on and 30 of them were postoperatively irradiated because of an advanced stage of the disease, residual growth after surgery, extranodal spread of the tumour or the presence of neoplastic emboli in the lymphatic vessels.

Tumours were staged according to UICC TNM classification,[14] and histopathological grade was defined according to WHO criteria.[15] The clinical and histopathological tumour characteristics are listed in Table 1.

All the 35 patients were eligible for the analysis of disease-free survival (DFS) and disease-specific survival (DSS), which were defined as the time intervals between the date of surgery and the date of disease recurrence/dissemination (DFS), or disease-related death or the last follow up (DSS). In 11 patients, the recurrent disease and/or distant dissemination were diagnosed during follow-up period, and 9/11 died of disease-related causes. Four patients died due to causes unrelated to the treated malignant disease and were censored at the last follow-up in the analysis of DSS. Median follow-up of those alive at the last follow-up examination was 28 months, range 23-40 months.

Group 2.

Cathepsin H concentration was determined in 21/45 male patients, aged 38 to 66 years (median, 53 years), with primary SCCHN, who were included in the study between June 1992 and August 1993.[13] Their tumour parameters are shown in Table 1. Seventeen patients were primarily operated on and 16 of them were postoperatively irradiated for the same indications as those in Group 1. Four patients received irradiation treatment alone. For more detailed information on the patients and therapy see Budihna *et al.*[13]

Eighteen patients were included in the analysis of DFS and DSS; three patients were lost to follow-up. In 11 patients, the disease recurrence/dissemination was diagnosed. The disease was the cause of death in nine patients and the disease-unrelated causes in four patients. Median follow-up of patients alive was 69 months, range 64-75 months.

Sampling and biochemical assay of cathepsin H

Group 1.

Five-ml samples of venous blood were collected on the day of surgery (Serum no.1) and 7-407 days (median, 55 days) after therapy (Serum no.2). Blood sampling was co-ordinated with the routine blood collection for preoperative and control laboratory tests. Thirty min. after withdrawal, blood was centrifuged at 1000xg/10 min. The serum was stored at -70°C until analysed.

Human CH concentration was analysed using a specific enzyme-linked immunoassay (sandwich ELISA; KRKA d.d., Novo mesto, Slovenia), developed at the Jožef Stefan Institute, Ljubljana, Slovenia.[16] Human liver CH was isolated and characterised as described,[4] and was used for immunisation of animals and as a standard for assay calibration curve. Utilising sheep polyclonal antibody for capture, and murine 2E3 monoclonal horseradish peroxidase-labeled antibody for detection, both raised to human antigen, the assay was able to detect a mature protein, a precursor molecule and enzyme-inhibitor complexes. The assay characteristics regarding linearity, recovery, within-run and between-run precision, and detection limit enable satisfactory application of the assay on serum and tissue samples.[16] The CH concentration was expressed in ng/ml of serum (ng/mls).

Briefly, sera in 1:4 dilution were added to the wells of microtiter plate that had previously been precoated with capture antibodies. After a 2h-incubation at 37°C, the wells were washed and filled with detection antibodies. After further 2 hrs. of incubation at 37°C, peroxidase substrate 3,3,5,5-tetramethyl benzidine (Sigma Chemical Co., St. Louis, MO) in the presence of hydrogen peroxide was added. The amount of degraded substrate, as a measure of bound immunocomplexed CH, was visualised by absorbance

at 450 nm, and the CH concentration was calculated from the calibration curve.

As controls, the results of CH concentration measurements in the sera of 30 healthy volunteers (13 females and 17 males, aged 21-49 years, mean 37 years) were used from the study of Kos *et al.* (with the permission of authors).[11] The serum collection, test kit and the time of biochemical analysis were exactly the same as in our study.

Group 2.

In 17 surgically treated patients, two tissue samples weighing 200-500 mg, representing matched pairs, were obtained from each tumour and the adjacent normal tissue. In four non-operated patients, only a tumour specimen was obtained during diagnostic endoscopy. The tissue cytosol was prepared as described.[13]

The cytosolic concentration of CH was determined by enzyme-linked immunosorbent assay (sandwich ELISA) using sheep immunoselective IgG as capture antibody, and rabbit peroxidase-labeled anti-CH IgG for detection, as described by Kos *et al.*[12] The CH concentration was expressed in ng/mg of total protein (ng/mgp).

Statistics

The results were analysed using a PC computer and BMDP software package (BMDP Statistical Software, Los Angeles, CA). All the tests were two-sided and the results were considered statistically significant at the probability level of 0.05.

The difference between the median concentrations of CH in match pairs of Serum no.1 and no.2, and of tumour and normal tissue samples were determined by the Wilcoxon signed rank test. The Mann-Whitney *U*-test was used to test the relation of Serum no.1 and tumour tissue concentration of the enzyme to clinical and histopathological prognostic factors, and to subsequent recurrence/dissemination of the disease, and to calculate the difference in serum levels of CH between controls and cancer patients. The relationship between CH concentration in Serum no.2 and the time of its collection was established by Spearman's rank correlation. In the survival analysis, Kaplan-Meier product limit method was used,[17] and the difference between groups was tested by the log-rank test.[18] Patients were grouped according to the cut-off concentration of CH, at which maximal difference in the survival rates was determined.

Results

Group 1.

A significantly elevated concentration of CH was measured in Serum no.1 as compared to Serum no.2. (8.9 vs. 8.0 ng/mls, P=0.04; Figure 1) or sera from healthy volunteers (8.9 vs. 4.9 ng/mls, P<0.0001). Among the clinical and histopathological prognostic factors under investigation, only the histopathological grade appeared to be related to the CH concentration as measured in Serum no.1

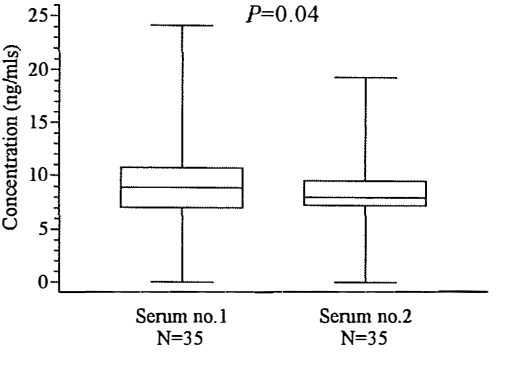

Figure 1. Concentration of cathepsin H in Serum no.1 and Serum no.2 (Group 1). The top and the bottom of the box represent the 25 and 75th percentiles, respectively, and the ends of the bars represent the range. The line in the box is the median value. N, number of samples.

Table 1. Clinical and histopathological characteristics of tumours and corresponding concentrations of cathepsin H in Serum no.1 (Group 1) and tumour tissue (Group 2).

Tumour characteristics	Group 1			Group 2		
	No. of patients	Concentration (ng/mls)[a]	*P*-value	No. of patients	Concentration (ng/mlp)[a]	*P*-value
Localisation						
Larynx	11	9.6 (6.1 - 24.1)	NS	12	1.0 (0.6 - 2.3)	NS
Non-larynx[b]	24	8.5 (0.0 - 15.7)	NS	9	0.7 (0.5 - 7.1)	
T-stage						
T_{1+2}	18	7.6 (0.0 - 15.7)	NS	5	0.9 (0.5 - 7.1)	NS
T_{3+4}	17	9.3 (4.7 - 24.1)		16	0.9 (0.5 - 2.3)	
N-stage						
N_0	19	8.9 (5.0 - 24.1)	NS	6	0.9 (0.7 - 2.3)	NS
N_{1-3}	16	8.9 (0.0 - 15.7)		15	1.0 (0.5 - 7.1)	
TNM-stage						
$Stage_{I+II}$	8	7.5 (5.0 - 10.2)	NS	2	(0.7 - 1.0)	-
$Stage_{III+IV}$	27	9.3 (0.0 - 24.1)		19	1.0 (0.5 - 7.1)	
Histopathologic grade						
G_{1+2}	28	9.1 (4.7 - 24.1)	0.06	17	0.9 (0.5 - 7.1)	-
G_3	3	5.0 (0.0 - 8.8)		2	(0.5 - 1.4)	
Unknown	4	-		2	-	
Extranodal tumour spread						
Negative	4	7.9 (4.7 - 14.7)	NS	4	1.3 (0.5 - 2.0)	NS
Positive	12	9.2 (0.0 - 15.7)		9	0.9 (0.5 - 7.1)	
Unknown	0	-		2	-	

[a] Median (range).
[b] Oral cavity, oropharynx, hypopharynx.
NS, not significant.

(G_{1+2} vs. G_3, 9.1 vs. 5.0 ng/mls, P=0.06) (Table 1). The duration of time interval between the completion of therapy and Serum no.2 collection did not influence the enzyme concentration (R_S=–0.12, P=0.50). In patients with subsequently diagnosed locoregional recurrence or distant dissemination of the disease, CH concentration in Serum no.1 was insignificantly lower than in those with no evidence of active disease at the last follow-up examination (8.4 vs. 9.1 ng/mls, P=0.45). At any concentration level of CH in Serum no.1 used as a cut-off concentration, a better survival of patients was associated with high enzyme concentration, but the differences in DFS- and DSS-rates between low and high enzyme groups did not reach the level of statistical significance. The difference in DFS rates was maximal at a cut-off concentration 10.7 ng/mls (74^{th} percentile; 60 vs. 89% at 2 years, P=0.13), as it was for DSS (65 vs. 86% at 2 years, P=0.25) (Figure 2A).

Group 2.

The CH concentration was significantly higher in normal tissue samples than in their tumour counterparts (2.2 vs. 0.9 ng/mgp, P=0.001) with tumour to normal tissue ratio of median concentrations 0.42. No correlation was observed between tumour CH concentration and the established prognostic factors (Table 1) or the presence of subsequent recurrence/dissemination of the disease (for details see Budihna *et al.*[13]). In the analysis of DFS and DSS, the maximal differences in survival rates were calculated using cut-off concentration 720 ng/mgp (39^{th} percentile). In

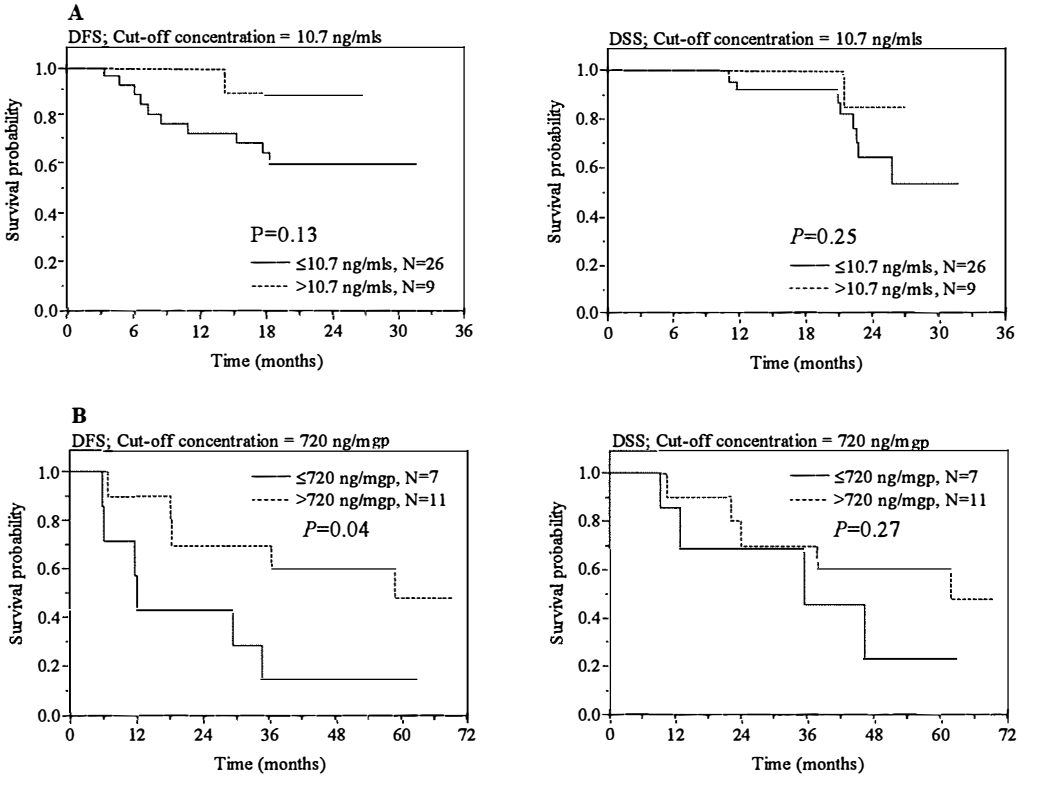

Figure 2. Disease-free survival (DFS) and disease-specific survival (DSS) of patients with respect to the cut-off concentrations of cathepsin H at which maximal differences in survival rates were calculated. A, Group 1; B, Group 2. N, number of patients.

both cases better survival was associated with a high concentration of CH, and the difference in DFS between the groups was statistically significant (DFS: 14 vs. 48% at 5 years, P=0.04; DSS: 23 vs. 60% at 5 years, P=0.27) (Figure 2B).

