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Editorial office
Radiology and Oncology
Institute of Oncology
Zaloška 2
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Slovenia
Phone: +386 1 4320 068
Phone/Fax: +386 1 4337 410
E-mail: gsersa@onko-i.si

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Breath-hold times in patients undergoing radiological examinations: comparison of expiration and inspiration with and without hyperventilation

Reinhard Groell, Gottfried J. Schaffler, Stephan Schloffer

Department of Radiology, University Hospital Graz, Austria

Background. *Breath-holding is necessary for imaging studies of the thorax and abdomen using computed tomography, magnetic resonance imaging or ultrasound examinations. The purpose of this study was to compare the breath-hold times in expiration and inspiration and to evaluate the effects of hyperventilation.*
Patients and methods. *Thirty patients and 19 healthy volunteers participated in this study after informed consent was obtained in all. The breath-hold times were measured in expiration and inspiration before and after hyperventilation.*
Results. *The mean breath-hold times in expiration (patients: 24±9sec, volunteers: 27±7sec) were significantly shorter than those in inspiration (patients: 41±20sec, $p<0.001$; volunteers: 62±18sec, $p<0.001$). Additional hyperventilation resulted in a significant increase (range: 40-60%, $p\leq0.005$) of the mean breath-hold times either in expiration and in inspiration and for both patients and volunteers.*
Conclusions. *Although breath-holding in expiration is recommended for various imaging studies particularly of the thorax and of the abdomen, suspending respiration in inspiration enables the patient a considerable longer breath-hold time.*

Key words: tomography, X-ray computer; magnetic resonance imaging; ultrasonography; breath holding

Introduction

Respiratory motion may degrade imaging studies particularly of the thorax and of the abdomen. Therefore breath-holding is crucial during thoracic and abdominal examinations using different modalities such as computed tomography, magnetic resonance imaging, digital angiography, or Doppler sonography.

Breath-holding in expiration is regarded to result in more consistent organ positioning and is therefore recommended for various imaging studies particularly of the abdomen.[1] However, in the clinical experience it is easier to hold the breath in inspiration than in expiration. This is of clinical importance when the clinician has to make the decision whether the examination should be performed in expiration or inspiration.

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Correspondence to: Reinhard Groell, MD. Department of Radiology, University Hospital Graz, Auenbruggerplatz 9, A-8036 Graz, Austria. Phone: +43 316 385 81611; Fax: ++43 316 385 3231; E-mail: reinhard.groell@kfunigraz.ac.at

Previous reports have shown that the maximum breath-hold time may be increased by hyperventilation and by administration of oxygen.[2,3] However, a comparison of breath-hold capabilities between expiration and inspiration has - to our knowledge - not been performed yet. This prompted us to prospectively evaluate and compare the breath-hold capabilities of patients and of healthy volunteers in expiration and inspiration without and after hyperventilation.

Patients and methods

The study population consisted of 30 outpatients and 19 healthy volunteers. The patients (15 female, 15 male; mean age: 64±15years, range: 31-85 years) were referred to abdominal ultrasound for various clinical reasons. The majority (n=17) of them were examined in routine screening. Six of the patients were smokers with a smoking history of more than ten pack-years. Four patients had a medical history of chronic obstructive pulmonary disease (COPD) and two of them additionally had chronic heart failure (CHF), the patients received medical therapy for these conditions. The healthy volunteers (8 female, 11 male; mean age: 32±5years, range: 23-43years) were employees of our institution. Two of them were smokers with a smoking history of more than ten pack-years. None of the volunteers had known diseases of the cardio-respiratory system.

All patients and healthy volunteers gave informed consent to the performance of this study. For the patients the measurements were performed while the patients were waiting for their ultrasound examination. During the study all participants were lying in the supine position. The patients were instructed to hold their breath in expiration ("breathe in, breathe out, hold your breath") and in inspiration ("breathe in, breathe out, breathe in, hold your breath"). Then the same respiratory maneuvers were performed following six deep inhalations of room air (corresponding to approximately 20-30 seconds of hyperventilation). A time span of at least 2 minutes

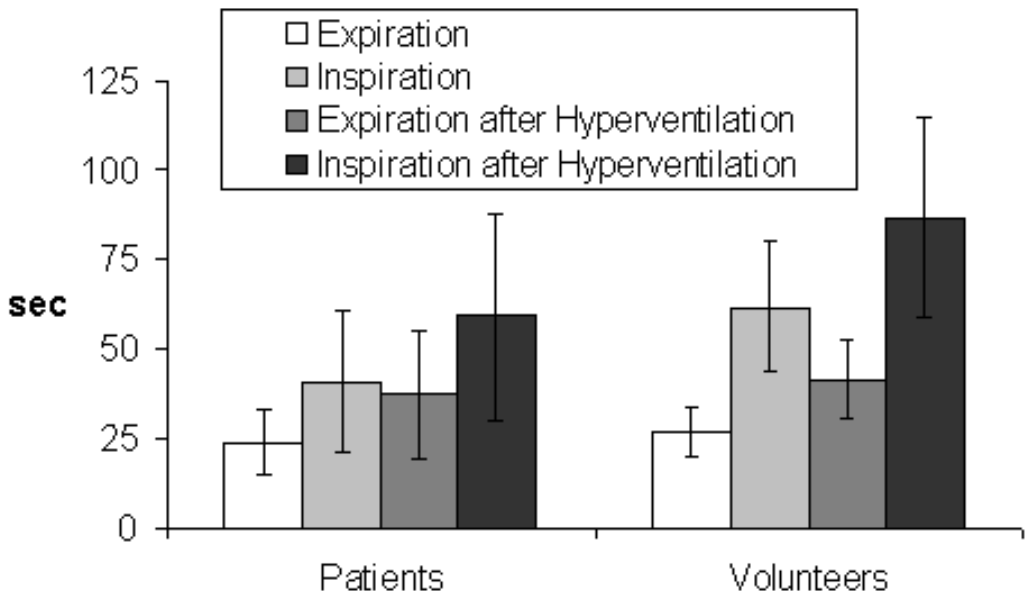

Figure 1. Mean breath-hold times in patients and healthy volunteers without and after hyperventilation.

was kept between the breath-holds. The order of inspiration and expiration was changed alternately among different subjects to minimize the effects of training.

Statistical comparison of different respiratory maneuvers was performed using a Student t-test with a 5% level of statistical security. Multivariate analysis was performed to evaluate the influence of age, sex, smoking history, cardio-pulmonary diseases, or of the order of examinations (expiration performed before inspiration or vice versa) on the measured breath-hold times.

Results

The mean breath-hold times in expiration were significantly shorter than those in inspiration both without hyperventilation (patients: 24±9sec vs. 41±20sec, $p<0.001$; volunteers: 27±7sec vs.62±18sec, $p<0.001$) and after hyperventilation (patients: 37±18sec vs. 59±29sec, $p<0.001$; volunteers: 42±11sec vs.87±28sec, $p<0.001$) (Figures 1,2). Hyperventilation resulted in a significant increase of the measured mean breath-hold times in expiration (patients: 24±9sec vs. 37±18sec, $p<0.001$; volunteers: 27±7sec vs.42±11sec, $p=0.005$) and inspiration (patients: 41±20sec vs. 59±29sec, $p<0.001$; volunteers: 62±18sec vs. 87±28sec, $p=0.002$). In expiration the mean breath-hold times were not statistically different ($p>0.23$) between patients and healthy volunteers either without or after hyperventilation. However, in inspiration the breath-hold times of the healthy volunteers were generally longer than those of the patients ($p<0.003$). Multivariate analysis revealed that in patients with COPD or CHF the breath-hold times without hyperventilation were not statistically different from those of patients without such diseases (Figure 2). After hyperventilation, the mean breath-hold times were lower in the patients with COPD or CHF (expiration: 21±10sec vs. 40±18sec, $p=0.02$; inspiration: 37±16sec vs. 62±30sec, $p=0.05$), however this comparison is limited by the small number of patients with COPD or CHF (total: n=4). While there were no significant sex differences in breath-holding without hyperventilation, after hyperventilation lower breath-hold times were observed in women than in men ($p<0.03$). Age ($p>0.2$), smoking history ($p>0.4$), or the order of examinations (expiration performed before inspiration or vice versa, $p>0.1$) showed no significant influence on the measured breath-hold times.

