RADIOLOGY AND ONCOLOGY

December 2001
Vol. 35 No. 4
Ljubljana

ISSN 1318-2099

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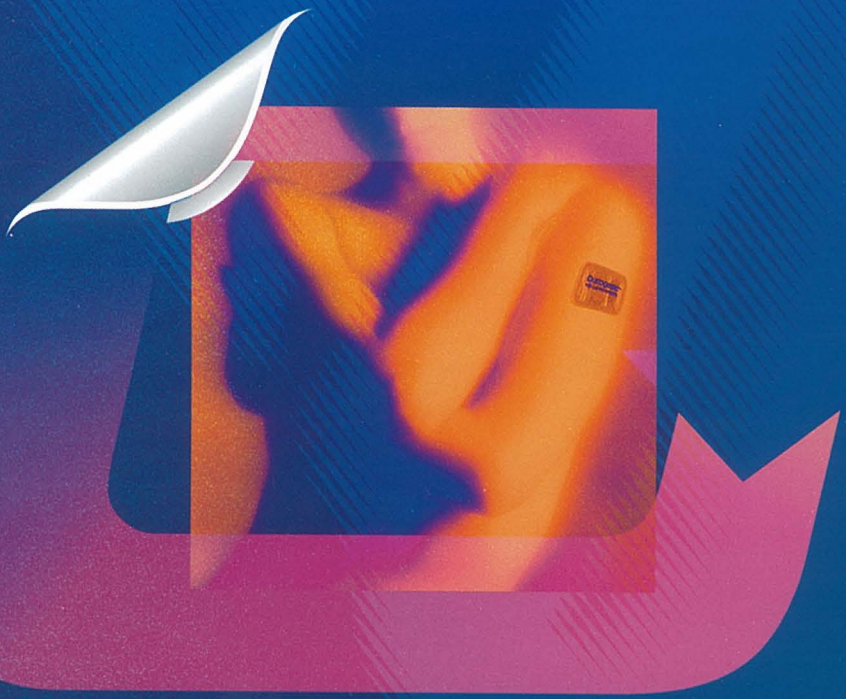

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RADIOLOGY AND ONCOLOGY

Editorial office
Radiology and Oncology
Institute of Oncology
Zaloška 2
SI-1000 Ljubljana
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December 2001
Vol. 35 No. 4
Pages 237-334
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.

Publisher
Association of Radiology and Oncology

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

Reader for English
Mojca Čakš

Key words
Eva Klemenčič

Secretaries
Milica Harisch
Mira Klemenčič

Design
Monika Fink-Serša

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

Published quarterly in 700 copies

Bank account number 02010-0090006751
Foreign currency account number
010-7160-900067/4
NLB d.d., Podružnica Ljubljana Center, Ljubljana
SWIFT: LJBASI2X

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

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

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

This journal is printed on acid-free paper

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

RADIOLOGY AND ONCOLOGY

Ljubljana, Slovenia
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Diagnostic value of pneumoperitoneum on plain abdominal film

Marija Frković, Tajana Klapan, Ines Moscatello, Marijan Frković

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Background. *Pneumoperitoneum is the presence of air outside the gut lumen as the hallmark of alimentary tract perforation. It can be spontaneous or traumatic in origin. The most frequent cause of spontaneous pneumoperitoneum is the perforation of gastric or duodenal ulcer and the aim of the study was to assess the diagnostic value of pneumoperitoneum on plain abdominal film.*

Patients and methods. *This is a retrospective study based on the diagnostic value of pneumoperitoneum on plain abdominal film, with the patient in upright, supine and sometimes left lateral decubitus position. The study included 79 patients who were admitted to our hospital during a 2-year period of time (1998-1999) and operated on for perforated gastroduodenal ulcer.*

Results. *Ten (12.66 %) of 79 patients underwent operation without radiological procedure. Sixty-nine (87.34 %) patients were examined radiographically and 53 (76.81 %) of them had signs of pneumoperitoneum initially on the plain film.*

Conclusions. *The most common cause of pneumoperitoneum was perforated duodenal ulcer in elderly male patients. The most frequent sign of pneumoperitoneum was the crescent shaped free air beneath the diaphragm.*

Key words: peptic ulcer perforation; pneumoperitoneum – radiography

Introduction

Pneumoperitoneum is the presence of air outside the gut lumen as the hallmark of alimentary tract perforation on plain film.[1] It can be spontaneous or traumatic in origin. The most frequent cause of spontaneous pneumoperitoneum is the perforation of gastric or duodenal ulcer[2] and that is the reason why we included these patients in our study. The aim of the study was to assess the diagnostic value of pneumoperitoneum on plain abdominal film.

The traditional sign of pneumoperitoneum is the crescent shaped free air beneath the diaphragm on erect chest seen on abdominal plain film. In this position, it is possible to detect as little as 1 to 2 ml of free air.[1,3]

The signs of pneumoperitoneum on supine abdominal film are:

Received 25 September 2001
Accepted: 6 October 2001

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- "the dog's cap sign" – air in Morison's pouch,[4]
- free air occurring subhepatically inferior and anterior to the liver as a linear collection of air parallel to the lower edge of liver,[5]
- the falciform ligament sign as a vertically oriented soft-tissue band parallel to the right border of the spine in the region of the thoracolumbar juncture,[6]
- "Wind's sign" or "lucent liver sign",[1]
- "the dome sign" – free air trapped under the middle part of the diaphragm,[7]
- "the double wall sign" or Riegler sign – when there is air in the peritoneal cavity and in the bowel lumen, the mucosal and serosal surfaces are simultaneously outlined,[1]
- "the sign of triangle" – the air between the loops of bowel,[7]
- "the football sign" – the air-distended peritoneum beneath the anterior abdominal wall,[1]
- and finally, rare signs of pneumoperitoneum, like visible lateral umbilical ligaments or urachus, free air in an inguinal or femoral hernia sac and pneumoscrotum.[8-10]

Some of the described signs are visible on Figures 1 and 2.

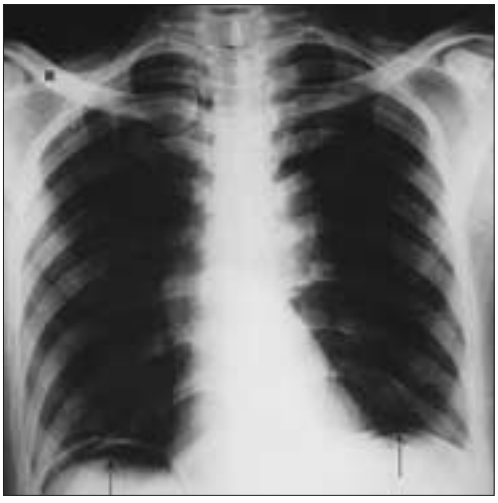


Figure 1. The sign of pneumoperitoneum on erect chest film – crescent shaped free air beneath the diaphragm (arrows).

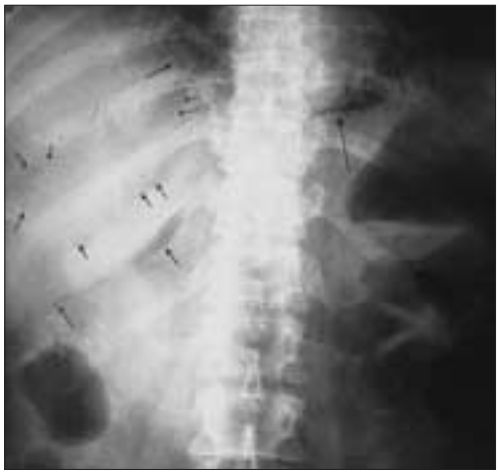


Figure 2. The signs of pneumoperitoneum on supine plain abdominal film (arrows): "Wind's sign" or "lucent liver sign", free air subhepatic as a linear parallel collection, the falciform ligament sign, "the dome sign".

Pneumoperitoneum may be a solitary plain film finding or it may coexist with pneumomediastinum or pneumoretroperitoneum, or both.[1,11,12]

It should be emphasized that there is a condition known as "benign" or "internistical" pneumoperitoneum – spontaneous pneumoperitoneum without peritonitis which usually has no clinical signs[13] and can be diagnosed only by plain abdominal film.[14,15] "Benign pneumoperitoneum" was described with gastric distension,[14.15] jejunal diverticulosis,[16] pneumatosis intestinalis17 and scleroderm,[18,19] and immunosuppressive therapy.[11,15] Such patients are treated by conservative therapy and very rare by surgical operation.[20,21]

The other conditions which can mimic the signs of pneumoperitoneum are: interposition of the colon or Chilaiditi syndrome, fat depositions, artifacts, intraabdominal abscess, intraperitoneal or internal hernia and volvulus, especially of the mobile caecum.

Pneumoperitoneum is usually diagnosed on plain abdominal film with the patient in upright, supine or left lateral position. Also, it

can be diagnosed on erect chest film or using ultrasound (US),[22] or computed tomography (CT).[23]

Patients and methods

During a 2-year period of time (1998-1999), 79 patients were admitted to our hospital and operated on for perforated gastroduodenal ulcers.

The abdominal plain films were taken in 69 (87.34 %) patients preoperatively, with the patients in upright (62 cases), supine (3 cases) and left lateral decubitus position (4 cases)).

In 5 (7.25 %) of 16 patients with suspected ulcer perforation but with normal findings on plain film, the additional contrast-study under diascopic control was performed.

We divided our patients in several groups, according to their age, sex, type of ulcers and signs of pneumoperitoneum.

Results

We reviewed retrospectively the hospital data of 79 patients who were admitted to our hospital in the 2-year period of time from 1998 to 1999 and operated on for perforated gastroduodenal ulcer. The data are given in Table 1.

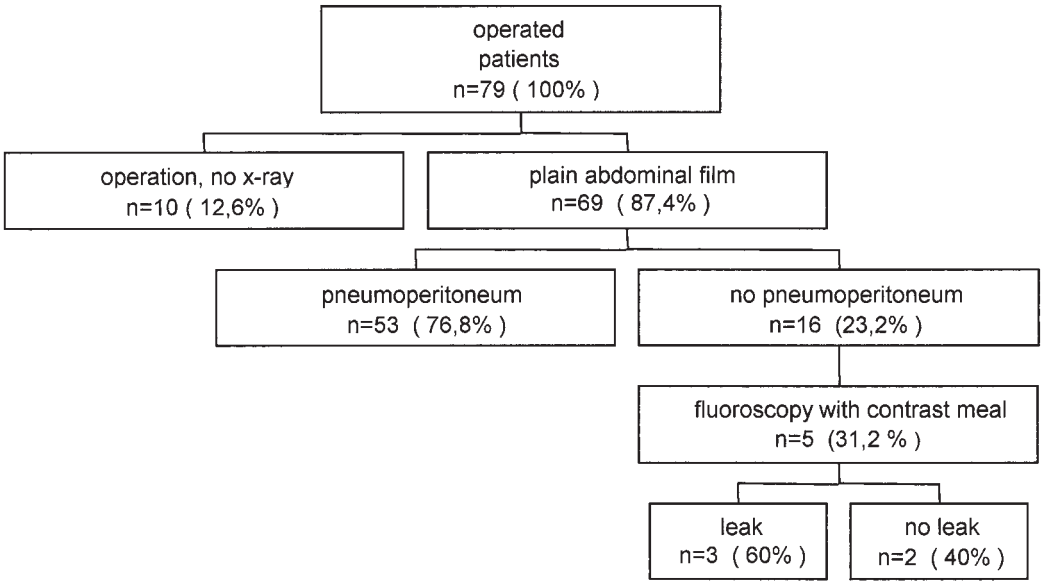
Ten (12.66 %) of them underwent an operation without radiological procedure.

We noticed that men had higher incidence of both ulcers than women, especially duodenal (M:F = 55:24). Also, it was interesting to note that women had equal incidence of both ulcers (Figure 3).

The distribution of pathologic findings, according to the age of patients is shown by linear chart (Figure 4). Apparently, most of the patients were 40 to 60 years old.

The most frequent sign of pneumoperitoneum was the crescent shaped free air beneath the diaphragm (49 cases or 92 %), whereas other known signs of pneumoperitoneum were mentioned and identified very rarely (Figure 5).

Table 1. Operated patients because of perforated gastroduodental ulcers and radiologic examins performed in emergency department



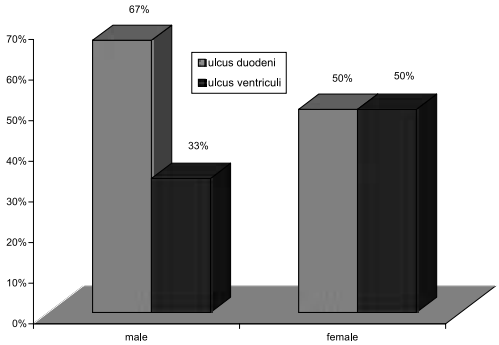


Figure 3. Type of perforated ulcer according to the sex.

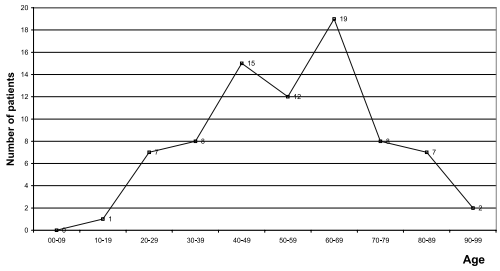


Figure 4. The age incidence of patients with perforated ulcers shown by chart.

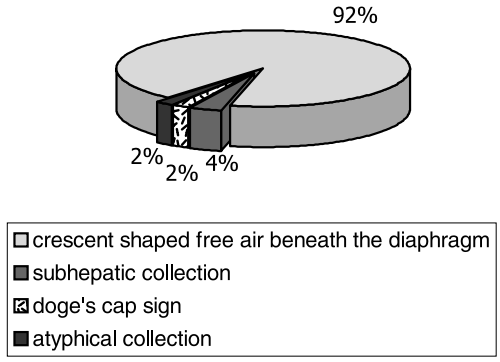


Figure 5. Identified signs of pneumoperitoneum on plain abdominal films graphically.

The other signs were: subhepatic collection (2 cases or 4 %), "doge's cap sign" or free air in Morison's pouch (1 case or 2 %) and atypical collection of intraperitoneal air between the bowel loops (1 case or 2 %).

Discussion

Most of radiologists describe the findings of pneumoperitoneum as positive in 60-85 % of cases.[13,24-26] So, our results are in corellation with their findings.

Also, we have to emphasize that abdominal plain films were analyzed by different radiologists in Emergency Department. Maybe, these results would be better if the plain films were analyzed by radiologists who are subspecialists in gastroenterology. The most frequent sign which we found was crescent shaped free air beneath the diaphragm. We think that the reason why it is so, is probably the fact that most of radiologists (ours and others) prefer that, whenever is possible, the abdominal plain film are made with the patients in upright position. It is the least dependent part of peritoneal cavity and free air can be easily detected. We believe that, if radiologists were able to recognize the signs of pneumoperitoneum, the position of patient would not matter at all. In that case, other signs of pneumoperitoneum, not only the crescent shaped free air beneath the diaphragm (Figure 5), would be recognized and identified on the plain abdominal films.

Since the policy of most surgeons is to recommend surgery in any patient with abdominal symptoms and suspected pneumoperitoneum, it is evident that the plain abdominal films can give valuable information.[24]

Should we perform the plain abdominal film in all patients?

The "board – like" rigidity which generally indicates an abdominal catastrophe needing laparotomy can be found in 83-93 % of these patients.[10] It means that a substantial number of patients with perforated ulcer are referred to roentgen examination even though laparotomy is indicated whatever the radiologic findings. Some proportion of patients with normal plain films operated on directly and without further investigations may support the impression that the plain films were taken in many patients "just to be sure".[24]

Very often, less experienced surgeons work in emergency departments and they need a plain abdominal films as a diagnostic support.

One argument that may support the practice of obtaining X-ray films in nearly all patients is related to the fact that the perforation occurs in elderly patients with often atypical clinical findings.[25,27,28]

Our investigation also confirms this (Figure 4).

The influence of age on a low proportion of pneumoperitoneum in young patients is difficult to explain. Seely[29] and Taylor[30] have suggested that the acute ulcers, which may be more common in young patients, can be expected to heal spontaneously.

Even in centers with unlimited resources, plain films supplemented by gastrointestinal contrast studies as needed, remain the modern standard for evaluation of patients who have suspected gastrointestinal perforation.

These widely available, easy to perform, and relatively inexpensive procedures are relatively sensitive and specific for evaluation of this problem. It is, therefore, crucial for radiologists to be familiar with the often subtle signs of gastrointestinal perforation on plain abdominal films.

Skills of plain film interpretation should not be permitted to erode in the environment of newer technologies.[31]

Although newer technologies like US[22] and CT[23] give possibillity for detection of pneumoperitoneum, they are not routinely used in emergency departments so that the signs of pneumoperitoneum detected by them are significant, but usually an incidental finding.

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Radiol Oncol 2001; 35(4): 243-7.

Can we rely on cancer mortality data? Checking the validity of cervical cancer mortality data for Slovenia

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[1]Epidemiology Unit, [2]Cancer Registry of Slovenia, Institute of Oncology, Ljubljana, Slovenia, [3]Institute of Public Health. Ljubljana, Slovenia

Background. *Valid inference on cervical cancer mortality is very difficult since – on the basis of death certificates – it is not always possible to distinguish between cervix, corpus and unspecified uterine cancer deaths. Our aim was to estimate the extent to which cervical cancer as the official cause of death reflects the true mortality from cervical cancer in Slovenia.*

Material and methods. *The data on 2245 deaths from cervix, corpus uteri, and uterus-unspecified cancers for the period 1985-1999 were linked to the Cancer Registry of Slovenia database from the mortality database of Slovenia.*

Results. *Officially, in the period 1985-1999, there were 878 cervical cancer deaths. The comparison of these causes of death with the cancer sites registered in the Cancer Registry revealed that they include only 87.7 % patients with a previous diagnosis of cervical cancer. Of 650 corpus uteri cancer deaths, 17. 1 % of patients were registered to have cervical cancer, and of 717 unspecified uterine cancer deaths, 31.4 % were registered. Taking into account the correctly identified cervical cancer cases among cervical cancer deaths and misclassified cervical cancer deaths as corpus uteri and unspecified uterine, the corrected number of deaths would be 1106.*

Conclusions. *When evaluating the impact of cervical cancer mortality from national mortality rates, the stated underestimation should be taken into account. However, this does not hold for some other cancers.*

Key words: cervix neoplasms – mortality; death certificates; registries; Slovenia

Received 5 November 2001
Accepted 25 November 2001

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Introduction

In our everyday practice, we often meet clinicians seeking the information on the official cause of death of their patients believing that this source is the most reliable. To prove that this is not always true we present our analysis of the data on cervical cancer mortality in Slovenia. Their validity is of special importance because mortality is the final measure of the effectiveness of cervical cancer screening programs in a country.[1]

It has already been pointed out that a valid inference on cervical cancer mortality is very difficult since – on the basis of death certificates – it is not always possible to distinguish between cervix, corpus and unspecified uterine cancer deaths.[2] To estimate the extent to which cervical cancer as the official cause of death reflects the true mortality from cervical cancer in Slovenia, all causes of death from uterine cancers (ICD 8 codes 180 and 182) were matched with the diagnosis registered in the Cancer Registry of Slovenia (CRS). The CRS has been operating since 1950 and is a unique basis for such epidemiological analyses.[3]

The official crude mortality rate from cervical cancer increased from 12.5/100,000 in 1953 to 16.3/100,000 in 1962. Since then, it was slowly decreasing (Figure 1). In 1968, it was the first time below 10/100,000. In 1997, it was 6.2/100,000 and in 1999, 5.1/100,000.[4,5]

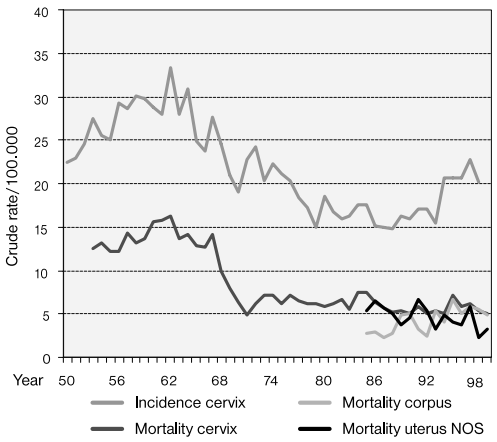

Figure 1. Incidence of cervical cancer and mortality from cervical, corpus and unspecified uterine cancer; 1950-99.

Material and methods

Data on 2245 deaths from cervix, corpus uteri, and uterus-unspecified cancers in the period 1985-1999 were linked to the Registry database from the mortality database of Slovenia.[5] During the calendar period under study, the eighth revision of the International Classification of Diseases (ICD) was used in the CRS, while the ninth and tenth in coding cancer deaths.[6,7,8] The classification of cancer deaths was thus re-coded according to the eighth revision. The linkage was based on PIN. The cause of death was compared to the diagnosis registered in the Registry. In case more than one cancer had been registered, the gynaecological cancer (ICD 8 codes 180-184) was considered as the cancer of interest irrespective of the status of cervical cancer at death of the patient.

Results

Officially, in the period 1985-1999, there were 878 cervical cancer deaths. The comparison of these causes of death with the cancer sites registered in the Cancer Registry revealed that they include only 770 (87.7 %) patients with a previous diagnosis of cervical cancer. But of 650 corpus uteri cancer deaths, there were 111 (17.1 %) patients registered to have cervical cancer and of 717 in whom unspecified uterine cancer was recorded as the cause of death, 225 (31.4 %) were registered to have cervical cancer. Taking into account the correctly identified cervical cancer cases among cervical cancer deaths and misclassified cervical cancer deaths as corpus uteri and un-

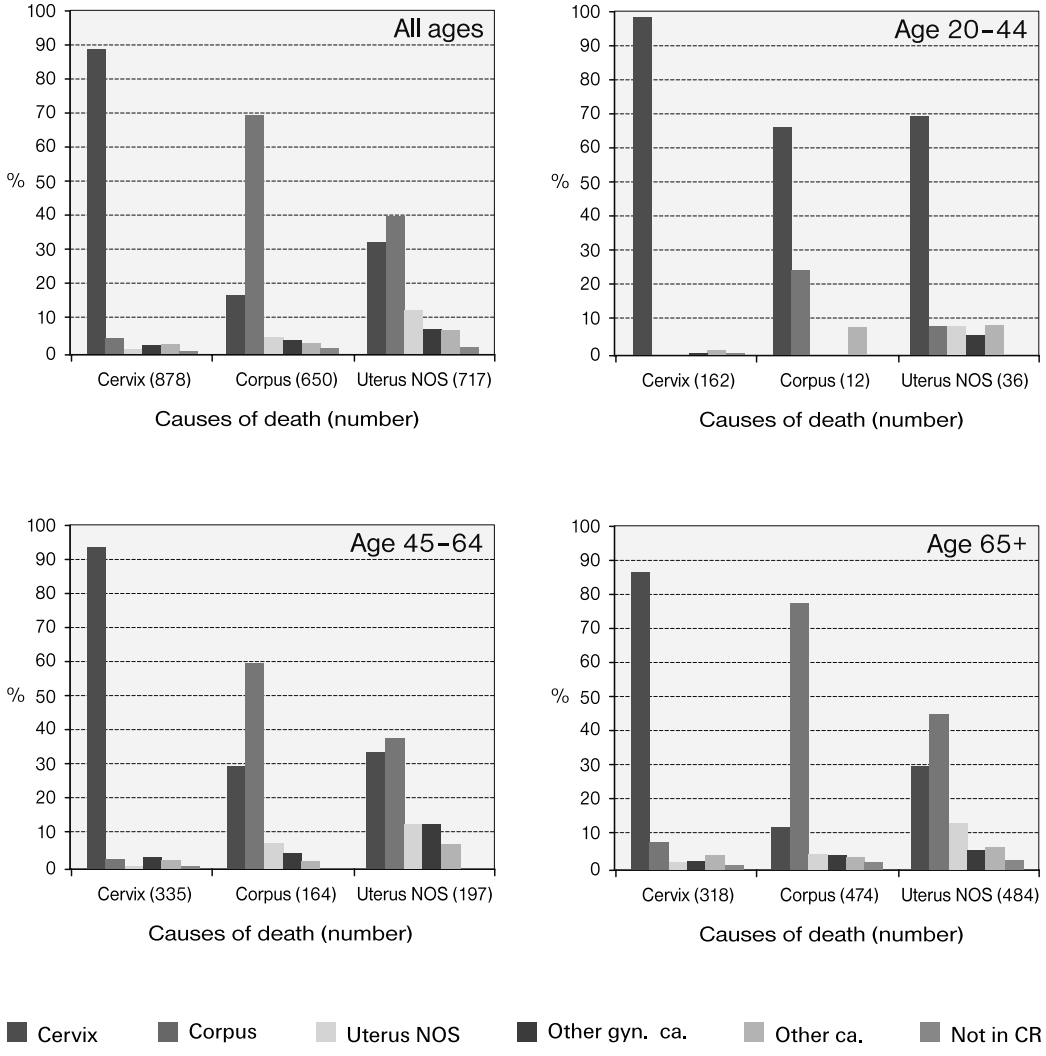

Figure 2. Uterine cancer as official cause of death by subsite, by cancer site registered in CRS, and by age; 1985-99.

specified uterine, the corrected number of deaths would be 1106.

Hence, the official cervical cancer mortality would be underestimated by 26 %. This underestimation differs by age groups. In women, aged 20-44 years, nearly all deaths from cervical cancer (158 of 162) were confirmed at the Registry. However, the death of 33 young patients who had been registered as cervical cancer cases was attributed to the corpus uteri (8) and uterus unspecified cancer (25). The official data on cervical cancer mortality would thus be underestimated by 17 % in this age group, while in the age group 45-64 years and in the oldest one for 23 and 33 %, respectively (Figure 2).

The trend of official and corrected cervical cancer mortality in the period 1985-1999 is presented in Figure 3. The shape of the time trend was not appreciably affected by the underestimation of mortality.

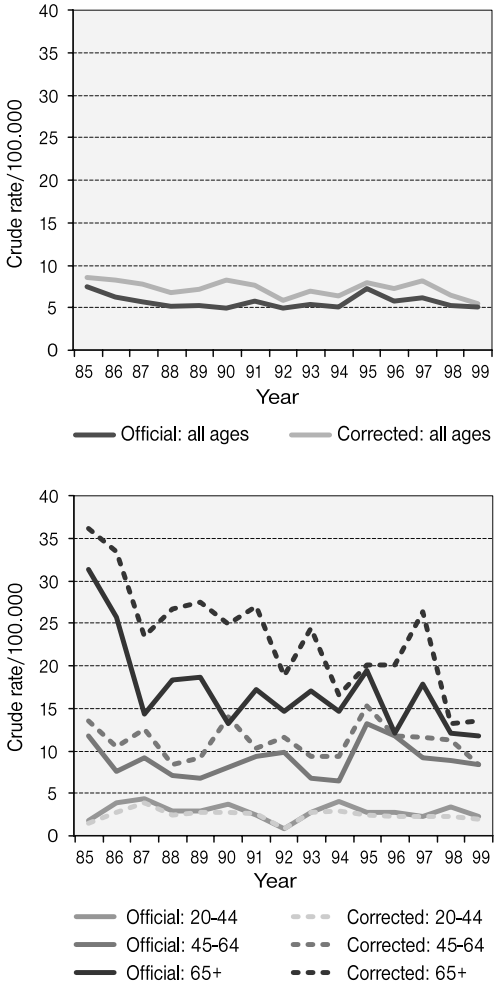

Figure 3. Trends in official and corrected cervical cancer mortality; 1985-99.

Discussion

Though mortality data are available for more countries than incidence data, they have several disadvantages. Death certification is less precise in terms of cause of death than the incidence data recorded by cancer registries.[9,10] In cervical cancer we face also the problem of erroneous cause of death statements and of a varying proportion (20-65 %) of cervix cancer deaths that are coded to "uterus not otherwise specified".[2,11]

Our study revealed that among "uterus not otherwise specified" cancers about a third are attributable to cervical cancer. Also, among cervical cancer deaths not all cancers have been diagnosed as this cancer. After combining the erroneous cause of death statements and inexact coding, the official mortality statistics underestimates cervical cancer deaths for about a quarter.

In mortality statistics the underlying cause of death is coded according to the rules of the International Classification of Diseases currently in use. In most patients who have ever been diagnosed with cancer, this disease is recorded on death certificate as the underlying cause of death. But it depends on the doctor, completing the death certificate, whether cancer site is determined properly and, consequently, properly coded in mortality statistics.

It has been shown that the degree of misclassification varies with cancer site, being greater for those that are more difficult to diagnose.[10] It is well known that the reliability of diagnoses recorded on death certificates depends on the place of death, being more accurate for those who died in hospitals and where the autopsy had been performed.

It was assumed that, in women below the age of 45 years, most deaths from uterine cancers are due to cervical neoplasm; so, many international comparisons take into account all uterine cancer deaths.[2,12,13] Our study provides a numerical estimate for this hypothesis: officially, there were 210 uterine cancer deaths in this age group in the period under study, of which only 192 were identified as cervical cancer cases in the CRS. Older women less often dye in hospitals than younger ones. This could also explain a greater proportion of misclassified cervical cancer deaths in older age group. Namely, in case of the patients who did not dye in the hospital, the physician, certifying death, was not always the family doctor or the doctor who treated the patient, but the one who was

on duty and did not know the medical history of the deceased in details; so, the cause of death may be less precise.

Our study suggests that the extent of misclassifications and improper or less precise coding of death causes have not changed appreciably in the time period observed, and the curve shape of the time trend in cervical cancer mortality was not considerably affected. But it may happen that by improving death certification reliability, changes in mortality from cervical cancer may lead to an apparent increase, as already experienced in some countries.[11]

Conclusion

When evaluating the impact of cervical cancer mortality drawn from national mortality rates, the underestimation as stated above should be taken into account. However, this is not true for some other cancers. In lung cancer, in the CRS during the data cleaning proces, we observe, each year, an overestimation of death officially attributed to lung cancer.