Discussion

Possible clinical significance of the cysteine proteinases as prognosticators of disease recurrence and patient survival based on their involvement in proteolytic processes leading to the invasion and metastasising of tumour cells.[6] The most investigated enzymes of cysteine class are CB and CL, whereas the role of CH in the invasive behaviour of cancerous cells and particularly its prognostic value is much less investigated.[19] In present study, the concentration of CH was measured in sera from patients with SCCHN (Group 1). The prognostic significance of the enzyme concentration was estimated and compared with updated results from the study of Budihna *et al.*,[13] who determined CH levels in tissue cytosols (Group 2).

In Group 1 patients, a significantly higher serum concentration of CH was measured compared to control sera of healthy volunteers. The same relationship was reported by Gabrijelčič *et al.*[8] for breast cancer and Kos *et al.*[11] for malignant melanoma, indicating that a markedly increased proportion of the

enzyme is secreted from the malignant cells rather than being routed to lysosomes as it is usual in normal cells. Besides, the concentration of CH in Serum no.2 was significantly reduced as compared to that in Serum no.1, although Spearman's statistics showed no correlation between Serum no.2 enzyme values and the time of its collection. It appears that the decrease in proteolytic activity in the treated area as a result of the resection/-destruction of the gross tumour burden results in a decrease in serum CH levels, which is most probably gradual, slowly approaching normal value, as it has already been established for aspartic proteinase cathepsin D (CD).[20]

The only correlation observed was between serum concentration of CH and histopathological grade, and even in that case it was of marginal statistical significance. A higher enzyme concentration was associated with a lower histopathological grade of tumours, i.e. with biologically less aggressive form of the disease. Bearing in mind the tumour-to-normal tissue relation of cytosolic concentration of CH, the established difference logically reflects successive steps in malignant transformation of the cell, from normal - with the highest enzyme concentration - to well, moderately and - finally - to poorly differentiated cell with the lowest enzyme concentration. To our knowledge, besides in head and neck cancer a decreased expression of CH in tumour tissue has been determined also in lung cancer (Schweiger *et al.*, unpublished data). On the other hand, Gabrijelčič *et al.*[8] demonstrated that CH concentration was higher in malignant than in non-malignant samples from breast cancer patients, and that it increased with the histopathological grading, which is just the opposite of what we have found in head and neck cancer. The results in glioma[9] and malignant melanoma[10] are consistent with those in breast cancer.[8] We can speculate that characteristics of the tissue of origin may also play a role in the expression of enzyme, resulting in the differences observed between various tumour types. Among murine tissues, the highest concentration of CH was found in the kidney, followed by vagina, liver, lung and spleen,[21] while much less is known about its distribution in human.

Apart from CH (present study), elevated serum values of CB[22] and aspartic proteinase CD[20] have also been reported in patients with SCCHN. Furthermore, in serial sampling of serum, CB has also proved to be a useful marker in following response to therapy in laryngeal carcinoma.[22] As observed by Krecicki and Siewinski,[22] in all 14 patients who failed after surgery a significant increase in CB activity was measured at least two weeks before clinical evidence of metastases or signs of recurrent disease. In our study, serum (i.e. Serum no.2) was collected also after therapy. However, due to a wide time span in its sampling, it is not possible to draw any conclusion on the potential role of CH as a marker of treatment response or early indicator of treatment failure.

The survival analysis revealed an indisputable trend towards a better prognosis with increasing serum and tissue levels of CH in both the analysis for DFS and DSS. There is only the study of Budihna *et al.*[23] on breast cancer that has recognised high tissue concentration of CB as prognostically superior. All other reports,[19] including that on the prognostic significance of serum CH in melanoma patients,[11] have correlated elevated serum and/or tissue levels of cysteine proteinases with worse survival. This difference could reflect not only the specific characteristics of individual cancer types but also the selection criteria for cut-off concentration. For example, in melanoma study[11] the same test kit was used for biochemical analysis of serum CH as in ours and the results of both studies are comparable. However, while we used the optimal cut-off concentration at which the maximal difference was calculated

between low and high CH groups, in the other study[11] a median value was chosen for this purpose. The difference in DFS-rates as calculated with respect to tissue cut-off concentration of CH was of statistical significance, which was not the case with its serum concentration. Nevertheless, the follow-up in Group 1 is probably too short to yield a more reliable estimation rather than a trend only.

For CH measurement enzyme-linked immunoassay was used. With the first version of the test kit, using polyclonal antibodies for capture and for detection,[12] the enzyme concentration in tissue cytosol was determined in Group 2 patients.[13] The results were in the range of the amounts obtained for cathepsins by protein purification and enzyme activity measurements.[24] When the same test kit was used for quantitative determination of CH in sera from patients with breast cancer,[8] enzyme levels were 50-100 times higher than those of CB and CL, suggesting that they were overestimated, most probably due to a non-specific reaction of polyclonal antibodies with other serum components. For that reason, a more sensitive and specific ELISA utilising mono- and polyclonal antibodies and capable of detecting distinct CH forms was developed.[16] It was used in the present study for the determination of CH concentration in the serum from patients with SCCHN, and previously by Kos *et al.*[11] in patients with malignant melanoma. The concentration ranges agree well between the studies and are consistent with the amounts reported for CB and CL.[11] The assay has already been in use for the analysis of CH concentration in tissue cytosols of the human heart, muscle and kidney.[16] The results were consistent with the levels determined in rats using an immunoassay based on polystyrene beads coated with anti-rat CH IgG,[21] and were approximately 50-times higher than those in patient sera. The ratio between cytosolic concentration of CH as measured in cancerous patients[13] and different human tissues[16] was about 10:1. However, when we attempted to use a modified poly-mono version of the assay for enzyme determination in tissue cytosols of patients in Group 1, inconsistency between CH concentrations as measured upon different dilutions of individual sample within the working range of the assay was observed and the analysis was not carried out.

To conclude, in this report we have shown that CH concentration in serum from patients with SCCHN was elevated as compared to normal serum from healthy volunteers. Further, considering the tissue concentration of CH as well, less aggressive forms of the disease were found to be associated with higher levels of the enzyme. In DFS and DSS analysis, a trend towards better prognosis was observed with high concentrations of CH as measured in both the serum and the tissue samples. Although our results should still be confirmed by a more extensive study with appropriate follow-up, it appears that also in SCCHN CH concentration might be of prognostic importance.

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Radiol Oncol 1999; 33(2): 153-7.

Chemical radioprotection (WR-2721) in patients with head and neck cancer

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Background and methods. *Amifostine was given as daily intravenous application (200mg/m2) 10-15 minutes prior to radiotherapy in 36 patients with locally advanced head and neck tumors to spare normal tissues, such as the salivary glands and oral cavity, from irradiation. Postoperative radiotherapy was carried out to a complete dose of 60 Gy given in 30 days, with single doses of 2 Gy. Side effects of radiotherapy were assessed using the WHO-criteria.*

Results. *According to the WHO-score, mucositis occurred in 10 patients (grade I) and 26 patients (grade II). Dysphagia was recorded in 10 patients as grade I and in 12 patients as grade II. Xerostomia was established as grade I in 14 patients and as grade II in 16 patients. Skin reactions were grade I in 9 patients and grade II in 13 patient. Drug-related toxicity was recorded in 12 patients: hypotension grade I and nausea grade I were observed in 3 patients, while vomiting grade I and grade II were documented in 3 and 1 patient respectively.*

Conclusions. *According to the data from the literature, we believe that the application of amifostine is feasible, and amifostine is an effective radioprotector decreasing both acute and late side effects in patients irradiated for head and neck tumors.*

Key words: head and neck neoplasms - radiotherapy; radiotherapy - adverse effects; radiation - adverse effects; amifostine

Introduction

Radiotherapy for head and neck tumors can produce significant acute and chronic side effects, such as mucositis and xerostomia, because in many cases most of the salivary glands as well as the major integral volume of the oral cavity are included in the irradiation portals.[1]

The degree and duration of radiation damage to the oral mucosa and to the salivary glands is related to the total dose of radiotherapy, to fractionation, to the volume of the treatment fields and the overall treatment time.[2-5] In some cases severe mucositis can lead to a pause in radiotherapy and therefore to treatment delay.6 In a particular situation every day of treatment delay will decrease the probability of remission rate.[6]

Received 30 June 1998
Accepted 15 December 1998

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On the other hand, xerostomia is one of the most severe and definitive side effects in patients with head and neck tumors. Xerostomia will significantly undermine the quality of life of those patients. In the past it had been impossible to avoid these side effects by modifying the schedule of radiation therapy or using oral mucosa protecting drugs.

After World War II, in 1949, investigations directed into finding an effective radioprotective agent were started at Walter Reed Army Institute of Research.[7] More than 4400 substances had been investigated, and in terms of side effects, WR-2721 or amifostine was the most effective and compatible agent.[7] Since 1995 the use of amifostine in medicine has been permitted in Germany. Hence, after the introduction of a new radioprotective agent there is a new approach to spare normal tissue.

Amifostine is a pro-drug and must be converted to the active free thiol by the membrane-bound alkaline phosphatase.[8,9] Yang *et al.* have shown that the number of membrane-bound alkaline phosphatase is significantly higher in normal than in tumor cells.[10] So amifostine will be dephosphorylated and activated faster in normal than in tumor cells.[11,12] During the first 30 minutes after infusion, the concentration of the agent in normal cells is 102 -103 times higher than in tumor cells.[12] This difference produces a selective protection of healthy cells while there is no protection of tumor cells, because of a very low concentration of the agent in tumor cells directly after infusion. In the cells amifostine works by direct protection of the DNA. The free thiol protects against radiation damage by acting as a free radical scavenger, and by donating hydrogen to repair damaged target molecules.[13-15] It is worth mentioning that so far the exact mechanism of action in radioprotection has not been fully understood. In the last years a series of normal tissues have been studied to evaluate tissue protection factors for amifostine.[16,17] Tissues with high protection factors are especially bone marrow (factor 3.0), immune system (factor 3.4), epidermis (factor 2.4), salivary glands (factor 2.0), oral mucosa (factor>1).[16,17] That is why amifostine is given recently as a radioprotector in patients with advanced head and neck tumors, because mucositis and xerostomia are severe acute and late side effects, which can decrease life quality.