Discussion

In various radiological modalities the optimal image quality is achieved when the patients hold their breath during the entire study. With the advent of fast imaging modalities such as spiral computed tomography or rapid magnetic resonance imaging more and more studies may potentially be acquired within a single breath-hold. Frequently the study time lies in the order of the breath-hold time with some variations in either direction. These variations, however, may considerably influence the success of the examinations. Thus, to optimize the quality of the examination, the radiologist has to consider the breath-hold capabilities of the patient. Breath holding in expiration is reported to allow a more reproducible organ positioning than breath holding in inspiration.[1] That is why many studies, particularly of the abdomen, should primarily be performed in expiration. Although thoracic studies usually benefit from maximal distention of the lungs as it occurs in inspiration, expiratory scans may be necessary e.g. in patients with obstructive lung diseases to document possible air trapping.[4]

Reviewing the literature, we found three radiological studies that evaluated the breath-hold capabilities of adults either in expiration

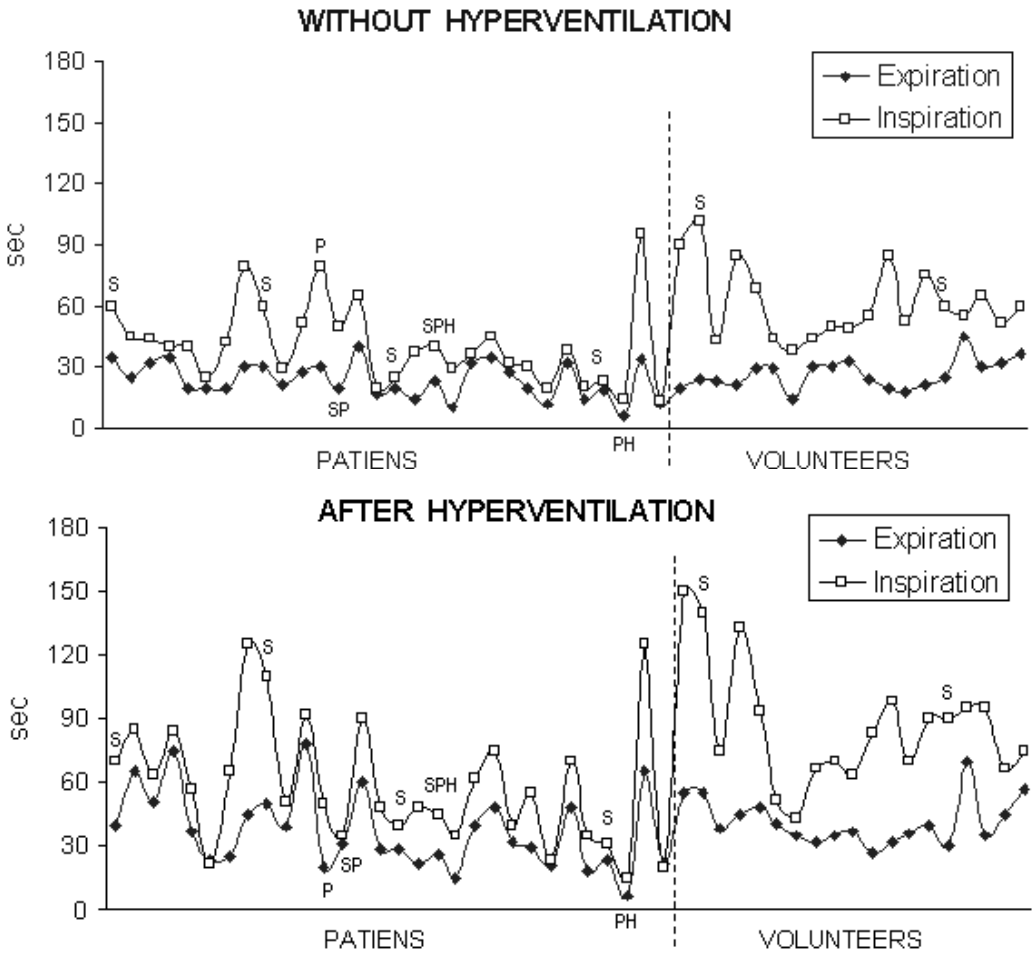

Figure 2a,b. Scatter graphs showing the breath-hold times in expiration/inspiration without (Figure 2a) and after (Figure 2b) hyperventilation. The data points are connected for each of parameter of ventilation. The graphs indicate smokers (S) with a history of more than 10 pack-years, patients with COPD (P), and patients with chronic heart insufficiency (H).

or in inspiration and two of these studies documented the benefit of hyperventilation and of administration of oxygen.[2,3,5] We found no report that compared breath-holding capabilities between expiration and inspiration that could be transformed to the conditions in a radiological setting. Some reports have investigated the physiological changes that occur during suspended respiration with special interest in oxygen saturation and heart rate, most of these studies were performed in divers.[6-8] As part of the physiologic diving reflex a decrease in the heart rate can be observed during breath-holding which was also observed by Gay and Marks.[2,5]

With and without hyperventilation the breath-hold times in inspiration exceeded those in expiration by approximately 50-130%. Although it is a wide-held belief that it is easier to hold the breath in inspiration than in expiration, the amount of these differences exceeded our expectations.

All of the patients investigated in this study were outpatients and none of them was

severely pulmonary-compromised, although four patients had a medical history of COPD and two of them additionally had CHF. The healthy volunteers were generally considerably younger than the patients, and most of them were physically active. This may explain the longer mean times in the group of volunteers than in the patient group. In our study population hyperventilation increased the maximum breath-hold capabilities in expiration and inspiration, however these effects were less pronounced in patients with cardiac or pulmonary diseases (Figure 1). This confirms the results of Marks et al. who demonstrated that the effects of hyperventilation were less beneficial in pulmonary-compromised patients while administration of oxygen resulted in increased breath-hold times even in pulmonary-compromised patients.[2] Similar to the observations of Gay et al. we found no significant influence of smoking on the maximum breath-hold times.[5]

In conclusion, suspending respiration in inspiration results in considerably longer breath-hold times when compared to breath-holding in expiration. The radiologist has to decide which respiratory maneuver is best suitable to optimize the performance of the specific imaging studies.

Acknowledgement

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Percutaneous drainage of abdominal fluid collections that require laparotomy or relaparotomy with ultrasound guidance

Damir Miletić[1], Miljenko Uravić[2], Željko Fučkar[2], Robert Glavaš[3], Dubravka Topljak-Polić[1]

[1]Department of Radiology, [2]Clinic for Surgery, [3]Department of Gastroenterology, Clinical Hospital Rijeka, Croatia

Background. *The aim of the study was to determine efficacy and reliability of percutaneous abdominal drainage in surgical patients and to evaluate intercostal approach to drain subphrenic collections.*

Material and methods. *Eighty-seven patients aged from 29 to 84 years (mean, 55.5 years) were percutaneously drained under the sonographic guidance due to the postoperative or nonoperated abdominal collection that would otherwise require laparotomy. Intercostal, subcostal, lateral and anterior approach with eight to 14 French catheters were used to evacuate abdominal collection.*

Results. *The intercostal approach was used to drain 31 (60.8%) of 51 subphrenic collections. The mean duration of drainage was independent of the intercostal or subcostal drainage route, but was significantly prolonged ($p<0.05$, Mann-Whitney U test) for purulent collections (median, 18 days; range 7-73 days) in comparison to hematomas, bilomas and other nonpurulent collections (median, 11 and 6 days, respectively). Sonographically guided percutaneous drainage was a definitive method in 92% patients, with 9.2% minor complications. Successful rate for subphrenic collections was even greater (96%).*

Conclusions. *Sonographically guided percutaneous drainage is the method of choice in the treatment of abdominal collections that require laparotomy. If the puncture site is at least two intercostal spaces lower than the dome of diaphragm and catheter is not introduced through the pleural effusion, intercostal drainage is equally efficient and not less secure than subcostal approach.*

Key words: sonography; abdomen; drainage

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Correspondence to: Damir Miletić, MD, PhD, Department of Radiology, Clinical Hospital Rijeka, Krešimirova 42, Croatia. Phone: + 385 51 65 83 85; E-mail: Damir.Miletic@medri.hr

Introduction

Despite the initial skepticism, percutaneous catheter drainage of abdominal collections is well established and widely accepted procedure by an interventional radiologist and surgeons.[1,2,3] Fluid collections may be drained under the sonographic, CT or fluoroscopic

guidance. The intercostal or subcostal approach may be used to drain subphrenic collections. Subcostal drainage route is generally recommended to avoid pleural transgression.[4] The intercostal approach is preferred to drain left subphrenic collections after splenectomy.[5]

The aim of our study is to determine the efficiency and reliability of the percutaneous abdominal drainage in surgical patients, as well as to compare the transpleural and extrapleural approach to drain both right and left subphrenic collections.

Material and methods

In five-years' period we planned 101 sonographically guided percutaneous drainages of the suspected abdominal collections in patients who were admitted at the surgery clinic. Only in 3 patients the intestinal interposition could not be avoided in any potential approach route, and percutaneous drainage was postponed or withdrawn. Percutaneous aspiration of the fluid content with or without instillation of antibiotics was a definitive method in 6 patients. In other five patients initial diagnostic needle aspiration confirmed solid lesion instead, and the aspiration biopsy followed.

Our study includes 87 patients, 46 male and 41 female, with percutaneous catheter drainage of abdominal fluid collection. The mean age of the patients in our study were 55.5±10.9 (SD), ranging from 29 to 84. Laparotomy or relaparotomy was planned due to clinical symptoms such as fever, abdominal pain, respiratory or intestinal problems, palpable mass, and CT or sonographic visualization of the fluid collection.