Due to misclassifications described in cancer mortality data and similar deficiencies in the data on suicide and other injuries and poisonings, infant mortality data and maternal mortality, the Institute of Public Health plans to delay the publishing of the official edition on mortality for at least one year. It is hoped that this time will be enough to link mortality data with other databases to render mortality data more reliable. But in monitoring long term trends in mortality this change will have to be taken into account.

Acknowledgement

We appreciate the financial support from the European Commission, contract grant number SI2.305757 (2000 CVG2-029).

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Radiol Oncol 2001; 35(4): 249-54.

Communication after laryngectomy

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Background. *Laryngectomy is the mode of treatment of the patients with advanced laryngeal and hypopharyngeal cancer. It affects many important functions, including speech.*
Patients and methods. *Various alaryngeal speech modes are available so that no laryngectomee should be left without a means of communication.*
Results. *There is a variety of artificial devices, including electronic ones that produce their own battery driven sound. Alternatively, the patient can learn a new form of voicing using a muscular segment of the upper esophagus as a source of sound (esophageal speech). A puncture can be created surgically through the esophageal wall and a prosthesis placed in it to divert pulmonary air into the esophagus and through the same muscular segment to produce sound.*
Conclusions. *Many factors influence the choice of an alternative to be used with a particular patient. In Slovenia, esophageal speech is the most frequently used alaryngeal speech mode.*

Key words: laryngeal neoplasms; laryngectomy; speech, alaryngeal; speech, esophageal

Epidemiology and etiology of laryngeal and hypopharyngeal cancer

Laryngeal and hypopharyngeal cancers are quite common in Slovenia. In 1995 they represented 1.9 % of all new malignant diseases in Slovenia. The incidence of laryngeal cancer was 9.1/100.000 inhabitants in men, and 0.5/100.000 inhabitants in women. The incidence of hypopharyngeal cancer was 4.5/100.000 inhabitants in men, and 0.3/100.000 inhabitants in women. In 55 % of patients with laryngeal cancer and only 12 % of patients with hypopharyngeal cancer, the disease was discovered in a localized stage. In all other patients, the malignant disease was in an advanced stage and required more aggressive treatment.[1]

Laryngeal and hypopharyngeal cancers usually occur in men aged 50-65 years with a long history of tobacco consumption frequently associated with alcohol abuse. The alcohol-related nutritional deficiencies could be involved in the etiology of these cancers.[1,2] As a result, the patients often present with notable co-morbidities. In addition, the socio-

Received 15 October 2001
Accepted 10 November 2001

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cultural level is rather poor in the majority of cases. This particular characteristic of laryngeal and hypopharyngeal cancer patients explains the delay in diagnosis and the problems linked to treatment compliance.

Laryngectomy and its consequences

Laryngectomy is a surgical procedure usually reserved for patients with advanced laryngeal or hypopharyngeal carcinoma or patients who fail radiation treatment.[3] Loosing the larynx means adapting to a living without some basics that characterize us as human. Respiration and speech are altered for ever; swallowing needs to be re-learned; smell and taste are attenuated; lifting, straining and coughing (all of which are dependent on a closed glottis) are compromised. Although there are numerous potential problems (emotional, psychological, physical, economic, social, surgical, and communicative), the inability to speak is considered the greatest of the difficulties the patient is faced by.[4]

Voice restoration after laryngectomy

After the removal of the larynx, the patient no longer has a source of sound for speaking. Currently, there are two categories of sound restoration: alternative "natural" sound sources and mechanical speech aids. The former category utilizes esophageal and tracheoesophageal speech, whereas the latter an electronic artificial larynx.[5]

Esophageal speech (ES)

ES traditionally has been the dominant approach to laryngeal speech rehabilitation. Some retrospective studies demonstrated a range of success from 12 % to 97 %.[6-11] In 1982, Gates *et al*. published the results from the first prospective investigation of ES acquisition, which showed that 26 % of their laryngectomy study group were able to acquire ES.[6] In a more recent prospective study, Hillman *et al*. found that only 6 % of their patients developed usable ES.[12]

ES is produced by compressing the air into the esophagus; the released air vibrates the pharyngeal-esophageal segment and produces the esophageal tone used for speech. The sound produced enters the oral cavity where it is articulated and shaped into words.

Generally, there are three primary methods used to teach esophageal speech: consonant injection, glossopharyngeal press, and inhalation. Regardless the method used, the goals are for the patient to be able to impound rapidly the air into the esophagus, expel it from there in a controlled manner, and produce fluent ES. Esophageal speakers have a much lower air reservoir (less than 100 cm^3) than is available to laryngeal speakers from the lungs (even > 5 litres). The small air supply will limit the esophageal speaker's ability to produce long utterances on a single charge of air.

The advantages of the ES are:

- The sound of ES is more natural and closer to the laryngeal voice.
- ES requires no dependence on mechanical instrument.
- The patient is able to achieve some measure of pitch and loudness control, and good esophageal speakers are able to vary these dynamically during speech.
- Both hands are free during speech.

ES has also some disadvantages:

- ES must be learnt and may take a long time to master it. Some patients may never learn to produce functional ES even after much effort.
- A person's ability to articulate clearly must be good, otherwise the intelligibility of ES may be poor.
- The patient may have difficulty being heard above back-ground noise.[5]

Tracheoesophageal speech (TES)

The tracheoesophageal puncture method, coupled with the use of the voice prosthesis, was introduced by Singer and Bloom in 1980.[13] The surgery may be performed at the time of the laryngectomy (primary procedure), or it may be performed at a later date (secondary procedure). Early studies, focused on carefully selected groups of patients who underwent the insertion of a prosthesis as a secondary procedure, reported success rates ranging from 56% to 93 %.[14-16] More recent studies, which have focused on the insertion of a prosthesis as a primary procedure, have reported acquisition rates ranging from 30 % to 93 %.[17-19]

In this approach, a small, silicone, valved prosthesis is inserted into a surgically created midline tracheoesophageal fistula. The uni-directional valved prosthesis is designed to maintain tract patency and protect against aspiration. The patient can divert pulmonary air from the trachea (by occluding the tracheostoma with a finger) through the prosthesis, thereby creating a sound in the pharyngoesophageal segment. The air pressures required to force open the slit of the valve range between 2 and 25 cm H_2O and depend on the rate of airflow from the lungs and the type of the device used.[20] Some patients may have considerable difficulty producing the pressures. In these cases, a lower resistance prosthesis is suitable. Special valves are available to avoid manual occlusion of the stoma. These valves close automatically when greater than normal thoracic pressures are present as when the patient wishes to produce speech.

There are still contraindications in the selection of patients for the prosthesis insertion: inability to care for the stoma, poor manual dexterity, a stenotic stoma, poor eyesight, esophageal stenosis, and poor patient's motivation.

The advantages of TES are:

- This technique can provide the most rapid restoration of nearly normal speech in most of the laryngectomized patients.
- TES is smooth and fluent because of the availability of pulmonary air.
- Loudness and pitch variation is possible.
- The approach is feasible in most of the laryngectomized patients and is also reversible if so desired.

The disadvantages of TES are:

- The insertion of the voice prosthesis requires another surgical procedure if not done together with the laryngectomy.
- Occasional aspiration due to poorly seated prosthesis, or poorly functioning prosthesis is possible.
- A buildup of candida deposits requires frequent cleaning.
- The functioning period of the prosthesis is limited.[5,21]

Artificial larynx (AL)

Previous reports of AL use among laryngectomy patients vary in many aspects. The estimates of AL use range from 5 % to 66 %.[6,22,23] This device uses electric power to drive a vibrator that provides the sound source. It generates a sound with approximately the same frequency as is the fundamental laryngeal frequency. One type of the device consists of a tube that delivers sound from the vibrator to the mouth. the sound is then articulated in the normal way. Another version consists of a hand held vibrator that is designed to deliver the sound through the skin when placed on the neck. Until recent years, the AL was considered to be the method of choice only for those patients who were unable to learn ES. Clinical experience has demonstrated that AL actually may be helpful in the acquisition of ES. AL may serve as a communication bridge until ES or TES training is initiated. Recent studies from the USA report that a majority of laryngectomees use AL – 55 %.[12]

The advantages of AL:

- It is easy to learn how to use it.

- AL provides adequate volume to be heard in noisy places.
- Volume and pitch control is possible.
- The speech with AL is intelligible when properly used.

Speech with AL has some disadvantages:

- AL has a noisy electronic sound that attracts attention.
- The neck type device cannot be used in the patients with heavily scarred neck.
- Moderate initial purchase cost and occasional additional cost for repair.
- AL requires very clear articulation to assure intelligibility.[5,21]

Characteristics of alaryngeal speech

Fundamental frequency (F0)

Most electronic speech aids have a manually adjustable F0. These are typically set to a low pitch for a male voice (about 100 Hz) and to a higher one for a female voice (about 200 Hz). Some have a variable frequency adjustment.

The F0 of the ES is usually lower than the average laryngeal F0. The results of different studies range from 57 Hz to 136 Hz.[24,25] The reasons for such a variety of the results are probably different measuring instruments used in different speech samples.

The F0 in TES is reported to be closer to normal laryngeal speakers, at least for male speakers, and ranges from 72 Hz to 134 Hz.[24,26] Some authors measured higher F0 in TES than in ES.[27]

Vocal intensity(VI)

The level of VI in users of AL is typical of normal laryngeal speakers during normal conversation or reading, ranging between 75 and 85 dB. The intensity range is somewhat reduced.[28] The intensity of the electronic vibrator is largely determinated by the design of the instrument.

The intensity of ES is usually lower in overall loudness than normal. The range of VI that esophageal speakers are able to produce is much lower than the intensity range of normal speakers (about 10 dB vs. 30 dB).[29]

The intensity of TES appears to be only slightly lower than the levels produced by laryngeal speakers. In some speakers, variation of intensity may be greater than normal.[30]

Temporal characteristics

There are little data on the temporal characteristics of speech with an AL. The few studies available indicate that reading rates are slower when using an AL compared to normal phonation or TES.[28] We might expect longer reading times because of the need for precise articulation to maintain an acceptable level of intelligibility.

In general, the patients using ES read slower than normal laryngeal speakers. Their reading rates are about 60-70 % of the rate of a normal speaker. They spend 30-45 % of their reading time in silence because they need to recharge air supply more frequently. They have a much shorter phonation time, typically less than 6 s, which is no doubt due to a small volume of air in the esophagus.[24,29,30]

Patients using TES also read at a slower rate than normal speakers but faster than esophageal speakers. Their slower rate may be due to difficulties in controlling the pharyngoesophageal segment and the need to articulate precisely. These speakers spend about 10-30 % of their reading time in silence. Their phonation time is longer than in esophageal speakers – about 12 s, but shorter than in normal speakers (in average 15-20 s).[24,29,30]

Intelligibility

Few studies have compared all three forms of alaryngeal speech. The intelligibility of speakers using AL ranges between 30 and 90 %. The average intelligibility is reported to be

about 60 %. The major cause of relatively low intelligibility is the failure to maintain the voicing distinction. Voiceless consonants tend to sound like voiced consonants.

The intelligibility of ES varies considerably, but in general, it is somewhat higher than in AL users. The average is about 70 %. Most of the deficiencies committed by esophageal speakers were voicing errors. Like in users of an AL, voiceless consonants were perceived as voiced.

TES also show considerable variation in intelligibility, but in general, they produce the most intelligible speech of the three forms of alaryngeal speech. The patients using TES do not have to use any structures in the vocal tract to insufflate the pharyngo-esophageal segment; therefore, they can maintain their normal (or develop near normal) patterns of articulation and more normal flow of speech.[31-33]

Speech rehabilitation after laryngectomy in Slovenia

According to a study performed in a group of members of an Association of laryngectomized subjects in Slovenia, 62 % of the subjects use ES, 8 % use TES, and 9 % of subjects use AL in every-day communication. About 18 % of 113 subjects who answered the questionnaire communicate using pseudo-whisper or writing. Only 2 % of subjects use AL and ES or TES. The laryngectomees assessed their satisfaction with their mode of communication using longitudinal analogue scale – 85 %.[34]

The results differ from the studies performed in the USA or Australia. Hillman et al. report that only relatively small percentages of laryngectomees developed usable ES (6 %) or remained nonvocal (8 %), and that a majority of the patients ended up as users of AL (55 %) or TES (31 %).[12] In Australia, the mode of communication after laryngectomy is about the same: 5.2 % patients use ES, 50 % AL, and 31.6 % TES.[35]

The reason for the difference in communication mode is a good access to speech therapy in Slovenia. Patients start learning ES two or three weeks after the surgery. The Association of laryngectomized subjects organizes free courses for better communication skills twice a year. Perry and Shaw report on very low referral rate to speech pathology in Australia.[35] In Slovenia, the voice prosthesis is inserted only in the patients who cannot learn ES.

Many factors influence the choice of the mode of speech rehabilitation in a particular patient. For a successful rehabilitation, a team approach is necessary. A team of professionals (ENT surgeon, phoniatrician, speech therapist, psychologist) meets the patient before laryngectomy to explain him/her the possibilities of voice restoration after the surgery. The rehabilitation is started as soon as the wounds are healed and continues in the following years. The whole team and the patient are aware that only a successful speech rehabilitation enables a good quality of life after laryngectomy.

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Radiol Oncol 2001; 35(4): 255-8.

Combined therapy for oral cavity and oropharyngeal squamous cell carcinoma: Depth of invasion as prognostic factor

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Background. *The aim of the study was to emphasize the importance of surgical management of squamous cell carcinoma (SCC) in the head and neck and to find the most important predictive factor for cervical lymph node metastasis and prognostic factor for survival. The use of multimodality therapy is being discussed as well.*

Patients and methods. *From June 1st, 1992 to May 31st, 1998, 154 patients with oral cavity and oropharyngeal SCC were admitted to the Department of Otorhinolaryngology and Cervicofacial Surgery in the Teaching Hospital of Maribor. The criteria for inclusion into the study were met by 142 patients, but only 62/142 patients entered the multimodality protocol (surgery and postoperative radiotherapy). These 62/142 patients were treated surgically and 49 of them were postoperatively irradiated, while 13/62 declined postoperative radiotherapy. Surgical specimen was evaluated for positive or negative lymph nodes, tumor margins and the depth of invasion. Tumor cells were stained for Ki67 proliferative factor.*

Results. *The depth of invasion was the most important predictive factor for the neck metastases in multivariate model including also the grade, pT and T. pN was found to be important in determining the overall survival using Cox regression model ($p < 0,05$). A statistically important discrepancy between N and pN classification was found. In 23 cases N was overrated and in 3 cases underrated. The overall 5-year disease specific survival was 55 %. Ki67 correlated with the grade of tumor differentiation. No statistically significant correlation was found with lymph node metastases.*

Conclusions. *The depth of invasion is the most important factor determining the occurrence of the neck metastases whereas the N status determines the survival.*

Key words: mouth neoplasms – therapy; oropharyngeal neoplasms – therapy; combined modality therapy; carcinoma, squamous cell; neoplasm invasiveness; survival analysis

Received 7 July 2001
Accepted 10 August 2001

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Introduction

The modality of treatment of oropharyngeal and oral cavity squamous cell carcinoma (SCC) is still a matter of debate. Almost all authors use the multimodality therapy approach, and only a few are willing to take a chance with the single modality therapy. Chemotherapy is becoming more and more used in the therapy of head and neck SCC, although its results are inconsistent and benefits not proven.

Regardless of the modality used, the survival of patients with head and neck SCC in the last 50 years has not improved as much as we would like, but the quality of life has, especially with the use of reconstructive procedures. In the management of the head and neck SCC, two questions emerge: 1. How to treat? 2. How much is enough (ultraradical multimodality therapy, close margin surgery)? The answers to these two questions should bring us closer to the final goal: the cure of the patient with less therapy and better quality of life.

We wish to present the results of multimodality approach to the treatment of patients with oral cavity and oropharyngeal SCC at the Department of Otorhinolaryngology and Cervicofacial Surgery in Maribor Teaching Hospital. We tried to identify the most important predictive factors determining regional metastasizing and locoregional prognosis as well as prognostic factor for survival.

Patients and methods

From June 1st, 1992 to May 31st, 1998, 154 patients with oral cavity or oropharyngeal SCC were admitted to our department.

The criteria for the inclusion into the analysis were: 1. histologically proven SCC in the oropharynx or oral cavity, 2. no meta- or synchronous tumors found at panendoscopy, 3. no prior oncological treatment, 4. resectable tumor.

The inclusion criteria for entering the multimodality protocol were satisfied by 142 patients. In 23 of 142 patients, the carcinoma was medically inoperable because of a significant co-morbidity or extent of the disease, whereas 57 patients declined primary radical surgical therapy.

Sixty-two (44 %) of 142 patients were treated surgically and 49/62 (80 %) under multimodality protocol: surgery and postoperative teleradiotherapy with doses ranging from 52 to 56 Gy, while 13/62 declined postoperative radiotherapy.

There were 58 (93 %) males and 4 (7 %) females. The average age of patients was 55.6 years. The youngest one was 31 and the oldest 78 years old.

All patients were preoperatively submitted to screening tests, panendoscopy and neck ultrasound, and had the tumor (re)classified according to cTNM.[1]

The radicality of tumor excision was proven with intraoperative frozen section. Tumor and neck specimens were sent to the Pathohistology Department for the final macro- and microscopical analysis. The following parameters were determined:

1. Depth of invasion measurable from virtual line connecting uninvolved tissue margins to the deepest tumor line.
2. Immunohistochemical detection of Ki-67 proliferating factor.
3. Thickness of tumor measured from the most superficial tumor line to the deepest line of invading tumor.
4. Lymph nodes identified in fibrofatty tissue obtained during neck dissection; the nodes were searched for metastatic cells and extracapsular spread by conventional histological technique and serial sections through lymph node.
5. Tumor classified according to pTNM.

Ki-67 was detected by Immunotech monoclonal antibody Ki-67 antigen (MIB-1) using

standard immunochemistry methods.[2] The average proportion of Ki-67 positive nuclei was calculated for each sample.

The data were analyzed using Statistica for Windows with Student t test, multivariate linear regression models, Kaplan Meier survival analysis and Cox regression analysis.

Results

Intra/transoral excision and comprehensive neck dissection were performed in 41/62 (66 %) patients, and commando operation in 21/62 (34 %) patients. All resections were R0.

The defect was reconstructed with myocutaneous flaps (platysma, PMMF, infrahyoid) in 10/62 cases (16 %), with forearm free fasciocutaneous flaps in 8 cases, local flaps in 13 cases, and primary suture or split thickness skin graft in 31 cases.

Of 62 patients, 51 (83 %) were with tumors classified as stage III or IV.

Bilateral and multilateral neck dissections were performed in 55 and 7 cases, respectively. In each neck specimen, more than 30 lymph nodes were isolated (average 39).

N+/pN0 conversion was observed in 15/62 (24 %) cases and overall 23/62 (37 %) necks were overrated (cN > pN).

Occult metastases were found in only 3/62 (5 %) cases. Using Student t test, we found statistical difference between N and pN for the same cohort of patients ($p = 0.019$).

Kaplan Meier survival curves for the patients treated with multimodality approach ($n = 49$) according to N stage are presented in Figure 1.

In order to identify factors influencing the rate of metastases in regional lymph nodes, we analyzed clinical and pathognomonical factors using multivariate linear regression. The depth of invasion, T stage, thickness and differentiation grade were analyzed. The only statistically important factor was the depth of invasion > 4 mm ($p = 0.0237$).

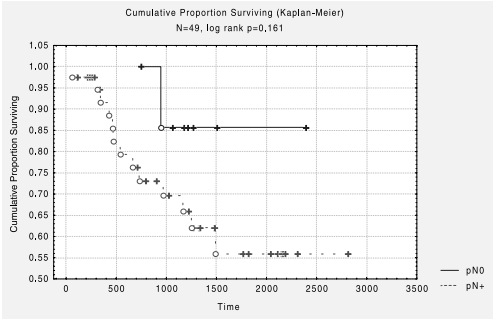

Figure 1. Kaplan Meier survival analysis according to pN stage.

The patients' survival was dependent on pN status ($p = 0.056$) in the model that included pN, pT, G and depth of invasion, using Cox regression for analysis.

A 5-year disease specific survival of the group of patients with oropharyngeal cancer was 74% and those with oral cavity 47 %. The overall disease specific survival was 55 %.

Of 62 patients, 16 died of disease, 3 were lost to follow up, 5 died from locoregional recurrence, 7 from distant metastases and 4 had regional recurrence without any evidence of primary tumor. All patients with regional recurrence (9/16) had positive neck status at the first treatment.

The incidence of locoregional recurrence in combined modality group and in single modality (surgery only) group was 16.3 % and 7.5 %, respectively. The distribution of advanced stage in both groups was statistically similar.

Ki-67 expression correlated to the grade of differentiation and had no statistically significant influence on node metastases. The results are preliminary and the independent research is being performed.

Discussion

In our analysis, we presented a "classical" treatment concept of SCC of the oral cavity or

oropharynx. The most surprising result is low incidence of occult metastases and a high rate of overrated necks. In 24 % of all cases, the clinical suspicion for metastases didn't prove correct. Regional disease is an important survival prognostic factor and Kaplan Meier survival curves are significantly different for pN0 and pN+ status.

The depth of invasion was found to be one of the most important factors determining the occurrence of regional metastases. The depth of invasion > 4mm means a 3 times higher probability of positive neck.[3,4] The depth of invasion is not included in TNM system. The invasion of tumor cells in the surrounding tissue is followed by angiogenesis and matrix lysine. Invasive and antigenic tumor potentials are connected and only together they have prognostic value. The depth of invasion is simple and evident in any gross evaluation of a tumor specimen.

The grade of differentiation does not prove to be the decisive factor in metastasis prognosis. As indicated by our preliminary results, the same holds true for Ki-67.

In our series, a large proportion of admitted patients with resectable and medically operable tumors (57/119) declined further surgical therapy. The reason for their decision is not clear from retrospective analysis. According to the results of some other authors, surgery as single modality treatment for advanced oral cavity or oropharyngeal cancer is definitely more successful than primary radiotherapy.[5,6] Most authors advocate postoperative irradiation in the cases of positive margins, perineural and perivascular spread in the case of extracapsular spread or in the case of multiple positive nodes.[7]

Conclusions

We believe that the depth of invasion should be included in every pathologist report of oropharyngeal and oral cavity SCC because it may influence the decision for further treatment and postoperative irradiation. The depth of invasion is as important for the prognosis as TNM classification. In our study, Ki-67 proliferative factor didn't correlate with metastatic potential of the SCC of the oral cavity and oropharynx.

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Radiol Oncol 2001; 35(4): 259-66.

Evolving strategies in the treatment of childhood rhabdomyosarcoma: Slovenian experience

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Background. *Neoadjuvant chemotherapy (Cht) has changed the treatment of rhabdomyosarcoma (RMS) in children. The purpose of our study was to review the children treated for RMS between 1974 and 1996.*
Patients and methods. *Fifty-one children, 1-15 years old, were included. Primary sites of tumour were: head and neck 15, orbit 6, genitourinary 12, extremity 9, torso 5 and paratesticular 4. Twelve patients were in stage I, 10 in stage II, 26 in stage III and 3 in stage IV. Of 43 histologically confirmed RMS 25 were embryonal, 13 alveolar, 1 botryoid, 1 spindle cell and 3 sarcoma NOS. In 8 patients, only fine needle aspiration biopsy (FNAB) was available. All patients had Cht, 29 neoadjuvant, 20 had surgery first, 40 had irradiation (RT), 2 stage IV patients had bone marrow transplant (ABMT). Multidrug Cht varied: VCR, AMD, and cyclophosphamide (VAC) were used in the 1970s, with Adriablastine (T2), methotrexat (MTX) and/or other drugs (T6, T11) in the 1980s, and in the 1990s, cyclophosphamide was replaced by ifosfamide (VAIA). The treatment was started with Cht in orbital and head and neck tumours and in the majority of genitourinary tumours, but surgery was first in paratesticular and in the majority of extremity tumours.*
Results. *The 3 patients with stage IV disease died. Of those with localised tumour, 34 (70%) were alive and well 5 years after treatment, 80% stage I, 75% stage II and 61% stage III. One patient died of heart failure, 3 of Cht toxicity and 1 of intercurrent disease.*
Conclusions. *The survival of our patients has improved during the last 2 decades and increased from 57 % to 70 % for patients treated after l985. It is now comparable to that in other centres. With the introduction of neoadjuvant Cht, surgery and RT have become more conservative and could sometimes even be abandoned, thereby reducing considerably the risk of late sequels. Orbital, genitourinary and paratesticular embryonal RMS of low stages have very good prognosis. Primary tumours of the extremities and head & neck, mainly of alveolar type, have poor prognosis. For alveolar type of RMS and stage IV tumours, the present treatment modalities, including ABMT, are not effective.*

Key words: rhabdomyosarcoma – therapy, child, survival analysis

Received 3 October 2001
Accepted 20 October 2001

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Introduction

When treating a child with a malignant tumour we try to achieve a cure with minimal risk for early, as well as late toxic effects of treatment.[1] This aim is easier to reach in early stages of the disease. The cure rate has significantly improved in the last decades with the introduction of multidrug chemotherapy (Cht). This is now used practically in all solid malignant tumours in children.[2,3] With the introduction of neoadjuvant Cht, surgery and radiation therapy have acquired more the role of adjuvant treatment may be more conservative and even be omitted in some cases after a complete response to Cht. The advantages are twofold: the response of the tumour can be evaluated in each particular patient, but not only statistically. Therefore, it can continue and be successful also after surgery. Moreover, Cht can be sufficient as the only treatment.[4,5] A morphological diagnosis to confirm malignancy is required before treatment as well as for the choice of proper Cht.[6] The aim of this study was to review the children with rhabdomyosarcoma (RMS), treated at the Hemato-oncological unit of the University Children's Hospital and at the Institute of Oncology in Ljubljana during a 23-year period.

Patients and method

Between l974 and l996, 5l children aged from l to l5 years (median 6) and registered at the Cancer Registry of Slovenia with the diagnosis of RMS, were managed by a team of physicians at the Hemato-oncological unit of the University Children's Hospital and the Institute of Oncology in Ljubljana. Five children with RMS, who were first treated and /or followed outside Slovenia, are not included. Evaluation of the tumour extent included clinical examination, plain radiography computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, technetium bone scan, bone marrow biopsy, spinal tap in patients with parameningeal disease and those with stage IV tumours. The tumours were staged according to the SIOP-UICC[7] as follows:

Stage I: Tumour restricted to the organ of origin	12
Stage II: Tumour invasion beyond the organ of origin	10
Stage III: Tumour with regional metastases	26
Stage IV: Tumour with distant metastases.	3

The following primary sites were defined:

Head & Neck	15
Parameningeal	8
pterigopalatinal fossa	1
nasopharynx	3
maxilla	4
face	5
ear lobe	1
oral cavity	1
Orbit	6
Genitourinary	12
vagina	4
prostate	1
(para)vesicular	7
Paratesticular	4
Extremity	9
lower	5
upper	4
Torso	5

There were 27 boys and 24 girls less than l6 years old at diagnosis (mean 6.9, SD 4,2). The diagnosis was provided by fine needle aspiration biopsy (FNAB) in 20 and by surgical biopsy in 31 patients prior to treatment. Of 43 histologically confirmed RMS, 25 were embryonal, 13 alveolar, 1 botryoid, 1 spindle cell and 3 sarcoma NOS. Multidrug chemotherapy varied. Vincristine, actinomycin D and cyclophosphamide (VAC) were used in the 1970s, VAC in combination with adriablas-

Table 1. Chemotherapy according to stage (51 patients)

CHT/ Stage	VAC	T2, VACA & VAIA	T6, T9 & T11	NP*
I	2	6	3	2
II	3	5	1	/
III	2	10	14	/
IV	/	/	/	3(ABMT)
Total	7	21	18	5

*CIVADIC

tine (T2 -protocol, VACA) and methotrexate (T6 or T11-protocols) in the 1980s. VACA or VAIA (cyclophosphamide replaced by ifosfamide) were used in the 1990s (Table 1).

All 51 patients received Cht; 29 neoadjuvant, 20 had surgery first, in 40, Cht was combined with irradiation (RT). Of the 3 stage IV patients, 2 had bone marrow transplant (ABMT) at first treatment and one had half body RT in combination with Cht (VAIA alternating with VP 16 and cisplatinum) and RT to all involved areas.

The treatment was started with Cht in orbital and head and neck and in the majority of genitourinary tumours, whereas surgery was the first treatment in paratesticular and in the majority of extremity tumours. Twenty-one patients had no surgery (2 stage I, 1 stage II, 15 stage III and the 3 stage IV patients). Surgery was delayed in 10 patients (5 genitourinary, 1 orbital recurrence, one extremity and one torso) (Table 2).

Forty patients received RT to primary or metastatic site (including the 2 who had ABMT). In 2 patients with maxillary primary tumours, RT was given at first treatment, while in 24 patients, after Cht and in 16 also after surgery. The doses of RT varied between 30 Gy and 50 Gy, depending on tumour stage and response to Cht as well as on the experience of an earlier study.[8] Two patients received 60 Gy.

The patients were followed between 5 and 26 years (median 12) from the diagnosis. None were lost from the follow-up. The survival was calculated from the date of the diagnosis until December 31st 2000, when the study was concluded, or until the date of death. It was presented in survival curves according to the Kaplan-Meier method.[9] The statistical significance was calculated using the log rank test.