Patients and methods

Since 1995 we have treated 36 patients with advanced head and neck tumors applying amifostine daily before radiotherapy. Amifostine at a dosage of 200 mg/m^2 was given 10-15 minutes before irradiation. The substance was administered as short infusion over a period of 15 minutes.

During application, blood pressure was measured before, during and after infusion. All patients had given their informed consent to therapy. Patients with a blood pressure below 100 mmHg were excluded. All patients were male. Their median age was 52 years (range: 42-66).

All patients had undergone a complete resection of the gross tumor and a unilateral or bilateral neck dissection. Only patients with primary tumors were investigated.

It is very important that the irradiation takes place within 30 minutes after infusion because the difference in concentration between healthy and tumor cells will decrease significantly in correlation with time. Radiotherapy was given in 5 fractions per week, with single doses of 2 Gy over 6 weeks, to a total dose of 60 Gy. At least 75% of the salivary glands and 2/3 of the oral cavity were included in the irradiation fields. Postoperative irradiation was carried out on a linear accelerator (6 MV) using opposed irradiation portals. The target volume was defined as previous gross tumor site includ-

ing the area of regional lymph nodes. The target volume was irradiated up to a total dose of 36 Gy, given according to the above described fractionation. Afterwards, the irradiation fields were divided into anterior photon and posterior electron fields to spare the radiosensitive spinal cord. The choice of posterior electron energy was based on the axial CT-imaging defining the distance between the skin surface and the spinal cord. Retrospective analysis of the irradiation technique shows an absolute dose homogeneity for the complete target volume and especially for the salivary glands and oral cavity.

The irradiated parts of these had been included within the 100% isodose. The median follow-up for the patients is calculated to be 17 months (range: 3-24 months) up to now. The grade of irradiation-related side effects of as well as drug toxicity were documented using the WHO-score.[18] During radiotherapy, every patient underwent clinical check up once weekly. After completed radiotherapy, the patients were followed up clinically every month to evaluate late effects after irradiation.

Results

Because of hypotension amifostine administration had to be discontinued in 12 patients. In every case the infusion could be completed within a few minutes without antihypotensive therapy. There were no cases of dizziness, sneezing or flushing, nor other allergic side effects observed. Nausea grade I was recorded in 3 patients, vomiting grade I and II in 3 and 1 patients, respectively. Hence, administration of amifostine proved to be feasible and non-toxic. Daily application of 200 mg/m^2 amifostine prior to radiotherapy entails no toxicity grade III or IV, according to WHO.

In terms of radioprotection, we have obtained the following results (Table 1):

Table 1. Different side effects according to the WHO-score in patients with and without amifostine

WHO- grade	Grade I	Grade II
Mucositis	10	26
Dysphagia	10	12
Dermatitis	9	13
Xerostomia	14	16

Mucositis grade I was documented in 10 patients, and grade II in 26 patients. Dysphagia grade I and II occurred in 10 and 12 patients, respectively. Dermatitis was evaluated as grade I in 9 patients, and as grade II in 13 patients. Xerostomia grade I was seen in 14 patients, and grade II in 16 patients. There were no cases of grade III or IV side effects due to high dose irradiation (60 Gy/30 days) recorded.

Discussion

In our investigation, the use of radioprotector amifostine resulted in a marked decrease in typical side effects related to high-dose radiotherapy for head and neck tumors. The results are in agreement with a number of other studies.

In 1994, Mc Donald *et al.* described a decrease in xerostomia during and after radiotherapy in 9 patients measuring the salivary function.[1] In this investigation a good feasibility of the drug was documented, but no sparing of acute mucositis during radiation was found when amifostine was administered at a dosage of 100 mg/m ^{2}.

Dendale and colleagues have investigated mucositis in rodents, using different doses of amifostine before irradiation.[19] In 3 groups altogether 24 animals got a total irradiation dose of 24 Gy, with single doses of 4 Gy after 40 mg/kg, 200 mg/kg and 400 mg/kg amifostine given intraperitoneally. The results were scored and there was no significant difference seen between the 3 doses of amifostine,

whereas the difference between any amifostine group and the crude radiotherapy group was highly significant.

Bohuslavizki *et al.* administered 500 mg/m^2 amifostine to patients with cancer of the thyroid before iodine therapy.[20] Although the number of patients was very small, xerostomia was calculated to be 37% in the group treated by radiotherapy alone (n=9), and none in the amifostine group (n=8).

From Munich we have got some preliminary results using amifostine in patients with ENT - relapses.[21,22] All the patients (n=40) received another dose of radiotherapy (40 Gy), combined with 350 mg/m^2 5-FU and 300 mg/m^2 amifostine in the state of recurrence. A mucositis grade I or II was documented in 4 patients only. Buenzel *et al.* have published some reliable data from a prospective randomized phase-II study including 39 patients.[23,24] One group was given radiotherapy to a complete dose of 60 Gy in 30 days with concurrent administration of carboplatin (70 mg/m^2, day 1-5 + day 21-25) with or without 500 mg amifostine. There was a highly significant decrease in the incidence of mucositis, xerostomia and thrombopenia noted in the patients who received amifostine.

The follow-up time was 12 months, the disease free survival rate was 79% in the amifostine and 64% in the radiochemotherapy group, respectively. Complete response was 72% in the amifostine and 43% in the control group. Hence, this could be regarded as convincing evidence that there is no shielding of tumor cells. Similar data were reported by Füller and colleagues.[25]

Because of those promising preliminary data, the RTOG started a prospective randomized phase III-study in patients with head and neck tumors stage III and IV. In the study, a radiotherapy dose of 70 Gy was given in 35 fractions with or without amifostine 200 mg/m^2.[26] In the amifostine group the rate of xerostomia was reduced significantly (p=0,0004), and the time to the onset of xerostomia was significantly longer (p=0,0001). The preliminary results in the literature have shown that there is no sparing of tumor cells by amifostine[27], so that up to now survival time and disease free survival time have not been significantly different in amifostine and control groups.

In their investigation, Mc Donald *et al.* administered 100 mg/m^2 amifostine.[1] While this dose failed to prevent the onset of mucositis, doses between 200 mg/m^2 and 300 mg/m^2 amifostine proved successful in reducing this side effect.

Dendale *et al.* have shown a dose dependent correlation between incidence of mucositis and dose of amifostine.[19] The optimal dose of amifostine still remains to be established.

In conclusion, considering our data we believe that amifostine is an effective radioprotector able to decrease acute as well as late side effects in patients irradiated for head and neck tumors.

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Radiol Oncol 1999; 33(2): 159-62.

Surgical treatment of advanced oropharyngeal cancer with preservation of the larynx

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Background and methods. *This retrospective study evaluates the oncological and functional results obtained in 61 patients with advanced oropharyngeal cancer who underwent extended tumor resection as a primary procedure or as a salvage surgery form.*

Results. *Although the oropharyngeal cancers involved the base of the tongue, or some of them extended to the lateral hypopharyngeal wall, the surgery was performed without total laryngectomy. The tumor extended to the vallecula and/or to the pharyngoepiglottic fold in five cases, which required supraglottic laryngectomy. The closure following the extended resection of the tumor was made with flap reconstruction in all patients. The preferred method was employing the pectoralis major myocutaneus flap. The survival rates were 75%, at 1 year and 31%, at 2 years and 25% from 2 to 5 years with recurrence of the disease. In one patient, the nasogastric tube could not have been removed, and another patient could be decannulated only after postoperative radiation because of the persistent oedema.*

Conclusions. *A satisfactory functional result was obtained in this series. In most of our patients, good functioning of larynx as well as voice preservation were secured.*

Key words: oropharyngeal neoplasms - surgery; organ sparing - methods; larynx - surgery

Introduction

The therapeutic approach to the patients with previously untreated advanced carcinoma of the oropharynx or of those presenting with recurrences after surgical and/or radiation failure presents numerous difficulties.[1-3] The question of quality of life is very important.

Received 6 June 1998
Accepted 10 December 1998

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Oncological success in the treatment of head and neck cancer is bought at a price of crippling of vital functions such as eating, breathing, speech and, furthermore, striking aesthetic deformity. Beside the strict oncological principles, and adequate resection of cancer with the preservation of laryngeal function is one of the main goals in our department. The present study of a series of patients is a review of the oncological and functional results of this kind of surgery performed in the last 6 years.

Material and methods

Sixty-one patients, 54 men and 7 women, underwent surgical procedure for advanced oropharyngeal cancer from January 1990 to January 1996. Their ages ranged from 40 to 71 years (mean 48 years). Twenty-eight patients had previous radiotherapy only, 9 had partial surgery and radiotherapy, and the remaining 24 patients presented with untreated carcinoma.

All tumors had their origin in the oropharynx. Invasion in oral cavity was very frequent. The tumor invaded the mobile tongue and had an extension to the floor of the mouth in 43 patients. There was extension to the mandible in 7 patients and in 17 other cases the tumor involved the gingival mucosa. In 17 patients, the tumor spread to the lateral wall or the lateral and posterior wall of the hypopharynx.

The clinical staging of the disease is reported according to the UICC TNM staging system[4] for head and neck tumors (Table 1). Resection of the base of the tongue with parts of the oral cavity without segmental resection of the mandible was performed in 13 patients. Composite resection of the oropharynx was required in 31 cases. Resection of the base of tongue with partial hypopharyngectomy was required in 4 patients. Composite resection of the oropharynx with partial hypopharyngectomy was performed in 8 patients. Partial horizontal laryngectomy was required in 5 patients because the tumor involved the vallecula or pharyngoepiglottic fold as well.

Neck dissection was performed in all patients. The terminology for describing neck dissection follows the publication by Robbins *et al.*[5] Radical neck dissection was indicated for clinically positive neck nodes in 29 patients, and, for clinically negative neck nodes, in 9 patients. Modified radical dissection was performed in 6 cases for N1 neck disease and in 18 cases for N0 neck disease. Selective neck dissection was done in one patient for N0 neck. In two patients, the neck dissection was bilateral. In the first case, ipsilateral radical neck dissection and, in the other side, modified radical neck dissection were used. In the second case, modified radical neck dissection was performed in both sides.

Table 1. TNM staging

Primary			Neck stage				Totals
stage	N0	N1	N2a	N2b	N2c	N3	
T1	0	0	0	0	0	0	0
T2	4	4	0	0	0	0	8
T3	3	0	1	1	1	0	6
T4	19	14	4	7	0	3	47
Total	26	18	5	8	1	3	61

Stadium I. -
Stadium II. 4
Stadium III. 7
Stadium IV. 54

Reconstruction of the defect was carried out by the transposition of the myocutaneous flap in 60 patients (58 pectoralis major, 2 latissimus dorsi) and a free microvascular flap was used in one patient (latissimus dorsi).

Postoperative radiation treatment was given to the patients who had no previous radiation.

Results

One patient had early postoperative medical complication as gastric perforation, three patients had aspiration pneumonia and, in two cases, pneumonia was observed. Twenty-seven patients had flap related complications, *e.g.*, flap necrosis dehiscence, infection and fistula formation.

Total flap loss occurred in one patient. This complication required a secondary reconstruction by latissimus dorsi myocutaneous flap. Partial flap necrosis occurred in 8 patients. Secondary repair was required in 4 patients. In 4 patients, the area of necrosis was minimal and did not require additional treatment. Orocutaneous fistula developed in 10 patients but in all it closed spontaneously with conservative management. There were 8 cases of minor wound complication on the neck or the donor site.