Seventy-five patients (86.2%) underwent recent abdominal surgery: of those, biliary surgery was performed in 33, gastrointestinal in 19, splenectomy in 10, liver surgery in 6, and other in 7 patients. The postoperative sonography within the first 10 days detected intraperitoneal collection. In other 12 patients (13.8%) without previous laparotomy intraperitoneal or retroperitoneal fluid collection was found by ultrasound or computed tomography. In this group we detected paracolic abscesses due to diverticulitis in 6 patients, and retrocecal appendicitis in one patient. The retroperitoneal penetration of the descendent colon carcinoma caused a huge abscess formation with compression to the bowel in one patient. Two collections appeared liquefied neoplasms; one was induced by the gastric ulcer perforation and the other by the missile penetration.

All percutaneous drainages were performed under the sonographic guidance. All specimens obtained by needle aspiration were submitted for cytology, aerobic and anaerobic cultures. Nonpurulent materials were also chemically analysed. Eight to 14 French catheters were used to evacuate abdominal collections. Generally, we used 8 to 10 French catheters for serous collections including bile, and for intercostal approach. Twelve to 14 French catheters were used for purulent and viscous collections. The complete procedures consumed 10 to 30 minutes of time, depending mostly on localization. Patients were regularly followed-up the 2nd and 7th day from percutaneous catheter placement, as well as immediately after the drainage stopped. The catheter was not removed before sonography or CT confirmed the complete evacuation of the infected collection. Incomplete drainage was tolerated in particular sterile hematomas if evacuation stopped and clinical response was favorable. In other noninfected collections we also insisted on complete evacuation before the catheter removal. In a case of catheter malfunction or absence of clinical improvement, the catheter position and function were revised with sonographically controlled instillation of 5-10 ccm of normal saline.

We used intercostal, subcostal, lateral, and

anterior approach with the patient supine, in the left or right decubitus position. The access route was elected by the previous sonographic examination. Intercostal drainage was performed as lower as possible to reach the collection, at least 2 intercostal spaces lower than the dome of the diaphragm was visualized. If the puncture line included pleural effusion, the catheter was not applied.

The linear array transducers of 5 MHz with the central canal to guide the needle were used in our series. The needle position was constantly monitored during the insertion, diagnostic aspiration and the guide wire placement if the Seldinger technique was used. We also used a trocar technique, especially in the intercostal approach. When properly positioned, catheters were secured in place by suturing to the skin.

Statistical differences were calculated with the Mann-Whitney U (MWU) test, and the analysis of variance (ANOVA).

The procedures were conducted in accordance with the ethical standards of the Helsinki Declaration of 1975.

Results

In five-years' period we performed 89 percutaneous catheter drainages of abdominal fluid collections in 87 patients under the sonographic guidance.

We detected 34 fluid collections (38.2%) in the right subphrenic space with or without subhepatic extension, 8 collections (9.0%) isolated in the subhepatic space, 17 collections (19.1%) in the left subphrenic space, 11 collections (12.4%) between the intestinal loops, 10 collections (11.2%) in the right/left paracolic gutter, and 6 collections (6.7%) in the retroperitoneum. Other rare locations included preperitoneal collections beneath the abdominal wall in 2 cases, and lesser sac abscess in one patient. In two patients we drained 2 different collections consecutively.

Aspirated material was macroscopically purulent in 31, sanguineous in 11, serous in 9, and undetermined in 21 collections. In 17 collections we aspirated bile. A Gram stain, aerobic and anaerobic cultures confirmed infected material in 47 (52.8%) specimens. Namely, 5 hematomas, one seroma, and 10 undetermined collections were infected. Fifty-eight specimens were submitted for a chemical analysis to confirm the origin of a fluid collection. Cytologic examination indicated malignant tumor in four specimens.

Of 51 subphrenic collections, intercostal approach was used to drain 31 (60.8%) collections, 20 out of 34 (58.8%) in the right and 11 out of 17 (64.7%) in the left subphrenic space. We selected intercostal approach (Figure 1) whenever it was the shortest route to reach the collection, but only if pleural effusion could be avoided on the puncture line. In 20

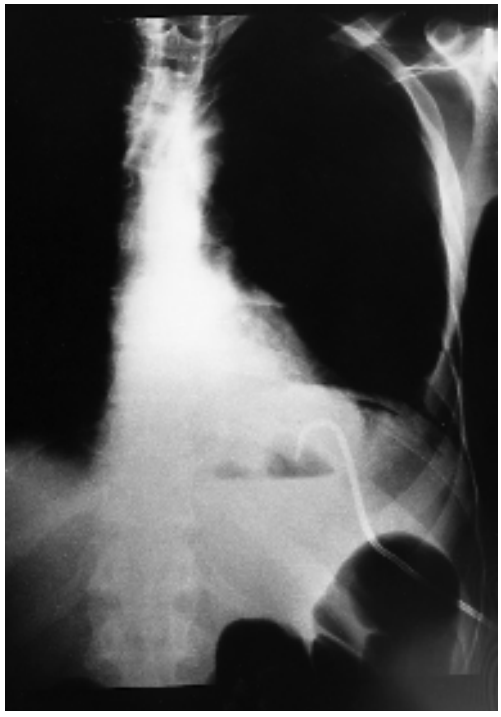

Figure 1. Intercostal approach to drain left suphrenic abscess formation. Note elevation of the left hemidiaphragm, and gas accumulation below.

(39.2%) subphrenic collections the extrapleural access route was preferred.

We preferred the lateral approach for percutaneous drainage of the collections in paracolic gutters, the posterolateral approach for the retroperitoneal collections, and the anterior approach for preperitoneal and lesser sac collections. The access route for the collections between the intestinal loops was selected individually to avoid small or large bowel transgression. Intestinal interposition was excluded by the real-time sonographic monitoring of peristalsis.

The median number of days of drainage for all abdominal collections was 13 days (range, 2-73 days). Significant variability regarding the duration of drainage of different fluid qualities was detected (Figure 2, ANOVA). The median drainage time for purulent collections was 18 days (range, 7-73 days), significantly longer ($p<0.05$, MWU) in comparison to hematomas (median, 11 days; range, 5-28 days). The drainage time for bilomas (median, 6 days; range, 2-17 days), seromas and other nonpurulent collections (median, 6 days; range, 3-13 days) was significantly shorter ($p<0.05$, MWU) compared to hematomas, and purulent collections. Patients stayed in hospital up to 20 days after the catheter insertion. If the drainage had to be prolonged (Figure 2), the outpatient care with regular weekly controls was preferred.

Although 8 and 10 French catheters were used in the intercostal approach, the mean duration of drainage was not significantly different in comparison to the subcostal drainage route where larger catheters (up to 14 French) were placed (median, 15 and 12 days, respectively).

Intra-abdominal collections were completely evacuated in 76 (87.4%) patients, including both patients with two different collections in the abdomen. In 4 (4.6%) patients with sterile hematoma evacuation was incomplete, but percutaneous drainage was a definitive method. Further resolution of residual coagulated blood products after the catheter removal was followed-up on ultrasound controls until the resorption is completed (up to 95 days). Sonographically guided percutanous drainage was entirely successful in 80 patients (92%) with 82 collections. Forty-nine

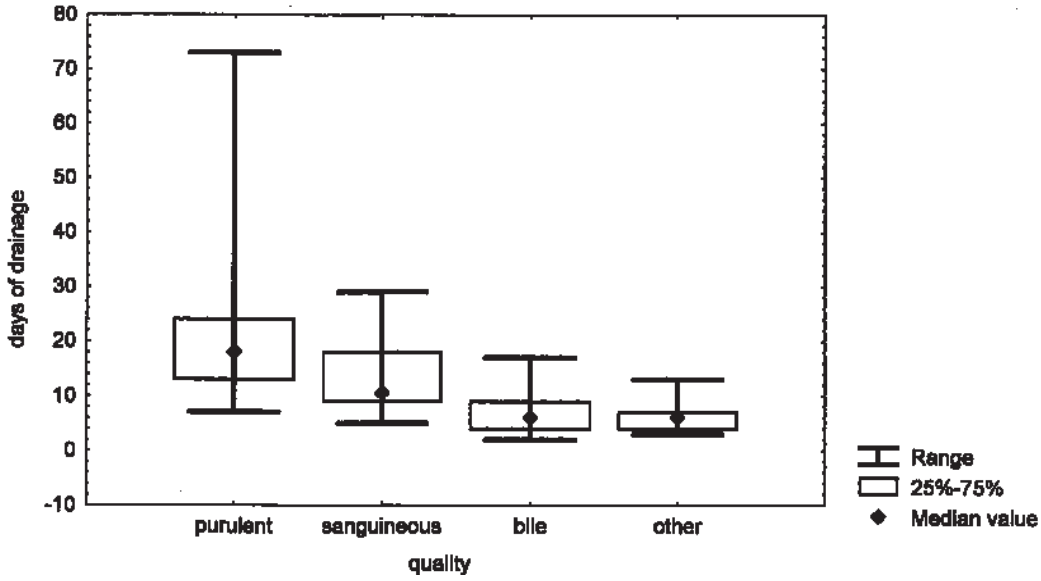

Figure 2. Drainage time variability for different qualities of abdominal collections.

of 51 patients with subphrenic collections (96%) were cured by percutaneuos catheter drainage.