Results

Sixty-seven % of all patients included in the study and 70 % of those with locoregional disease were alive and well more than 5 years from diagnosis. The survival of patients treated before 1985 was 57 % (12/21) and 70 % (21/30) of those treated after 1985. Of the 17 patients who died, 13 died of tumour, 4 of complications and one, 18 years after diagnosis, of an intercurrent disease without tumour. Toxicity due to Cht was the cause of death in

Table 2. Treatment according to primary site (48 patients, stage IV excluded)

	Chemotherapy			Surgery			RT*
Site	total	1th	2nd	total	1th	later	total
Orbit	6	5	1	2	1	1	6
Head& Neck	13	5	8	6	6	/	13
Genitourinary	12	7	5	10	5	5	8
Extremity	7	4	3	6	3	3	6
Torso	6	5	1	2	1	1	4
Paratestis	4	/	4	4	4	/	/
Total	48	26	22	30	20	10	37

*radiotherapy

2 patients treated for recurrence and in one stage IV patient. Cardiotoxicity was the cause of death in one patient with stage III alveolar RMS of the anterior thoracic wall 8 years after completed treatment. At the time of death, a recurrence was also present. She was 3 years old at diagnosis. She was treated by Cht according to T2 protocol, including adriablastine and concomitant RT to the thoracic wall (4500 Gy given in split courses during 3 months).

The survival of the patients with stage I and stage II was very good, while it was significantly worse for the patients with stage III and stage IV (Figure 1). None of the stage IV patients survived, all had multiple bone metastases. All patients with orbital tumours and those with paratesticular tumours survived. Of the l2 patients with genitourinary tumours, one with primary tumour of the prostate died. Patients with primary tumours of the head and neck, torso and extremities did poorly. The patients with paratesticular, orbital and genitourinary primary tumours did significantly better than those with tumours of other primary sites (Figure 2). The survival was not different in those patients in whom treatment was started with Cht compared to those who received Cht postoperatively (Figure 3). Furthermore, there was no difference in survival between those who had no surgery, delayed surgery or surgery as first treatment (Figure 4). The outcome of the disease in patients who had more intensive Cht

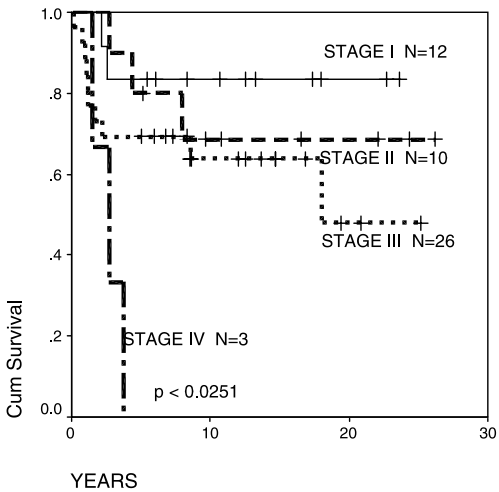
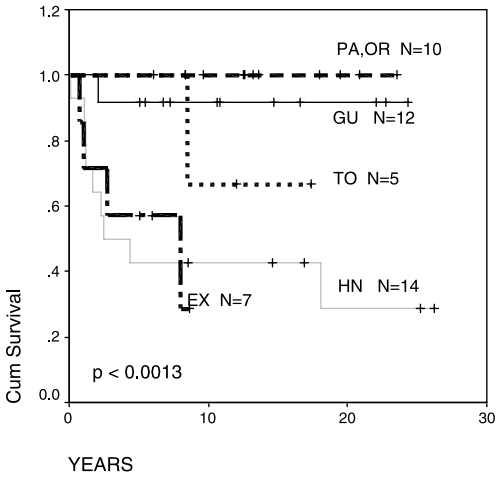


Figure 1. Disease free survival by stage in 51 patients.



PA = paratesticular, OR = orbit, GU = genitourinary, TO = torso, EX = extremity

Figure 2. Disease free survival by tumor site in 48 patients

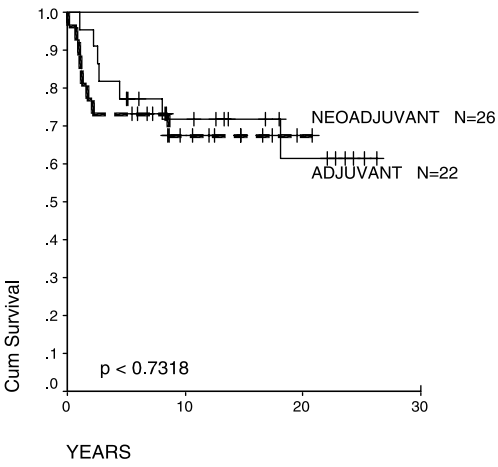


Figure 3. Difference in disease free survival between patients with adjuvant and neoadjuvant chemotherapy (48 patients)

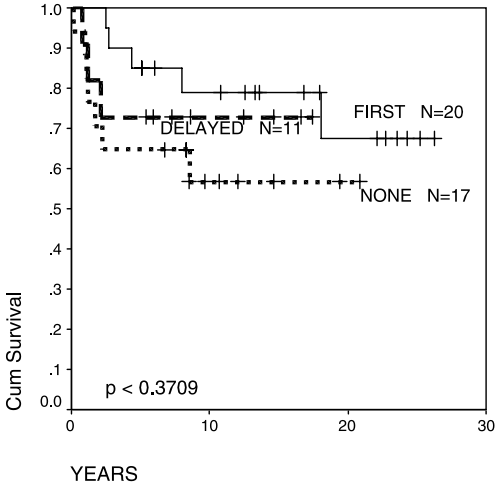


Figure 4. Disease free survival by surgery in 48 patients

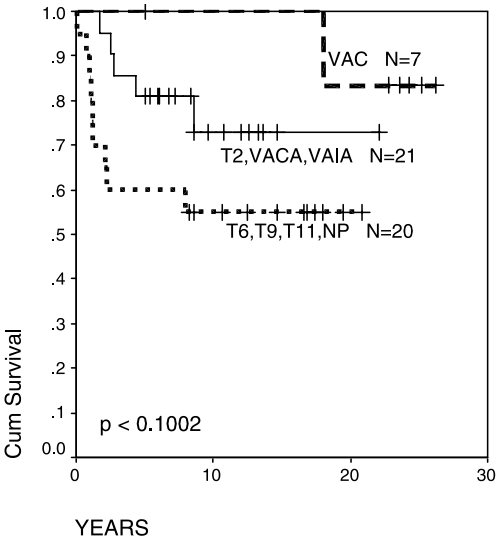


Figure 5. Disease free survival by type of chemotherapy in 48 patients.

was not better compared to those who had VAC (Figure 5). The morphological type of RMS did not significantly influence survival (Figure 6). There were two late deaths after recurrence, both in patients with alveolar type of RMS.

Discussion

In our series of patients with RMS the distribution of sex, age and primary sites is similar to that of other reported studies. The same is true for the overall 5-year survival, which has improved in Slovenia during the last two decades from 57 % to 70 %.[10,11] The stage of the disease and the site of the primary tumour significantly influence the outcome, as known. Recently, a staging classification, based on the experience of the IRS group, was proposed, with some low stage tumours classified as unfavourable and some tumours of higher stages with expected favourable outcome.[12] In our experience, this has not been entirely confirmed. Patients with primary parameningeal tumours, classified as unfavourable, regardless of the stage, do extremely poorly (only one of the 8 survived) as also reported by others. All 7 patients with vesicular and paravesicular primary tumours, however, are among survivors (Table 3). Only 2 of them had tumours with less than 5 cm in diameter, all embryonal RMS. All patients with genitourinary primary tumours, except one with a primary tumour of prostate, survived.

The influence of tumour morphology on survival could not be statistically confirmed in our small series; however, it was noted that the patients with the alveolar type of tumour did poorly (Figure 6). This finding is consistent with other reports.[13,14] Fourteen patients had alveolar RMS, including 3 stage IV patients and only 5 out of the remaining 11 with locoregional disease are among survivors. Of the 6 who died, 5 had local recurrence (one died of cardiotoxicity with the tumour still present). They died 3, 4, 6 and 8 years after diagnosis. The results of the IRS studies II and III differ regarding the influence of tumour morphology on survival. It has therefore been suggested that the primary site, correlated to the histological type, is the prevailing and independent prognostic fac-

Table 3. Patients with primary tumours of the bladder or tumours invading the bladder

Pt I	Sex	Age in years	Year of diagnosis	Prim. site	Stage	Type	Surgery	RT	Cht	Late effects
I.P.	M	5	1976	paravesicular	T2 N1 M0	S	TR	48Gy	VAC	elevated FSH, soft tissue atrophy, recurrent cystitis
M.P.	M	2	1978	bladder	TlN0M0	E	Cystectomy	none	VAC	stoma, recurrent uroinfections
K.Ž.	F	5	1984	paravesicular	T2N0M0	E	STR	46	T11	soft tissue atrophy, amenorrhoe
M.J.	F	1.5	1990	paravesicular	T2N0M0	E	STR	none	T2	none
R.U	M	7	1993	paravesicular	T2N0M0	E	STR	30*	VACA	none
B.K.	M	2	1994	bladder	T2N0M0	E	none	37.5	VACA	none
A.M.	F	2	1995	bladder	TlN0M0	E	TUR	39**	VACA	none

*Hyperfractionation, **Transperineal implant, Ir 192, TR = Total resection with partial cystectomy, TUR Transuretral resection, STR Subtotal resection, S = spindle cell RMS, E = embrional RMS, FSH = follicle simulating hormone

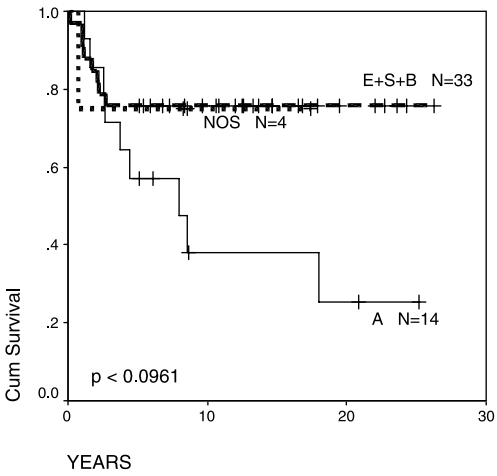


Figure 6. Disease free survival by morphological type in 51 patients. (A = Alveolar, E = Embryonal, B = Botroyd, S = spindle cell, NOS= RMS not otherwise specified)

tor.[15] It might also be possible that the morphology of the tumour did not influence the outcome significantly because the patients with alveolar tumours received more intensive Cht. In our series, the primary site and the type of the tumour were correlated (Table 4). All tumours of the extremities were of the alveolar type and all genitourinary and paratesticular tumours were of nonalveolar type.

Table 4. Primary site versus morphological type

Primary site	Alveolar	Embryonal and other
Orbit	1	5
Head & neck	6	9
Genitourinary	0	16
Paratestis	0	4
Extremity	5	0
Torso	2	3
Total	14	37

The survival of patients in our study was not influenced by different schedules of Cht (Figure 5), which was probably too aggressive for some groups of patients. At least 3-drug combinations were used, often 4 or even more. ABMT has not been reported as successful in patients with RMS,[16] which was also true in our 3 stage IV patients who all had bone metastases.

The group of patients with bladder and paravesicular tumours, presented in Table 3, reflects the development of treatment approaches during the two decades, for the entire series. Radical surgery and high dose RT

were applied in the 1970s and very aggressive and Cht with more conservative surgery and lower doses of RT in the 1980s. Later, surgery and RT were more conservative, while 4-drug Cht was still used. With equal results in terms of survival, the consequences of treatment are considerably reduced.

The survival curves for those patients who were first treated with surgery and those who had neoadjuvant Cht and delayed, more conservative surgery are identical (Figure 6). The patients who had no surgery at all did not do significantly worse, even with the inclusion of the patients with high-risk parameningeal tumours.

Conclusions

During the period under investigation, the methods of treatment of children with RMS have evolved. With the introduction of neoadjuvant Cht, surgery and RT have become more conservative or were even abandoned, thereby reducing the risk of late sequelae. The survival of our children with RMS has improved during the period under investigation and is now comparable to that in other centers. Orbital, genitourinary and paratesticular embryonal RMS of low stages have very good prognosis and may be cured with conservative treatment and less toxic chemotherapy. Primary tumours of the extremities and head and neck, mainly of alveolar type, have poor prognosis with present treatment modalities. Hopefully, new Cht schedules, will prove more effective. For alveolar type of RMS and stage IV tumours the present treatment modalities, including ABMT, are not effective; therefore, new approaches should be considered.[17]

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Radiol Oncol 2001; 35(4): 267-72.

The technique of craniospinal irradiation of paediatric patients in supine position

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Background. *Postoperative radiation therapy has significant impact on local control and overall survival of paediatric patients with brain tumours but an irradiated volume is often a controversial issue. Our aim was to describe a new technique of craniospinal irradiation as a postoperative treatment in patients with the risk of relapse of brain tumours as well as to estimate the side effects of such craniospinal irradiation.*

Patients and methods. *In the last 4 years, 17 paediatric patients under 15 years of age with medulloblastoma (8) ependymoma (6) and glioblastoma (3) received postoperative craniospinal axis radiotherapy by a new technique developed in our departments. This technique is based on irradiation in supine position with the use of asymmetric jaws of the linear accelerator.*

Results. *Radiotherapy was well tolerated and dose-reduction was not needed in any case. Skin reactions were mild in all patients. The gastrointestinal and haematological toxicity was mild to moderate (WHO grade I-II).*

Conclusion. *The proposed new technique of craniospinal irradiation is advantageous in terms of side effects and could be recommended to be widely used. Craniospinal irradiation in supine position is an alternative method to the treatment in prone position. The evaluation of the effectiveness was limited by a short follow-up interval.*

Key words: brain neoplasms – radiotherapy; radiotherapy – methods; supine position; child

Received 26 October 2001
Accepted 3 November 2001

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Introduction

The primary treatment of choice of the brain tumours is surgery. Combination of treatment modalities is preferred in the treatment of medulloblastomas, ependymomas and gliomas. Postoperative radiation therapy has significant impact on local control and overall survival. In general, postoperative radiotherapy has been reported to improve the outcome. Meta-analyses of the patients irradiated postoperatively have revealed a prolongation of the 5-year survival rate by 20-30 % (ependymomas) and 60 % (medulloblastomas). An irradiated volume is often a controversial issue, especially regarding irradiation of the spinal axis after resected intracranial ependymomas and gliomas. In this study, we report a new technique for craniospinal irradiation developed in our departments.[1-6]

Patients and methods

Between January 1997 and March 2001 a total number of 17 (medulloblastoma 8, ependymoma 6, glioblastoma 3) paediatric patients aged under 15 years (median age 6.6 years) were treated in the Departments of Radiation Oncology, St. Anne's Hospital and Masaryk Memorial Cancer Institute in Brno. Surgical resection was performed in all patients. All tumours were histologically proved and were localized infratentorially in the posterior fossa. In the indicated cases, chemotherapy was administered after radiotherapy. All of the patients were irradiated with a dose of 24-36 Gy to the whole craniospinal axis and with a dose of 50-54 Gy to the tumour bed (30-36 Gy "high risk", 24-30 Gy "standard risk" group). In cases with residual tumour a total irradiation dose up to 58-60 Gy was delivered. A new radiotherapy technique using asymmetric jaws of the linear accelerator was employed in all patients.

The development of modern accelerators enables the use of asymmetric jaws in whole brain irradiation in order to minimise the risk of damage of critical areas in close proximity to the target volume, and to diminish the risk of overdosage at the border of the adjacent fields in the area of the irradiated spinal canal (Figure 1).[7-10]

The definition of the planning the target volume:

1) Whole brain: this volume encompasses the whole brain with 1 cm safety margin. The lower limit of the frontal area must be 5 mm below the frontal sinus and 1 cm below the temporal lobes. In front of vertebra C_2, 5 mm are required.
2) Spinal axis: the inferior limit must be vertebra S_{2-4}. The lateral safety margin of 5 mm is required regarding the lateral process. ICRU 50 point of the whole brain is on the mid axis of the target volume and that of the spinal axis is on the axis of medullar cord (Figure 2). Treatment planning was based on a series of about 20-25 consecutive CT slices. The use of three-dimensional treatment planning is a standard method.

In the whole brain and cervical spine irradiation (with the caudal border C_3-C_4), two opposite lateral fields were chosen with shielding blocks of the eye bulbs; the spinal cord was irradiated with two direct posterior fields. After reaching 33 % and 66 % of the planned dose, the size and the borders of the adjacent fields in the area of the spine were modified (Figure 3). It was necessary to include the whole vertebral volumes in the irradiated volume in order to diminish the risk of postirradiation scoliosis of the spine. Patients were lying in supine position and were fixed by a vacuum body immobiliser and ORFIT head mask. With the use of the vacuum body immobiliser, the applied depth dose decreased by 1-2 %. This irradiation technique caused a tolerable increase of the superficial skin dose with regard to the total dose applied to the planning

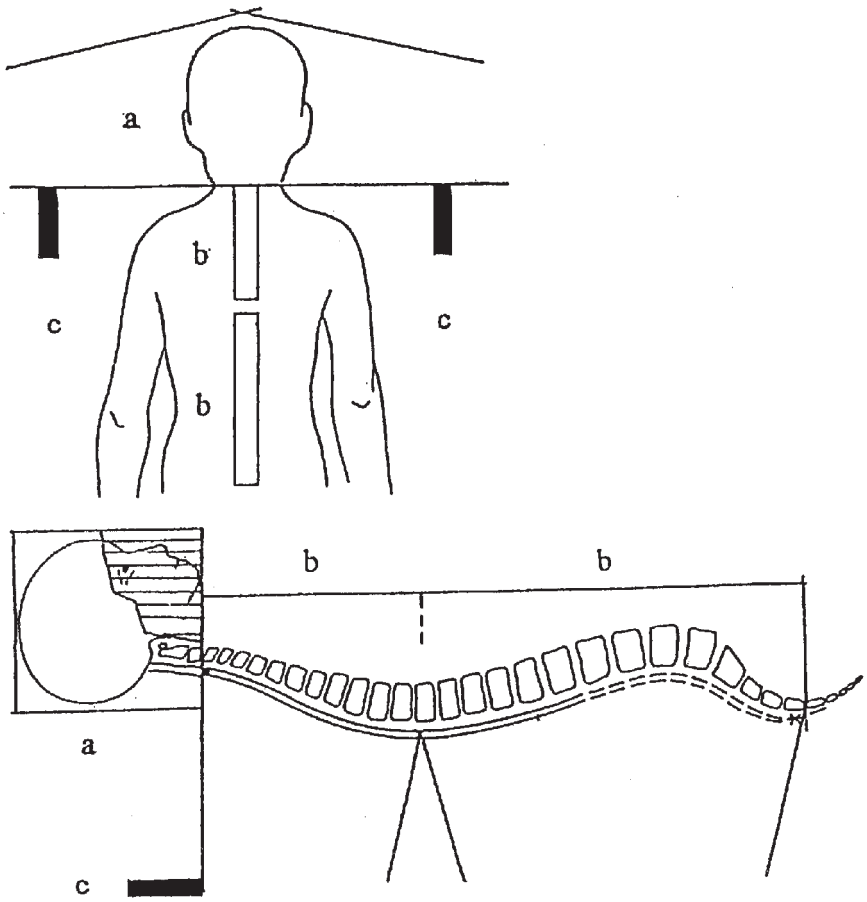

Figure 1. Modified technique of craniospinal irradiation with the use of two opposite lateral fields (individual shielding blocks of the eye bulbs and the face part of the skull) (a), and two direct fields (b), with the use of the asymmetric jaws (c). (Linear accelerator, X-ray, 6 MV).

target volume. Irradiation was performed using standard fractionation (5 fractions per week) with a single dose of 1.5-1.8 Gy for craniospinal axis by photon beam (6MV) of the linear accelerator. It was necessary to determine exactly the position of the child on the treatment table by laser beams and the optical pointer of the irradiation device.

Results

The prescribed dose of irradiation was delivered to all patients; dose reduction was not necessary in any of them. In October 2001 (date of evaluation), 8 out of 17 patients (47 %) were without any sign of disease and had no serious problems. Two patients presented significant neurological symptoms. Local relapse occurred in one patient, but was successfully treated by surgery. Six patients (35 %) died of local recurrence (5 of these 6 patients had partial resection).

Radiotherapy was well tolerated. Skin reactions were mild and were of grade 1 (WHO) in 15 (88 %) patients and of grade 2 in two (12 %) patients. Within three months after the completion of radiotherapy, these reactions disap-

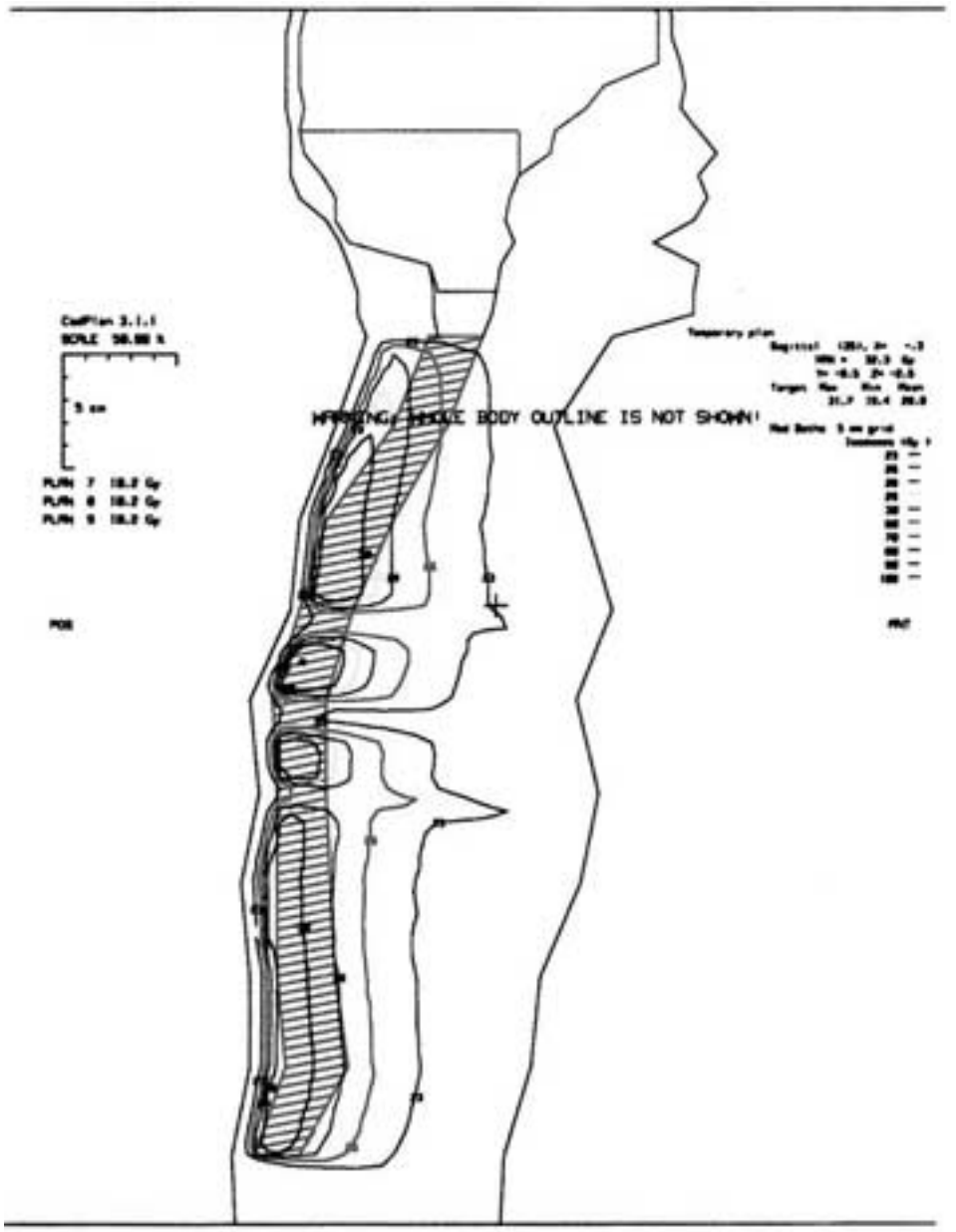

Figure 2. Isodose plan of irradiation spinal axis with two changes of the borders of the adjacent fields.

peared. The gastrointestinal and haematological toxicities were mild to moderate in all patients (WHO 1-2 gr.). Leucopenia grade I occurred in 70 % of patients, grade II in 18 %, trombocytopenia grade I in 35 %, diarrhoea grade I in 47 % and grade II 23 %, nausea in 47 %.

Further evaluation of the effectiveness of our therapy is not feasible due to the small number of patients and short follow-up interval.

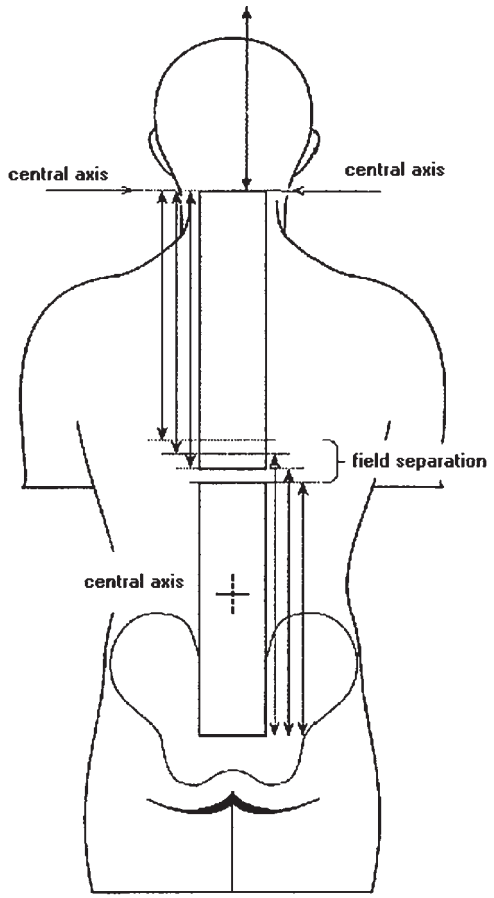

Figure 3. The radiation fields – scheme of the changes of the size.

Discussion

Irradiation of the craniospinal axis is a part of the treatment modality of a number of malignant diseases. Patients are usually treated in prone position which is not as comfortable, reproductable, and as easily maintained as supine position.[5-7]

Acute skin, haematological and gastrointestinal reactions were comparable with those in patients irradiated in prone position.[11]

To minimise anaesthesia related risks, the irradiation in supine position would be preferable to standard prone position. The treatment in supine position would be more comfortable for adult patients as well.[12]

In this study, we presented a technique of craniospinal axis radiotherapy with the use of asymmetric jaws of the linear accelerator in order to minimise the risk of irradiation induced toxicity to healthy tissue and to overcome the risk of overdosage at the adjacent fields. It is necessary to use a three-dimensional treatment planning. The assessment of the effectiveness is limited by the short follow-up interval.[13,14]

In conclusion, our technique of craniospinal axis irradiation is advantageous in terms of tolerability and side effects. We believe that it deserves to be widely used.

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Radiol Oncol 2001; 35(4): 273-6.

Case report

Anal ultrasound in the diagnosis of anal carcinoma

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Background. *We present a case of adenocarcinoma of the anal canal to demonstrate the usefulness of the anal ultrasound (AUS) in the diagnostics.*
Case report. *AUS was performed with the use of Bruel&Kjaer scanner type 3535 with an axial 7.0 MHz endoprobe. Examination was performed in left lateral position.*
AUS allowed for exact assessment of the depth of infiltration of the anal wall by the tumor, and precisely visualized its location and echotexture. Assessment of the perianal lymph nodes and tumor spread into adjacent tissues was also possible.
Conclusions. *AUS is a valuable imaging method in assessing the depth of invasion of anal carcinoma and gives valuable information before deciding on the choice of treatment.*

Key words: anus neoplasms – ultrasonography

Introduction

Anal tumors account for about 2.5-5 % of all malignant tumors of the colon. Their precise staging is important with regard to different methods of treatment. The depth of penetration within the wall of the anal canal is poorly recognized by digital examination. We present the ultrasound images and usefulness of anal ultrasound (AUS) in staging of anal canal carcinoma.

Received 13 September 2001
Accepted 30 September 2001

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Case report

An 83 year-old man complaining of anal bleeding and constipation observed for one year was admitted to proctologic outpatient clinic. Per rectum examination revealed an abnormal, mobile mass on the anterior wall of the anal canal, with the diameters of 2.5×2 cm.

During anoscopy, a specimen was taken and the histopathologic diagnosis revealed adenocarcinoma. Prior to AUS, an enema was done. AUS was performed to assess the stage of the tumor that was essential to choose a treatment method.

For anal ultrasonography, Bruel&Kjaer scanner, type 3535 with axial endoprobe of a frequency of 7.0 MHz and covered by a plastic cone with the external diameter of 17 mm

was used. The cone was filled with a few milliliters of degassed water, covered with a condom, and then introduced into the anal canal up to the depth of 5 cm. At the mid anal level, at a depth of 2 cm, a tumor was visualized originating from the mucous of the anterior and right wall, infiltrating a distal end of the internal anal sphincter (Figure 1). The tumor mass was getting larger towards the anal orifice and was infiltrating the subcutaneous part of the external anal sphincter at its anterior wall. At the low anal level, its diameter on axial image reached 15×13mm (Figure 2). The tumour's echotexture was not homogenous with anechoic areas representing most likely degeneration. No enlarged lymph nodes were visualized in the perianal tissues and the surrounding structures were not invaded.

According to the sonographic classification (uTN), the stage of the disease was defined as uT2N0. The tumor's size less then 2.5 cm, its mobility on rectal examination and lack of enlarged lymph nodes in perianal tissues were all the signs that spoke in favor of the local excision of the tumor. A polypoid lesion on the anterior wall at the low anal level, 1-2 cm from the anal orifice, of the size of 2.5×1cm and degenerating in the center was visible during operation. The result of the histopathologic examination of the interoperative specimen was adenocarcinoma mucinosum partim gelatinosum in adenoma villotubulare ani. A malignant infiltration was found in the superficial muscular layer within a transition zone of the anal canal.