Fifty- six patients were decannulated between the 3rd and 30th postoperative day (mean 13.5). They were released of nasogastric tube between 10th and 90th day (mean 22 days). In five cases, when the resection of the oropharyngeal cancer required supraglottic laryngectomy, the nasogastric tube was removed between the 33rd and 85th day after surgery (mean 60 days) and they were decannulated between the 30th and 85th day (mean 64 days).

One patient remained dependent on feeding tube because normal swallowing function was not restored. In this case, a second primary tumor in the brain was detected.

The survival rates were 75% at 1 year, 31% at 2 years and 25% at 2 to 5 years. The death was due to early recurrence of the disease. Forty-one of the 61 patients died. One was lost from the follow-up in the early postoperative period, and another died from cardiorespiratory disease (Table 2). Survival distribution of study population was analised by the Kaplan- Meier method (Figure 1,2).

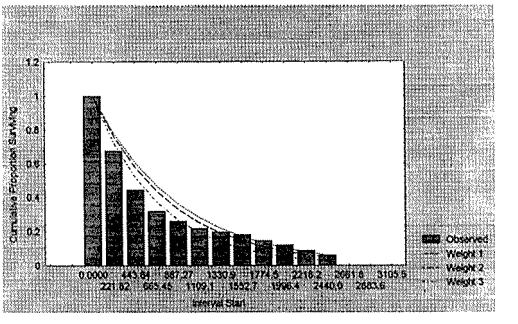

Figure 1. LS estimates of survivorship function. Model exponential Note: Weights: 1=1, 2=1. N, 3=N (I) H (I).

Local failure was the most frequent cause of death. Thirty-four patients had recurrence above the clavicles. Three patients presented with lung and one patient with brain metastases. Two multiplex primary malignant tumors were observed in the oesophagus.

Table 2. Outcome of previous therapy and salvage surgery

Previous care	Date of surgical procedure					
	1990-1995 61 patients 1 year follow up		1990-1994 48 patients 2 years follow up		1990-1993 40 patients from 2 to 5 years follow up	
	disease free survival					
Previously untreated	20/26	77%	6/19	31%	4/15	26%
Previously treated	20/35	74%	9/29	31%	6/25	24%
Total	46/61	75%	15/48	31%	10/40	25%

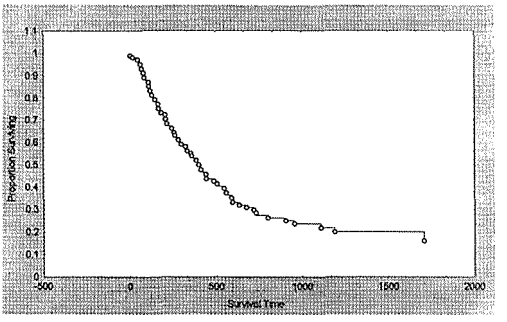

Figure 2. Survivorship function.

Discussion

The extension of the tumor to the vallecula or lateral wall of the hypopharynx and pharyngoepiglotic fold does not require total laryngectomy. Replacement of the lack of tissue with any type of flap following partial laryngectomy provides the motility of the preserved hypopharyngeal structures and oral tongue. It is necessary to provide the sensory component of the reflex mechanism by preserving the superior laryngeal nerve and its internal branches. The intact innervation of the larynx prevents aspiration. The relief of pain after surgery was marked by all of the patients. In the present study, there was no significant higher rate of postoperative morbidity after radiation failure than in the group of previously untreated patients.[7,8]

The survival rates were similar to those of Marcial and Brennan.[1,9] There was no significant difference of survival between the group of patients who underwent previous surgery or radiation and those who were previously untreated.[9,10]

It is the authors' opinion that extended tumor resection without associated laryngectomy provides excellent palliation of symptoms and offers acceptable survival results and quality of life.

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Radiol Oncol 1999; 33(2): 163-8.

Intensity-modulated radiotherapy with a multileaf collimator

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In intensity-modulated radiation therapy (IMRT), a small number of spatially-modulated beam ports is used to deliver a uniformly high dose to the target region, while simultaneously producing a better sparing of normal tissues than is possible with conventional, non-modulated beam delivery. When intensity-modulation is carried out with a multileaf collimator rather than with physical compensators, any required intensity pattern can be produced with a minimal planning effort on the part of the physicist or dosimetrist, and with reduced manipulation on the part of the therapists. The paper describes the recent implementation of multileaf collimator-based intensity-modulated radiation therapy at McGill University in Montreal.

Key words: radiotherapy-methods; radiotherapy planning, computer-assisted; radiotherapy dosage; multileaf collimator,

Introduction

Intensity modulated radiation therapy (IMRT)[1-3], carried out with multileaf collimators (MLC)[4-6] in combination with 3D planning systems which have the capability for inverse planning[7-9], represents the current forefront in radiotherapy. Since MLCs can be retrofitted to existing linear accelerators and controlled with readily available manufacturer hardware and software components, it is neither difficult nor excessively expensive to make the transition from conventional to intensity-modulated radiation therapy.

In our department, the MLC-IMRT treatment procedure is typically separated into the following six distinct steps: (a) definition of beam geometry for a prospective patient; (b) calculation of intensity-modulation matrices for each radiation field; (c) determination of MLC leaf sequencing for each field; (d) calculation and evaluation of the dose distribution; (e) verification of the beam delivery sequence with respect to machine outputs and the MLC sequence; and (f) patient treatment.

In this paper we describe each of the six steps involved in the MLC-IMRT process, and, as an example, present a typical head and neck treatment with the technique.

Received 7 November 1998
Accepted 12 January 1999

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Materials and methods

Patient simulation and treatment planning

In preparation for treatment planning, the patient is scanned on a dedicated radiotherapy CT-simulator (Picker-5000; Cleveland, OH). The complete set of axial CT images covering the tumour volume and neighbouring structures is transferred to a virtual simulation and image processing workstation (Picker VoxelQ/AcQSim; Cleveland, OH) for the localization and delineation of critical organs and target structures. The beam geometry (treatment isocenter, field sizes, gantry angles, special shielding, etc.) is determined using anatomical landmarks visible on digitally reconstructed radiographs (DRRs) and beam's- eye-views (BEVs) of the previously delineated target and critical organ data.

The axial CT slices together with the beam geometry are then transferred via a computer network to our treatment planning system (CadPlan, version 3.12; Varian, Palo Alto, CA) for calculation of the appropriate isodose distributions. For all our IMRT treatments to date we have used beam configurations common to standard techniques (e.g., three fields for head and neck, four-field box technique for prostate, etc.) in order to facilitate the transition from conventional, non-modulated therapy to MLC-IMRT.

Calculation of the intensity modulation matrix

Once the beam parameters have been established, we instruct the CadPlan system to create intensity-modulation matrices for each field of the treatment plan. Currently, these intensity matrices serve to compensate for irregularities in the patient's contour; however, in the future a complete inverse-planning package will provide the appropriate intensity modulation matrices to optimise the preplanned sparing of critical structures and dose homogeneity within the target volume.

Calculation of the leaf sequence

A leaf sequence algorithm[10,11] translates an intensity-modulation matrix into a set of MLC leaf configurations delivered at the treatment machine. The leaf sequence algorithm that we use has been developed in-house; however, most modern commercial treatment planning systems now have the ability to write leaf sequences. Our algorithm is designed to write the step-and-shoot type leaf sequences, implying that the leaves move to a given configuration, a prescribed number of monitor units is delivered for this configuration, the leaves then move to the next configuration, followed by the delivery of the prescribed number of monitor units, and so on.

The algorithm for the calculation of the leaf sequence proceeds as follows. At 1 cm intervals throughout the intensity-modulation matrix, profiles of the intensity along the direction of leaf motion are extracted. These profiles represent the intensity to be delivered under each of the 26 leaf pairs of the MLC. Each 2D intensity profile is then partitioned, or "sliced", into a number of regularly spaced intensity levels. The positional coordinates of the intercepts of a given intensity level with the intensity profile form a set of positions at which that particular MLC leaf pair must be placed during beam-on to deliver the given intensity level. This process is repeated for all intensity levels and then for all leaf pairs. Once completed, the resultant set of leaf pair positions is sorted to establish a sequence of segments that can be delivered efficiently. A percentage monitor unit setting (or "dose index") is calculated for each segment from the knowledge of the particular dosimetric characteristics of the linear accelerator. The leaf sequence is then transferred to the Varian MLC Dynamic Beam Delivery (DBD) Toolbox workstation, which controls the 52-leaf MLC of our linear accelerator (Clinac-2300 C/D; Varian, Palo Alto, CA).

Generation and verification of isodose distribution

In order to ensure accurate treatment planning, we reintroduce the MLC segments, determined by the leaf sequence program, into the CadPlan system in the form of separate blocked fields which are weighted according to the calculated "dose index" and calculate a new dose distribution. If there are no errors in the leaf sequence calculation, the dose distribution calculated from the "dose-index" weighted fields will agree closely with the dose distribution calculated with the original intensity matrices, leading to the conclusion that the proposed MLC sequence is adequate for the treatment. The actual treatment is carried out only after full agreement between the two calculated dose distributions is found and the beam delivery verified.

Verification of beam delivery

Prior to patient treatment the leaf sequence for each field is subjected to two dosimetric tests. The first test is a film measurement of the planar dose distribution resulting from the delivered leaf sequence for each individual field consisting of multiple subfields. This measurement confirms the proper orientation of all fields and allows us to verify that the beam intensity delivered to selected points is consistent with the intensity matrix.

The second test is a central axis measurement of the cumulative dose resulting from the leaf sequence for all fields. This test is carried out with a calibrated ionization chamber at the treatment isocenter depth in a tissue equivalent phantom.

Patient treatment

The actual patient treatment is similar to conventional type treatments. The main difference is that, instead of placing a physical compensator in the beam path for each field, the therapist first loads a leaf sequence file into the MLC control computer and then programs in the total monitor unit setting pertaining to that leaf sequence. The Varian DBD ToolBox software apportions the appropriate percentage of monitor units according to the "dose-index" to each segment of the leaf sequence.

The MLC segments are delivered at the nominal operating dose-rate of the linac (~400 MU/minute). During the time that the MLC leaves are changing position, however, the dose-rate drops to zero allowing the leaf movement under "beam off" conditions before returning to the nominal dose-rate for the delivery of the next MLC segment. Typically, the delivery of an MLC intensity-modulated leaf sequence requires 20%-50% more beam time than would the same treatment produced with physical compensators. However, this loss of beam time is amply made up by the radiation therapists not having to enter the treatment room to change the compensators, blocks, or wedges for each individual field used in the treatment plan.

Results and discussion

The first treatment with MLC-IMRT at the McGill University Hospital Centre was carried out in April 1998 and within six months 15 patients were treated with the technique. We now give an example of a typical MLC-IMRT treatment. The patient was treated for a base of tongue carcinoma with a dose of 44 Gy using an isocentric set-up consisting of two half-blocked lateral parallel-opposed 6 MV photon beams with a collimator setting of 11x10 cm^2. An additional 16 Gy boost (sparing the spinal cord) was delivered with the same beam geometry. The lower neck region was treated with a single anterior half-blocked field to 50 Gy prescribed at a depth of 3 cm. Intensity-modulation was desirable for the lateral fields to compensate for the uneven surface of the neck region, which under standard conditions would have

required the construction of custom-built compensators. Before calculating the intensity matrices for each field, the BEV and DRR modes were used to position shielding for the protection of the base of skull as well as the eyes and nose.