Only in four patients the surgical procedure was necessary, but even those patients benefited from the parcutaneous drainage. Namely, body temperature and white blood cell count decreased, while the overall condition improved. In the first patient with the complete bowel obstruction due to the huge extraluminal abscess formation and compression we evacuated 900 ccm of purulent and necrotic material. This procedure facilitated the normal evacuation of the colon, and a patient became fit for surgery. Carcinoma of the descendent colon penetrating to the retroperitoneal space was diagnosed with the barium enema after the percutaneous drainage (Figure 3). Right subphrenic collection in the second patient was a result of an intestinal perforation due to carcinomatous peritoneal dissemination, and percutaneous drainage preceded the operative resection. In both cases previously existed ileus subsided. Continuous daily drainage of 500-700 ccm of bile in the third patient indicated postoperative biliary fistula that required a surgical revision. Retrocecal abscess due to perforated appendicitis in the fourth patient was drained, and appendectomy followed.

In another two patients abdominal collections appeared liquefied neoplasms on cytology, and percutaneous drainage was only a palliative procedure with the evident clinical improvement. Finally, one patient with hematemesis died the 3rd day after the catheter placement. The abscess adjacent to the left hepatic lobe was diagnosed on CT and US, and sonographically guided percutaneous drainage without hepatic transgression fol-

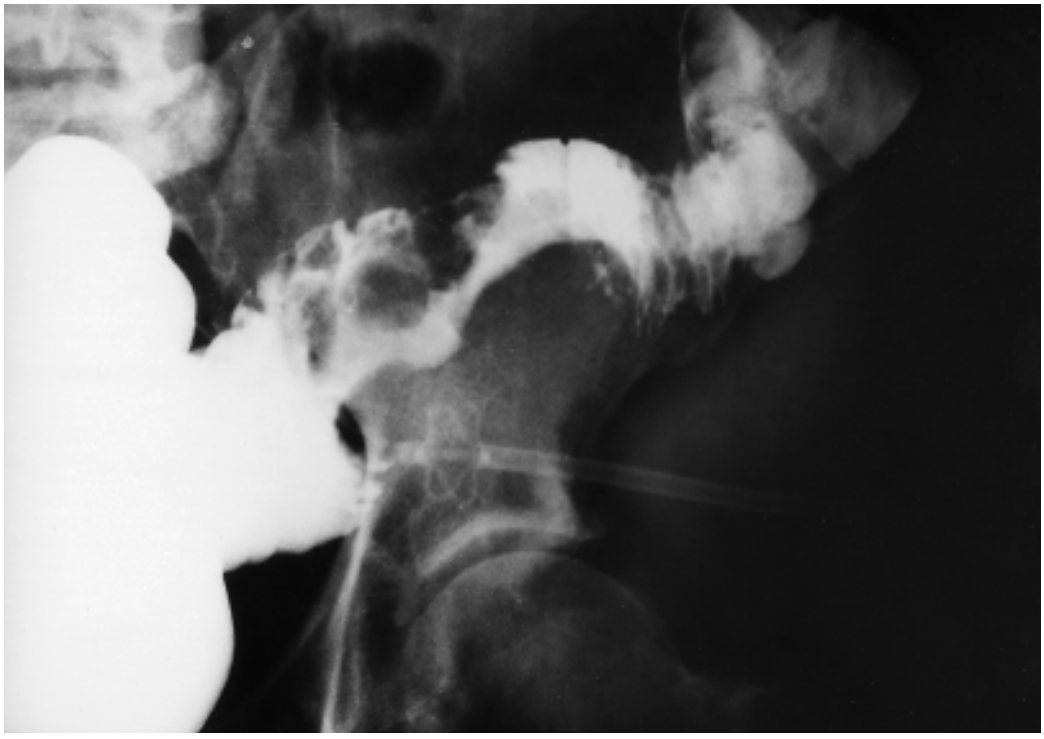

Figure 3. Percutaneous drainage of the retroperitoneal collection due to colon carcinoma invasion. Note irregular segmental stenosis of the colon. Catheter was introduced above the inguinal ligament to the left iliac fossa.

lowed. We evacuated 600 ccm of purulent material. At autopsy, a bleeding gastric ulcer with localized perforation was detected. The abscess cavity was localized to the lesser sac, and a catheter was placed in the proper position.

We detected 8 (9.2%) minor complications in our series, including superficial skin infection in one case, chills immediately after the catheter placement due to bacteremia in 2 patients, and intestinal content leakage near the subcostally introduced catheter in one patient with intestinal fistula. In one patient left subphrenic abscess after splenectomy recurred, and percutaneous drainage had to be performed again. The second attempt was followed by complete resolution of the fluid collection.

In two patients with intercostal approach concomitant pleural effusion increased in size, without clinical impairment. Aspirated pleural fluid was sterile. Only in one patient asymptomatic pneumothorax was detected after the intercostal catheter insertion.

Life-threatening complications of the sonographically guided PAD were not detected in our patients.

Discussion

In our series 80 out of 87 patients with abdominal fluid collections were cured by percutaneous drainage. Even 96% of all subphrenic collections were cured without further surgical intervention. Complications of the percutaneous catheter drainage of abdominal collections at surgery department in our patients were minor, incomparable with the reported morbidity and mortality rate (19.4% and 4.8%, respectively) of the surgical drainage of subphrenic abscesses.[6]

High successful and low complication rate in comparison to the previous reports[2,5] could be explained by the following: (1) five patients with the incomplete evacuation of the sterile hematoma who did not need further intervention were included in the success group (2) in three patients we canceled the procedure due to the equivocal bowel interposition (3) in 6 patients where needle insertion through the pleural effusion was unavoidable, we elected percutaneous aspiration of the subphrenic collection and instillation of antibiotics if the material was purulent. Percutaneous aspiration was repeated in 2 patients with the reaccumulation of the fluid content. Sonographic guidance provided a reliable differentiation between subphrenic and pleural accumulation due to the multiplanar imaging and real-time monitoring of diaphragmatic movement.

According to McNicholas et al. subcostal (extrapleural) drainage of subphrenic collections could be difficult only on the left side, especially after splenectomy.[7] In our experience this problem appears on both right and left side. Namely, subphrenic inflammation usually causes the ipsilateral diaphragmatic relaxation and diminishes its respiratory movement. Therefore, liver and spleen are withdrawn from the costal arch, and the inspiration is frequently not very helpful in subcostal approach. Although McNicholas et al. consider right-sided subphrenic collections easier to drain subcostaly, liver parenchyma was frequently interposed and unavoidable in subcostal approach in our patients, especially if subphrenic collection did not extend to the Morison's pouch. We elected the intercostal approach whenever the collection could be reached avoiding pleural effusion, if it was the shortest drainage route.

In our experience, definitive diagnosis did not need to be established before the percutaneous puncture of an abdominal fluid collection visualized on CT or sonography. The initial aspiration confirms the presence of a collection, determines whether the collection is infected and the material liquefied enough to be drainable, and establishes a safe route for the subsequent catheter insertion.[4] We di-

agnosed diverticulitis, large bowel carcinoma and retrocecal appendicitis on barium studies after the drainage was completed.

According to our results, the drainage time for hematomas was significantly longer than for bilomas and other nonpurulent collections, and in this group we had 4 incomplete evacuations. One could speculate if catheter drainage of hematomas is really justifiable, or needle aspiration should be sufficient. We recommend the catheter placement when a symptomatic collection is visualized even if sanguineous content is aspirated. Namely, five out of 11 hematomas in our series were infected, and even patients with sterile hematomas benefited from the procedure. Small, asymptomatic early postoperative collections are probably seromas or hematomas, requiring only ultrasound follow-up.

Conclusively, sonographically guided percutaneous drainage is the method of choice for the treatment of symptomatic postoperative abdominal fluid collections. This method is also successful in nonoperative treatment of abdominal collections that otherwise requires explorative laparotomy. In our experience, it was a definitive method in the treatment of the perforative diverticulitis. If the puncture site is at least two intercostal spaces lower than the dome of diaphragm and a catheter is not placed through pleural effusion, the intercostal drainage route for subphrenic collections is not less secure than the subcostal approach. If pleural effusion is unavoidable, only percutaneous puncture and aspiration without the catheter placement should be performed.

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Case report

Ultrasound-guided central venous cannulation in patient with radical dissection on both sides of neck: case report

Alan Šustić[1], Robert Cerović[2], Mirna Juretić[2]

[1]Department of Anesthesiology and ICU, [2]Department of Maxillo-facial surgery, University Hospital Rijeka, Croatia

Background. *Difficult and complicated surgical procedures in elective oncologic patients often require central venous (CV) catheterization. In certain number of cases, relevant anatomical changes, caused by basic disease, impede, or even prevent the use of standard "blind" CV cannulation. In such cases ultrasonography can be used successfully as an adjunctive method during the CV cannulation.*
Case report. *We present the patient with a radical dissection on both sides of neck and consequent important anatomical changes; the ultrasound-guided CV cannulation is successfully performed in this patient.*
Conclusions. *Ultrasound as a supporting method for CV catheterization in oncological patients has potential benefits. Sonography as an important adjunct method in central venous cannulation is recommended in difficult cases.*

Key words: sonography; catheterization, central venous

Introduction

Difficult and complicated surgical procedures in elective patients require the central venous (CV) cannulation for monitoring and for parenteral alimentation in the intraoperative and postoperative period. In certain number of cases relevant anatomical changes, caused by basic disease, impede, or even prevent the use of the standard CV cannulation. In such cases ultrasonography can be used successfully as an adjunctive method during the CV cannulation.[1-3] We present the patient with a radical dissection on both sides of the neck and consequent important anatomical changes; the ultrasound guided CV cannulation is successfully performed in this patient.