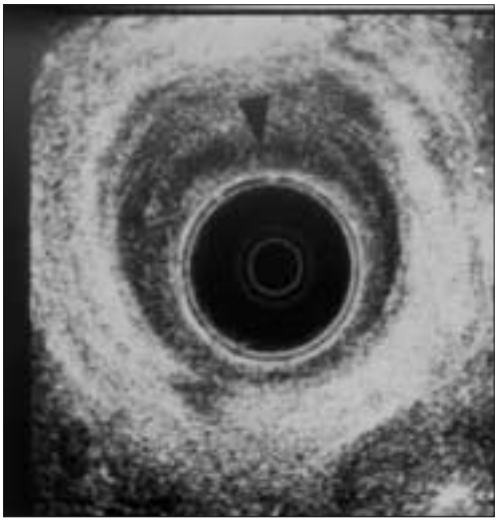

Figure 1. Adenocarcinoma of the anal canal. In the anterior (between 11 and 3 o'clock), a tumor invaded distal end of the internal anal sphincter (see the arrow).

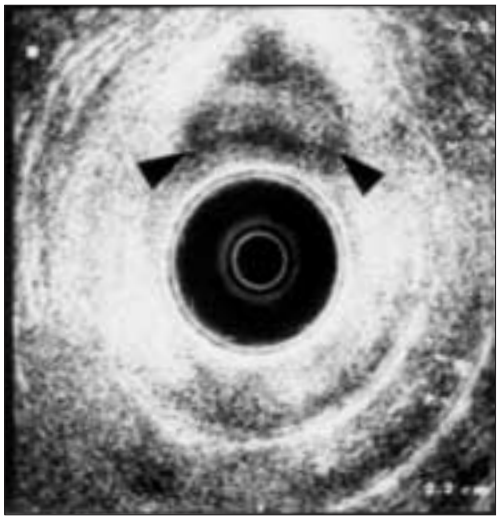

Figure 2. Adenocarcinoma of the anal canal. At low anal level, the tumor has the diameters of 15×13mm (arrows) and invades subcutaneous part of the external anal sphincter.

Discussion

The presented case of adenocarcinoma of the anal canal is rare, not only because of its prevalence but also its histologic type. Among the most frequent epithelial neoplasms, such as carcinoma planoepitheliale, basocellulare and mucoepidermale, adenocarcinoma is the most rarely diagnosed.[1] Additionally, anal canal carcinomas most frequently occur in patients between 55-60 years of age, and mostly affect women (ratio 2:3).

The staging of the anal canal cancers is important in planning treatment strategies

(which include local excision, abdomino-perineal resection of the rectum, radiotherapy, chemotherapy).[1-3] The TNM classification system currently used is based on the result of rectal examination, where only the margins of the tumor around the anal circumference and its proximal and distal ends, are assessed without assessing its mobility and evaluating the depth of penetration of the tumor into the canal wall. There is a small number of reports presenting the results of imaging methods for anal tumors and they are mostly addressed to low rectal cancers invading the anal canal. AUS in anal tumors diagnostics enables to define precisely the location of the tumor and its relation to the anal levels and walls. It is possible to assess local advancement with AUS because the layered structure of the anal canal is visible on AUS image.[4-8] Similarly as rectal tumors, anal carcinomas are also staged according to uTN classification, where „u" means that ultrasonography was used to determine the staging. In:

1) uT1 tumor is limited to submucosa and mucosa
2) uT2 is limited to sphincters
3) u T3 infiltrates perirectal tissues,
4) u T4 invades surrounding structures.

N0 and N1 mean lack or metastatic regional lymph nodes, respectively.

An anal carcinoma in AUS image appears as hypoechoic mass, most commonly not homogenous with areas of degeneration and with irregular outlines. Biopsy is necessary to confirm final diagnosis. In literature, single publications can be found on the accuracy of AUS in anal tumors staging, which is said to be almost 86 %.[2,3,9] In a study by Novell F. et al.[3] accuracy of AUS in local staging of anal canal cancers was 85.7 %: in a group of seven patients with anal carcinomas, sonographical assessment caused downstaging in only one case. In another study[9], AUS was performed on a group of 30 patients with anal cancers. AUS accurately assessed the depth of invasion by tumors and their relation to the surrounding structures.

The sonographic diagnosis influenced the choice of treatment methods. In the presented case, the staging of the anal cancer and its relation to the anal sphincters was not problematic at all. Lymphadenopathy is found in 25 % of cases with anal tumors. It is said that sensitivity of endosonography in visualizing enlarged lymph nodes is 83 %. Lymph nodes larger than 3 mm are already visible on AUS.[5,8] The accuracy of endosonography and endoluminal magnetic resonance imaging (MRI) for lymph node staging is 62-83% and 39-95 %, respectively.[10] The accuracy of computed tomography (CT) is between 22-73 %.[10] The studies comparing AUS and MRI show that MRI is inferior to AUS in N staging, although the specificity of both is low.[10-12] The size appeared to be unreliable criterion[10,12] whereas their echogenicity appeared to be more reliable. Hypoechoic lymph nodes representing metastases were predicted with a sensitivity of 72 %.[6]

When the diagnostics of malignant diseases is concerned, AUS is currently used before surgery of rectal tumors. Its ability to assess the contraction activity of striated muscle in the so-called "dynamic examination" is important.[13] For the anal canal tumors diagnostics, it is of a great value and, comparing to the endosonographic diagnostics of rectal tumors, AUS will probably have greater value due to less problematic and more equivocal criteria of diagnosis for each stage of the anal carcinomas. In postoperative follow-up, AUS may allow an early diagnosis of local recurrence in perianal tissues before they are evident on clinical examination.[14] In case of primary radiation or chemotherapy, AUS may be used to assess the tumor response to treatment from the changes of its size and echotexture.[2,3] A fine needle aspiration biopsy under sonographic control of an abnormal lesion is also possible to perform.[3] Simplicity, availability, non-invasiveness of

AUS together with excellent images of anal cancers and a precise assessment of their invasiveness are the advantages of this method, which should be carried out in addition to digital and anoscopy examinations and should have its place in the diagnostic algorithms of the malignant diseases of the anal canal, as it already has for rectal cancers.

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Radiol Oncol 2001; 35(4): 277-91.

First experience with a novel luminescence-based optical sensor for measurement of oxygenation in tumors

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Background. *The purpose of this preliminary study was to evaluate a novel luminescence-based fiber-optic sensor (OxyLite system) for the measurement of partial pressure of oxygen (pO_2) in tumors and for the detection of changes in pO_2 as a function of time. The new method was used simultaneously with the laser Doppler flowmetry method for the measurement of relative tissue perfusion.*

Materials and methods. *Blood perfusion and pO_2 were measured continuously via fiber-optic sensors inserted into SA-1 tumors in anesthetized A/J mice. The changes in blood flow and oxygenation of tumors were induced by transient changes of the parameters of anesthesia and by injection of a vasoactive drug hydralazine.*

Results. *Both optical methods used in the study successfully detected the induced changes in blood flow and pO_2. The measurements of pO_2 were well correlated with measurements of microcirculatory blood perfusion. In the majority of pO_2 measurements, we observed an unexpected behavior of the signal during the stabilization process immediately after the insertion of the probe into tumor. This behaviour of the pO_2 signal was most probably caused by local tissue damage induced by the insertion of the probe.*

Conclusion*. The novel luminescence-based optical oximetry can reliably detect local pO_2 changes in tumors as a function of time but some aspects of prolonged pO_2 measurement by this method require further investigation.*

Key words: sarcoma experimental-blood supply; laser-doppler flowmetry; oxygen; luminiscence

Received 3 August 2001
Accepted 28 August 2001

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Introduction

Over many years, abundant evidence has been accumulated that the oxygenation status in experimental and clinical tumors can influence the response of these tumors to various therapies, e.g. radiotherapy, hyperthermia, oxygen-dependent chemotherapy, photodynamic therapy and cell-mediated immunotherapy.[1-3] The oxygenation status and hypoxia in particular are also important for the development of malignant growth and for progression and outcome of the disease.[3] The ability to measure oxygenation status in individual tumors would be very valuable for the selection of appropriate therapy, treatment planning, and for prediction of the treatment outcome.

Many different methods have been developed to measure different physiological parameters related to tumor oxygenation or to tumor blood flow.[4,5] In general, tumors are poorly oxygenated in comparison to normal tissues.[1,3,5] The measurement methods should therefore enable an accurate assessment of very low oxygen levels. Extreme intratumoral heterogeneity in oxygenation and blood flow found in many experimental and clinical tumors require an evaluation of oxygenation and blood flow in different regions within the same tumor. Methods suitable for continuous measurement are particularly useful. They enable monitoring of changes in oxygenation at one location. This could be useful for at least two reasons. First, the effectiveness of treatment procedures targeting tumor blood flow and oxygenation in individual tumors could be evaluated. Second, the susceptibility of individual tumors to oxygenation- and blood flow-dependent therapy could be evaluated before the therapy is applied. It is also desirable that the measurement method of choice is noninvasive.

In the present preliminary study, we used two optical measurement methods, which fulfill some of the requirements mentioned earlier in this text. One of them, the time-resolved luminescence-based optical oximetry, presents a new approach for the measurement of oxygen partial pressure in a tissue and is an alternative to the well-established polarographic needle oximetry technique.[6,7] This method has only recently become commercially available (OxyLite instrument, Oxford Optronix, U.K.). There are two major advantages of this new method over standard polarographic method. First, the luminescence-based sensor does not consume oxygen; therefore, to monitor the changes in oxygenation as a function of time, it could be kept in one place in a tissue. Second, its accuracy is inversely proportional to oxygen content in the tissue, which makes this method of particular interest for the measurement of oxygenation in tumors where low oxygen content is typically encountered. The other optical method used in our study, the laser Doppler flowmetry, is not a new technique, but it has not been used extensively for measurements in tumors. Laser Doppler flowmetry enables the monitoring of microcirculatory blood perfusion changes. Although microcirculation and oxygenation in a tissue are related, simultaneous use of both methods can give more information about oxygen supply to a tissue than separate use of a single method. Both methods are minimally invasive and require only thin optical fibers to be inserted into tissue.

Before a new method can be used with confidence in experimental or clinical studies, its characteristics and limitations need to be known and understood. The use of luminescence-based oximetry has been so far documented in very few reports. The main goal of the present study was to get the first experience and to evaluate the usefulness of this new optical method for a continuous measurement of tumor oxygenation and for the detection of short-term oxygenation changes. The measurements were performed on SA-1 tumors in A/J mice under different conditions modifying blood flow- and oxygenation.

Materials and methods

Animals and tumors

The female A/J mice purchased from Rudjer Bošković Institute, Zagreb, Croatia, were used in our study. The mice, 10 to 12 weeks old, were kept in standard animal colony at 22°C and were fed and watered *ad libitum*. The experimental tumor line that we used was SA-1 fibrosarcoma (The Jackson Laboratory, Bar Harbor, USA). Tumor cells for the inoculation of solid tumors were obtained from the ascitic form of SA-1 tumor in A/J mice. Approximately 5×10^5 of viable cells were re-suspended in 0.1 ml NaCl solution (0.9%) and transplanted under the skin. Solid subcutaneous tumors were grown dorsolaterally on the right flank of mice. Experiments were performed 8 to 10 days after the transplantation when the tumors reached the size of approximately 100 mm^3. The size of the tumors was calculated using the ellipsoid formula as $V = \pi abc/6$ where a, b, and c are three mutually perpendicular tumor diameters measured by a vernier caliper. At the end of the experiments, the mice were euthanized under anesthesia by cervical dislocation. The experimentation on mice was conducted in accordance with the pertaining legislation and was approved by the Veterinary Administration of Ministry of Agriculture, Forestry and Food of Slovenia (permit number 323-02-156/99).

Anesthesia

All experimental procedures and measurements were conducted on anesthetized mice in order to eliminate pain and discomfort in mice and to minimize movements of non-restrained mice during long-lasting measurements. Anesthesia was induced and maintained by inhalant anesthetic isoflurane (Flurane-Isoflurane, Abbot Labs, U.K.). The gas mixture of oxygen O_2 and nitrous oxide N_2O (flow of each 0.6 l/min) containing isoflurane at 1.7% concentration was delivered to the mouse via a miniature facemask. While anesthetized, the animals were kept on an automatically regulated heating pad to prevent hypothermia. Rectal temperature was kept as close as possible to 37°C with variations of up to 0.5°C during single measurements and with the contact surface temperature of the heating pad below 39°C.

Oxygenation and blood flow measurement

Partial pressure of oxygen, pO_2, was measured by the OxyLite 2000 instrument (Oxford Optronix Ltd., Oxford, U.K.), a commercially available implementation of a novel time-resolved luminescence-based optical oximetry. The instrument has two independent channels for measuring pO_2 and temperature. The diameter of precalibrated optical probes is 230 μm. A thin wire thermocouple temperature sensor (diameter less than 100 μm) is attached to each pO_2 probe, which allows online temperature correction of pO_2 measurements. The principles of this luminescence-based optical method are described in more detail elsewhere.[6,8] Briefly, pulses of blue light emitted by a LED diode are carried via an optical fiber to ruthenium chloride luminophore, which is incorporated in a silicone rubber that is used to immobilize the tip of the probe. The tip of the probe is placed inside the tissue where oxygenation is to be measured. The incident light pulses induce pulsatile fluorescence of ruthenium molecules. The fluorescence decays in time because of collisions between the oxygen and the ruthenium molecules. The life-time of the excited fluorescence is inversely proportional to pO_2 in the part of the tissue that is in contact with the tip of the probe. The pO_2 can therefore be calculated from the measured life-time of the fluorescence using the so-called Stern-Volmer relation.[8]

Relative blood perfusion was monitored using a two-channel OxyFlo 2000 laser

Doppler instrument (Oxford Optronix Ltd., Oxford, U.K.). Even though laser Doppler flowmetry (LDF) can be applied entirely non-invasively, we used thin invasive probes (diameter 200 μm) in order to assess the perfusion inside the tumor. LDF is an optical method used to monitor local microvascular blood flow in a tissue. Extensive literature exists on its theory and application.[9,10] Briefly, when a tissue is illuminated by a coherent laser light, the light scatters on different tissue structures. When photons are scattered on moving structures, their wavelength is slightly changed. This is the so-called Doppler shift effect, which can be measured. The predominant moving structures in a tissue at rest are red blood cells. Their movement, which results from blood flow, can be detected by means of the Doppler shift effect. The output signal of LDF is proportional to the red blood cell perfusion which is defined as the number of the red blood cells multiplied by the mean velocity of these cells that move in the tissue sampling volume. The constant of proportionality between the perfusion and the detected LDF signal is unfortunately different for each location even within the same tissue. This means that all LDF measurements are intrinsically of relative nature and are quantified in arbitrary blood perfusion units (BPU).

Both instruments OxyLite and OxyFlo were connected to OxyData data acquisition unit (Oxford Optronix Ltd., Oxford, U.K.), which enabled data storage to a PC via a SCSI connector. All signals were sampled and stored at the frequency of 20 Hz.

Measurement protocol

Anesthesia in the mouse was started in the induction chamber at a concentration of isoflurane of 3 %. The mouse was then placed on the heating pad in prone position. No physical restriction was used. The anesthetic gas was delivered via a miniature facemask. The concentration of isoflurane was reduced to 1.7 %, which provided stable anesthesia. Rectal and surface temperature probes were attached for control of the core temperature of the mouse and of the surface temperature of heating pad. Approximately four minutes after the induction of anesthesia, the pO_2 and the LDF probes were inserted into the tumor through small superficial incisions in the skin. The probes were inserted through the incisions, pushed a few millimeters further into the tumor and then slightly withdrawn in order to minimize the pressure of the tip of the probe on the surrounding tissue. Although an exact positioning of the probes was not possible, one pair of pO_2 and LDF probes was inserted in a peripheral region of the tumor and the other in a central region of the tumor. Data recording was started normally about five minutes after the beginning of anesthesia. Special care was taken throughout the measurement not to move the probes or the mouse in order to minimize the movement artifacts in recorded signals.

A typical measurement lasted between one and two hours. In order to evaluate the applicability of both measurement methods for the detection of the changes in the blood perfusion and oxygenation in tumors, different procedures were applied. These procedures to induce changes in blood flow and oxygenation were performed only after the recorded signals had been stable for at least ten minutes. Normally, the stability of all signals (two pO_2 and two LDF) was reached between 20 and 40 minutes after the start of anesthesia.

The effect of hydralazine

An arteriolar vasodilator hydralazine (HYZ) was injected i.v. at a dose of 2.5 mg/kg of mouse weight. The solution for injection was prepared from powdered HYZ (Hydrazinophthalazine, Sigma Chemical Co., U.S.A.) by

dissolving it in sterile physiological saline (0.9 % NaCl). The mice in the control group were injected with sterile physiological saline only.

The effect of anesthetic concentration

Normal anesthesia was maintained by delivering isoflurane at a concentration of 1.7 % in a steady flow of O2 (0.6 l/min) and N2O (0.6 l/min). For the purpose of evaluation of the effect of anesthetic concentration on the blood flow and oxygenation in tumors, the concentration of isoflurane was increased to 3% for three minutes and then returned to the normal level of 1.7 %.

The effect of euthanasic procedure

In order to evaluate the validity of measurements, pO_2 and blood perfusion were also monitored during euthanasia of mice at the end of experiment. First the delivery of O_2 was eliminated from anesthetic gas mixture while maintaining the flow of nitrous oxide and isoflurane. Within two minutes after this procedure, the mice stopped breathing.

Results

A typical measurement

Both pO_2 and blood perfusion were measured at two locations in each tumor. In most cases the data recording was started immediately after all four probes were inserted into the tissue and, in few cases, the data recording was started just before the probes were inserted. All this occurred within five minutes after the start of anesthesia. The measurement lasted between one and two hours. Figure 1 shows an example of typical signals recorded simultaneously from one tumor during the first 40 minutes of measurement. Several important features characteristic of all pO_2 and LDF measurements can be observed.

After the insertion of the probes, the value of pO_2 in all measurements varied rapidly (within one minute). It decreased from initially high level to zero or close to zero value. We call this a *decrease phase* which cannot be observed in Figure 1 because, in this case, the data recording was started after the insertion of the probes. Following the decrease phase, two different types of pO_2 recordings were observed. An example of the first type, we shall call it a type I pO_2 signal, is shown in Figure 1a. In the type I measurements, the pO_2 value remained at zero or close to zero for a period of time which varied between different measurement locations, but in the majority of measurements, it lasted about five minutes. We call this a *zero pO_2 phase*. Following the zero pO_2 phase, the value of pO_2 in the type I measurements entered the *increase phase* during which the value of pO_2 was slowly increasing. The increase phase lasted on average between 15 and 20 minutes. After the increase phase, pO_2 value stabilized and remained mostly unchanged thereafter unless some oxygenation-modifying procedure was applied. We shall refer to this final stage in type I pO_2 measurements as to a *plateau phase*. In most type I measurements, pO_2 continued to increase very slowly even during the plateau phase but this increase was much slower than the increase during the *increase phase*. The type I pO_2 measurement was found in approximately 70 % of all measurement locations in tumors.

In about 30 % of all pO_2 measurements performed, pO_2 in tumors remained at zero or close to zero level for the entire period of observation after the initial *decrease phase*. An example of this second type of pO_2 recording, we shall call it a type II pO_2 signal, is shown in Figure 1b. In comparison to the type I measurements, the type II measurements are characterized by a complete absence of the *increase* and the *plateau phases* (compare the top two graphs in Figure 1).

It is very important to note that, in some cases, both the type I and the type II pO_2

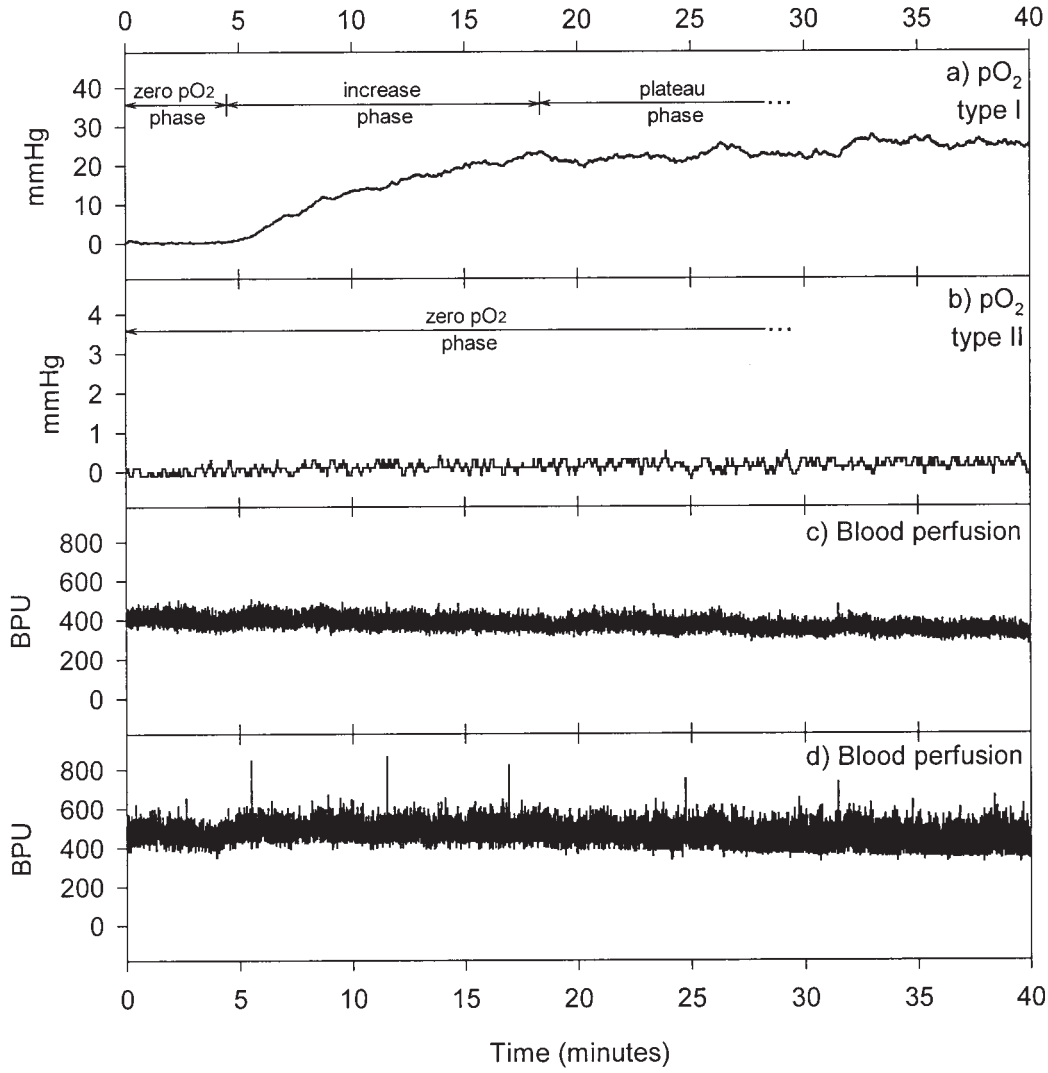

Figure 1. A typical measurement consisting of two pO_2 (a, b) and two LDF measurements (c, d) recorded simultaneously in the same tumor. The two distinct types of pO_2 measurements can be identified (a, b). The first 40 minutes of recording from a single tumor are shown.

measurements were encountered within the same tumor, as in the case presented in Figure 1. In other cases, both pO_2 measurements in tumor resulted in either of the two types, the type I or the type II measurement. In order to obtain a representative pO_2 value for each type I measurement, we averaged the raw pO_2 signal in the plateau phase over a period of five minutes. It is again important to note that these averaged pO_2 values in the type I measurements varied extremely between tumors and also from one location to another within the same tumor, from as little as 1 mmHg to more than 40 mmHg in a few cases. All tumor pO_2 values at rest including the averaged type II values were pooled to-

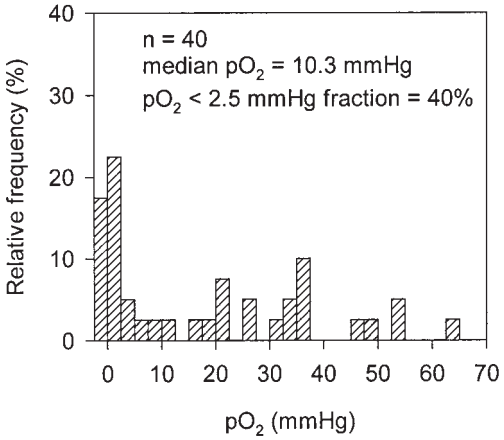

Figure 2. Distribution of all tumor pO_2 values measured at rest (n = number of measurements).

gether and are presented in a histogram in Figure 2. The median pO_2 value of 40 measurements from 28 tumors was 10.3 mmHg. A fraction of pO_2 values below 2.5 mmHg was 40 %.

Contrary to the pO_2 signals, only one type of LDF measurements was identified in tumors (Figure 1c and 1d). All recorded LDF signals became stable soon after the insertion of probes. A zero or close to zero blood perfusion was never encountered, which again is contrary to the pO_2 measurements. Due to the nature of LDF technique, the blood perfusion measurements are extremely sensitive to any kind of movement of probes relative to the surrounding tissue. The "smeared" blood perfusion signals in Figure 1 is a movement artifact caused by a quasi-periodic breathing of the mouse. This is shown in more detail in Figure 3. The spikes in Figure 3 correspond to inhalation and the "valleys" in between the spikes correspond to exhalation. The true blood perfusion is at the lower edge of the "smeared" blood perfusion signals in Figure 1. The amplitude of the quasi-periodic component in LDF signal, which could be even bigger than the true perfusion component of the signal, depended on the position of individual LDF probes relative to the direction of tumor movement caused by respiration. Because of this, substantial differences in the relative amplitude of the quasi-periodic component in comparison to the true perfusion level in different locations within the same tumor were common (compare Figures 1c and 1d).

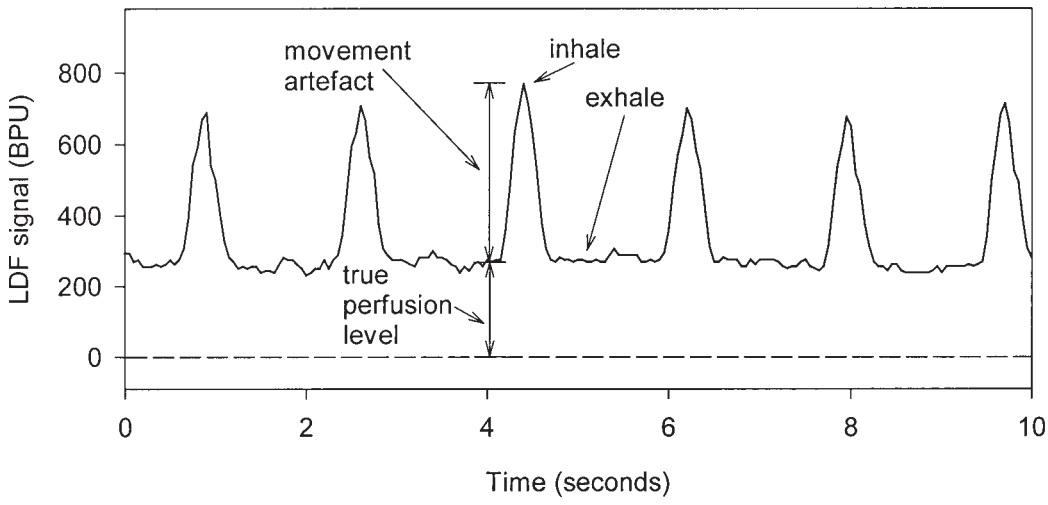

Figure 3. A close-up of a typical blood perfusion signal measured by LDF. The movement artifact caused by quasi-periodic respiration of the mouse is shown.