Figure 1 shows the digitally-reconstructed radiograph (DRR) generated for the patient from a series of axial CT slices and used for field placement and delineation as well as for comparison with portal images in the verification of patient positioning prior to dose delivery.

Dose distributions for the initial portion of the IMRT treatment are shown in Figure 2; part (A) for the midseparation sagittal plane, part (B) for an axial slice through the target volume as indicated by the dashed line in part (A). The distributions were calculated with the CadPlan 3D treatment planning system and are normalized to 100% at the dose maximum in the target volume. The total dose of 44 Gy, delivered in 22 fractions, was prescribed to the 96% isodose surface which covers the target volume. The dose uniformity in the treated volume is considerably better than that achievable without the use of intensity-modulated beams.

Figure 3 displays the four irregular subfields which were used to produce the intensity-modulation for the right lateral beam for the treatment of Figure 2. The irregular fields were produced and the dose was delivered with a multileaf collimator and a step-and-shoot method using the dynamic MLC option on our linear accelerator. For the right lateral field the basic irregular subfield shown in part (A) delivered the 85% of the total dose, while the other three subfields shown in parts (B), (C), and (D) each delivered 5% of the total dose.

The patients treated with the IMRT technique have an unusually mild skin reaction compared to reactions normally observed in patients treated with standard techniques. We believe that the lack of wedges, blocking trays, and compensators, all of which may increase the skin dose, is to be credited for the reduction in skin reaction.

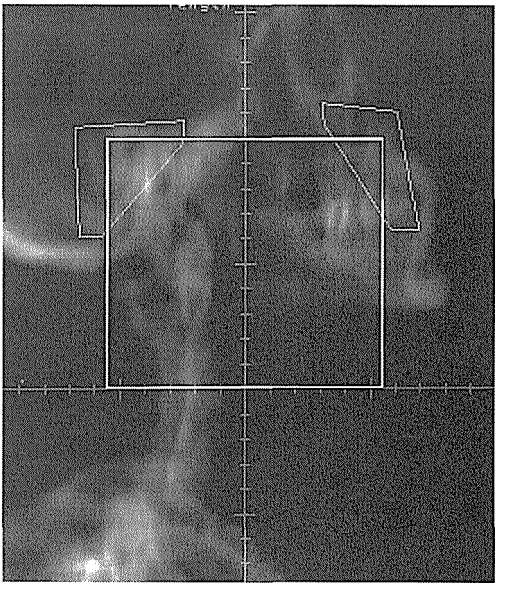

Figure 1. A digitally reconstructed radiograph (DRR) for a typical patient treated with intensity-modulated beams at McGill University.

Summary

Intensity-modulated radiation beams produced with a multileaf collimator are rapidly becoming an essential component of modern radiation therapy. With a wise purchase of equipment, the investment in time and money required for the successful implementation of intensity-modulated radiotherapy into the clinic can be maintained at a reasonable level and yield a substantially improved patient treatment when compared to standard techniques.

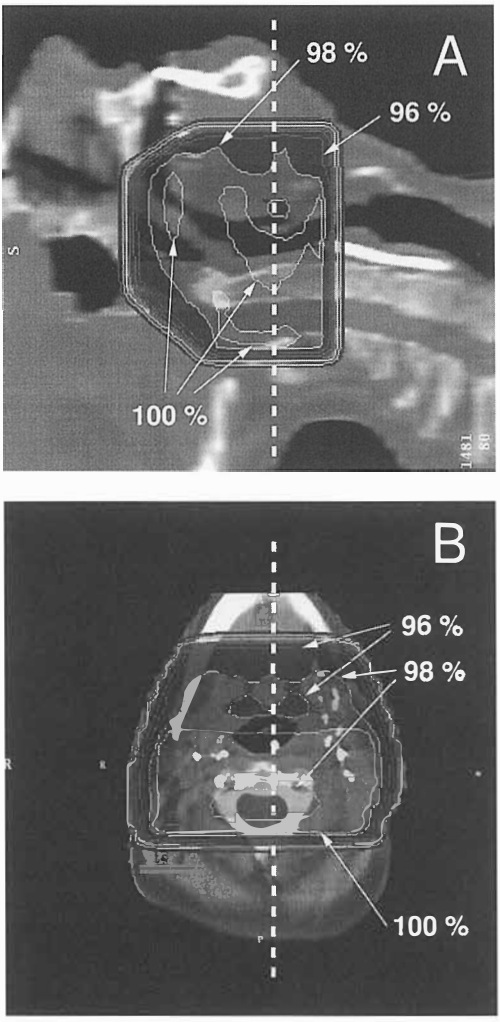

Figure 2. Dose distributions for a typical patient treated for a base of tongue carcinoma with intensity-modulated 6 MV beams. Part (A) is for the midseparation sagittal plane, part (B) for an axial plane defined with the dashed line in part (A). The distributions are normalized to 100% at the dose maximum in the target volume.

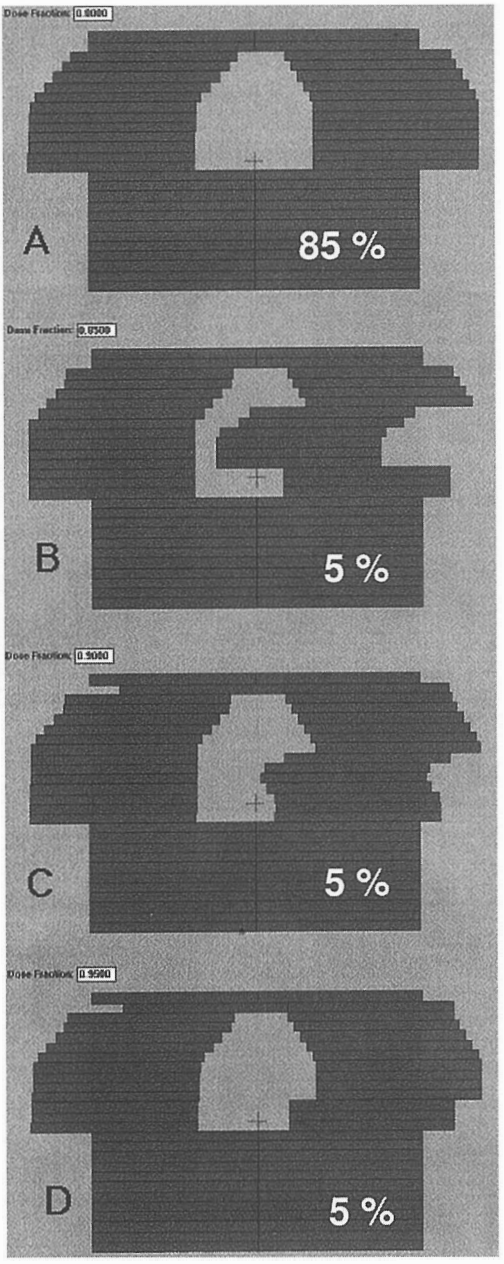

Figure 3. The four irregular subfields which were used to deliver the intensity-modulated right lateral beam for the dose distribution of Figure 2. The basic irregular subfield of part (A) delivers 85% of the total dose, the other subfields deliver 5% each.

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Radiol Oncol 1999; 33(2): 87-94.

Evaluacija spektra pri pri intrarenalni arterialni Doppler preiskavi pri zdravih otrocih

Cvitković Kuzmić A, Brkljačić B, Galešić K

Izhodišča. Namen raziskave je bil določititi značilnosti spektra pri Dopplerjevi preiskave intrarenalnih arterij zdravih otrok. Merili smo pospeševalni čas (AT) in rezistenčni indeks (RI).
Bolniki in metode. Vrednosti RI in AT smo primerjali med skupinami otrok različne starosti, vrednosti RI pa z vrednostmi pri odraslih. Pri ultrazvočni preiskavi intrarenalnih arterij z dvojnim Dopplerjem smo uporabili ultrazvočni aparat Acuson 128XP10. Preiskavo smo opravili na 150 otrocih (300 ledvicah), ki niso imeli kliničnih ali patomorfoloških sprememb urinarnega trakta. Otroci so bili glede na starost razvrščeni v 3 skupine: (1) 52 otrok je bilo starih od 2 do 6 let; (2) 48 otrok nad 6 do 11 let; (3) 50 otrok pa nad11 do 16 let.
Rezultati. V skupini I je bila povprečna vrednost RI (+/- 1.S.D.) 0,70 (+/- 0.03), v skupini II 0,625 (+/- 0,025) in v skupini III 0,585 (+/- 0,03). Vrednosti AT so bile od 0,04 do 0,09 sekund, povprečna vrednost pa je bila pri vseh treh skupinah enaka 0,07 (+/- 0,01) sekund.
Zaključki. Pri mlajših otrocih je RI znatno višji kot pri starejših in pri odraslih; po 6. letu starosti postanejo vrednosti RI pri otrocih enake vrednostim odraslih. RI=0,70, ki velja kot mejna vrednost za povečan upor v ledvičnem žilju pri odraslih, velja tudi pri otrocih, starejših od 6 let. Z analizo pospeševalnega časa lahko pri otrocih in odraslih na enak način ugotovimo zožitev glavne ledvične arterije.

Radiol Oncol 1999; 33(2): 95-9.

Ultrazvočno diagnosticiranbiliarni ileus - prikaz primera

Vidmar D, Repše S

Izhodišča. Kot diagnostično metodo izbora smo do nedavnega uporabljali rentgensko pregledno sliko trebuha, v novejšem času pa se vsebolj uveljavlja ultrazvok, ki v dovolj izkušenih rokah hitreje pokaže ileus, njegov vzrok in tudi mesto obstrukcije.
Prikaz primera. Prikazujemo primer bolnika, ki je bil sprejet na oddelek za abdominalno kirurgijo zaradi hudih bolečin v trebuhu in bruhanja. Pregledna rentgenska slika trebuha je pokazala znake obstrukcijskega ileusa in zračni holangiogram. Ultrazvočni pregled trebuha je potrdil stanje ileusa ozkega črevesja, prikazal pa je tudi etiologijo ileusa in mesto obstrukcije - kamen v distalnem delu ileuma. Poleg tega je pokazal patološko spremenjen žolčnik, nalepljen na dvanajsternik. Bolnik je bil operiran, pooperativni potek je minil brez zapletov.
Zaključki. Z ultrazvokom je možno ne le postaviti diagnozo ileusa, temveč tudi prikazati žolčne kamne kot vzrok ter mesto obstrukcije.

Radiol Oncol 1999; 33(2): 101-9.

Magnetnoresonančno slikanje parotidnih žlez pri bolnikih s Sjögrenovim sindromom: kvantitativno in kvalitativno ocenjevanje kontrastno ojačanih posnetkov

Stiskal M, Szolar D, Steiner E, Hitzelhammer J, Preidler KW, Czembirek H

Izhodišča. Študijo smo izvedli z namenom, da bi ocenili diagnostično uporabnost neojačanega in s kontrastom ojačanega magnetnoresonančnega slikanja parotidne žleze pri bolnikih s Sjögrenovim sindromom.

Bolniki in metode. Sedemindvajset bolnikov s Sjögrenovim sindromom smo slikali z magnetno resonanco brez ojačanja in s 'spin-echo' tehniko po aplikaciji kontrastnega sredstva GdDTPA. Morfološke ugotovitve magnetnoresonančnega slikanja smo primerjali z meritvami intenzivnosti signala na T1 in T2 obteženih slikah, narejenih brez kontrastnega ojačanja, in na pokontrastnih T1 obteženih posnetkih z ojačanjem. Kvantitativne in kvalitativne podatke bolnikov smo primerjali s podatki zdravih oseb (n=12).