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Correspondence to: Alan Šustić, M.D., Ph.D., Department. of Anesthesiology and ICU, Clinical Hospital Rijeka, T. Strižića 3, Rijeka 51 000, Croatia. Phone: + 385 51 21 84 07; Fax: + 385 51 21 84 07; E-mail: Alan.Sustic@mamed.medri.hr

Case report

In a 37 years old male patient, planocellular carcinoma of tongue was diagnosed six months before the present hospitalization. At that time the radical dissection on the right side of the neck was performed, as well as the

suprahyoid dissection on the left side, with the extirpation of a tumor and the resection of mandible. The plastic operation of the defect was done with a free skin-bone flap taken from the left leg. Now the patient was hospitalized due to multiple palpable lymph nodes on the left side of the neck.

The preoperative computerized tomography (CT) and the ultrasonography (US) detected multiple metastatic deposits on the left side of the neck with a complete obliteration and destruction of the left internal jugular vein in the middle part of the neck, and the displacement of the left carotid artery caused by metastatic processes. A somewhat smaller secondary deposit was found immediately above and beside the entrance of jugular vein into the subclavian vein.

By the above-mentioned technique an important segment of the right-sided subclavian artery and vein, displaced upwards craniodorsaly, can be visualized.

As with subsequent diagnostic methods no other metastatic deposits were found, the radical dissection of the left side of the neck was indicated. The preanaesthetic examination informed us of the patient's relatively good state of health, with normal laboratory findings, ASA III. Because of the magnitude of surgical intervention and the necessity of the perioperative and the postoperative monitoring, as well as parenteral nutrition, the central venous catheterization was indicated. Because of important anatomical and pathoanatomical changes, the preoperative right-sided subclavial catheter, under sonographic monitoring, was decided upon. Ultrasonic apparatus Hitachi 405 EUB, with linear transducer (5 MHz) was used sterilized as recommended by the manufacturer. Immediately before the cannulation by supraclavial approach, the displaced subclavial vein was visualized with a few transverse and vertical scans. Finding the best vertical section through the vein, we located the direction, as well as the route of a punction needle, and so we could begin the cannulation with two lumens CV catheter (16 Gauge, Arrow Inc.) with the introductor obeying the rules of a Seldinger technique. Without displacing the transducer we monitored the whole procedure and after the completed cannulation, again visualized the vein with CV catheter in it, by a few transversal and vertical scans (Figure 1).

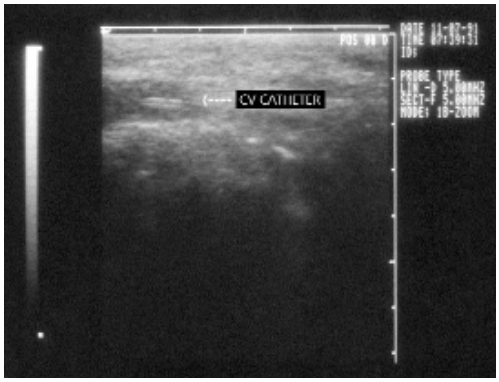

Figure 1. Ultrasonographic transversal presentation of v. subclavia (linear transducer; 5 MHz). Double echo in vein presents central venous catheter.

With the convex transducer (5 MHz) via suprajugular access, we visualized vena anonyma (Figure 2) and with the convex transducer (3 MHz) by a standard echocar-

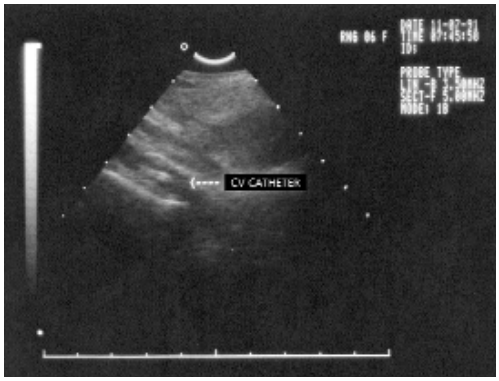

Figure 2. Ultrasonographic transversal presentation of v. anonyma (convex transducer; 5 MHz). Double echo in vein presents central venous catheter.

diographic approach, right heart, respectively. Subsequently, through a catheter, a bolus of 10 ml 0,9% NaCl was applicated; presented us with so-called "hand-made ultrasonic contrast" in the right atrium and we confirmed the right position of a central venous catheter.

Once the position of a catheter was determined, the affixation followed, without the need of radiologic control.

Discussion

The central venous catheterization in our patient was imperative because it permitted the intra- and postoperative monitoring of CVP, as well as the parenteral nutrition with highly caloric parenteral infusions and high-osmolality drugs. Blood samples for laboratory analysis could be taken from CV catheter as well.

Although brachial veins were in consideration for catheterization, due to the expected long-term need for the CV catheterization as well as potential thrombosis of the brachial or femoral vein, we decided for a standard subclavial route. In this very high-risk patient for "blind" (anatomically guided) cannulation we performed the CV cannulation by using the ultrasound guidance. With the ultrasound-guided technique an anesthesiologist (operator) can be oriented in anatomic relationships immediately before the cannulation.[1-4] He is able to monitor the position of the needle, guide-wire and catheter in the central vein during the procedure as well. By the described technique, the whole catheter, its loops or eventual knots, as well as the tip, can be visualized and there is no need for the postoperative confirmation by radiography. Besides, a real-time ultrasound guidance technique, which is cheap, quick and easy to perform, improves the success rate, reduces the number of passes and gives us the possibility of the early detection of some later complications in the CV cannulation (e.g. arterio-venous fistulas or pseudo-aneurysms).[2,5]

This report highlights the potential benefits of ultrasound as a supporting method for the CV catheterization in oncologycal patients. Summing the personal experience and the data from relevant literature, we can recommend sonography as an important adjunct method in central venous cannulation in difficult cases.[1-4]

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Case report

Bifocal primary intracranial germinoma in a child. Case report

Aleš Koren

Clinical Institute of Radiology, Clinical Center Ljubljana, Slovenia

Background. *Bifocal primary intracranial germinal tumors are rare. Only 5-10% of all germ cell tumors are found both in the suprasellar and pineal region.*

Case report. *In presented patient we found two primary intracranial germinomas in pituitary and pineal gland that were successfully operated. Radiological properties of germinomas and differential diagnosis are discussed.*

Conclusions. *Although the definite histological diagnosis cannot be achieved by computer tomography and/or magnetic resonance images alone, a detailed analysis of neuroradiological images is useful for predicting the histological diagnosis.*

Key words: computed tomography; magnetic resonance; germinoma, intracranial

Introduction

Germ cell tumors located in the central nervous system (CNS) represent less than 4% of the intracranial tumors and affect primarily children and young adults. These tumors frequently arise in the suprasellar and pineal region and in the midline structures around the third ventricle. Germ cell tumors can be divided as germinoma and nongerminomatous tumors regarding the histology. Only 5-10% of all germ cell tumors are found both in the suprasellar and pineal region[1] mostly as germinomas.

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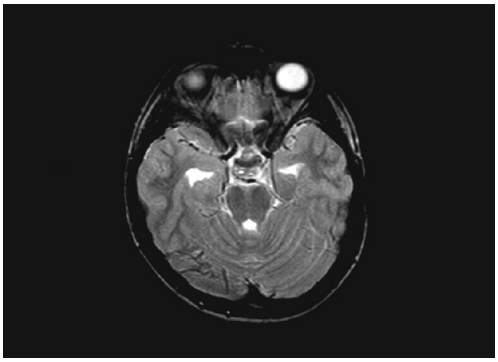
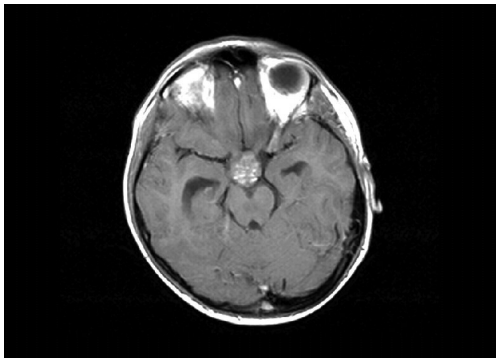
Correspondence to: Aleš Koren, M.D., Clinical Institute of Radiology, Clinical Center Ljubljana, Zaloška 7, 1000 Ljubljana, Slovenia; Phone +386 1 543 15 30; Fax: +386 1 433 1044.