The effect of hydralazine

Within one minute after the injection of HYZ, all type I pO_2 and all blood perfusion signals in tumor started to decrease (example in Figure 4b, c, d). In five to ten minutes after the injection, these signals reached the lowest level. On average, pO_2 decreased by 80 % (n = 13) and blood perfusion decreased by 50 % (n = 17). HYZ also induced a decrease in respiration rate and an increase in depth of breathing in mice, thus resulting in increased amplitude of the movement artifact in LDF signals. In most tumors treated with HYZ, type I pO_2 signals and blood perfusion signals started to recover very slowly approximately half an hour after the injection of HYZ. In the type II pO_2 signals (Figure 4a) no change was observed after the injection of HYZ. No significant changes were observed either in the type I pO_2 signals or in the blood

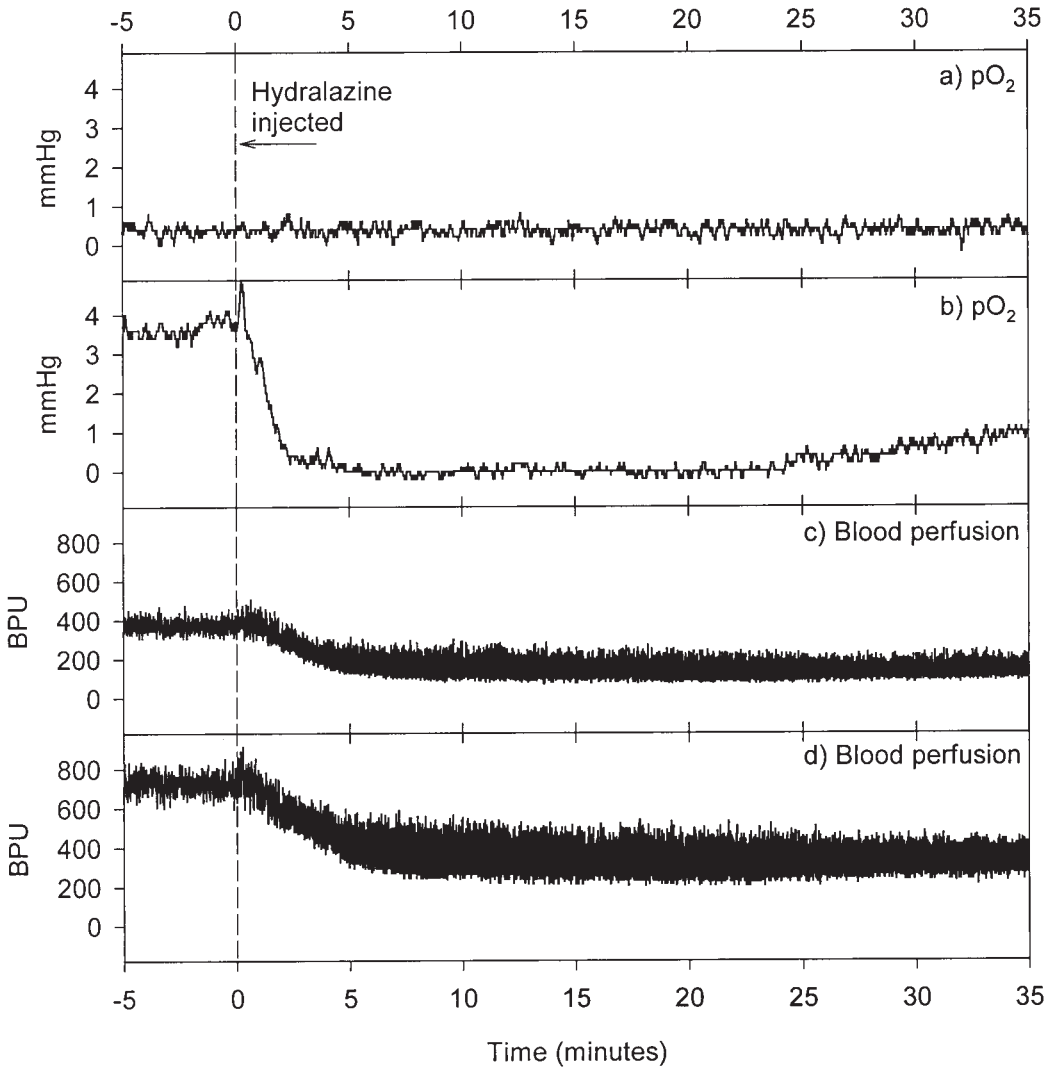

Figure 4. The effect of hydralazine on tumor pO_2 (a, b) and blood perfusion (c, d). The vertical line shows when hydralazine (dose 2.5 mg/kg) was injected. All recordings are from the same tumor.

perfusion signals in control animals which were injected with physiological saline (example not shown). The difference in the decrease of type I pO_2 values in tumors between HYZ-treated and control mice was highly significant ($p < 0.001$; Mann-Whitney rank sum test) as was also the difference in the decrease of blood perfusion between HYZ-treated and control mice ($p < 0.001$; Mann-Whitney rank sum test).

The effect of anesthetic concentration

An attempt was made to modify the blood perfusion and oxygenation in tumors by a transient increase and subsequent decrease of isoflurane concentration in the inhalation mixture. An example of the results in one tumor is shown in Figure 5. About one minute and a half after the increase of isoflurane concentration from 1.7 % to 3 %, the blood perfusion started to decrease, as can be seen in both LDF signals in Figure 5c and d. With the increase of isoflurane concentration the respiration in mice to become slower and jerkier, which resulted in an increased amplitude of the movement artifact in LDF signals. The decrease of blood flow was closely followed by a decrease in the type I pO_2 signals (Figure 5a). When isoflurane concentration was returned to normal level of 1.7 %, the blood perfusion started to increase within a few seconds. After a delay of about 1.5 minutes, the type I pO_2 signal also started to increase. While the LDF signals asymptotically approached the pre-treatment value, the type I pO2 signals usually approached the pre-treatment level after an overshot as can be seen in Figure 5a. No changes at all were seen during the described procedure in the type II pO_2 signals (Figure 5b).

The effect of euthanasic procedure

For the purpose of validation of the measurements, pO_2 and blood perfusion were also monitored during euthanasic procedure. At the end of each measurement, the flow of oxygen to the inhalation mixture was terminated while maintaining the flow of nitrous oxide and isoflurane. A typical example of the effect of this procedure on pO_2 and blood perfusion can be observed in Figure 6 which shows the last four minutes of recorded signals in one tumor. The type I pO_2 signals and blood perfusion signals rapidly decreased. This occurred within one minute after the shutdown of oxygen flow to the vaporizer. The decrease of pO_2 always occurred approximately ten seconds before the decrease of blood perfusion. The bottom level of pO_2 reached after the death was always close to zero in the range of -0.6 to +0.3 mmHg. The type II pO_2 signals (Figure 6b), which were close to zero value during the whole period of observation, remained unchanged during this procedure. In the majority of cases, the bottom level of blood perfusion reached after death was slightly above zero value.

Discussion

The time-resolved luminescence-based optical method used in our study is a new method that has only recently become available on the market. Its use has been reported by few authors so far.[6,7,11-13] Most authors report on evaluation of the new method by comparing this method to other techniques for measuring tissue oxygenation, in particular to the well-established polarographic oximetry. By many researchers, the polarographic oximetry and its implementation in Eppendorf Histograph instrument is considered to be the "golden standard" for pO_2 measurements in experimental and clinical tumors. A generally good correlation between the results of polarographic method and the new optical method was found in tumors,[6,13] but there were also discrepancies between the two methods in certain conditions and in different tissues.[11,13] These discrepancies arose from differences be-

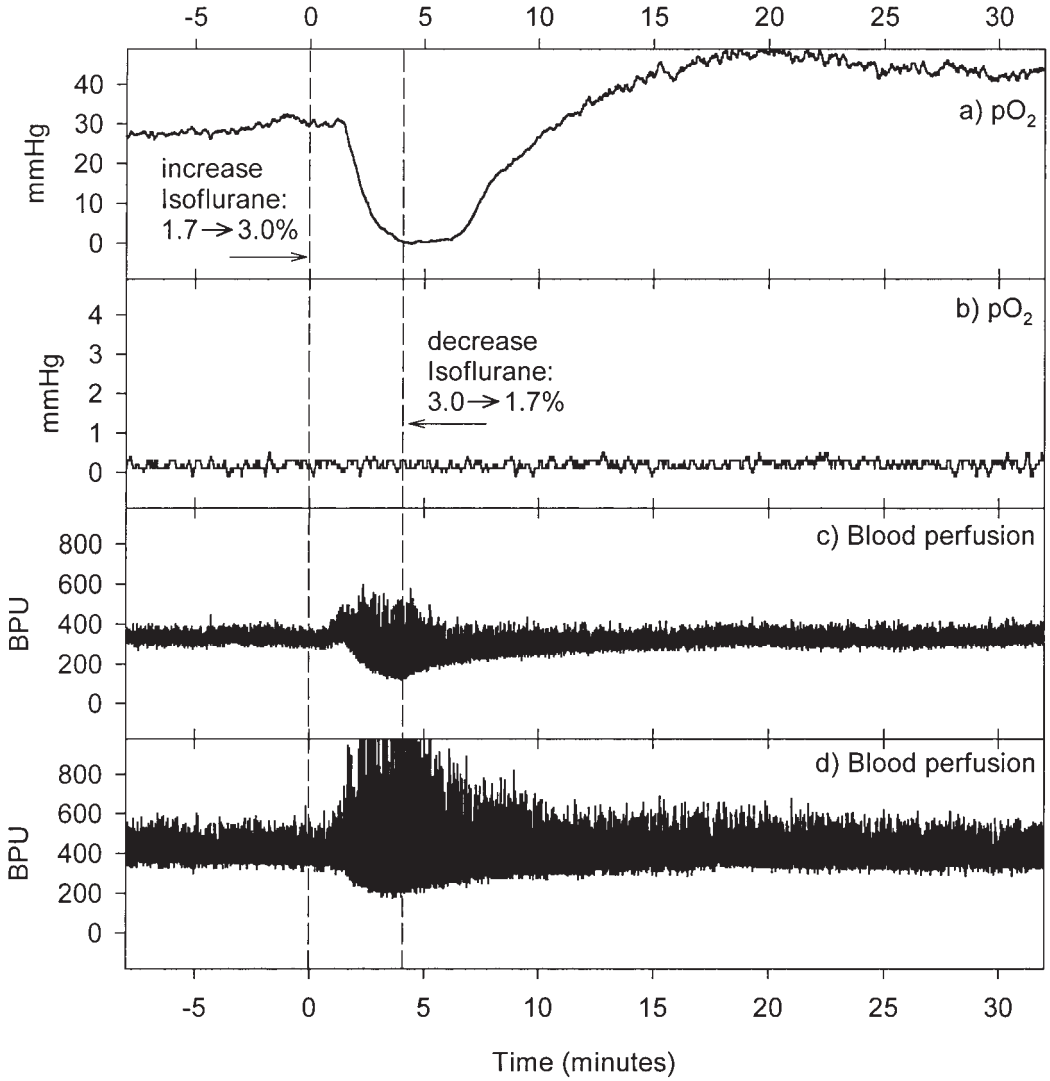

Figure 5. The effect of transient change in anesthetic concentration on tumor pO_2 (a, b) and blood perfusion (c, d). The two vertical lines show when concentration of isoflurane was changed. All recordings are from the same tumor.

tween the two methods such as the underlying physical principle of measurement, dimension of the probe, tissue sampling volume, and consumption of oxygen by the sensor.

A typical measurement

In our study, individual values of pO_2 measured with the novel luminescence-based method at rest prior to any blood perfusion- and oxygenation-modifying procedure were scattered in the range of 0 to above 40 mmHg. The great inter- and intratumoral variability in oxygenation observed in our study is in agreement with the well-documented inter- and intratumoral variability of many experimental and clinical tumors.[14] The histogram of all measured pO_2 values at

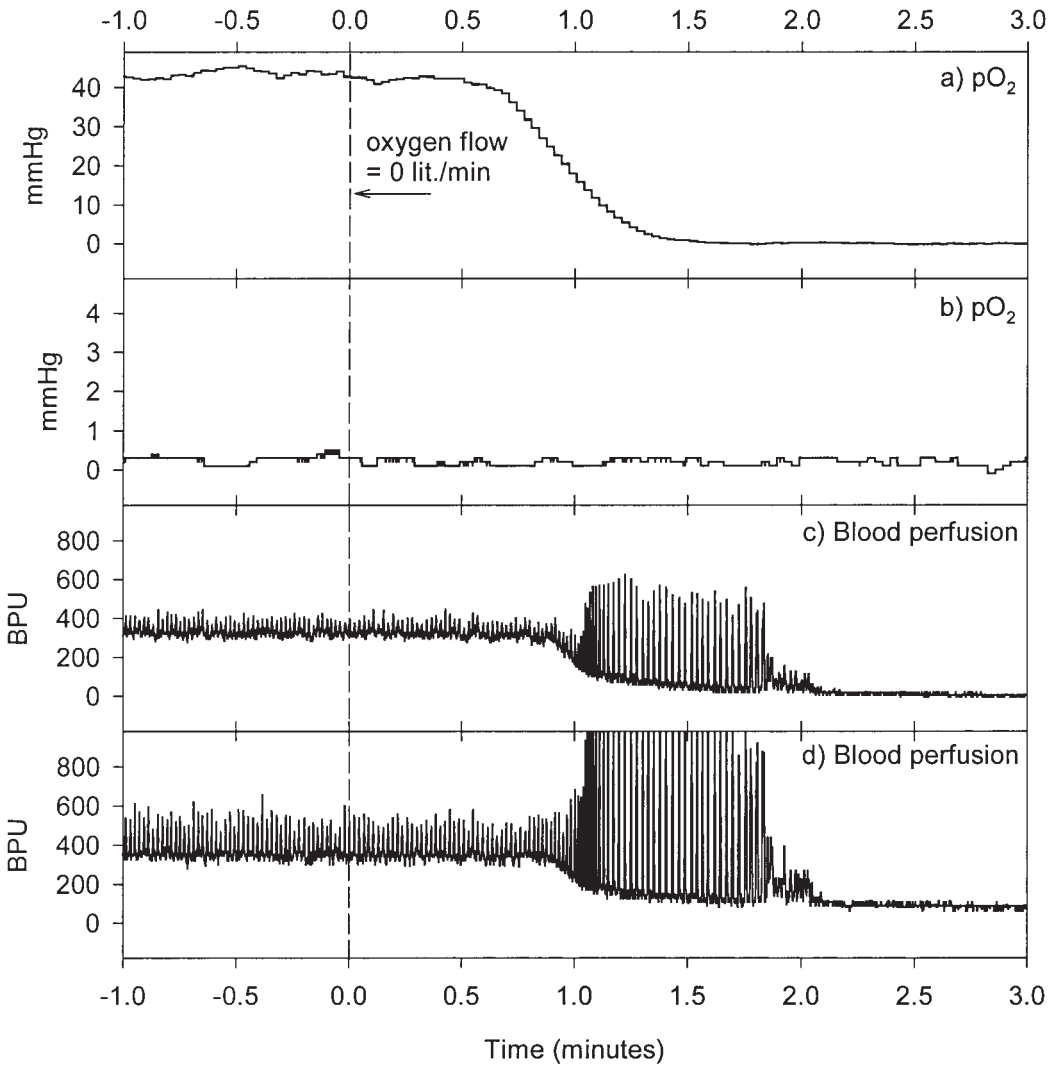

Figure 6. The effect of euthanasic procedure on tumor pO_2 (a, b) and blood perfusion (c, d). The vertical line shows when oxygen supply to inhalation mixture was terminated. All recordings are from the same tumor.

rest (Figure 2) shows a commonly encountered distribution of pO_2 values in tumors with the majority of pO_2 values close to zero. However, we did not expect the multiphase behavior found in the type I pO_2 measurements (Figure 1a).

The most probable reason for the observed phenomenon is the direct effect of the insertion of the probe on the tissue oxygenation. Steinberg *et al*. evaluated the injury caused by the insertion of polarographic pO_2 probe into tumor tissue in different tumor models.[15] They found clear histological evidence of tissue destruction and extravasation of erythrocytes around the insertion channel in tumor tissue caused by the insertion of probe. On the other hand, Schramm *et al*. provided histological evidence for compressed microves-

sels in the vicinity of the tip of polarographic sensor inserted into the rat muscle tissue.[16] The capillary compression increases the perfusion resistance of the passing erythrocytes, decreases the oxygen-carrying capacity of capillaries and, thereby also the oxygenation of the tissue in direct contact with the probe. It is also possible that the insertion of the probe into the tissue causes the vasoconstrictive reaction and subsequent deoxygenation of the tissue.[13]

The following hypothesis may explain the type I pO_2 signals. The insertion of the probe undisputedly injures the tissue in the immediate vicinity of the insertion channel. Some capillaries are ruptured and others can be occluded; a vasoconstrictive reaction in the tissue can occur. All these physiological changes can decrease the oxygen delivery to the tissue in the immediate vicinity of the tip of the probe. This tissue might therefore be severely deoxygenated immediately after the insertion of the probe. This deoxygenated state probably corresponds to the *zero pO_2 phase* in the type I pO_2 measurements (Figure 1a). In the period that follows, this tissue could be re-oxygenated due to gradual restoration of microcirculation. Gradual reoxygenation is probably reflected in the *increase phase* in the type I pO_2 measurements. In case of the type II pO_2 measurements, pO_2 of the tissue is most probably so low that no further deoxygenation and subsequent reoxygenation could be observed after the insertion of the probe.

The multiphase behavior of the type I pO_2 signals was never experienced in our blood perfusion measurements. This difference between generally well correlated LDF signals and type I pO_2 signals can be explained by a much larger tissue sampling volume in case of LDF measurement than in pO_2 measurement. LDF samples a tissue volume of the order of a cubic millimeter. The actual tissue sampling volume for the new time-resolved luminescence-based method is unknown, but is much smaller than the tissue sampling volume of LDF. The measured pO_2 values reflect local oxygenation in a very small part of tissue surrounding the tip of the probe. It is believed that this method samples pO_2 in the cells and intercellular space in direct contact with the tip of the probe, which means a volume of about 400 cells only.[7] The tissue farther away from the insertion channel, which is not affected by the insertion itself, should therefore contribute significantly to the LDF signal but not to the pO_2 signal.

To our knowledge, the unexpected multiphase behavior of pO_2 has been so far reported only in a recent paper by Seddon *et al*..[13] Our results are in excellent agreement with their results even though Seddon *et al.* performed their measurements on non-anesthetized and physically restrained mice. Other authors using OxyLite system have not reported this phenomenon.[6,7,11,12]

The effect of hydralazine

The results of our study showed that hydralazine at a dose of 2.5 mg/kg significantly reduced blood flow and oxygenation of SA-1 fibrosarcoma tumors in A/J mice. The effect was seen in all tumors in case of LDF measurements and in case of all type I pO_2 measurements (Figure 4). The amplitude and dynamics of the decrease in blood perfusion after the injection of HYZ obtained in our study are in direct agreement with the results obtained with LDF by other authors in various mouse tumor models after the injection of HYZ.[17-20] Our measurements of pO_2 by means of the novel time-resolved luminescence-based optical oximetry showed a pronounced decrease of pO_2 after the injection of HYZ. On average, pO_2 decreased by 80 % from the pretreatment level. This decrease was well correlated to the decrease in blood flow measured by LDF. Our results are also in agreement with the results of Okunieff *et al*.[21] who showed that metabolic rate in experi-

mental mouse tumors as measured by ^{31}P-NMR spectroscopy was significantly decreased by HYZ at a dose similar to ours. In their study the decreased metabolism caused by the lack of oxygen was demonstrated by the decrease of organic phosphates and increase of inorganic phosphates. Hydralazine is an effective peripheral vasodilator that has been used in the treatment of hypertension in humans.[22] It relaxes arteriolar smooth muscle, thereby effectively reducing the peripheral vascular resistance and decreasing blood pressure. In these conditions, the organism is trying to maintain normal blood flow in vital organs and tissues by "stealing" the blood flow in less vital tissues.[21] This "steal phenomenon" is responsible for the demonstrated decrease in blood perfusion in tumors. In our preliminary study we provide direct evidence of markedly decreased tumor oxygenation caused by the decrease in blood perfusion after the injection of hydralazine.

The effect of anesthetic concentration

Anesthetics undoubtedly affect a number of parameters of physiological conditions in mice. Isoflurane used in our study is a recommended anesthetic for small animals due to its minimum side effects, stable anesthesia, and wide safety margin. Isoflurane produces little or no depressant effect on cardiovascular system but it causes some respiratory depression.[23] Nitrous oxide, which was used together with oxygen to deliver isoflurane to anesthetized mice, has no significant effects neither on cardiovascular nor respiratory system.[23] Despite these facts, it was shown that a transient increase in concentration of isoflurane from 1.7 % (concentration used for maintenance of long-term stable anesthesia) to 3 % (concentration used for induction of anesthesia) produced a significant decrease both in the blood perfusion and in oxygenation of tumors (Figure 5). Both variables decreased with similar dynamics. When isoflurane concentration was returned to normal level, there was a delay between the increase of blood perfusion increase and that of pO_2. This can be explained by the delivery-limited oxygen consumption in low pO_2 conditions. All additional oxygen delivered by the increasing blood flow was readily consumed until oxygen delivery became abundant. When this happened, pO_2 also started to increase. The demonstrated effect of the change of anesthetic concentration indicates that: i) anesthetic conditions should be kept as constant as possible during the prolonged measurements of tumor blood perfusion and oxygenation; and ii) the values of tumor pO_2 measured under anesthesia are probably not entirely representative of the pO_2 in non-anesthetized conditions.

The effect of euthanasic procedure

Tumor pO_2 and blood perfusion were monitored during euthanasic procedure in order to verify if the measurements were valid. The results were as expected: the blood perfusion and type I pO_2 signals both decreased when oxygen supply to inhalation mixture was terminated (Figure 6). It is noteworthy to mention that the decrease in pO_2 preceded the decrease in blood perfusion by several seconds. This is a consequence of the decreased oxygen delivery in the presence of the still functioning blood flow. The value of type I pO_2 signals in all measurements dropped to zero level (as it should at death). However, in the majority of LDF measurements, there was still some residual blood perfusion signal present after death. This fake "blood perfusion" signal experienced in the absence of true blood flow is the so-called *biological zero* signal which is usually observed in laser Doppler measurements. The principle of laser Doppler flowmetry is based on the measurement of movement of red blood cells. In case of biological zero, the movement detected is predominantly Brownian motion

(thermally induced random motion) of various structures in the tissue.[9] In the laser Doppler signal picked up from the living tissue, the ever-present biological zero component is outweighed by a much stronger component originating from the true blood flow. In our measurements in tumors, the biological zero level detected after the death of the mice was normally less than 5 % of the total signal level detected in tumor at rest.

Conclusions

The first very important finding of the present study is that two distinct types of pO_2 signals were encountered in tumors. Only the type I measurements resulted in pO_2 values different from zero and, only in the type I measurements, the effect of oxygenation-modifying procedure could be seen. It is however possible that a procedure, which increases oxygenation, might convert some of the type II pO_2 measurements to the type I measurements. The second very important finding is that it takes a considerable amount of time before the type I pO_2 signals stabilize in the *plateau phase*. Reliable measurements of pO_2 changes can only be performed after the signal has entered the plateau phase. But it remains to be seen whether and to what extent the pO_2 value measured in the plateau phase represents the true pO_2 as it was before insertion of the sensor.

In our preliminary study using a novel time-resolved luminescence-based method for measuring the tissue oxygenation in combination with a well-established laser Doppler flowmetry, we have shown that both methods can be effective in the detection of local oxygenation and blood perfusion changes in tumors. Good correlation between the signals of both methods was found as it should be found since oxygenation in tissue depends on tissue microcirculation. It is important to note that these two methods are essentially showing different things and that their respective tissue sampling volumes are very different. Therefore, the results of one method can only supplement, but not replace the results of the other. Based on our results, we conclude that the interpretation of some aspects of pO_2 measurements with the novel luminescence-based method requires further investigation.

Acknowledgements

This study was supported partly by the Ministry of Education, Science and Sport of the Republic of Slovenia (grants J2-2222-1538, J3-3489-0302-01) and partly by the European Commission and the 5th Framework Programme (grant QLK 3-99-00484, CLINIPORATOR project). The authors wish to thank Simona Kranjc, M.Sc., of the Institute of Oncology, and Blaž Podobnik, B.Sc., of the Faculty of Electrical Engineering for their technical support in the course of the study.

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Persistent chromosomal aberrations in somatic cells in testicular cancer patients after different therapies

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Background. *The damage due to radiation or chemotherapeutic agents has been estimated successfully for the last 35 years from the numbers of the chromosome changes. This finding may serve as biological dosimeter. The aim of the study was to find persistent chromosomal aberrations in somatic cells in testicular cancer patients after different therapies.*

Patients and methods. *This prospective study includes 60 patients with testicular tumours. With respect to the histological results and various therapies that they were given they were divided into four groups. Prior to treatment, we did not detect any deviations either in the genome picture of our patients or in that of the subjects of the control group without malignant disease. The changes in the genome of individual cells after therapy were detected by the following tests: structural chromosomal aberrations (SCA) test, sister chromatid exchange (SCE) test and micronucleus (MN) test performed on binuclear lymphocytes.*

Results. *Immediately after the completion of treatment, chromosomal aberrations were inhibited, with the exception of dicentrics which persisted. Chemotherapy is less detrimental to the genome picture than radiotherapy and causes different types of chromosome changes. From the cytologic and mutagenetic points of view, irradiation proved to be more aggressive to patients than chemotherapy. Six months after the completed treatment, the mitotic activity was found to be nearly normal; but the chromosome damage persisted and was higher than before therapy and in the fourth group of patients who had been only operated on.*

Conclusions. *After irradiation as well as after chemotherapy the genome was repaired, because the damaged cells had died away. Considering that in the observed patients, only small tissue-cellular complex responded to radiation and that the number structural chromosome changes, predominantly unstable aberrations, such as dicentrics, was rather high, we assume that the repair of the genome will be faster in these patients.*

Key words: testicular neoplasms – therapy; chromosomal aberations; micronuclei

Received 19 September 2001
Accepted 27 September 2001

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Introduction

Patients with seminoma tumours received postoperative irradiation[1,2] or postoperative adjuvant chemotherapy with paraplatinum.[3,4] Patients with nonseminoma tumours may be treated by postoperative chemotherapy with BEPV5 or by surgery alone. The choice of treatment modality depends on the stage of disease, and toxic effects of treatment.[1,6-9]

The damage due to radiation or chemotherapeutic agents has been estimated successfully for the last 35 years from the numbers of the chromosome changes in the culture of lymphocytes in the peripheral blood.[10] The exposure of cells to ionising radiation can be recognised from a higher number of structural chromosome changes. This finding may serve as biological dosimeter. The technique most often used for the identification of these changes uses the following steps: preparing the cells exposed to radiation under in vitro circumstances and counting the chromosomal changes.[11-14]

Patients and methods

Subjects

This prospective study included 60 patients with testicular tumours, aged between 15 and 35 years, who had not been previously treated for any malignant disease. With respect to the histological test results and various therapies applied (surgery, radiotherapy, chemotherapy) we divided them into four groups. Mutagenetic tests were performed at diagnosis, immediately after the completion of treatment and six months after it.

The stage of the disease in patients with testicular tumour was defined according to TNM classification from the year 1992 (29).

Treatment approaches

Group I

All patients in the first group received postoperative irradiation. Radiotherapy was performed by an 8 MeV linear accelerator, using X rays. The irradiated area involved the iliac and paraaortal lymph nodes on the same side as the malignant process. The radiation field comprised an area from Th 10 to L5 and had a width of 10-11cm, AP and PA, and the inguinal lymph nodes on the same side, from the upper edge of L5, 1 cm medially, involving also the entire postoperative scar only up to a depth of 3 cm and only AP. On the back, the radiation field was the same, extending up to L5 and from there to the sacrococcygeal bone. The total radiation dose for Stage I was 30 Gy and for Stage II 36 Gy; the daily dose was 1.5 Gy. Thirteen patients were irradiated with 30 Gy and only two with 36 Gy.

Group II

The patients of the second group had a postoperative adjuvant chemotherapy with paraplatinum. A single dose (paraplatinum 300-400 mg/m^2) was administered in short infusion. The patients in the second group received one, two or three cycles of paraplatinum in doses from 450 mg to 750mg, depending on the body surface area. The total dose of paraplatinum thus ranged between 450 mg and 2250 mg per individuum.

Group III

Patients with nonseminoma tumours underwent surgery, orchidectomy of the affected testis followed by the adjuvant chemotherapy according to BEPV schedule: cisplatin 20 mg/m^2 i.v., days 1-5; bleomycin 15 mg/m^2 i.v., 1st and 2nd day; etoposide 100 mg/m^2 i.v., days 1-3; - vinblastine 3 mg/m^2 i.v,. 1st and 2nd day.

Patients had two, three or four cycles of chemotherapy according to BEPV schedule. Seven patients had four cycles, four patients had three cycles and four patients had two cycles of chemotherapy.

Group IV
All patients in this group underwent only surgery, i.e. orchidectomy of the affected testis, followed by lymphadenectomy or observation only.

Cytogenetic studies

We used peripheral blood lymphocytes as target material. Blood samples were taken simultaneously for the following three tests.

Structural chromosomal aberrations (SCA)
We used standard in vitro lymphocyte cultures for structural chromosomal aberration analysis. We added 0.3 ml of heparinised whole blood to 5 ml of Chromosome med 1A-GIBCO culture medium. The first in vitro cell division cycle was established with the addition of 5 μg/ml of BrdU-Sigma. The hypotonic procedure was performed with 0.075 mol/l potassium chloride, whereas fixing was carried out in a mixture of glacial acetic acid and methyl alcohol at a ratio of 1:3. The cell suspension was pipetted onto cold glass slides. The specimens were air-dried and stained with Giemsa-Sigma. The maximum analysed for every test subject were the very first 200 in vitro metaphases. The chromosome analysis was carried out exclusively in the metaphases of the first division cycle, identified by homogeneously stained chromosomes. Structural damages to chromosomes were categorised as chromosomal breaks, acentric fragments, dicentrics and ring chromosomes. Gaps were not included in the total number of chromosomal aberrations.[16]

Sister chromatid exchange (SCE)
The same culture medium was used as in the first test. We prepared in dark 72-hour lymphocyte cultures with the addition of 10 μg/ml BrdU and carried out the procedure according to Kato.[17] We analysed 50 cells per subject, counted SCE and presented them as average numbers per cell. The range of SCE frequencies was also recorded for every subject.

Micronucleus test (MN)
For this test, 3 μg/ml of cytochalasin B-Sigma was added to each in vitro lymphocyte culture in the 43rd hour of cultivation. We used the Fenech-Morley method. Hypotonic procedure was omitted. The specimens were stained with May-Grunwald and Giemsa. We analysed the cells with clearly blocked cytokineses (CB cells), i.e. binuclear cells. We examined 500 cells per person and presented the results as the number of micronuclei per 500 CB cells. For technical reasons, the MN test was not performed on the control group – environmental exposure.[18]

Statistical data processing

The median value of all parameters in the four groups of patients and for all three measurements was presented graphically (Windows Microsoft Excel). The changes in the genome picture were analysed for each treatment modality using the overall analysis of all three measurements (Friedman's test)[19] and the analysis of two measurements (Wilcoxon's test).[19] The Kruskal-Wallis (KW) test and Mann-Whitney (MW) test were used for the comparison of the median values of the areas between the two groups; the difference between groups is statistically significant if $p < 0.025$, since Bonferroni's correction must be taken into account.[20] A personal computer and the SPSS programme package (SPSS for Windows, Version 8.0.1) was used for statistical data processing that was carried out at the Institute for Biomedical Informatics of the Faculty of Medicine, University of Ljubljana.