Rezultati. Vrednosti T1 in T2 na posnetkih, narejenih brez kontrastnega ojačanja, so bile pri bolnikih s Sjögrenovim sindromom bistveno manjše kot pri zdravih osebah (T1: 62±4%, T2: 71±2% od osnove). Kvantitativna analiza posnetkov magnetnorezonančnega slikanja s kontrastnim ojačanjem je pokazala, da se je intenzivnost signala pomembno povečala pri vseh bolnikih s Sjögrenovim sindromom. Celo pri 4 bolnikih, pri katerih magnetnoresonačno slikanje ni odkrilo morfoloških sprememb, je bila intenzivnost signala pri slikanju s kontrastnim ojačanjem bistveno večja. (34±3% proti 17±3%;p<.05). Stopnja morfoloških sprememb je bila sorazmerna s kvantitativnimi podatki. Občutljivost magnetnoresonančnih posnetkov je bila 85 %, specifičnost pa 100 %.

Zaključki. S paramagnetnimi kontrastnimi sredstvi je mogoče zbrati več diagnostično pomembnih podatkov, zlasti pri bolnikih brez očitnih morfoloških sprememb, kar je razvidno iz bistveno močneje kontrastno ojačanega tkiva kot pri zdravih osebah. Stopnja morfoloških sprememb je v sorazmerju s kvantitativnimi podatki, zbranimi iz kontrastno ojačanih magnetnoresonančnih posnetkov, ne pa tudi s podatki iz nativnih magnetnoresonančnih posnetkov.

Radiol Oncol 1999; 33(2): 111-7.

Pozitronska emisijska tomografija (PET) v klinični praksi

Klutmann S, Bohuslavizki KH, Brenner W, Kröger S, Bleckmann C, Mester J, Henze E, Clausen M

Izhodišča. Pozitronska emisijska tomografija (PET) je neinvazivna slikovna diagnostična metoda, pri kateri uporabljamo izotope, ki sevajo pozitrone. Ti izotopi so vezani na biološke prenašalce in z njimi in vivo ocenjujemo metabolizem. Najbolj pogost radiofarmak, ki ga pri tej preiskavi uporabljamo, je s F-18 označen analog glukoze imenovan fluor-18-fluorodeoksiglukoza (F-18-FDG).

Zaradi omejenih finančnih sredstev, ki jih namenjajo državni proračuni za zdravstvo in zaradi visoke cene PET raziskav se je pokazala potreba, da bi natančneje opredelili indikacije za PET preiskave v klinični praksi. Tako je bila v nekaj zadnjih letih organizirana vrsta strokovnih sestankov, da bi določili, kakšno mesto imajo PET preiskave pri različnih boleznih v nevrologiji, kardiologiji in onkologiji. Nastali so seznami indikacij za PET preiskave v klinični praksi, ki so koristni tako za specialiste nuklearne medicine kot za specialiste drugih področij, kjer potrebujejo to diagnostično metodo.

Zaključki. Zaradi racionalizacije dela v klinični praksi sezname indikacij za PET preiskave že uporabljajo državne zdravstvene inštitucije in zavarovalnice pri odločitvi o refinanciranju omenjenih preiskav pri posameznem bolniku.

Radiol Oncol 1999; 33(2): 119-26.

Ocenjevanje ledvične funkcije iz očistka endogenega kreatinina in renografijo z ^{131}I-hipuranom pri onkoloških bolnikih pred kemoterapijo

Štabuc B, Hajdinjak T, Cizej TE

Izhodišča. Koncentracija kreatinina v serumu in očistek endgenega kreatinina (CrCl) sta merili, ki se pogosto uporabljata za oceno ledvične funkcije pred predpisovanjem nefrotoksične kemoterapije. V raziskavi smo občutljivost in specifičnost ocene glomerularne filtracije brez zbiranja urina z uporabo Cockroft-Gaultove formule iz koncentracije serumskega kreatinina (CrCo) primerjali z določitvijo očistka hipurana (HC) z renografijo.
Bolniki in metode. Vključenih je bilo 47 bolnikov, starih od 27 do 73 let. Pri vseh smo simultano določili koncentracijo kreatinina v serumu, CrCl, CrCo in HC pred zdravljenjem s kombinirano kemoterapijo s cisplatinom (CDDP) in pri 31 bolnikih še pred tretjim krogom kemoterapije. Koncentracije kreatinina v serumu in urinu smo določali z automatiziranim biokemijskim analizatorjem Hitachi 911. CrCl smo izračunali iz pretoka urina in razmerja med koncentracijo kreatinina v serumu in urinu ter standardizirali glede na telesno površino. Iz vrednosti serumske koncentracije kreatinina smo ocenili CrCo z uporabo Cockroft-Gaultove formule. HC smo ocenili z renografijo z ^{131}I-hipuranom in rezultate ovrednotili glede na normalne vrednosti Oddelka za nuklearno medicino, upoštevaje starost preiskovancev. Za statistično analizo smo uporabljali določitev korelacijskih koeficientov po Pearsonu in t-test s 95% intervalom zaupanja.
Rezultati. Občutljivost koncentracije kreatinina v serumu, CrCo in HC za napoved znižanega CrCl (<78 mL/min/1.73m^2) je bila 41%, 68% in 46%, specifičnost pa 95%, 71% in 76%. Vrednost CoCr za oceno zni_anega CrCl (občutljivost) je bila statistično značilno boljša (p=0.03) od HC. Vrednost CoCr za napoved normalnega CrCl (specifičnost) se ni statistično pomembno razlikovala od HC (p=0.3).
Zaključki. CrCo in/ali HC ne moreta nadomestiti CrCl za ocenjevanje GFR pri bolnikih, zdravljenih z nefrotoksično kemoterapijo.

Radiol Oncol 1999; 33(2): 127-36.

Napovedni testi za izid zdravljenja tumorjev po kemo in radioterapiji

Čemažar M

Predstavljeni so testi za napovedovanje izida zdravljenja tumorjev po kemo- in radioterapiji in poškodb normalnih tkiv po radioterapiji. Pregled je osredotočen na tiste teste, pri katerih je treba celice gojiti v pogojih *in vitro*. Poleg tega članek obravnava tudi korelacijo med napovednimi testi in izidom zdravljenja različnih vrst raka ter poškodbami normalnih tkiv.

Radiol Oncol 1999; 33(2): 169-76.

Radiol Oncol 1999; 33(2): 137-42.

Biokemično dokazan recidiv karcinoma prostate stadij T3N0 z ali brez adjuvantne radioterapije

Lee RJ, Middleton AWJr, Schaeffer CS, Middleton GW, Sause WT

Izhodišča. Bolniki, pri katerih žlezni karcinom prostate prerašča ovojnico prostate ali pa zajema semenjak (vesiculo seminalis), imajo slabšo prognozo. ätevilne študije so obravnavale učinek adjuvantne radioterapije na klinični izhod bolezni, toda redke so proučevale vpliv takšnega zdravljenja na biokemične znake bolezni, (kot je koncentracija prostata specifičnega antigena - PSA), ki kažejo na ponovitev in razsoj bolezni.

Metode. Naredili smo retrospektivno analizo 100 bolnikov s karcinomom prostate, kjer je karcinom preraščal ovojnico ali pa zjemal semenjak (stadij T3). Pri vseh je bila narejena prostatektomija. 31 bolnikov je bilo nato zdravljenih še z obsevanjem ležišča prostate, 69 bolnikov pa ni bilo obsevanih. Bolniki so bili obsevani glede na odločitev zdravnika. Neugodni napovedni dejavniki niso bili enakomerno razporejeni v obeh skupinah , več jih je bilo v skupini z radioterapijo. Povprečna doba sledenja je bila 60 mesecev.

Rezultati. 5- in 10-letno preživetje brez biokemičnih znakov bolezni je bilo pri bolnikih z radioterapijo 64% in 31%, pri bolnikih brez radioterapije pa podobno 55% in 30% (p = 0,76). Edina razlika, ki smo jo našli med obema skupinama bolnikov, je bila, da so imeli bolniki z adjuvantno radioterapijo zančilno boljšo lokalno kontrolo bolezni, ki je bila po 10 letih 95%, pri bolnikih brez radioterapije pa le 65% (p = 0,03). Ko smo primerjali samo bolnike, ki so imeli s karcinomom zajete semenjake, smo videli, da se je enako pogosto javljal biokemično dokazan recidiv ne glede na radioterapijo. Bolniki niso imeli okvarjene potence, če je bila narejena prostatektomija ob ohranitvi živcev, tudi če so bili kasneje obsevani.

Zaključki. Adjuvantna radioterapija po prostatektomiji pri bolnikih s karcinomom prostate T3 značilno zmanjša klinični lokalni recidiv bolezni, toda ne vpliva na biokemično dokazan recidiv in na celokupno preživetje. Učinek adjuvantne radioterapije bi bilo potrebno dokazati s kliničnimi randomiziranimi študijami.

Radiol Oncol 1999; 33(2): 143-51.

Katepsin H pri ploščatoceličnem karcinomu glave in vratu

Strojan P, Budihna M, Šmid L, Svetic B, Vrhovec I, Kos J, Škrk J

Izhodišča. Oceniti napovedno vrednost katepsina H (KH) pri bolnikih s ploščatoceličnim karcinomu glave in vratu (PCKGV).

Material in metode. Koncentracijo KH smo izmerili v vzorvic seruma 35 bolnikov, odvzetih ob operaciji (serum št.1) in 7 - 407 dni (mediana 55 dni) po zdravljenju (serum št.2). Uporabljali smo kvantitativni encimskoimunski test (ELISA; KRKA d.d., Novo mesto, Slovenija). Koncentracija KH, ki so jo v serumu 30 zdravih prostovoljcev izmerili Kos in sod. (Clin Cancer Res, 1997), je služila kot kontrola. Napovedno vrednost serumske koncentracije KH smo primerjali z izsledki raziskave Budihne in sod. (Biol Chem Hoppe-Seyler, 1996) o napovedni vrednosti tkivne koncentracije KH. Za to priložnost je bilo preživetje bolnikov iz omenjenega poročila ponovno evaluirano.

Rezultati. V serumu št.1 smo izmerili statistično značilno višjo koncentracijo KH kot v serumu št.2 (8.9 vs. 8.0 ng/mls, P=0.04) oz. v serumu zdravih prostovoljcev (8.9 vs. 4.9 ng/mls, P<0.0001). Koncentracija KH v serumu št.1 je bila statistično mejno odvisna od histopatološkega gradusa (G_{1+2} vs. G_3, 9.1 vs. 5.0 ng/mls, P=0.06), medtem ko povezanosti z drugimi uveljavljenimi napovednimi kazalci oz. pojavom ponovitve/razsoja bolezni nismo zasledili. Čas vzorčenja seruma št.2 ni vplival na koncentracijo KH v teh vzorcih. Nagnjenost k boljšemu preživetju bolnikov pri višjih koncentracijah KH v serumu št.1 smo opazili tako pri analizi preživetja brez znakov bolezni (PBB) kot tudi pri analizi za bolezen specifičnega preživetja (BSP). Največja razlika v deležu preživelih med bolniki z nizko oz. visoko vsebnostjo KH je bila zabeležena pri razmejitveni koncentraciji 10.7 ng/mls (PBB: 60 vs. 89%, P=0.13; BSP: 65 vs. 86%, P=0.25). Rezultati, ki se nanašajo na tkivne koncentracije KH so istosmiselni, pri čemer smo največjo razliko med skupinama z nizko oz. visoko koncentracijo KH izračunali pri razmejitveni koncentraciji 720 ng/mgp (PBB: 14 vs. 48%, P=0.04; BSP: 23 vs. 60%, P=0.27).