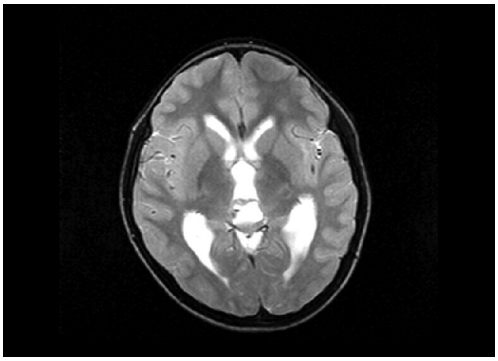
We present a case of isolated primary bifocal germinoma of CNS in a child.

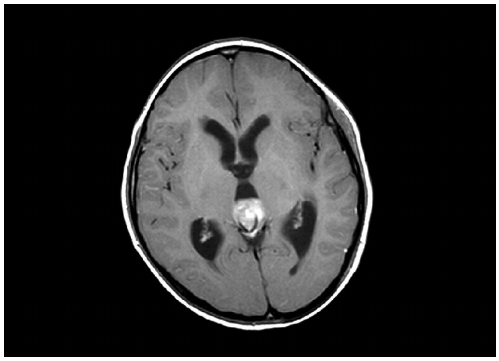
Case report

An eleven-year-old boy with nocturnal disuria was admitted to our hospital because of vomitting and frontal and retro orbital headache, which was depended on the position of the head. There were no vertigo or visual disturbances. We found signs of premature puberty, bilateral papillary edema and raised serum concentrations of PRL. The computed tomographic (CT) scan showed homogenous, well-delineated suprasellar tumor with obstructive hydrocephalus. An additional pineal region tumor was found on magnetic resonance images (MRI) of the brain. Both tumors had similar neuroradiological characteristics (Figures 1-4). We didn't









Figures 1,2. MRI (SE T2WI) in axial plane. Well-delineated oval lesions in sella turcica and pineal gland. Peripheral solid part is isointense, central cystic part is hyper intense. Hydrocephalus.

Figures 3,4. MRI (SE T1WI) in axial plane. Solid part of tumors is isointense with cortex.

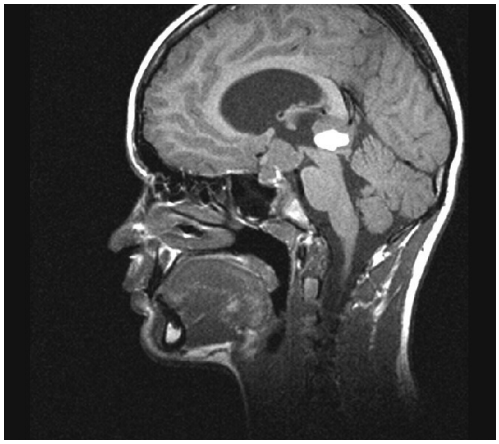
find similar lesions in testicles or elsewhere in the body.

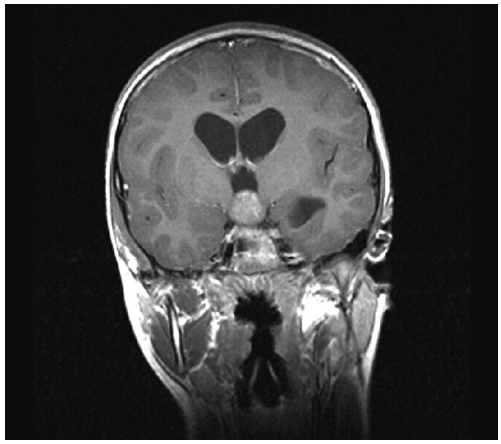
The patient had two operations. Surgeons resected first pituitary lesion. The macroscopically gray tumor had a peripheral solid vascular part and a central soft avascular one. The tumor compressed both optical nerves and the inferior part of optical chiasm and grew into infundibulum and through sellar diaphragm into sella turcica (Figure 5). Hypophysis was completely destroyed. The pineal tumor was resected by the second operation (Figure 6). This tumor grew from the third ventricular wall posteriorly in the pineal region to both basal veins and was macroscopically similar to the previously reseceted suprasellar tumor (Figures 7,8). The histological examination of both specimens showed mature germinoma with the positive reaction to alkaline phosphatase. All other tumor markers were negative.

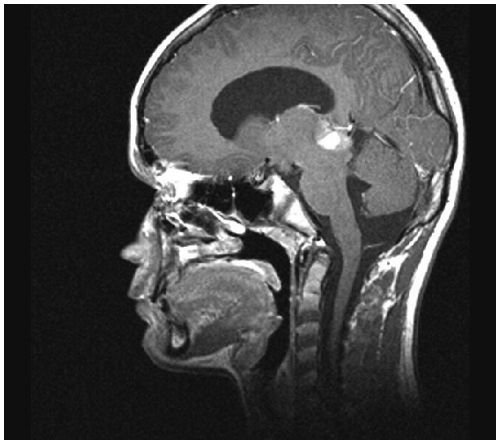
The patient had upper gaze failure and diabetes insipidus postoperatively. Cerebrospinal liquor for malignant cells was negative. No tumor was found on postoperative MRI (Figures 9,10). The additional radiotherapy of the third ventricular region with 4000 cGy was performed. The boy was treated with a substitute hormonal therapy and antiepileptic prophylaxis. The child is in complete remission 15 months after the diagnosis.

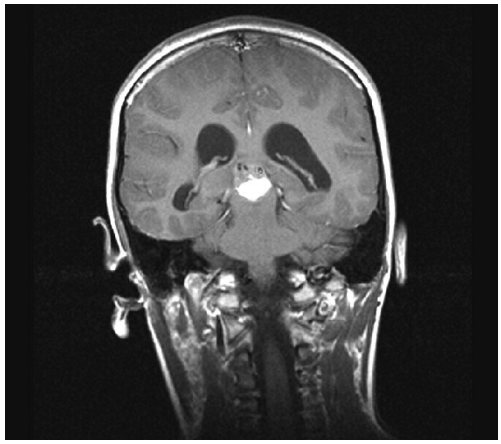
Discussion

Intracranial germ cell tumors are a heterogeneous group of lesions that occur in children and young adults. Within the classification of









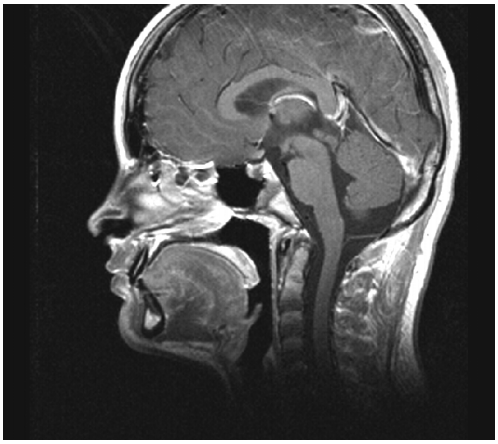
Figures 5,6. MRI (SE T1WI) in sagital plane before and after administration of gadolinium contrast media (Gd CM). Solid part of tumors enhance homogeneously after administration of Gd CM. Note compression of surrounding structures. No communication between lesions is seen.

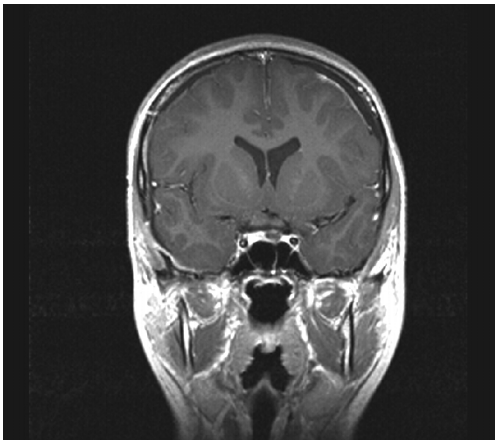
Figures 7,8. MRI (SE T1WI) in coronal plane after administration of Gd CM. Midline position of tumors with impression of third ventricular floor.

intracranial germ cell tumors, there are a variety of different tumor types which carry different prognoses. The most recent World Health Organization classification of germ cell tumors is as noted in Table 1. The most frequent histological type is germinoma (65%) and it holds the best prognosis[2] with over 90% 5 year survival rate.[3-5] They are located usually in the pineal or the suprasellar region.[6] Lesions in infundibulum alone are also described.[7] Rarely they appear in thalamus and basal ganglia or other intracranial loca-

Table 1. WHO classification of intracranial germ cell tumors

5.0	Germ cell tumors
5.1	Germinomas
5.2	Embryonal carcinoma
5.3	Yolk sac tumor
5.4	Choriocarcinoma
5.5	Teratoma
5.5.1	Immature
5.5.2	Mature
5.5.3	Teratoma with malignant transformation
5.6	Mixed germ cell





Figures 9,10. Ppostoperative control MRI (SE T1WI) in sagital and coronal plane after administration of Gd CM. There is no residual tumour.

tions. Pineal germinomas have strong male predominance in contrast to suprasellar germinomas that are more frequent in females. Bifocal lesions in these regions are found in 5-10%.[1] It is unclear whether they represent actual spread of the tumor or the simultaneous development of tumor in two sites.

Clinical presentations of germinoma are dependent on the localization of lesions. Tumors in pineal area can present with hydrocephalus, visual symptoms, obtundation, pyramidal tract signs and ataxia. Suprasellar tumors often produce diabetes insipidus and a pituitary hormonal dysfunction.