Results

Prior to treatment, we did not detect any deviations neither in the genome picture of our patients nor of the subjects of the control group without malignant disease. (Table 1, Fig. 1-6).

Immediately after the completion of treatment, the mitotic activity was observed as well as a significant increase in the frequency of chromosomal aberrations. In the patients treated with radiotherapy, this was mainly due to an increased number of dicentrics (Fig. 2). Chemotherapy affects the genome to a lesser degree than radiotherapy. Usually, the percentage of structural chromosome changes is higher after chemotherapy ($p = 0.005$) (Fig. 1A), but still lower than after irradiation $p = 0.005$ (Fig. 1). The types of chromosomal changes in the observed patients were different, with the unstable chromosome changes predominating ($p = 0.005$) (Fig. 2). After chemotherapy, the number of dicentrics is not significantly different ($p = 0.08$) (Figure 2). The number of MN is higher after irradiation than chemotherapy ($p = 0.005$) (Fig. 5), although after chemotherapy according to BEPV schedule and chemotherapy with paraplatinum, the numbers of micronuclei differ significantly ($p = 0.005$) (Fig. 5). A significantly higher number of SCE was noted after chemotherapy than after radiotherapy ($p = 0.001$) (Fig. 6).

Six months after the completion of treatment, the mitotic activity was found to be mainly normal, but in comparison to the fourth group that was treated only by surgery, a large percentage of chromosomal aberrations persisted (Fig.1).

The analysis of genome changes immediately after the completion of therapy and six months later showed statistically significant difference in chromosome changes in the first group ($p = 0.01$) and in the third group

Table 1. The median value of all parameters in four groups of patients and for all three measurements

	measurement	group I	group II	group III	group IV
Me of % SCA/200 cells	1	2	1,5	1,5	1,5
	2	50	4,5	6	1,5
	3	9	3	3,5	1,5
Me of dicentrics/200 cells	1	0	0	0	0
	2	17	0	0	0
	3	6	0	0	0
Me of acentric fragments/	1	1	0	0	0
200 cells	2	21	2,5	2	0
	3	8	2	2,5	0
Me of chromosomal breaks/	1	3	3,1	2	2,9
200 cells	2	5	5,1	4,9	3
	3	5	4,1	4	3
Me ofMN/500CB cells	1	5	6	6	4
	2	19	8	12	4
	3	12	7	10	4
SCE/50 cells	1	6,2	6,4	6,4	6,2
	2	6,4	7,9	8,3	6
	3	6,5	7,4	7,2	6

Me = median value, No = number, SCA = structural chromosomal aberrations, MN = Micronucleus test, SCE = sister chromatid exchange

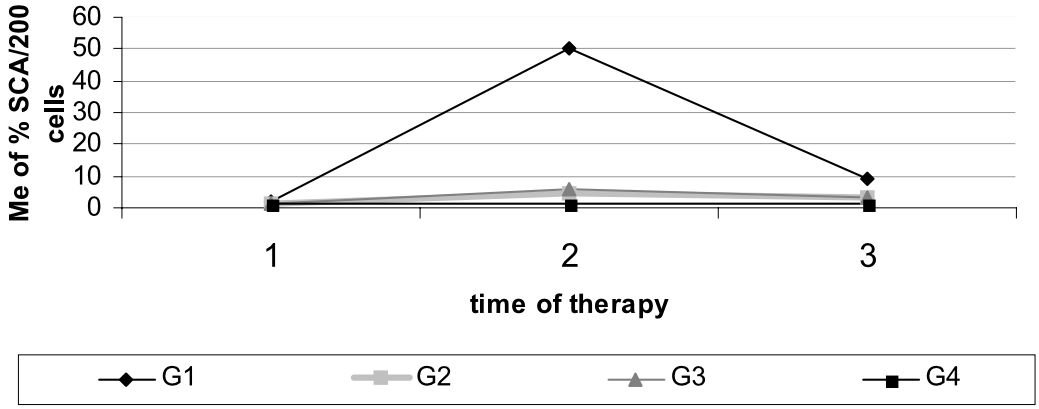

Figure 1. Median value of the chromosomal aberrations of patients with testicular tumour (p = 0.005).

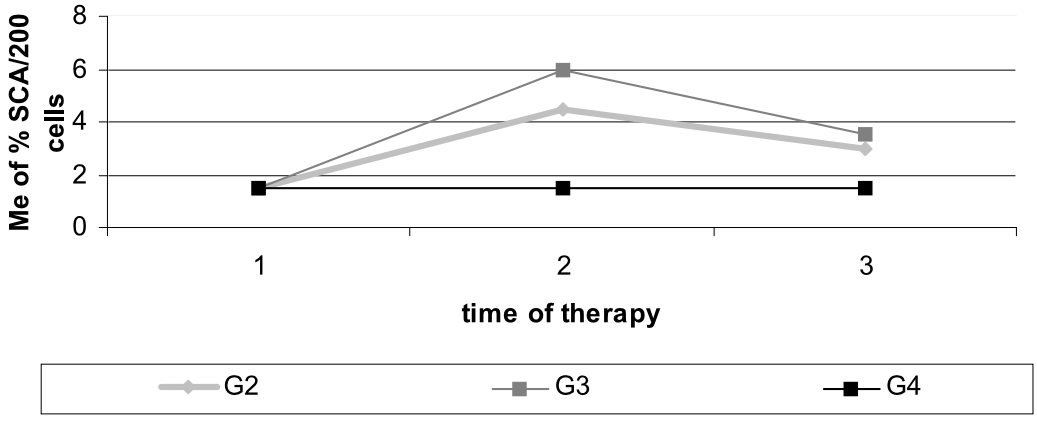

Figure 1a. Median value of the chromosomal aberrations of patients with testicular tumour (p = 0.005).

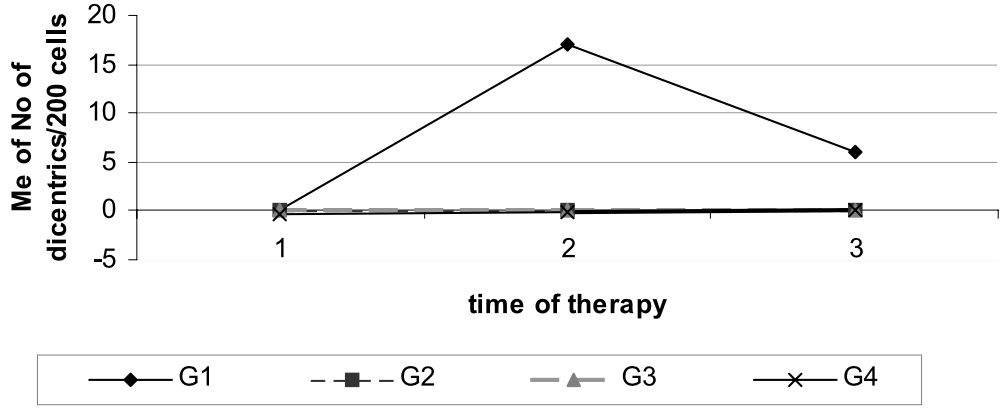

Figure 2. Median value of the dicentrics of patients with testicular tumour (p = 0.005).

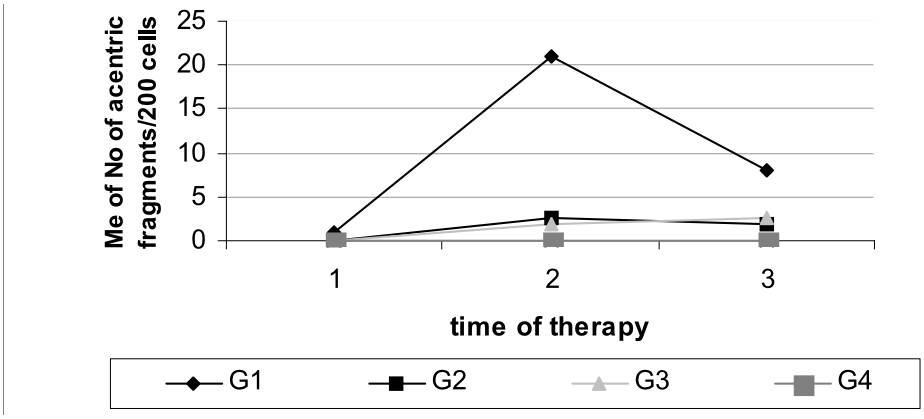

Figure 3. Median value of the acentric fragments of patients with testicular tumour (p = 0.005).

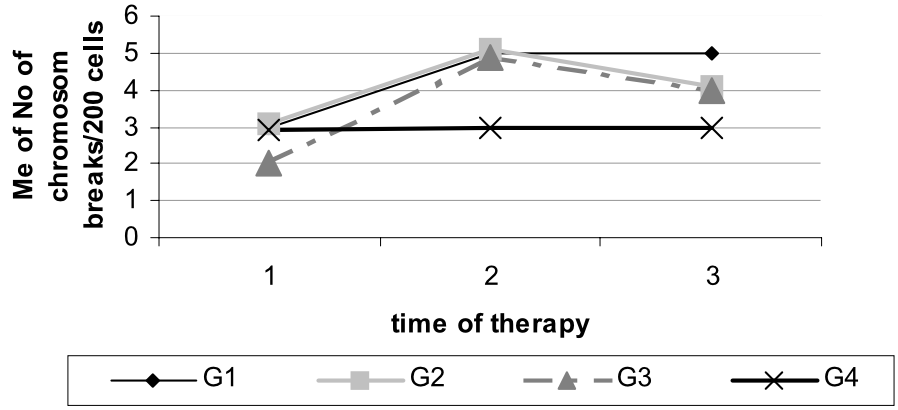

Figure 4. Median value of the chromosomal breaks of patients with testicular tumour.

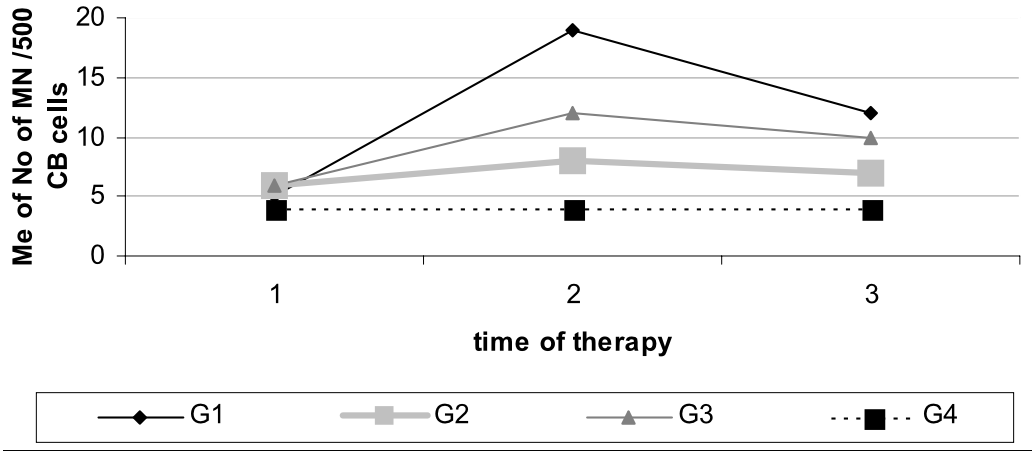

Figure 5. Median value of the MN of patients with testicular tumour (p = 0.005).

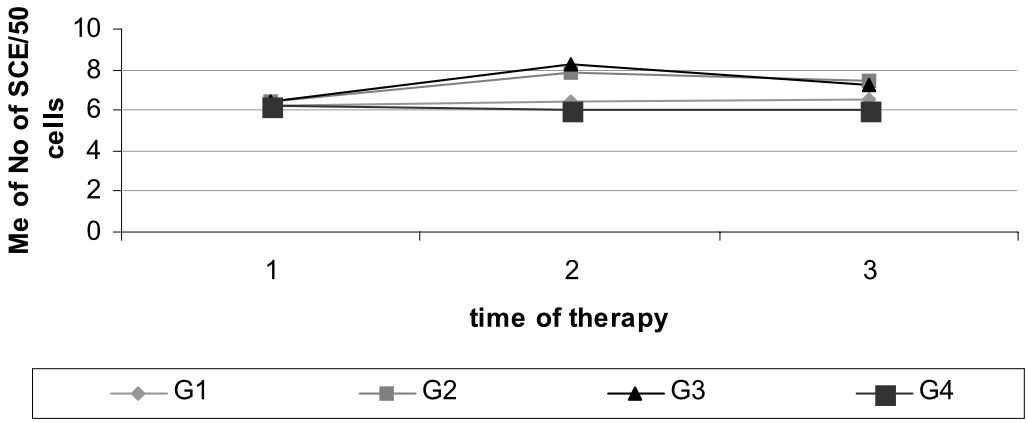

Figure 6. Median value of the SCE per cell of patients with testicular tumour (p = 0.001).

(p = 0.004). The comparison of numbers of chromosomal changes prior to the therapy and six months later was statistically significant in groups I to III (p = 0.001).

The number of micronuclei was also significantly higher in all the first three groups. It was the highest in the first group of patients treated by radiotherapy. Only in this group, the number of micronuclei was found to be significantly decreasing six month after the therapy (p = 0.001). Six months after therapy, MN values in the patients treated by radiotherapy and in those treated by chemotherapy according to BEPV schedule were significantly higher compared to pre-exposure levels.

Six months after chemotherapy, the number of SCE was still higher than the starting values (p = 0.001).

Discussion

Prior to treatment, we did not detect any deviations neither in the genome picture of our patients nor of the subjects of the control group without malignant disease.[21] The changes inside the genome were observed after chemotherapy as well as after radiotherapy.[22,23] From cytologic and mutagenetic points of view radiotherapy is more aggressive than chemotherapy, mostly because of a much higher level of unstable chromosomal changes, such as dicentrics. In irradiation, the mitotic activity in lymphocytes is significantly, yet only temporarily inhibited, whereas in chemotherapy, the inhibition of mitotic activity in vitro depends on the chemotherapeutic treatment scheme.

In a certain lapse of time after treatment, the genome picture is repaired. The time of repair depends on the length of exposure of the tissue-cellular complex and the type of damage. The genome is repaired because the damaged cells have died away. A great number of unstable aberrations, such as dicentrics, predominate in the patients treated by radiotherapy. These unstable cells are so severely damaged after radiotherapy that they are unable to survive and die shortly. In irradiated patients, a fast repair of the genome is mainly due to the following two reasons: the first is that a small tissue-cellular complex responds to radiation, and the second is that the structural chromosome damages predominate, especially unstable aberrations, such as dicentrics. The differences in the genome of individual cells are still possible. They may induce the development of oncogene and, consequently, of sec-

ondary tumour due to primary tumour treatment.[24]

The analysis of the treatment results in patients treated by chemotherapy showed that the difference between the chemotherapy according to BEPV schedule and the chemotherapy with paraplatinum was very small. In our patients, the only difference between both chemotherapy schedules was the number of micronuclei. This difference was statistically significant at all parameters in comparison to the first group been treated only by radiotherapy or the fourth group treated only by surgery.

Similar results were published also in other studies reporting of significantly higher effect of irradiation on the genome than that of chemotherapy [25,26] and of significantly higher effect of chemotherapy on the genome in comparison to the genome of the patients treated by surgery alone.[27]

Six months after the completed therapy, the number of cytogenetic changes was lower and not statistically significant any more. Monochemotherapy with paraplatinum is a new method of adjunct treatment of patients with an early stage of seminoma and is just being introduced at our Institute. The patients included in this study are the first to be treated with this method in Slovenia.[28,29] The advantages of paraplatinum monotherapy, lie primarily in short hospitalisation time, thereby also in shorter overall treatment time, as well as short-lived and mild side effects of chemotherapy,[3,4] as was also confirmed in our study.

In conclusion, the following question remains: Can the chromosome changes after chemotherapy, such as chromosome and chromatide breaks that take a longer time for the genome repair, also induce the development of oncogene and, consequently, of secondary tumours, considering that the cells with that kind of damage have more possibilities for surviving?

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Radiol Oncol 2001; 35(4): 303-8.

Legislation on the protection of experimental animals

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The aim of this paper is to establish the current situation in the field of legislation on the protection of experimental animals in Slovenia. The protection of experimental animals has been regulated by the provisions of the Protection of Animals Act.[1] On the basis of this act, the Instructions on Conditions for the Issuing of Authorisations for Experiments on Animals for Scientific and Research Purposes[2] and the Rules on the Ethics Commission for Experiments on Animals [3] have been used.

The basic protection of experimental animals is provided for by a system of permits for experiments on animals. Permits for experiments on animals are granted by the administrative authorities responsible for veterinary medicine in cases where experiments are urgently required for medical, veterinary medical, or scientific and research purposes and the results are expected to produce important new knowledge, or when the suffering of animals is ethically acceptable in comparison with what the experiment is expected to achieve; where, in cases of basic research, experimental aims cannot be achieved by any other method or procedure, the experiment is performed on the minimum possible number of animals of the lowest neurophysiological sensitivity and a method is used that causes the minimum level of suffering, pain or lasting harm. Staff involved in the execution of experiments or in the care and nursing of animals, the premises for the accommodation or rearing and provision of animals, and the installations and devices used must all comply with the set conditions.

With the adoption of the Act, which has been harmonised with EU regulations, legislation on the protection of experimental animals has been put into effect. As laid down by the act[1], the implementing regulations will lay down the conditions for the issuing of permits for experiments on animals, the procedure, documentation, records, reports and responsibilities of experts on the protection of animals, and staff and other conditions relating to the execution of experiments and procedures involving animals.

Key words: animals, laboratory; animal husbandry; animal rights; legislation

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This paper was presented at the "Laboratory Animals in Biomedical Research" seminar, Ljubljana, 27 October 2001.

Received 19 October 2001
Accepted 25 October 2001

Introduction

The above cited parts of the legislation on the protection of experimental animals in Slovenia have been reviewed. The protection of animals used for experimental purposes is an increasingly important area. In most developed European countries, extensive legislative activities in this area began back in 1986. With the adoption of the Protection of Animals Act[1] (which came into effect on 18 December 1999), legislation regulating the protection of experimental animals finally entered into force in Slovenia.

The act not only provides protection for animals against cruelty but also lays down the principles of animal welfare. In the general provisions section, the act defines public responsibility for the protection of animals and their life, health and welfare by stressing the following basic principle: "No man shall be allowed to cause suffering, illness or death to an animal without a well-substantiated reason."

Data from the legislation in force in Slovenia

The field of protection of experimental animals in Slovenia is regulated by the Protection of Animals Act[1], the Instructions on Conditions for the Issuing of Authorisations for Experiments on Animals for Scientific and Research Purposes[2], and the Rules on the Ethics Commission for Experiments on Animals.[3] All animals with a developed nerve system that enables them to feel painful external influences must be dealt with under the terms of this act. The provisions of the act must always be taken into consideration when working with vertebrate animals and, depending on their level of sensitivity, with all other animals. There is a special chapter in the act: Experiments on Animals (Articles 21-24). Basic protection for experimental animals is provided for by a system of permits for experiments on animals which are issued by the administrative authorities, competent for veterinary medicine.

Experiments on animals may only be carried out by organisations registered to perform experiments on animals and granted permission by the Veterinary Administration of the Republic of Slovenia (VARS). Any organisation performing experiments on vertebrate animals must appoint an animal protection expert with the appropriate qualifications. The expert provides the explanations needed in order to obtain approval for the experiment and is responsible for ensuring that experiments are carried out in compliance with the law.

Permission for an experiment is granted only if the experiment is truly necessary, and if:

- the experiment is required for medical, veterinary or general, scientific and research purposes, it is expected that the results will yield new and important findings, and it is expected that animal suffering will be ethically acceptable in comparison with what the experiment is expected to achieve;
- it is basic research;
- the goal of the experiment cannot be achieved by any other methods and procedures;
- the lowest possible number of animals with minimal neuro-physiological sensitivity and methods that cause the lowest amount of suffering, pain or permanent injury are used in the experiment;
- the animal is anaesthetised before the start of the experiment, unless the pain caused by the experiment is lower than the pain caused by anaesthesia or the anaesthetisation of the animal is against the purposes of the experiment;
- the animal will be properly treated or killed after the experiment is completed, if suffering cannot be prevented;

- the persons who carry out experiments or take part in them and the persons who take care of the animals, including duties of a supervisory nature, have attained the appropriate level of education;
- the animals used in the experiment come from well-organised and registered breeding establishments; in exceptional cases, when the experiment is necessary for the preservation of an animal species and the animal is the only one suitable for the experiment or when it cannot be bred, the competent authority may issue permission for the experiment in compliance with the Regulation on the Protection of Endangered Animal Species.4 Experiments on equine animals, bi-ungulates, dogs or cats may only be performed when it is not possible to achieve the envisaged aim by experimenting on other animals.[2]

A special permit from VARS is required for each individual experiment. The person who will conduct the experiment must have the proper qualifications.[1] Only persons with adequate qualifications in veterinary and human medicine, biology and animal husbandry may perform experiments on animals.[2] An authorisation is not necessary for experiments that have been prescribed by a legal act or ordered by a court of law or a competent inspector on the basis of a legal act, or in the execution of vaccinations or diagnostic investigations, the collection of blood or other material, or the detection of injuries and diseases.[1] The performance of experiments on animals in order to test chemical agents used in war, cosmetic preparations, alcohol or tobacco products, or of experiments in which muscle-paralysing agents are used and performed without the use of anaesthesia are prohibited.[1] Surgical operations for educational purposes which cause suffering and harm to or even the death of the animal are also prohibited. These can be performed only exceptionally by university or scientific and research institutions in cases where they are necessary in the course of the regular training of doctors of human or veterinary medicine, biologists or pharmacists, and where their objective cannot be achieved by any other means of training (films, photographs, models, etc).[1,2]

The Rules on the Ethics Commission for Experiments on Animals lay down the procedure of examining and processing opinions on the necessity of a certain experiment in order to grant permission to carry it out on an animal.[3] The rules lay down in detail the composition, tasks, competence and method of work of the ethics commission for experiments on animals.

Immediate inspection and control of the implementation of the law and other regulations and international agreements on the protection of animals against suffering is carried out by veterinary inspection and control services. The Veterinary Practice Act[5] and the Protection of Animals Act[1] define the competence of the veterinary inspection and control service to order measures to protect animals against suffering, and the penalties for violations of the legislation. A veterinary inspector has the right and duty to prohibit the performance of any procedures on animals which are not permitted or which violate the provisions of the Protection of Animals Act, order such experiments to be discontinued, or prohibit the carrying-out of experiments on animals which are carried out in violation of the provisions of the act, or when any existing deficiencies have not been eliminated by the prescribed deadline. The performance of experiments on animals in violation of the regulations is punishable by a fine under the Protection of Animals Act[1] and, when premeditated, is considered as having caused suffering to the animal in question, which as such is punishable under the Penal Code.[6] The penalty envisaged for such an offence is a prison sentence of up to three months.

A fine of between SIT 100,000 and 150,000 under the act[1] is imposed on an individual carrying out an experiment on animals in violation of Article 21 of the Act (without permission and in an organisation that has not been registered for such an activity) or when experimenting on animals in violation of Article 22 of the act (procedures for educational purposes without an approval). A fine of between SIT 150,000 and 10 million is imposed on a legal entity for the same violation.

A fine of between SIT 150,000 and 10 million is imposed on an individual who conducts painful procedures on vertebrate animals without the use of appropriate anaesthesia or when conducting experiments on vertebrate animals in violation of Article 23 of the Act (when the organisation in question fails to appoint an expert on the protection of animals). A fine of between SIT 25,000 and 500,000 is imposed on the responsible person of the legal entity which committed such a violation.

Discussion

There is direct and indirect links between animals and human beings – they are of vital importance to us. We should never forget that animals are living creatures with senses and are capable of expressing their feelings. We should handle them in a humane manner and with a due level of responsibility. Well-regulated legislation is of great importance for animal protection. Major progress at the international level in the legal regulation of the protection of experimental animals was made in the 1980s. An important turning point was the 1986 Convention of the Council of Europe on the Protection of Animals Used for Experimental and Other Scientific Purposes.[7] A contribution towards increased protection for experimental animals was also made by the European Union (then the European Economic Community) with the adoption of Directive 86/609/EEC[8], which is essence does not differ from the European Convention. The objective of the Directive and of the European Convention is to ensure that the provisions on the protection of experimental animals are harmonised in the national legislation of the member states. The European Convention[7] and the European Union Directive[8] define in different chapters the essential principles of use of animals in experiments and the conditions for rearing and accommodating the animals, with the minimum recommended housing areas for individual animal species and the micro-climatic conditions. By incorporating the proposed standards, any departures from the norm will be eliminated; by prescribing conditions for the rearing, accommodation and use of animals in experiments, measures for the protection of experimental animals will be made uniform. Most importantly, by granting mutual recognition to the test results of experiments performed on animals, the unnecessary duplication of experiments will be prevented.

Slovenia is undergoing accelerated legislative activities to protect experimental animals. Experiments on animals carried out for scientific and research purposes have been regulated and subject to restrictions in Slovenia since 1985, when the Instructions on Conditions for Granting Authorisations for Experiments on Animals for Scientific and Research Purposes were issued.[2] These instructions include certain provisions that were laid down later on by the European Convention [7] and EU Directive.[8] According to the instructions, any organisation intending to use animals in experiments that cause pain, suffering or lasting harm must obtain the approval of the competent administrative authority, provide conditions in which such experiments may be carried out, restrict the use of domestic animals in experiments and for educational purposes, and keep a protocol of the experiment and a report on the experi-

ment by the person in charge of it. A drawback to these instructions was that the penalties they prescribed were low and the execution of inspection and control ineffective.

By adopting the Protection of Animals Act[1], progress has been made in regulation of the protection of experimental animals. The act includes some of the basic provisions of EU Directive 86/609/EEC[8] as follows:

- Experiments on vertebrate animals may be performed only when it is anticipated that the suffering of the animal will be ethically acceptable in the light of the envisaged results.
- Experiments on animals may only be performed by organisations registered to carry out experiments on animals and duly authorised by the competent administrative authority.
- Experimental animals may only originate from an organised and registered rearing establishment for the rearing of experimental animals;
- The person carrying out the experiments shall have appropriate professional qualifications.
- The person carrying out the experiments shall keep records on the number and species of animals used and the type of experiment, and notify the competent administrative authority thereof.

Slovenia has the Rules on the Protection of Experimental Animals, which transpose all other provisions of the EU Directive.[8] The rules lay down the detailed conditions for granting permits to carry out experiments on animals, the procedure, documentation, records, reports and obligations of professionals relating to the protection of animals, staff and other conditions for the execution of experiments, and the procedures relating to the use of animals in experiments. The rules lay down detailed conditions regarding the establishments involved in the rearing, supply and use of animals in experiments, and the premises, equipment and staffing thereof. The rules include provisions concerning the planning of experiments under consideration of the 3R concept (1R – replacement, or replacement of a planned method with another; 2R – reduction, or selection of an experiment that requires fewer animals; 3R – refinement, or the conducting of an experiment to perfection so as to guarantee its success) and the use of alternative methods. The EU Directive[8] and the draft rules include the 3R concept in the following provisions:

- An experiment may not be repeated when any other scientifically satisfactory method is available that enables attainment of the envisaged result and does not require the use of animals.
- When an experiment is urgently necessary, the selection of animal species must be carefully studied. An experiment must be chosen which requires the minimum number of animals, and animals with the minimum neuro-physiological sensitivity, which causes the minimum pain, suffering, stress and lasting harm, and which in all probability will give satisfactory results.
- All experiments must be planned so as to avoid stress, avoidable pain and suffering on the part of experimental animals.
- Experiments causing lasting pain to animals are subject to a special application to the competent authority and their use must be justified.

Efforts have been made in Slovenia to ratify the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123).[7]

Data on the use of experimental animals in European countries (including Slovenia) shows a decline in the use of animals in experiments. The annual number of experimental animals used in Slovenia in the past three years has totalled approximately 22,000 (in 1996 it was still 30,000, and between 1993 and 1995 it was 33,000). Most of the experi-

ments are carried out on laboratory rodents by the chemical and pharmaceutical industries for substance testing in compliance with applicable legislation, regulations and international agreements. Institutes and laboratories of the faculties of medicine, veterinary medicine, biology, zootechnics and physiology use animals for basic research or scientific and research activities; these account for nearly one fifth of all experimental animals used. To a lesser extent, animals are also used in the diagnosis of disease and for education and training purposes. An important role in reducing the number of experimental animals is played by the applicable legislation, the replacement of animals with alternative methods (and a legal requirement to do so), the required permits for conducting experiments, ongoing staff training, successful co-operation between research institutions and researchers at national and international levels, active associations and societies, and increased responsibilities of the commissions for the protection of animals.

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Radiol Oncol 2001; 35(4): 309-15.

Standardisation of laboratory animals for biomedical research in Poland

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Increasing demands for biomedical research require laboratory animals of good quality. Simultaneously, the scientists aim at limiting the number of sacrificed laboratory animals. Some international organisations like ICLAS, FELASA, SOLAS deal with the promotion of proper breeding and care of laboratory animals. In 1997' The Animal Protection Act' was adopted by Polish Parliament. The standardisation of laboratory animals for biomedical research in Poland is the main task of the Commission on Biology of Laboratory Animals P.A.Sci. and Polish National Committee for Collaboration with ICLAS. In 2000, the Department of Genetics and Laboratory Animal Breeding, M. Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw was designed as National Reference Centre for SPF Animal Breeding.