Zaključki. Naši rezultati posredno dokazujejo specifično vlogo KH v procesih invazije in zasevanja pri PCKGV. Pri tej vrsti raka bi serumska in zlasti tkivna koncentracija encima lahko imeli tudi napovedno vrednost.

Radiol Oncol 1999; 33(2): 153-7.

Kemični radioprotektor (WR-2721) pri bolnikih s karcinomom glave in vratu

Wagner W, Radmard A, Boyomo AB

Izhodišča in metode. Da bi zaščitili zdravo tkivo ustne votline in žlez slinavk, smo 36 bolnikom z napredovalim rakom glave in vratu 10-15 minut pred obsevanjem intravensko injicirali 200mg/m^2 amifostina. Bolnike smo postoperativno obsevali do skupne doze 60 Gy, z dozami po 2 Gy v 30 dneh. Stranske učinke radioterapije smo ovrednotili po merilih WHO.
Rezultati. 10 bolnikov je imelo mucositis I. stopnje, 26 bolnikov pa mucositis II. stopnje. Disfagijo I.stopnje smo opazili pri 10 in II.stopnje pri 12 bolnikih. Kožno reakcijo I.stopnje smo zabeležili pri 9, II.stopnje pa pri 13 bolnikih. Toksične sopojave amifostina smo opazili pri 12 bolnikih: hipotenzijo I.stopnje in slabost I.stopnje pri 3 bolnikih, bruhanje I.stopnje pri 3, II.stopnje pa pri 1 bolniku.
Zaključki. Na osnovi naših podtakov in podatkov iz literature zaključujemo, da je uporaba amifostina kot radioprotektorja izvedljiva, in da amifostine ščiti zdrava tkiva pri obsevanju tumorjev glave in vratu.

Radiol Oncol 1999; 33(2): 159-62.

Kirurško zdravljenje raka na orofarinksu z ohranjenim larinksom

Balatoni Z, Élō J, Kótai Z

Izhodišča in metode. V retrospektivni raziskavi smo poskušali z onkološkega in funcionlanega vidika oceniti rezultate zdravljenja 61 bolnikov z napredovalim rakom na orofarinksu, ki so že prestali razširjeno resekcijo tumorja kot primarno zdravljenje ali pa kot reševalno terapijo.
Rezultati. Čeprav je večina rakov na orofarinksu zajemala tudi koren jezika ali se je razširila do stranske stene hipofarinksa, nismo naredili totalne laringektomije. V 5 primerih se je tumor vrasel v območje epiglotisne valekule ali faringoepiglotisa, zato je bila potrebna supraglotisna laringektomija. Po obsežni resekciji tumorja smo pri vseh bolnikih opravili rekonstrukcijo z režnjem, pri kateri smo najbolj pogosto uporabili reženj velike pektoralne mišice. Preživetje je bilo po 1 letu 75%, v času od 2 do 5 let pa je znašalo 31%, vendar s precej pogosto zgodnjo ponovitvijo bolezni. Pri enem bolniku nismo mogli odstraniti nazogastrične sonde, drugmu pa smo zaradi obsežnega in trdovratnega edema lahko odstranili kanilo šele po pooperativnem obsevanju.
Zaključki. V tej seriji bolnikov smo glede funkcionalnosti dosegli zadovoljive rezultate. V večini primerov smo pri bolnikih ohranili funkcionalnost farinksa in sposobnost govora.

Radiol Oncol 1999; 33(2): 163-8.

Z večlistnim kolimatorjem modulirana jakost polj v radioterapiji

Curtin-Savard A, Parker W, Vuong T, Podgoršak EB

V radioterapiji uravnavamo enakomerno visoke doze v tarči z modulirano jakostjo polj, pri čemer potrebujemo le majhno število prostorsko moduliranih sevalnih polj. S to tehniko lažje ohranimo normalno tkivo kot z običajnim nemoduliranim obsevanjem. Po modulaciji z večlistnim kolimaterjem fizik, dozimetrist in radioterapievt lažje in z manj manipuliranja z aparaturami dosežejo zaželeno porazdelitev polja kot z običajnim fizičnim kompenzatorjem. Članek opisuje pred kratkim vpeljano tehniko modulacije jakosti sevalnega polja s pomočjo večlistnega kolimatorja na Univerzi McGill v Montréalu.

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.

Radiotherapy

June 6- 10, 1999

ESTRO course on Imaging for Target Volume Determination in Radiotherapy, will take place in York, UK.

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

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Diagnostic radiology

June 7-9, 1999

The ESO training course will take place in Moscow, Russia.

Contact ESO Office for Russia and Community of Independent States, Blokhin Cancer Research Centre, L. Demidov, Kashirskoye shosse 24, 115478 Moscow, Russia; or call +70 95 3241184/3241504; or fax +70 95 3241504

Breast cancer

June 15-16, 1999

The ESO training course "Breast Reconstructive and Cancer Surgery" will take place in Milan, Italy.

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

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Oncology through the ages

June 16-19, 1999

The International Conference on "Oncology Through The Ages: Historical, Philosophical and Ethical Aspects" will take place in Olympia, Greece.

Contact ESO office for Balkans and Middle East, N. Pavlidis, E. Andreopoulou Medical School, Department of Medical Oncology, University Hospital of Ioannina, 45110 Ioannina, Greece; or call +30 651 99394 or +30 953 91083; or fax +30 651 97505; or contact Amphitrion Congresses, 7 Sygrou Avenue, 11743 Athens, Greece, or call +30 1 9249701; or fax +30 1 9249836; or e mail congress@amphitron.gr

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Breast cancer

June 17-19, 1999

The "1st Milan Breast Cancer conference" will be offered in Milan, Italy.

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

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Lung cancer

June 17-19, 1999

The 1st International Workshop on Lung Cancer will take place in Athens, Greece.

Contact Ginis Vacances S.A., 23-25 Ermou Str., Athens 10563, Greece; or call +30 1 3250 401 / 3241 217; or fax +30 1 3237 703; or e-mail gins@travelling.gr

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Radiotherapy

June 20-24, 1999

ESTRO course of Conformal Radiotherapy in Practice will take place in Amsterdam, Netherlands.

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

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Radiophysics

July 21-23, 1999

DOSGEL '99 1st international Workshop on Radiotherapy Gel Dosimetry will be held in Lexington, KY, USA

Contact DOSGEL '99 Workshop Secretariat, Att.

Clive Baldock, Centre for Medical and Health Physics, Queensland University of Technology, GPO Box 2434, Brisbane Q 4001, Australia; or fax +61 7 3864 1521; or e-mail c.badlock@qut.edu.au; or see internet http://mednet.qut.edu.au/gels/

Radiotherapy

July 26-27, 1999

International Symposium on Radiation-Induced Lung Damage, will take place in Dresden, Germany

Contact W. Dörr, M. Baumann, Th. Hermann, Klinik und Poliklinik für Sthralentherapie und Radioonkologie Medizinische Fakultät Carl Gustav Carus, Fetscherstrasse 74, D- 01307, Dresden, Germany; or call +49 351 458 3373; or fax +49 351 458 4339; or e-mail doerr@rcs.utz.tu-dresden.de

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Radiophysics

August 29 - September 2, 1999

ESTRO course on Physics for Clinical Radiotherapy, will be held in Leuven, Belgium.

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

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Oncological therapy

September, 1999

The ESO training course "Locoregional Therapy and drug Targeting" will take place in Athens, Greece.

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

Lung cancer

September 1-4, 1999

6th Central European Lung Cancer Congress will take place in Budapest, Hungary

Contact Coopcongres, P.O. Box 434, H- 1371 Budapest 5, Hungary; or call +36 1 209 4876; or fax +36 1 466 9051

Histopathology

September 1-5, 1999

The ESO training course "Theoretical and Diagnostic Histopathology-PCTDH (Part II), Systematic Pathology: A" will take place in Alexandroupolis.

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

Lung cancer

September 2-5, 1999

The International Conference on Controversies in the Management of Lung Cancer will take place in Halkidiki, Greece.

Contact Forum International Congress Organizers, 18 Mitropoleos str. GR-546 24 Thessaloniki, Greece; or call +30 31 257128 / 243588; or fax +30 31 231849; or e-mail gsamaras@med.auth.gr; or see internet http:// www.med.auth.gr/conf/cancer/eng

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Biological therapy

September 10-11, 1999

The ESO training course "Biological Therapy - News on Cytokines, Monoclonal Antibodies and Genetic Vaccines" will take place in Vienna, Austria.

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

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Oncology

September 12-16, 1999.

The ESTRO 18 / ECCO 10 Congress will be offered in Vienna, Austria.

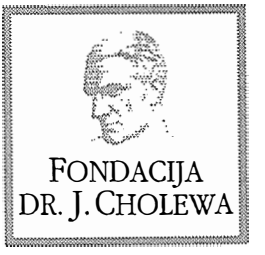
Contact the FECS office, av. E. Mainier 83, B-1200 Brussels, Belgium; or call +32 2 775 02 01; or fax: +32 2 775 02 00

Melanoma

September 20-21, 1999

The ESO training course will take place in Budapest, Hungary.

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



FONDACIJA DOC. DR. J. CHOLEWE

v skladu s pravilnikom za vzpodbujanje študija in znanstveno raziskovalnega dela za leto 1999, razpisuje:

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2. **denarne podpore za znanstveno raziskovalno delo s področja onkologije.**

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Prijave s kratkim opisom predvidenega študija ali raziskovalnega dela, skupaj z življenjepisom in bibliografijo, pošljite do 20. junija 1999 na naslov:

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Prosimo vas, da vlogi priložite soglasje ustanove v kateri delate in ustanove katero nameravate obiskati.
Dodatne informacije dobite na telefon 322-861 vsak torek in četrtek med 12.00 in 13.00 uro.
Kandidati bodo o izboru pisno obveščeni 60 dni po zaključenem razpisu.

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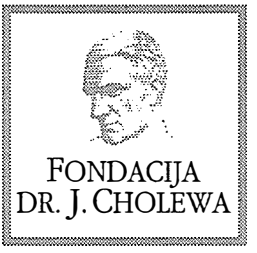

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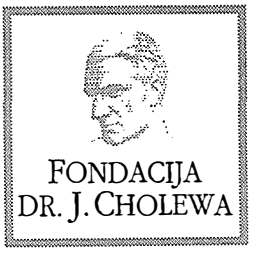


Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education - A Report for the Second Quarter of 1999

In the second quarter of 1999 the Foundation continued with its previously outlined activity. As the expected economic outlook for the Republic of Slovenia is remaining more or less stable, the Foundation is ever more increasing its efforts in informing and educating would-be sponsors about its goals and already accomplished results. The financial situation of the Foundation is continuing to be sound, despite larger expenses that occured in 1998, in comparison to 1997. Almost all of the expenses in the past year have already been planned in the programme for 1998, thanks to the efforts of the Scientific Council of the Foundation. It should also be stressed that on several occasions the Executive Council of the Foundation efficiently assured the smoothness in the day to day affairs during the whole of 1998.

In the recently unveiled financial report for the 1998 a clearcut presentation of the expenses was given. In the past year the Foundation co-sponsored the „1st Congress of Slovenian Surgeons" in Maribor, Slovenia, the „Seminar on the Diagnostics of Malignant Lymphomas", organized by the Institute of Oncology in Ljubljana, Slovenia, the „Breast Cancer School", organized by the Institute of Oncology in Ljubljana, and the meeting of „Europa Donna" Association in Ljubljana. It also co-sponsored the already traditional and established „Hepato-Biliary School" in Ljubljana, as well as two „Oncological Weekend" meetings that took place in Slovenia. It co-sponsored grants to five physicians in order to advance their studies in various reputed oncology centers around the world, as well as congress registration fees and other expenses to several applicants from Slovenia.