Germinomas are macroscopically solid, quite homogenous tumors with a possible soft or partly cystic central part. They can seed by cerebrospinal liquor to other part of brain or meningeal surface.[8,9] Germinomas are composed of more than one cellular type in 10%,[10] their unspecific histological tumor marker is placental alkaline phosphatase.[11]

The neuroimaging characteristics of germinomas and nongerminomatous germ cell tumors are similar enough to limit diagnostic certainty, and either tissue confirmation or measurement of specific tumor markers are needed for the diagnosis. In addition, germ cell tumors in the pineal region cannot be definitively separated on the basis of neuroimaging characteristics from other tumors such as pineoblastomas, pineocytomas or gliomas. In the suprasellar region germinomas may be difficult to separate from other lesions which infiltrate the surrounding brain mimicking gliomas and histiocytomas.

Germinomas are radiologicaly well delineated, oval or lobular and expansive or partly infiltrative tumors.[6] The proportion of water to tumor cells determines their radiological morphology. The tumor can be isodense to hyperdense on CT and isointense to hyperintense on T1WI and T2WI on MRI. The solid part of the tumor shows isointense signal to cortex and intense opacification after the application of gadolinium contrast media Gd CM[3] (Figures 1-8). Germinomas are quite homogenous and uncapsular with no calcinations and other inclusions or important cystic and/or hemorrhagic areas.[12]

The treatment for germ cell tumors has become somewhat divergent as recommendations for the treatment differ between pure germinomas and other forms of germ cell tumors.[13] The craniospinal radiation of germinomas together with local doses of 4000 cGy showed excellent results.

Although a definite histological diagnosis cannot be achieved by CT and/or MRI alone, the detailed analysis of neuroradiological im-

ages is useful for predicting the histological diagnosis.

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Artifacts and non-osseous uptake in bone scintigraphy. Imaging reports of 20 cases

Guido M. Weiner, Lars Jenicke, Vika Müller, Karl H. Bohuslavizki

Department of Nuclear Medicine, University Hospital Eppendorf, Hamburg, Germany

Background. *Numerous possible artifacts may render the evaluation of bone scans difficult.*
Case reports. *This article provides a pictorial survey of both typical and extraordinary pitfalls in bone scintigraphy, which are caused by increased or reduced tracer accumulation of soft tissue or bone.*
Conclusions. *One should be aware, that in individual patients, "artifacts" in bone scintigraphy lead to formerly unknown diagnoses, and the diagnostic and therapeutic procedure may be influenced decisively.*

Key words: radionuclide imaging; bone; ^{99m}Tc-diphosphonates; artifacts; benign bone diseases; malignant bone diseases

Introduction

Bone scintigraphy is commonly used for the diagnosis and staging of both benign and malignant bone diseases.[1] After the intravenous injection and the initial distribution through the whole body by simple perfusion, the radiotracers used commonly, i.e. ^{99m}Tc-labelled diphosphonates such as HDP or MDP in a standard dose of 600-800 MBq, diffuse into the extracellular space. The initial local distribution is predominately influenced both by the blood flow and the vascularization of the perfused region. Thus, early acquisitions reflect perfusion and blood pool images. Due to its affinity to calcium, diphosphonates are then bound in a simple physicochemical way to bone structures within some 3 hours. Then, the regional skeletal uptake is determined both by the initial perfusion and the metabolism of the bone.[2] Bone scans taken after a three to five hours interval after injection usually provide a good contrast between soft tissue and skeletal structures, since the background activity of the soft tissue is as less as 2-10%.[3]

In order to ensure proper scan reading a thorough indication and a detailed medical history are mandatory prior to the injection. All relevant past illnesses and therapies must be included (drainages, biopsies, surgery). An inspection and an examination at least of the region under investigation should be performed routinely. In most cases these simple

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Correspondence to: Karl H. Bohuslavizki, M.D., Ph.D., Department of Nuclear Medicine, University Hospital Eppendorf, Martinistr. 52, D-20246 Hamburg, Germany. Phone: +49 40 42803-4047; Fax: +49 40 42803-9330; E-mail: bohu@uke.uni-hamburg.de

things help to avoid misinterpretations. If there still remains doubt on the interpretation, additional spot images or SPECT-imaging may be required for clarification.

Apart from physiological, age-related or typical pathological patterns, numerous causes of artificial tracer distribution patterns are known, which have to be taken into account when reading bone scans.[4] The main causes for artifacts are contaminations of the skin or of the clothes, paravenous or subcutaneous injection sites.[5] However, causes for artifacts in bone scans are numerous, and case reports have been published dealing with hyperhidrosis,[6] foreign body[7] and constitution,[8] etc. McAffee and Silberstein[9] published a survey of various causes of non-osseous uptake.

Case reports

The following bone scans are intended to give an overview of numerous artifacts, which have been collected over the last few years in a large university-based nuclear medicine department. Figures 1-7 show artifacts, e.g. technical causes, reduced tracer uptake etc., Figures 8 and 9 display malignant soft tissue tumors, Figure 10 shows inflammation, Figures 11-17 display non-inflammatory artifacts, e.g. lymphatic edema, adipositas, trauma, anatomical variants etc, and Figures 18-20 depict artifacts associated with the renal system. Unless otherwise noted, all images were acquired 3 hours after injection using a standard dual-headed whole-body gamma camera (Bodyscan, SIEMENS, Erlangen, Germany) equipped with low-energy high-resolution collimators.

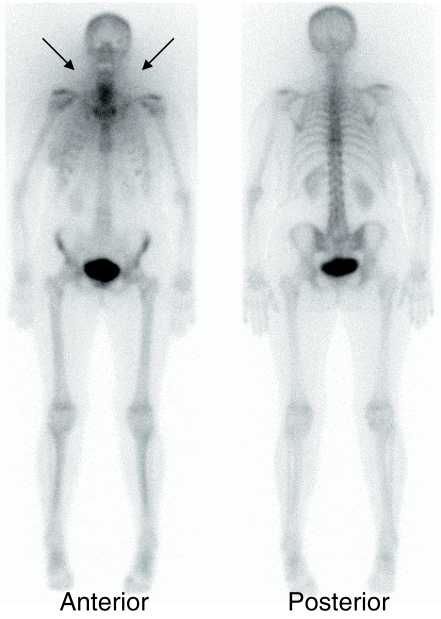

Figure 1. Diffuse radiation surrounding the neck resulting from a septa penetration of the high energy radiation (364 keV) of ^{131}I three weeks after radioiodine treatment.

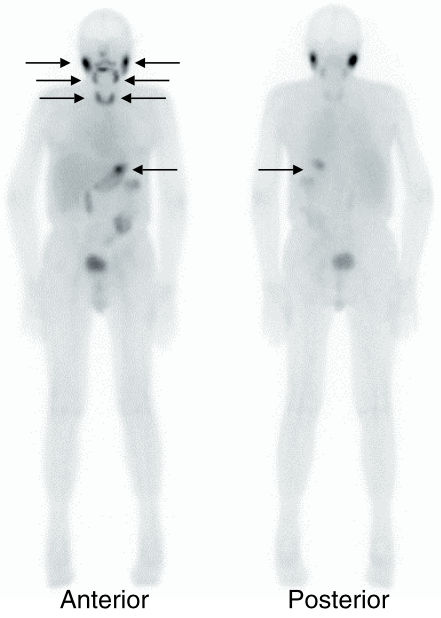

Figure 2. Uptake of free ^{99m}Tc-pertechnetate within the salivary glands, thyroid gland, gastrointestinal tract, and the urinary tract caused typically by absent radiolabelling of the diphosphonate complex of HDP.

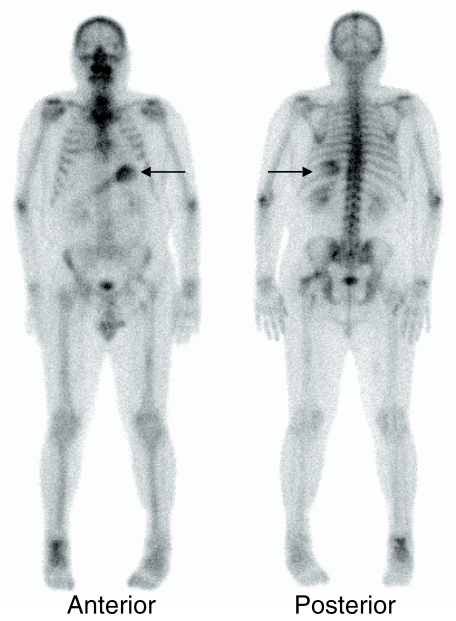

Figure 3. Bone scintigraphy following 3 hours after salivary glands scintigraphy. Note, uptake of free ^{99m}Tc-pertechnetate in the stomach.