The department consists of five integral parts: (1) bank of SPF (Specified Pathogen Free) inbred mouse and rat strains, (2) experimental animals laboratory, (3) health monitoring laboratory, (4) genetic laboratory, (5) anatomo-pathological laboratory.

Trends in development of laboratory animal science in Poland and Europe are overlapping. The standardisation of laboratory animals according to international recommendation seems to be sufficient in leading centres.

Key words: laboratory animals; SPF mice research; Poland

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The paper was presented at the seminar "Laboratory Animals in Biomedical Research", held in Ljubljana 27 October 2001.

Received 23. October 2001

Accepted 29 October 2001

Introduction

Permanently increasing demands GLP (Good Laboratory Practice) laboratory rules as well as Polish law regulations require laboratory animals of good quality for biomedical research.[1] Simultaneously, the scientists aim at limiting the number of sacrificed laboratory animals. The use of animals in biomedical research provokes considerable conflicts and discussion. The international co-operation and collaboration is necessary to solve the problem. Some international organisations deal with the promotion of proper breeding and care for laboratory animals.

The following international organisations are the best known within Europe and worldwide:

1. The International Council on Laboratory Animal Science, ICLAS
2. Federation of European Laboratory Animal Science Associations, FELASA
3. Gesellschaft für Versuchstierkunde, GV-SOLAS
4. Institute for Laboratory Animals Resources, ILAR
5. Fund for Replacement of Animals in Medical Experiments (FRAME) founded as a scientific charity.

In Europe, two intergovernmental organisations have adopted regulations for protection of laboratory animals: The Council of Europe, CE and The European Community, EC.

Although the aims of these organisations are not the subject of this presentation, it should be mentioned that their statutory activities are based on the concept of "Three R-s", namely: **R**eduction, **R**efinement and **R**eplacement.

The standardisation of laboratory animals for biomedical research in Poland is the main task of the Commission on Biology of Laboratory Animals P.A.Sci. and Polish National Committee for Collaboration with ICLAS.

The breeding of high quality experimental animals is not possible without progress in the field of laboratory animal science and appropriate political decisions.

Background of laws and policies that impact protection of animals in Poland (including laboratory animals)

In 1928, the regulation referring to the problem of protection of animals was issued by the President of the Republic of Poland. (The first comparable act has been in force in England since 1924.)

In 1957, the regulation issued by the Ministry of Justice and the Ministry of Interior described the general requirements to receive a licence to experimentation on animals and possible penalties.

In 1959, the Ministry of High Education issued a regulation concerning the problem of using animals for scientific purposes.

Today's legislation

In 1986, in Strasbourg, 11 members of Council of Europe signed 'The Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes'.

In September 1997, the Polish Parliament adopted the 'Animal Protection Act' to harmonise Polish legislation with European Community. General requirements for animal care and use were based on the above mentioned Convention.

In September 1999, the State Committee for Scientific Research appointed National Ethics Committee on Animal Experimentation. Its members are representatives of biological, medical, veterinary sciences and humanities. Simultaneously, 17 Local Ethics Committees were approved.

In November 1999, the Government issued an act that listed 85 scientific insti-

tutes, medical and veterinary faculties, universities as well as pharmaceutical firms that are allowed to perform experiments on animals.

Progress of laboratory animal sciences and alternatives

Polish Commission on Biology of Laboratory Animals P.A.Sci.

Since 1962, the Polish Commission on Biology of Laboratory Animals has been acting under the auspices of Polish Academy of Sciences. The organisation is an excellent place of contact between researchers and people dealing with the breeding of laboratory animals.

In the period 1964-1993, the Polish journal "Laboratory Animals" was published under the supervision of the Commission on Biology of Laboratory Animals. In the last issue, the Convention signed in Strasbourg was published in Polish translation.

The journals 'Comparative Medicine' formerly 'Laboratory Animal Science', 'Laboratory Animals' as well as the Japanese journal 'Experimental Laboratory Animals' are also available in Poland.

Membership in ICLAS

The long-lasting collaboration of P.A.Sci with ICLAS has resulted in the participation of Polish National Representative in the Governing Board of ICLAS. There are three laboratory animal breeding centres in Poland fulfilling the criteria to function as an ICLAS Reference or Monitoring Centre: 'The ICLAS Reference and Monitoring Centre System is a regional, international and inter-institutional collaborative network in line with ICLAS policy'.[2,3]

In 1978, the Reference Centre for Histocompatibility Testing in Mice was designated at the Institute of Oncology in Warsaw. Ten years later (1988), ICLAS Regional Monitoring Centres for Microbiology and Genetics were designated at the Institute of Oncology in Warsaw and at the Institute of Immunology & Experimental Therapy in Wrocław (Poland).

In 2000, the Department of Genetics and Laboratory Animal Breeding in the M. Skłodowska-Curie Memorial Cancer Center and the Institute of Oncology in Warsaw was designated as National Reference Centre for SPF Animal Breeding.

Development of alternatives in Poland

The end of 1980s was the period when Polish scientists were stimulated to develop alternatives mainly in pharmacology and toxicology testing. It has resulted in Poland's participation in EU Fifth Framework Programme in 1999 and numerous joint publications. INVITOX ON LINE service is available from Medical University of Warsaw server. The 13th ESTIV INVITOX (European Society for Toxicology in Vitro) Workshop will be held in Poland in 2004.[4]

Presentation of the National (Polish) Reference Centre for SPF Animal Breeding

The Department of Genetics and Lab. Animal Breeding consists of five integral parts:

1. bank of SPF (Specified Pathogen Free) inbred mouse and rat strains,
2. experimental animal division,
3. health monitoring laboratory (microbiology),
4. genetic laboratory,
5. anatomo-pathological laboratory,

– bank of SPF inbred mouse and rat strains

SPF animals are an essential tool in the investigations in oncology, immunology, and transplantology. SPF mice became indispensable models in oncology because their

longer life span allows to develop numerous, spontaneous tumours with rare different histological patterns. The incidence of chemically or irradiation induced neoplastic tumours is significantly higher when SPF animals are used.

Three inbred strains of rats and 18 of mice (7 inbred and 11 congenic resistant strains) are maintained in the barrier system in SPF condition.[5] They are bacteriologically, virologically and parasitologically controlled. Breeding division consists of 12 breeding rooms, each equipped with lock-chamber. The doors of lock-chambers are blockaded. Room temperature around 22°C, higher pressure and 12 h light/dark are regulated automatically and controlled for each room separately. There are 15 exchanges of air per hour. The air is filtrated by three filters, the last one is the absolute filter. Pelleted food (LABOFEED H), cages with bedding material and bottles with water are sterilised in double-door autoclaves and transferred through the barrier into the clean area. The entrance to the breeding part is allowed only to the employed, specially trained personnel after taking a shower and donning sterile medical suits.[6]

Pelleted food LABOFEED H is produced under the supervision of the Institute of Animal Physiology and Nutrition P.A.Sci.

Animal breeding is carried out according to the single line system and breeding protocols are kept in Excel computer program. The animals are registered at the Committee on Standardised Nomenclature for Inbred Strains of Mice and at the National Academy of Science, NW Washington DC 20418. The symbol is W.

Figure 1. A trained and properly dressed personnel takes care for animals.

– experimental animal division

Conditions in the division for experimental animals are the same as in the SPF breeding part, however animals are maintained in the "semi barrier" system. Experimental animals at this division are kept at the least MD (Minimal Disease) standard. The researchers are allowed to enter the animal and operating rooms after conforming the hygienic rules.

– health monitoring

The health monitoring includes parasitological, bacteriological and virusological control according to the recommended international rules.[7,8,9]

Bacterial colonies cultured from nasopharynx, eye, caecum, colon and vagina are identified on standardized diagnostic media. Bacteriological identification is performed using BioMerieux API kits (French production). Mycoplasma sp. and virusological infections are verified by ELISA – (Enzyme-linked immunosorbent assay) using Perimmune MAT kits (American production). As a rule, three mature animals are sacrificed for examination three times per month.

The list of microorganisms (viruses, bacteria and mycoplasma, ecto- and endoparasites) tested has been published.[10]

– genetic control

In 1981-1998, genetic monitoring including morphological, biochemical and immunological markers was being performed. Five mor-

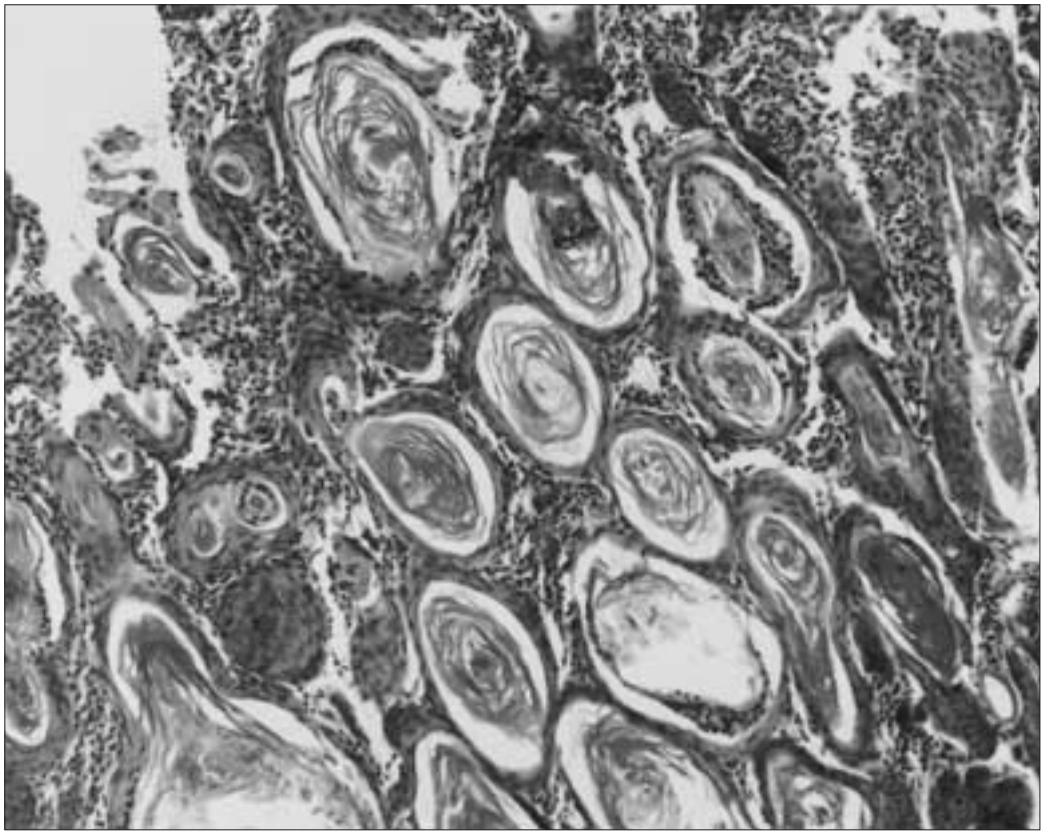

Figure 2. Irradiation-induced mammary gland tumour – *adenoacanthoma*.

phological and 9 biochemical markers were used for genetic monitoring in rats, whereas 4 morphological, 15 biochemical and 9 immunological markers in mice.[10,11,13] Genetic profiles were constructed for all maintained animals. Since 1999 genetic control of mice from seven inbred strains and one congenic resistant line by random amplification of polymorphic DNA (RAPD) method has been performed.[14] Ten arbitrary primers were tested. Moreover, genetic profiles (chromosome No 1 – 8) were constructed for these strains using microsatellite markers.

– anatomo-pathological and histopathological control

Emphasis is being placed on the incidence of spontaneous and radiation-induced tumours.[15,16] The data obtained from 7 inbred strains (AKR/W, BALB/cW, BN/aW, CBAT6/W, C3H/W, C57BL/10PhW, DBA/2W) are collected. The observed tumours are histologically classified. H&E staining as well as histochemical and immunohistochemical methods are routinely used. The incidence of mammary gland, lung, liver and haematopoietic system tumours was analysed.

Today's trends and problems

The mouse strains with defined genetic background are used in fundamental researches. The trends in the development of laboratory animal science in Poland and Europe are overlapping. In oncology, the inbred strains, recombinant inbred strains and recombinant congenic strains are the most preferred.

The high cost of mice and rats supplied from the breeding reference centres to other institutes reduces the number of animals per project, but parallely discourages the investigators to new experiments.

Besides, in practice it is very difficult to prove researchers' ignorance of law and laboratory animal science and punish them especially when an experiment is failed.

Conclusion

Standardisation of laboratory animals according to international recommendations seems to be sufficient in leading centres. However, the general state of laboratory animals breeding in Poland still needs improvement and additional funds.

The law regulation is respected by the institutes conducting fundamental researches; however, it has rather resulted from the awareness of scientists than effectiveness of law regulations.

In future, high priority for development of modern genetics, immunology, oncology and others may cause seeing laboratory animals more as research tools than as companions in scientific investigation.

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Radiol Oncol 2001; 35(4): 237-42.

Diagnostična vrednost pneumoperitoneja pri rentgenskem slikanju abdomna na prazno

Frković M, Klapan T, Moscatello I, Frković M

Izhodišča. Pneumoperitonej imenujemo prisotnost zraka izven širine lumna črevesa in je najvažnejši dokaz perforacije črevesa pri rentgenskem slikanju abdomna na prazno. Perforacija je lahko spontana ali pa posledica poškodbe. Najpogostejši vzrok spontane perforacije je razjeda želodca ali dvanajsternika. Zato je bil namen naše raziskave preveriti diagnostično vrednost pneumoperitoneja pri rentgenskem slikanju abdomna na prazno.
Bolniki in metode. V retrospektivni raziskavi smo analizirali 79 bolnikov, ki so bili v dvoletnem obdobju (1998-1999) napoteni v našo bolnico in operirani zaradi perforirane razjede želodca ali dvanajsternika. Preverjali smo diagnostično vrednost rentgenske slike abdomna na prazno, predhodno narejene na bolnikih v stoječem polažju, leže na hrbtu in leže ne levem boku.
Rezultati. Deset (12,66 %) od 79 bolnikov je bilo operiranih brez predhodne radiološke diagnostike. Pri 16 (87,34 %) bolnikih smo naredili radiološke preiskave, 53 (76,81 %) pa jih je imelo začetne znake pneumoperitoneja pri slikanju abdomna na prazno.
Zaključki. Najpogostejši vzrok pneumoperitoneja je bila perforirana razjeda dvanajsternika pri starostnikih. Najpogostejši rentgenološki znak pneumoperitoneja pa je bila srpasta kolekcija zraka pod trebušno prepono.

Radiol Oncol 2001; 35(4): 243-7.

So podatki o umrljivosti za rakom verodostojni - analiza podatkov o umrljivosti zaradi raka materničnega vratu

Primic Žakelj M, Pompe Kirn V, Škrlec F, Šelb J

Izhodišče. Uradni podatki o umrljivosti za rakom materničnega vratu so manj zanesljivi, saj v zdravniških poročilih o vzroku smrti mesto izvora raka v maternici ni vedno dovolj natančno opredeljeno. Želeli smo oceniti, v kolikšni meri objavljeni podatki o umrljivosti za rakom materničnega vratu v Sloveniji odražajo dejansko umrljivost za to boleznijo.
Gradivo in metode. 2245 smrti, pri katerih je bil v podatkovni zbirki Inštituta za varovanje zdravja v letih 1985-1999 kot vzrok smrti naveden rak materničnega vratu, telesa ali maternice, smo računalniško povezali s podatkovno zbirko Registra raka za Slovenijo.
Izsledki. V obdobju 1985-1999 je po uradnih podatkih za rakom materničnega vratu umrlo 878 žensk, v Registru raka je bilo od njih prijavljenih s to boleznijo le 87,7 %. Med ženskami, ki naj bi umrle zaradi raka materničnega telesa, jih je 17,1 % imelo v resnici raka materničnega vratu, od 717, pri katerih je bil kot vzrok smrti naveden le rak maternice, pa je 31,4 % dejansko imelo raka materničnega vratu. Če med vzroki smrti upoštevamo pravilno opredeljen rak materničnega vratu, dodatno pa še napačno ali premalo natančno opredeljene primere, je zaradi raka materničnega vratu umrlo 1106 žensk, kar pomeni, da so uradni podatki o umrljivosti za rakom materničnega vratu podcenjeni za 26 %.
Zaključek. Uradni podatki o umrljivosti za rakom materničnega vratu podcenijo dejansko stanje, kar je treba upoštevati pri vrednotenju bremena te bolezni. To pa ne velja za vse rakave bolezni.

Radiol Oncol 2001; 35(4): 249-54.

Sporazumevanje po laringektomiji

Hočevar-Boltežar I, Žargi M

Izhodišča. Pri bolnikih z napredovalim rakom grla in spodnjega žrela je kirurška odstranitev grla najbolj primeren način zdravljenja. To zdravljenje pa negativno vpliva na številne pomembne bolnikove funkcije in tudi na govor.
Bolniki in metode dela. Po odstranitvi grla je možnih več različnih načinov tvorbe glasu, tako da se vsak laringektomirani bolnik lahko nauči katerega od teh načinov govornega sporazumevanja.
Rezultati. Znanih je več različnih, tudi elektronskih naprav, ki s pomočjo lastnih baterij proizvajajo zvok. Drugo možnost predstavlja ezofagalni govor, pri katerem se bolnik nauči uporabljati mišični segment na prehodu v zgornji požiralnik kot generator glasu. Kirurško možnost govorne rehabilitacije po laringektomiji pa predstavlja vstavitev proteze v kirurško narejeno fistulo med zgornjim požiralnikom in sapnikom. Proteza usmeri zrak iz pljuč v zgornji požiralnik, glas pa nastane pri prehodu zraka skozi že omenjeni mišični segment.
Zaključki. Številni dejavniki vplivajo na izbiro najprimernejšega načina nadomestnega govora pri posameznem bolniku. V Sloveniji največ laringektomiranih bolnikov uporablja ezofagalni govor.

Radiol Oncol 2001; 35(4): 255-8.

Kombinirano zdravljenje ploščatoceličnega karcinoma ustne votline in ustnega žrela. Globina tumorske invazije kot napovedni dejavnik

Čizmarevič B, Lanišnik B, Didanovič V, Kramberger K

Izhodišča. V raziskavi smo nameravali ugotoviti pomembnost kirurškega zdravljenja ploščatoceličnega karcinoma glave in vratu ter najti najpomembnejši napovedni dejavnik za širjenje karcinoma v vratne bezgavke in določiti, kateri dejavnik v največji meri vpliva na preživetje bolnikov.

Bolniki in metode. Od 1. 6. 1992 do 31. 5. 1998 smo na Oddelku za otorinolaringologijo in cervikofacialno kirurgijo Učne bolnišnice v Mariboru obravnavali 154 bolnikov s ploščatoceličnim karcinomom ustne votline in ustnega žrela. Kriterijem naše retrospektivne študije je ustrezalo 142 bolnikov, a le 62 je bilo primernih za kombinirano zdravljenje s kirurgijo in pooperativno radioterapijo. Od 62/142 operairanih bolnikov je bilo 49 tudi pooperativno obsevanih, 13/62 bolnikov pa je odklonilo nadaljnje zdravljenje. Na kirurškem resektatu smo ugotavljali, ali tumor zaseva v vratne bezgavke, ali je bil izrezan v zdravo in kakšna je bila globinska invazija tumorja. Prav tako smo na tumorskih celicah določali proliferativni faktor Ki67.

Rezultati. V multivariantni analizi, ki je vključevala tudi stopnjo diferenciacije tumorja, klinični in patološki stadij tumorja (T in pT), je bila globina tumorske invazije najvažnejši napovedni dejavnik za širjenje karcinoma v vratne bezgavke. Ob uporabi Coxovega regresijskega modela pa je bila patohistološka opredelitev prizadetosti bezgavk (pN) pomemben dejavnik za preživetje bolnikov ($p < 0,05$). Našli smo statistično pomembno razliko med klinično in patološko opredelitvijo prizadetosti vratnih bezgavk (N in pN). V 23 primerih so bile bezgavke napačno ocenjene kot prizadete s tumorskimi celicami, v 3 primerih pa so bile spregledane. Celotno 5-letno preživetje bolnikov je bilo 55 %. Izražanje Ki67 je bil odvisno od stopnje diferenciacije tumorja in statistično značilne povezave s širjenje karcinoma v vratne bezgavke nismo ugotovili.

Zaključki. Pri bolnikih s karcinomom ustne votline in ustnega žrela je bila globina tumorske invazije najvažnejši napovedni dejavnik za zasevanje karcinoma v vratne bezgavke, preživetje bolnikov pa je bilo odvisno od prizadetosti vratnih bezgavk s tumorskimi celicami.

Radiol Oncol 2001; 35(4): 259-66.

Spremenjeni načini zdravljenja rabdomiosarkoma pri otrocih, slovenske izkušnje

Pohar-Marinšek Ž, Anžič J, Jereb B

Izhodišča. Neoadjuvantna kemoterapija (KT) je spremenila način zdravljenja rabdomiosarkoma (RMS) otrok. Namen naše študije je bil analizirati rezultate zdravljenja pri otrocih, ki so bili zdravljeni v letih med 1974 in 1996.

Bolniki in metode. V študijo smo vključili 51 otrok med enim in 15 letom starosti. Primarni tumorji so bili v področju glave in vratu pri 15, v orbiti pri 6, v genitourinskem področju pri 12, na udih pri 9, na trupu pri 5 in paratestikularno pri 4 bolnikih. Dvanajst bolnikov je imelo bolezen s stadijem I, 10 s stadijem II, 26 s stadijem III in 3 s stadijem IV. Med 43 histološko potrjenimi primeri RMS je imelo 25 bolnikov embrionalni podtip, 13 alveolarni, 1 botrioidni, 1 vretenastocelični in 3 sarkom brez nadalnje opredelitve. Pri 8 bolnikih je bila morfološka diagnoza opredeljena le iz vzorca aspiracijske biopsije s tanko iglo (ABTI). Vsi bolniki so prejeli KT, 29 neoadjuvantno, 20 je bilo najprej operiranih, 40 je bilo obsevanih (RT), 2 bonika z boleznijo v stadiju IV sta imela presaditev kostnega mozga. Kombinirana KT se je v različnih obdobjih razlikovala: z VRC, AMD in ciklofosfamidom (VAC) smo zdravili v 70-tih letih, kombinirano z Adriablastinom (T2), MTX in/ali drugimi kemoterapevtiki (T6, T11) v 80-tih letih, ciklofosfamid smo zamenjali z ifosfamidom v 90-tih letih (VAIA). S kemoterapijo smo pričeli pri tumorjih orbite, glave in vratu in pri večini tumorjev genitourinskega področja. Operacija je bila na prvem mestu pri paratestikularnih tumorjih in pri večini tumorjev na udih.

Rezultati. Vsi trije bolniki z boleznijo v stadiju IV so umrli. Med bolniki z lokaliziranimi tumorji je bilo 34 (70 %) živih in brez znakov bolezni 5 let po pričetku zdravljenja: 80% bolnikov s stadijem I, 75 % s stadujem II in 61 % s stadijem III. En bolnik je umrl zaradi odpovedi srca, 3 zaradi posledic KT in eden zaradi spremljajoče bolezni.

Zaključki. Preživetje otrok z RMS se je v Sloveniji izboljšalo v zadnjih dveh desetletjih iz 57 % na 70 % po letu 1985 in je sedaj primerljivo s preživetjem v centrih po svetu. Z uvedbo neoadjuvantne KT sta postali kirurgija in RT bolj ohranitveni (konzervirajoči) in se jima včasih lahko celo izognemo, s čimer zmanjšamo možnost neželenih posledic. Embrionalni RMS, ki zraste v orbiti, genitourinskem ali paratestikularnem področju ima v nizkem stadiju bolezni dobro prognozo. Tumorji, ki zrastejo na udih in v področju glave in vratu, večinoma alveolarnega tipa, imajo slabo prognozo. Za alveolarni tip RMS in IV stadij bolezni današnji način zdravljenja ni učinkovit, vključno s presaditvijo kostnega mozga.

Radiol ●ncol 2001; 35(4): 267-72.

Obsevanje kraniospinalnega predela pri otrocih, ki ležijo na hrbtu

Šlampa P, Seneklova Z, Simicek J, Soumarova R, Burkon P, Burianova L

Izhodišča. S pooperativnim obsevanjem lahko večkrat preprečimo lokalno ponovitev bolezni in podaljšamo preživetje otrok z možganskimo tumorji. V članku smo želeli predstaviti nov način pooperativnega obsevanja kraniospinalnega predela pri otrocih, ki so bili operirani zaradi možganskega tumorja; želeli pa smo tudi opisati morebitne stranske učinke takšnega zdravljenja.
Bolniki in metode. V štirih letih smo pooperativno obsevali kraniospinalno področje pri 17 otrocih, starih manj kot 15 let. 8 jih je imelo meduloblastom, 6 ependimom in 3 glioblastom. Obsevali smo jih z novim načinom, ki smo ga razvili na našem oddelku. Bolniki so ležali na hrbtu in ne na trebuhu, kakor je sicer običajno. Pri obsevanju smo uporabili nesimetrične čeljusti linearnega pospeševalnika.
Rezultati. Bolniki so radioterapijo dobro prenašali, pri nobenem ni bilo potrebno zmanjševati obsevalne doze. Pri vseh bolnikih so bile kožne reakcije blage. Prav tako so bili gastrointestinalni in hematološki učinki blagi ali zmerni (stopnje I ali II po klasifikaciji SZO).
Zaključki. Nov način obsevanja kraniospinalnega področja bolnika ležečega na hrbtu povzroča malo stranskih učinkov, zato ga priporočamo. Je alternativna metoda glede na običajno obsevanje, ko leži bolnik na trebuhu. Ker je opazovana doba naših bolnikov kratka, je oceana učinkovitosti takšnega obsevanja (vpliv na lokalno ponovitev bolezni in preživetje) še nezanesljiva.

Radiol ●ncol 2001; 35(4): 273-6.

Diagnostična ultrazvočna preiskava anusa za ugotavljanje analnega karcinoma. Prikaz primera

Sudoł-Szopińska I, Szczepkowski M, Jakubowski W

Izhodišča. Opisujemo primer bolnika z adenokarcinomom, s katerim želimo prikazati uporabnost ultrazvočne preiskave v diagnostiki analnega karcinoma.
Prikaz primera. Ultrazvočno preiskavo smo opravili z odčitalcem *Bruel&Kjaer*, tip 3535 z endoskopsko sondo s frekvcenco 70 MHz. Preiskava je bila opravljena na bolniku, ki je ležal na levem boku. Z ultrazvočno preiskavo anusa smo natančno izmerili globino infiltracije tumorja v steno anusa ter ugotovili lokacijo in ultrazvočne značilnosti tkiva. Z ultrazvočno preiskavo smo ocenili tudi prizadetost perianalnih limfnih bezgavk in razširjenost tumorja v sosednja tkiva.
Zaključki. Ultrazvočna preiskava anusa je zelo primerna metoda za ugotavljanje globine infiltracije analnega karcinoma in za zbiranje podatkov, ki so pomembni pri odločitvi o načinu zdravljenja.

Radiol Oncol 2001; 35(4): 277-91.

Prve izkušnje z novo luminescenčno optično metodo za merjenje oksigenacije v tumorjih

Jarm T, Lešničar H, Serša G, Miklavčič D

Izhodišča. S to uvodno študijo smo želeli predvsem ugotoviti uporabnost nove luminescenčne optične metode (sistem OxyLite) za merjenje delnega tlaka kisika v tumorjih (pO_2) in za časovno spremljanje sprememb pO_2. Hkrati s to novo metodo smo uporabili optično metodo laser Doppler, s katero lahko merimo relativni pretok krvi v tkivu.

Materijal in metode. Meritve smo opravili na tumorjih SA-1 pri miših A/J, ki so bile med poskusi anestetizirane. Za časovno zvezno zajemanje meritev pO_2 in pretoka krvi smo uporabili senzorje v obliki optičnih vlaken, ki smo jih vstavili v tumor. S spreminjanjem koncentracije vdihanega anestetika in z uporabo vazoaktivne učinkovine hidralazin smo izzvali spremembe v pretoku krvi in oksigenaciji.

Rezultati. Rezultati so pokazali, da sta v splošnem obe merilni metodi uspešno zaznali spremembe v pretoku krvi oziroma pO_2. Izmerjene spremembe pO_2 in spremembe v mikrocirkulaciji so bile v tesni korelaciji. V večini meritev pO_2 smo naleteli na nepričakovan potek signala med umirjanjem izmerjene vrednosti takoj po vstavitvi merilne sonde. Tak potek signala je najverjetneje posledica lokalnih poškodb tkiva, ki nastanejo med vstavljanjem sonde v tumor.

Zaključek. Nadaljne reziskave bodo potrebne za podrobnejšo osvetlitev tega vidika meritev z novo luminescenčno optično metodo za merjenje oksigenacije.

Radiol Oncol 2001; 35(4): 317-24.

Radiol Oncol 2001; 35(4): 293-302.

Prisotnost kromosomskih sprememb v somatskih celicah pri bolnikih s tumorji mod po različnih načinih zdravljenja

Bilban Jakopin C in Bilban M

Izhodišča. Raziskave v zadnjih 35 letih so pokazale, da so spremembe v genomu posameznih celic povzročene tako zaradi zdravljenja s citostatiki, kot zaradi obsevanja z ionizirajočimi žarki. Te spremembe v celici nam lahko služijo kot biološki dozimeter. Namen raziskave je bil najti prisotnost kromosomskih sprememb v somatskih celicah pri bolnikih s tumorji mod po različnih načinih zdravljenja.

Pacienti in metode. V prospektivno študijo smo vključili 60 bolnikov s tumorji mod, ki smo jih glede na histološke izvide in načine zdravljenja razdelili v štiri skupine. Pred zdravljenjem ni bilo odstopanj v genomski sliki v primerjavi z enako kontrolno skupino pregledovancev brez malignoma. Vpliv zdravljenja na genom bolnikov smo ugotavljali s pregledom genomske slike pred zdravljenjem, po zdravljenju in šest mesecev kasneje s priznanimi citogenetskimi testi, s katerimi smo pogledali strukturne spremembe kromosomov – SCA, izmenjavo med sestrskimi kromatidami – SCE in mikronukleus – MN pri dvojedrnih limfocitih.