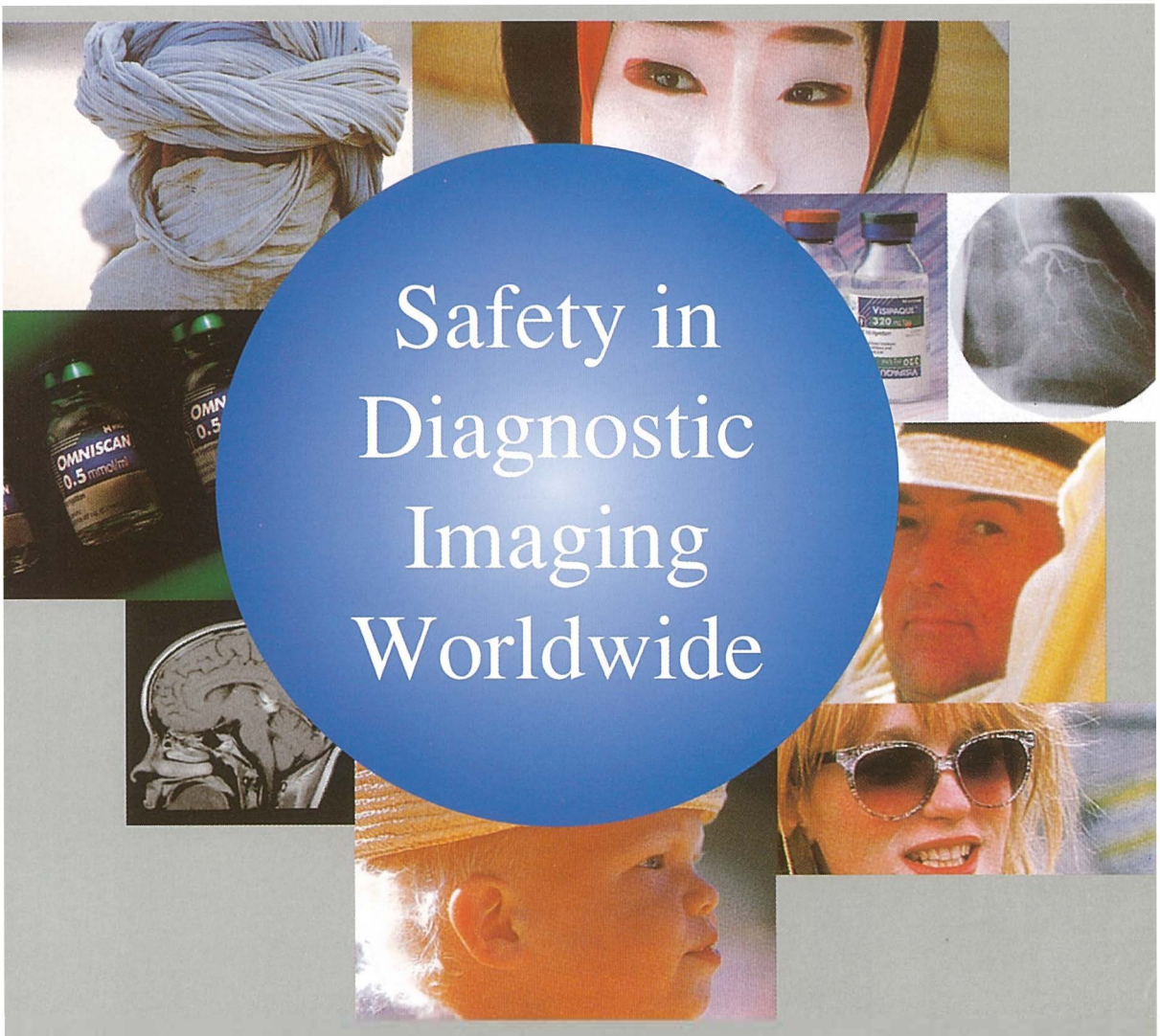
The Foundation continues to support the regular publication of „Radiology and Oncology" scientific journal. This journal is gaining in international importance, with its impact being noted more and more frequently. The Foundation also supports the regular publication of the „Challenge ESO Newsletter", a newsletter of the European School of Oncology from Milan, Italy, which is being distributed worldwide free of charge to physicians interested in the problems of cancer in countries with limited resources. Both these journals are being edited and published in Ljubljana, Slovenia.

For the first time the Foundation sponsored a grant to a physician from an important regional hospital to study closely some of the problems associated with surgical oncology for one month at the Institute of Oncology in Ljubljana, Slovenia. This seems to be a practical and efficient approach to foster an intellectual exchange of the existing and anticipated knowledge in the field of oncology in this country. The Foundation will continue to support this new and practical approach, together with the sponsoring of the regional professional meetings within Slovenia that will probably take place in the near future.

In the future the Foundation also plans to continue its cooperation with the „Mali Vitez" Foundation. It plans to strongly enhance the cooperation with „Slovenian Association for Fight Against Cancer", especially in its efforts, together with government authorities and other agencies, to help in the establishment of adequate and efficient primary prevention and early cancer diagnostics infrastructure. In this setting, the training and adequate preparation of personnel involved in this activity is unquestionably of paramount importance.

The Foundation plans to continue with its outlined activity. It is becoming increasingly efficient, especially after it was successfully registered according to the new goverment regulations. Together with the administrative help provided recently, this will help it in achieving its stated goals.

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



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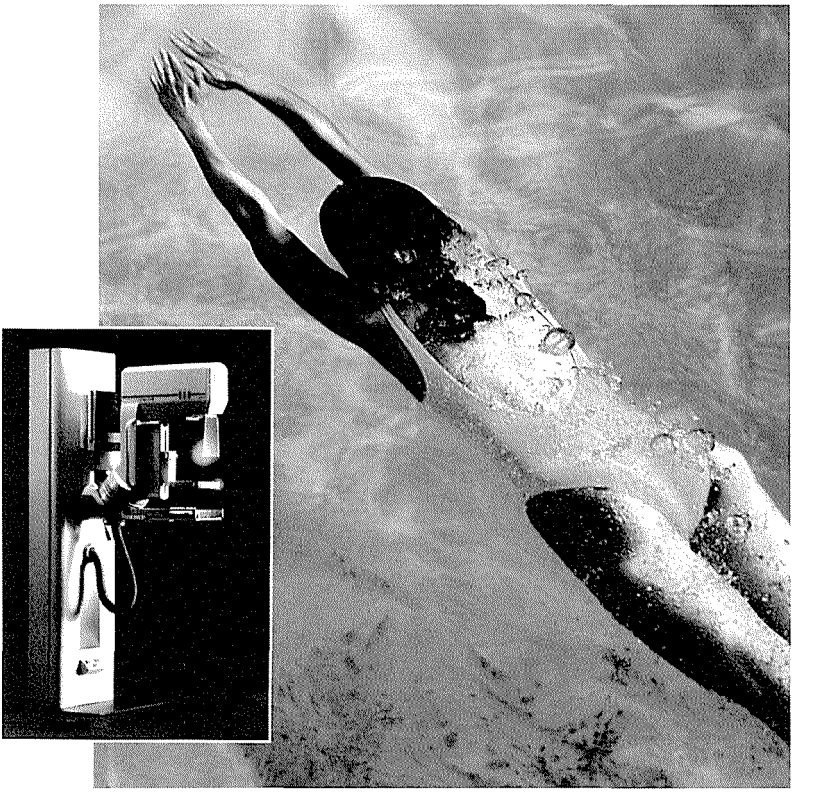

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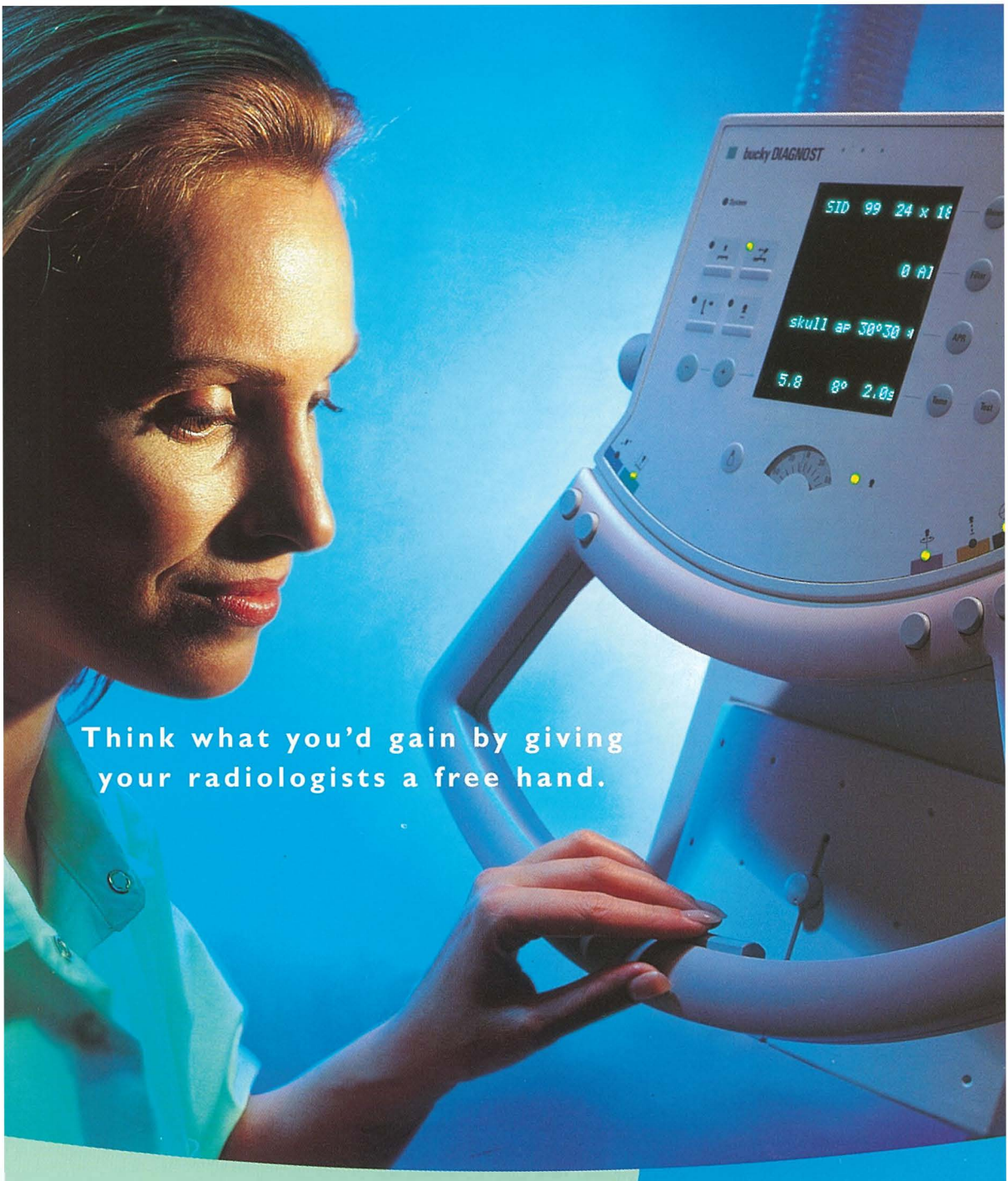

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