Rezultati. Takoj po končanem obsevanju smo ugotovili močno inhibicijo mitotske aktivnosti limfocitov in zvečano število nestabilnih kromosomskih sprememb (dicentrikov). Kemoterapija je vplivala na genomsko sliko v manjšem odstotku, tip kromosomskih sprememb je bil drugačen. S citološko mutagenetskega vidika se je pokazalo, da je samo obsevanje bolj agresivno od kemoterapije.

Šest mesecev po zaključenem zdravljenju je mitotična aktivnost večinoma normalna, vendar pa je še vedno zelo velik odstotek sprememb glede na začetne vrednosti in v primerjavi z bolniki, ki so bili le operirani.

Zaključek. Tako po obsevanju kot po zdravljenju s citostatiki pride do normalizacije genoma, kajti poškodovane celice odmrejo. Glede na to, da je pri obsevanih bolnikih le majhen tkivni celični volumen izpostavljen obsevanju, pri kemoterapiji pa je celotni organizem izpostavljen kemijskemu agensu, upravičeno pričakujemo hitrejšo popravo genoma pri obsevanih bolnikih.

Radiol Oncol 2001; 35(4): 303-8.

Zakonodaja o zaščiti poskusnih živali

Ornik D, Pogačnik M

Namen pričujočega dela je bil ugotoviti stanje na področju zakonodaje o zaščiti poskusnih živali v Republiki Sloveniji.
Zaščita poskusnih živali je urejena z določili Zakona o zaščiti živali. Na njegovi podlagi se uporablja Navodilo o pogojih za izdajo dovoljenja za poskuse na živalih v znanstveno-raziskovalne namene in Pravilnik o etični komisiji za poskuse na živalih.
Osnovna določila zaščite poskusnih živali so v sistemu izdaje dovoljenj za poskuse. Dovoljenje za poskuse na živalih izdaja upravni organ, pristojen za veterinarstvo, če so poskusi nujno potrebni in nujni iz medicinskih, veterinarsko-medicinskih ali znanstveno-raziskovalnih razlogov in se pričakuje, da bodo rezultati prinesli pomembna nova spoznanja, oziroma je trpljenje živali etično sprejemljivo v primerjavi s pričakovanim dosežkom; nadalje tudi če gre za temeljne raziskave, če se poskusnih ciljev ne da doseči z drugimi metodami in postopki, in če se poskus izvede z najmanjšim možnim številom živali z najnižjo nevrofiziološko občutljivostjo in metodo, ki povzroča najmanj trpljenja, bolečin in trajnih poškodb. Osebje za vodenje in izvajanje poskusa ter za nego in oskrbo živali, prostori za bivanje oziroma rejo in oskrbovanje živali ter naprave in priprave morajo izpolnjevati predpisane pogoje.
Z sprejetjem zakona, ki je usklajen z evropskimi predpisi, se je začelo urejati tudi področje zaščite poskusnih živali. Kot določa zakon bodo izvršilni predpisi določali natančnejše pogoje za izdajo dovoljenj za opravljanje poskusov na živalih, o postopku, dokumentaciji, evidenci, poročilih in o obveznostih strokovne osebe za zaščito živali, kakor tudi o kadrovskih in drugih pogojih za izvajanje poskusov ter o postopku z živalmi po končanem poskusu.

Radiol Oncol 2001; 35(4): 309-15.

Standardizacija laboratorijskih živali za biomedicinske raziskave na poljskem

Szymańska H, Krysiak E, Piskorowska J, Woszczyński M, Czarnomska A

Vedno večje potrebe po biomedicinskih raziskavah zahtevajo kakovostne laboratorijske živali. Hkrati pa si znanstveniki prizadevajo za omejitev števila žrtvovanih živali. Nekatere mednarodne organizacije, kot so ICLAS, FELASA ali SOLAS, se ukvarjajo z propagiranjem primerne reje in oskrbe laboratorijskih živali. Leta 1997 poljska vlada je sprejela Zakon o zaščiti živali. Standarizacija laboratorijskih živali za biomedicinske raziskave na Poljskem je poglavitna naloga Komisije za laboratorijske živali Poljske akademije znanosti in Poljskega narodnega komiteja za sodelovanje z ICLAS-om. Leta 2000 je bil Oddelek za genetiko in rejo laboratorijskih živali Centra za zdravljenje raka Maria Skłodowska Curie in Onkološkega inštituta v Varšavi oblikovan kot Narodni referenčni center za rejo laboratorijskih živali SPF.
Oddelek je sestavljen iz petih delov: 1. Banka SPF; 2. Laboratorij poskusnih živali; 3. Laboratorij za nadzor zdravja; 4. Genetski laboratorij; 5. Anatomsko-patološki laboratorij. Trendi v razvoju znanosti o laboratorijskih živalih na Poljskem in v Evropi so si podobni. Standarizacija laboratorijskih živali se zdi po mednarodnih priporočilih zadostna v vodečih centrih.

Radiol Oncol 2001; 35(4): 317-24.

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.*

Chemotherapy

December 12-14, 2001.

The "5th International Symposium on Clinical Febrile Neutropenia" will be offered in Brussels, Belgium.

Contact Mrs. Martine Hazard and call +32 2 541 3201; or fax +32 2 541 3202; or e-mail martine.hazard@bordet.be

Radiation therapy

February 6-8, 2002

The symposium "Cardiovascular Revascularization Therapy" and satellite meetings will take place in Washington, DC, USA.

Contact Cardiovascular Research Institute, CRT 2002 Registration, 110 Irving Street, NW, Suite 6D, Washington, DC 20010, USA; or cal +1 202 877 7947; or fax +1 202 877 8141; or see http://www.radiationline.com

Lung cancer

March 7-9, 2002.

The "2nd World Conference on Clinical Cooperative Research for Lung Cancer" will be offered in Brussels, Belgium.

Contact European Lung Cancer Working Party, c/o Prof. J.-P. Sculier, Institute Jules Bordet, 1, rue Heger-Bordet, B-1000 Brussels; or call +32 25 39 04 96; or fax +32 25 34 37 56; or e-mail 101473.1044@compuserve.com; or see http://www.elcwp.org

Radiotherapy

March 10-14, 2002

The ESTRO teaching course "Radiotherapy Treatment Planning: Principles and Practice" will take place in Dublin, Ireland.

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

Lung cancer

March 14-15, 2002

The IASLC international workshop "Early Invasive Lung Cancer. New Diagnostic Tools & Treatment Strategies will be offered in Turin, Italy.

Contact Organising Secretariat, CCI Centro Congressi Internazionale srl, Via Cervino 60, 10155 Turin, Italy; or call +39 011 244 69 16; or fax +39 011 244 69 00; or e-mail info@congressiefiere.com

Gastroenterology and hepatology

March 14-16, 2002

The teaching course "Gastroenterology and Hepatology" will take place in Las Croabas, Puerto Rico.

Contact Program Coordinator, John Hopkins University, Turner 20/720 Rutland Avenue, Baltimore, Maryland 21205, USA; or call +1 410 955 2959; or fax +1 410 955 0807; or e-mail cmenet@jhmi.edu; or see http://www.med.jhu.edu/cme

Brachyherapy

March 24-26, 2002

The ESTRO teaching course "Endovascular Brachytherapy" will take place in Vienna, Austria.

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

Breast cancer

April 5-6, 2002

The teaching course "Current Concepts in the Multidisciplinary Management of Early-Stage Breast Cancer" will take place in Baltimore; Maryland, USA.

Contact Program Coordinator, John Hopkins University, Turner 20/720 Rutland Avenue, Baltimore, Maryland 21205, USA; or call +1 410 955 2959; or fax +1 410 955 0807; or e-mail cmenet@jhmi.edu; or see http://www.med.jhu.edu/cme

Thoracic surgery

April 11-12, 2002.

The "5th International Meeting on General Thoracic Surgery" will be offered in Barcelona, Spain.

Contact RCT, C/Aulestia i Pijoan, 12 Baixos 98012, Barcelona, Spain, or call +34 93 415 69 38; or fax +34 415 69 04; or e-mail rct@rct-congresos.com

Radiation physics

April 14-18, 2002

The ESTRO teaching course "Physics for Clinical Radiotherapy" (Extra edition) will take place in Izmir, Turkey.

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

Radiation oncology

April 21-25, 2002

The ESTRO teaching course "Radiation Oncology: A Molecular Approach" will take place in Santorini, Greece.

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

Radiotherapy

April 21-25, 2002

The ESTRO teaching course "Dose Determination in Modern Radiotherapy: Beam Characterisation, Calculation and Verification" will take place in Perugia, Italy.

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

Radiotherapy

May 9-11, 2002

The Annual Brachytherapy Meeting GEC/ESTRO will take place in Antalya, Turkey.

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

Nuclear medicine

May 12-15, 2002.

The "4th International Congress of the Croatian Society of Nuclear Medicine" will be offered in Opatija, Croatia.

Contact Prof. Damir Dodig or Mr. Božidar Kasal, Nuclear Medicine Congress Secretariat, KBC Rebro, Kišpatičeva 12, 10000 Zagreb, Croatia, or call +385 1 24 21 851; or fax +385 1 24 21 874; or e-mail bkasal@public.srce.hr; or see http://jygor.srce.hr/nuclmedzg-rebro/

Radiation therapy

May 15-19, 2002

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

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

Radiotherapy

June 2-6, 2002

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

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

Breast cancer

June 5-7, 2002

The "4th Milan Breast Cancer Conference" 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; or see http://www.cancerworld.org

Radiology

June 9-11, 2002

The "UK Radiological Congress 2002 UKRC 2002" will take place in London, UK.

Contact UKRC Secretariat, PO Box 2895, London, W1A 5RS, UK; or call +44 (0)20 7307 1410; or fax +44 (0)20 7307 1414; or e-mail conference@ukrc.org.uk

Bronchology and bronchoesophagology

June 16-19, 2002.

The "12th World Congress for Bronchology" and the "12th World Congress for Bronchoesophagology" will be offered in Boston, USA.

Contact Congress Secretariat. Tufts University School of Medicine. Office of Continuing Education, 136 Harrison Avenue, Boston, MA 02111, USA, or call +1 617 636 6509; or fax +1 617 636 0472; or see http://www.aabronchology.org

Brachytherapy

June 16-20, 2002

The ESTRO teaching course "Modern Brachytherapy Techniques" will take place in Lisbon, Portugal.

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

Clinical oncology

June 20-22, 2002.

The "3rd International Anglo-Croatian Symposium on Clinical Oncology" in collaboration with "51 Radiotherapy Club" (UK) meeting will be offered in Dubrovnik Cavtat, Croatia.

Contact Dr. Fedor Šantek, Executive Secretary; Medical school, Clinic of Oncology and Radiotherapy, University Hospital Centre Rebro, Kišpatiјeva 12, Zagreb, Croatia; or call +385 1 4552 333.

Radiotherapy

June 23-27, 2002

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

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

Radiotherapy

June 23-27, 2002

The ESTRO teaching course "Imaging for Target Volume Determination in Radiotherapy" will take place in Coimbra, Portugal.

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

Oncology

June 30 - July 5, 2002.

The "18th UICC International Cancer Congress" will be offered in Oslo, Norway.

Contact Norwegian Cancer Society, P.O. Box 5327 Majorstua, N-0304 Oslo, Norway, or call +47 22 59 30 00; or fax +47 22 60 69 80; or e-mail cancer@oslo2002.org

Radiology

July 1-5, 2002

The "22nd International Congress of Radiology (ICR 2002)" will take place in Cancun, Mexico.

Contact B.P. Servimed, S.A. de C.V., at Insergentes Sur No. 1188 50 piso, Col. Del Valle, 03210 Mexico DF, Belgium; or call +525 575 9931; or fax +525 559 9407; or e-mail fmricr@servimed.com.mx

Clinical Oncology

August 4-9, 2002

The "Masterclass in Clinical Oncology" will take place in Montecatini Terme, Italy.

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

Radiotion physics

August 25-29, 2002

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

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

Radiobiology

August 25-29, 2002

The ESTRO teaching course "Basic Clinical Radiobiology" will take place in St. Petersburg, Russia.

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

Prostate cancer

September 1-3, 2002

The ESTRO teaching course "Brachytherapy for Prostate Cancer" will take place in Utrecht, the Netherlands.

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

Lung cancer

September 1-4, 2002

The "8th Central European Lung Cancer Conference" will be offered in Vienna, Austria.

Contact Conference Secretariat, Mondial Congress, Faulmanngasse 4, A-1040 Vienna, Austria; or call +43 1 588 04 0; or fax +43 1 586 91 85; or e-mail congress@mondial.at

Lung cancer

September 8-12, 2002

The "IASLC Workshop on Progress and Guidelines in the Management of Non Small Cell Cancer" will be offered in Bruges, Belgium.

Contact Secretariat, P. van Houtte, Dept. Radiotherapy, Institute Jules Bordet, Rue Heger-Bordet 1, B-1000 Brussels, Belgium; or call +32 2 541 3830; or fax +32 2 538 7542; or e-mail paul.van-houtte@bordet.be

Medical physics

September 9-13, 2002

The "10th International Congress on Boron Neutron Capture Therapy" will take place in Essen, Germany.

Contact Dr. Ray Moss with e-mail moss@jrc.nl

Radiation therapy

September 17-21, 2002

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

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

Oncology

September 29 - October 3, 2002.

The "2nd World Assembly on Tobacco Counters Health" will be offered in New Delhi, India.

Contact Convenor, WATCH 2002, 509-B, Sarita Vihar, New Delhi 110 044, India; or call +91 11 694 4551; or fax +91 11 694 4472; or e-mail cancerak@del6.vsnl.net.in; or see http://www.watch-2000.org

Radiation therapy

October 6-9, 2002

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

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

Radiation oncology

November 10-16, 2002

The ESTRO teaching course "Evidence-Based Radiation Oncology: Methodological Basis and Clinical Application" will take place in Tenerife, Spain.

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

Radiation oncology

March 15-19, 2003.

The "2nd International Conference on Translation Research and Pre-Clinical Strategies in Radiation Oncology, ICTR 2003" will be offered in Lugano, Switzerland.

Fax +41 91 820 9044, or e-mail jbernier@pop.eunet.ch, or see http://www.osg.ch/ictr2003.html

Lung cancer

August 10-14, 2003.

The "10th World Conference of the International Association for the Study of Lung Cancer" will be offered in Vancouver, Canada.

Contact 10th World Conference of Lung Cancer, c/o International Conference Services, 604-850 West Hastings, Vancouver BC Canada V6C 1E1, or call +1 604 681 2153; or fax +1 604 681 1049; or e-mail conference@2003worldlungcancer.org

Radiation therapy

September 21-25, 2003

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

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

Radiation therapy

October 19-23, 2003

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

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

Radiation therapy

September 12-16, 2004

The 23rd Annual ESTRO Meeting will be held.

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

Radiation therapy

October 3-7, 2004

ASTRO Annual meeting will be held in Atlanta, USA.

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

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

Please sent information to the Editorial office, Radiology and Oncology, Zaloška 2, SI-1000 Ljubljana, Slovenia.

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Supplement 1/2001
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Editors greatly appreciate the work of the reviewers who significantly contributed to the improved quality of our journal.

Author Index 2001

Supplement 1/2001

Subject Index 2001

Supplement 1/2001

Note

One issue of Radiology & Oncology, Volume 35, 2001, was published only in Slovenian as Supplement 1 and was intended exclusively to the students of School for Health Professionals at the University of Ljubljana. Hence, all indexes for the above Supplement (reviewers', author's and subject's) are quoted separately and are not included into the indexes of regular issues.

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

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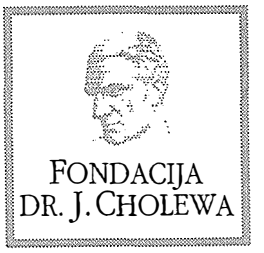


Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education - A Report for the Final Quarter of 2001

The activity of the "Dr. J Cholewa" Foundation for cancer research and education continued its activity throughout the remaining weeks and months of the year 2001 as it was outlined during the last meeting of its Executive council. The change in the donors' attitude towards the Foundation in the last months prior to this report caused some concern and the ways how to adapt to the new circumstances were seriously discussed. Some of the new initiatives were taken into consideration and it is hoped that it will be possible to review some tangible results of the activity under discussion in the near future.

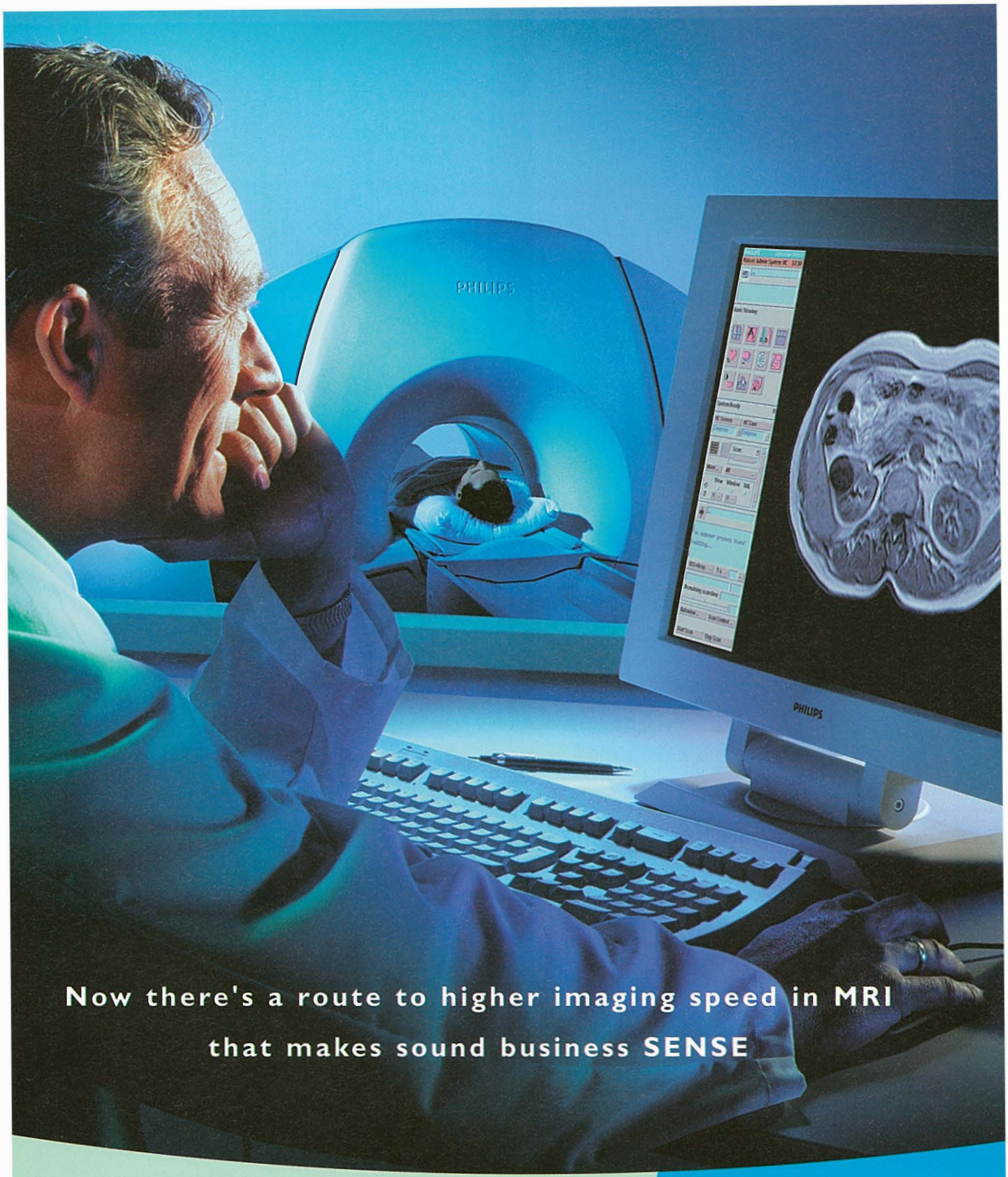
The Foundation is planning to intensify its contacts with some of the established donors and it also plans to attract new ones to its cause. It is believed that considerable interest exists in collaboration with the Foundation among the executive officers and managers of various companies, especially those with the interests in pharmacology development and production and in banking. The activity of the Foundation is by now well established as a result of a relatively long and substantial experience, and through the hard work of its members it offers a certain guarantee that the means from the possible donations will be spent in an efficient and impartial way.

The Foundation continues to support the regular publication of "Radiology and Oncology" international scientific journal that is edited, published and printed in Ljubljana, Slovenia. The support for the publication of the "Challenge Newsletter" is still to be re-evaluated and the decision should be taken in due time.

The Foundation has received a number of grant applications and it has considered the merits of the proposed research work thoroughly. The support is to be given to research work in the field of oncology originating in Slovenia with regard to the quality and to several other important aspects presented in proposals for the grants. A decision was also taken that a number of grants is to be awarded to experts from various parts of Slovenia in order to attend various conferences and meetings in the field of oncology in Slovenia and around the world.

The Foundation is considering to invite new members to its Executive council in order to better represent and understand the present urgencies and advances in the continuos development of cancer research and education in Slovenia. It will thus continue to pursue its stated objectives with new vigour and objectivity.

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



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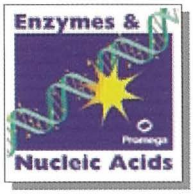

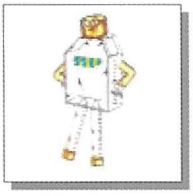

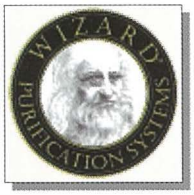

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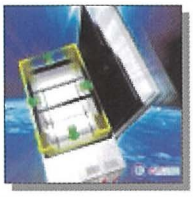

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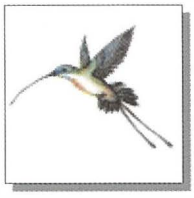

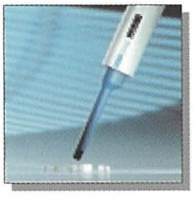

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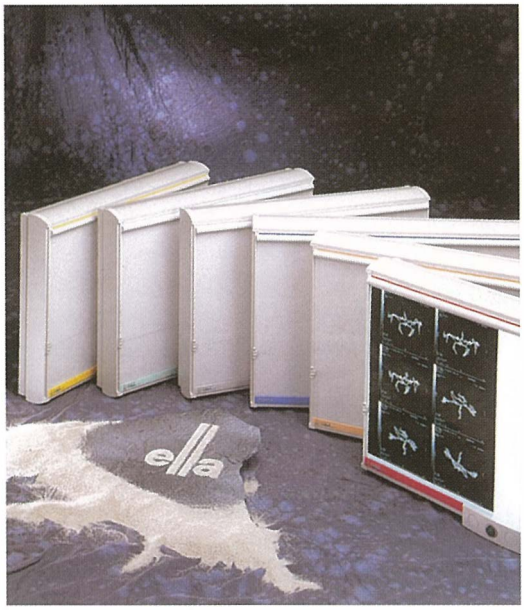

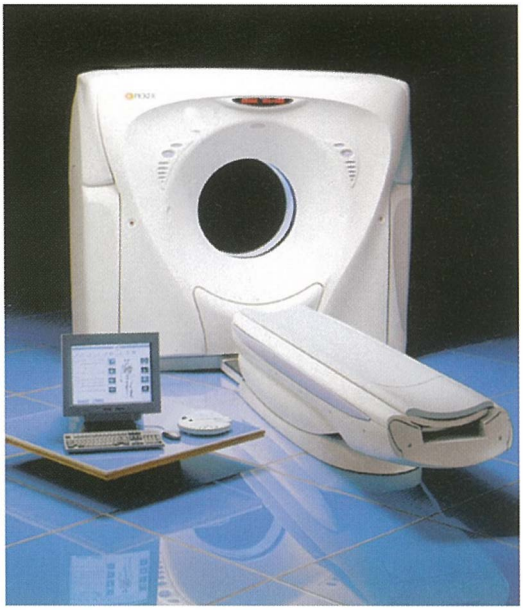

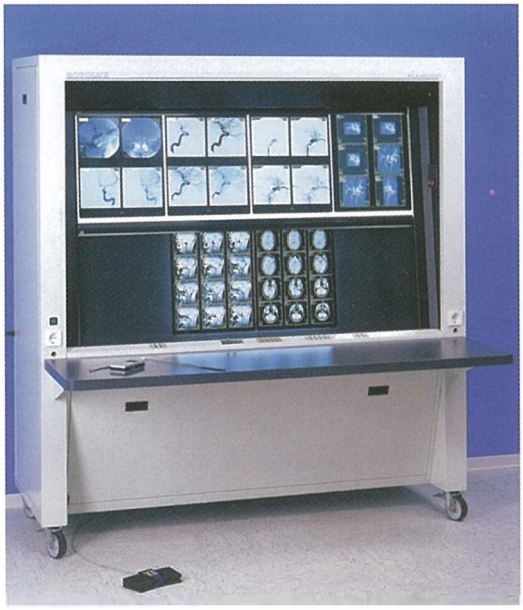

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kriptokokni meningitis	*prvi dan 400 mg, nato od 200 do 400 mg na dan*
vzdrževalno zdravljenje	*200 mg na dan*

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Manuscript written in English should be submitted to the Editorial Office in triplicate (the original and two copies), including the illustrations: *Radiology and Oncology,* Institute of Oncology, Zaloška 2, SI-1000 Ljubljana, Slovenia; (Phone: +386 1 432 00 68, Tel./Fax: +386 1 433 74 10, E-mail: gsersa@onko-i.si). Authors are also asked to submit their manuscripts on a 3.5" 1.44 Mb formatted diskette. The type of computer and word-processing package should be specified (Word for Windows is preferred).

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

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

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

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

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

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

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

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

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

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

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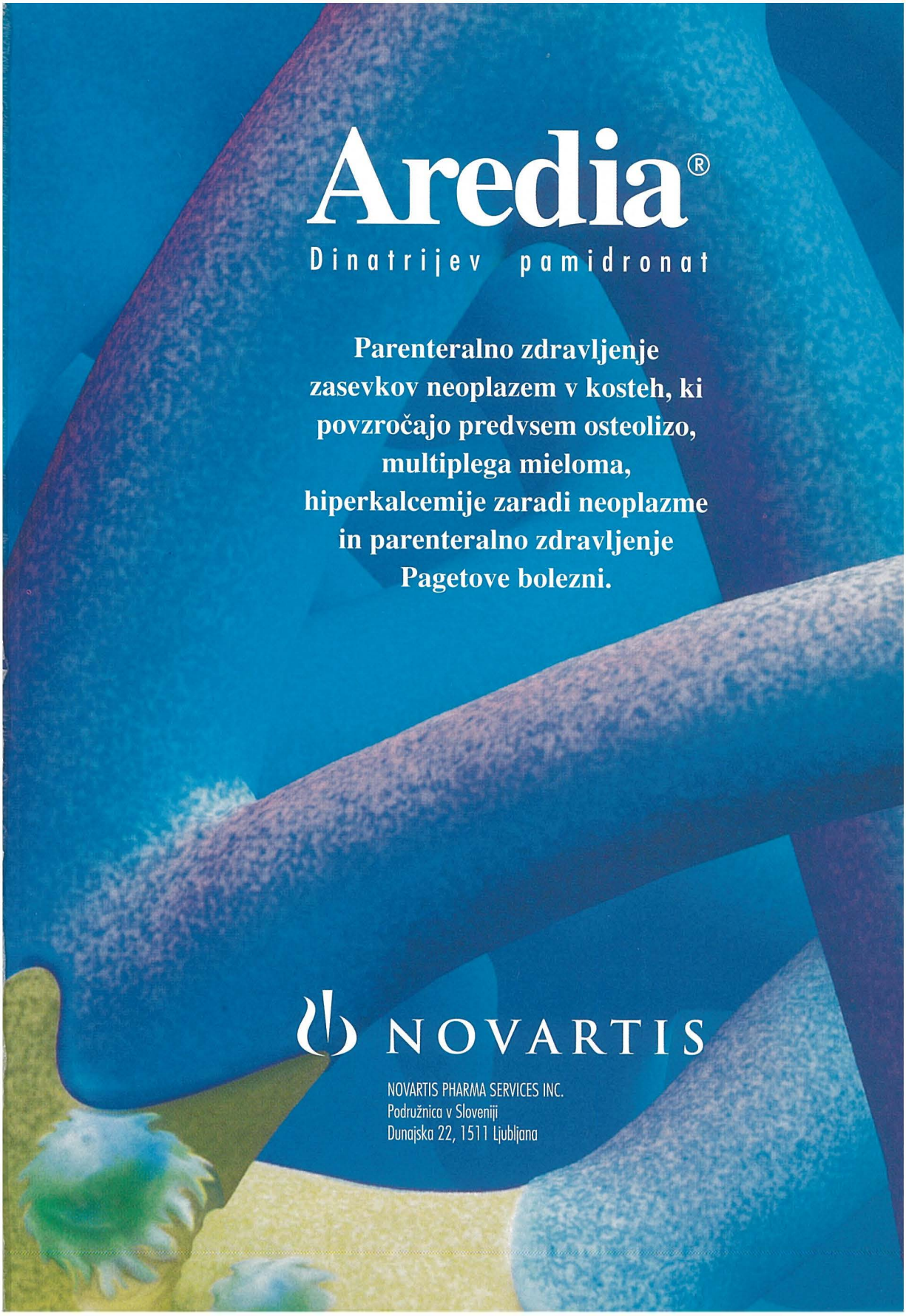
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