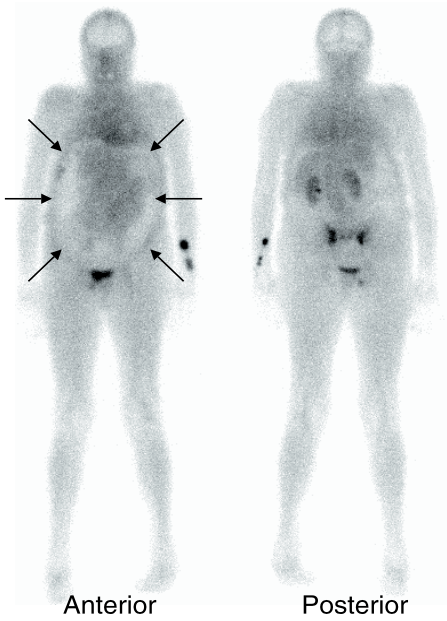

Figure 4. Blood pool images acquired 5 min after i.v. injection of ^{99m}Tc-HDP. Note a ringlike reduced tracer uptake in the anterior view in a patient with ascites.

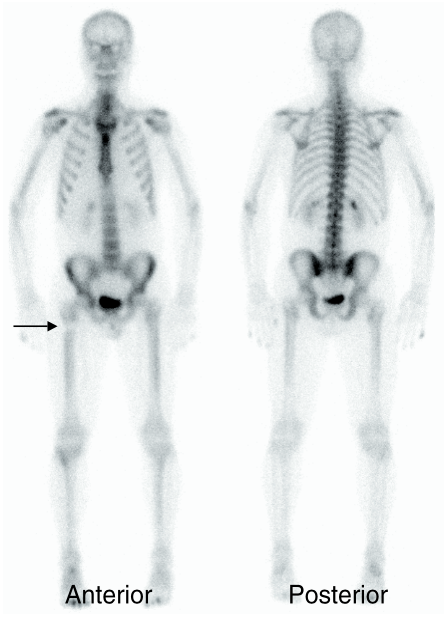

Figure 5. Cold spot in projection to the right proximal femur in the anterior view as a result of attenuation due to a purse in the trouser pocket.

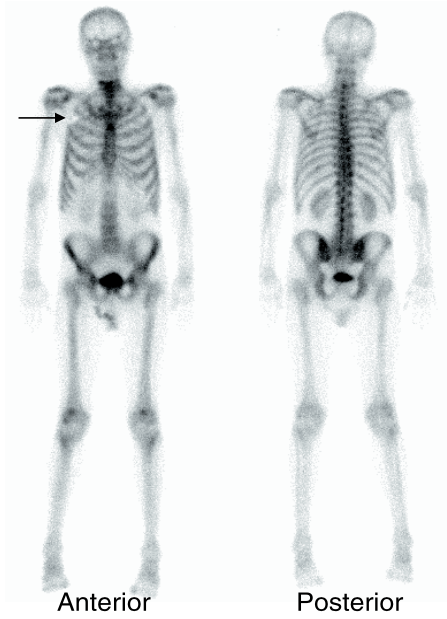

Figure 6. Cold spot on the right upper thoracic wall as a result of attenuation due to an implantated pacemaker.

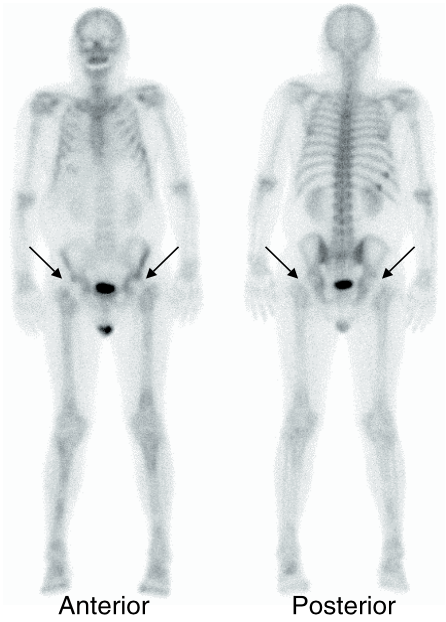

Figure 7. Symmetric cold spots on both femoral heads in a patient with bilateral hip prosthesis.

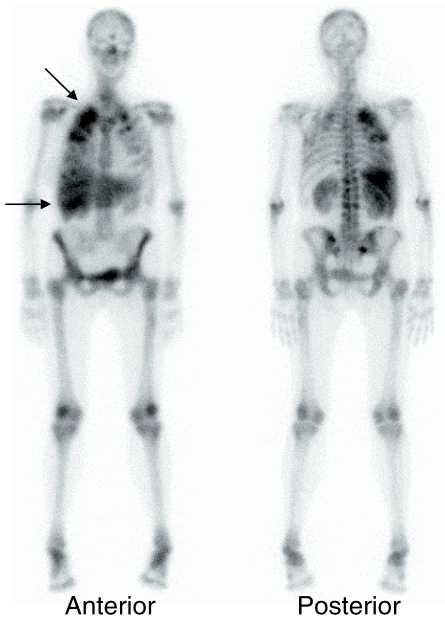

Figure 8. Massive increased tracer uptake in the soft tissue of the lung and the liver caused by calcifying metastasis in a patient with breast cancer.

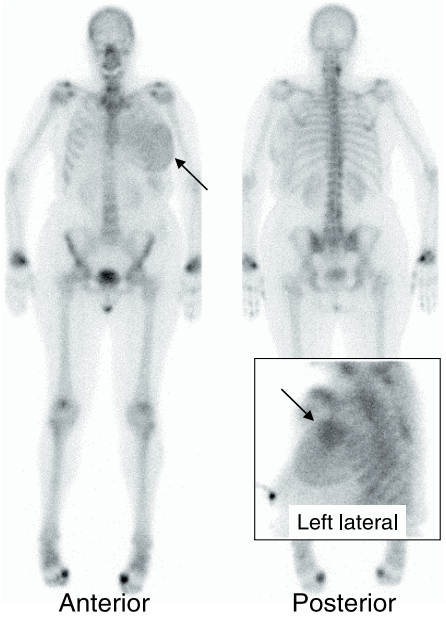

Figure 9. Massive soft tissue accumulation in the left breast in a patient with an inflammatory breast cancer detected by bone scanning.

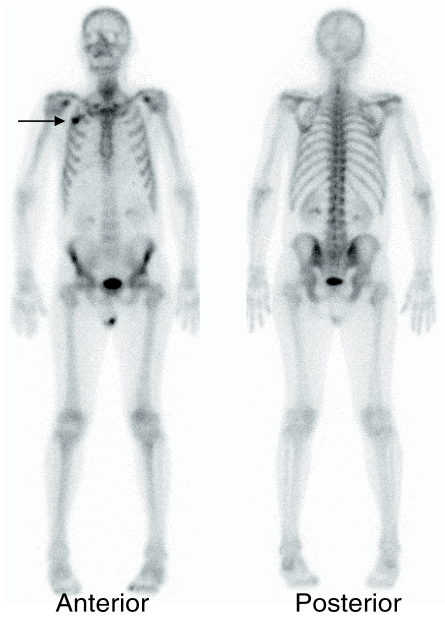

Figure 10. Focal tracer accumulation in the right upper thoracic wall caused by an infected tip of a Buelau-drainage.

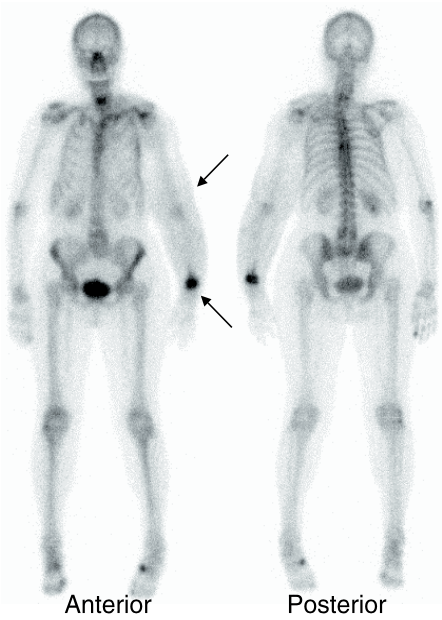

Figure 11. Increased tracer uptake in the complete left arm caused by lymphatic edema following axillary lymph node dissection. Note, paravenous injection of the ^{99m}Tc-HDP at the left wrist.

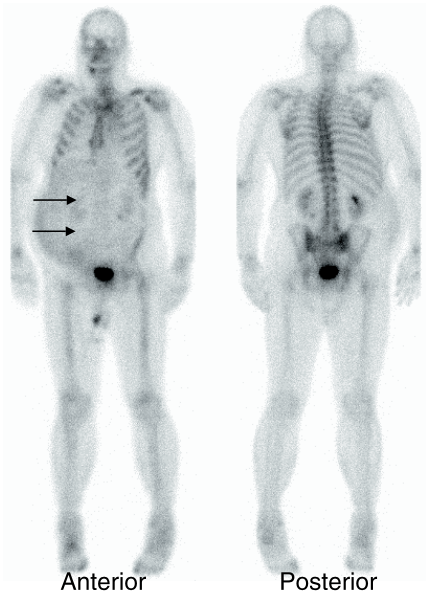

Figure 12. Attenuation of photons caused by massive abdominal fat in a male subject, which renders the interpretation of the lumbar spine difficult in the anterior view.

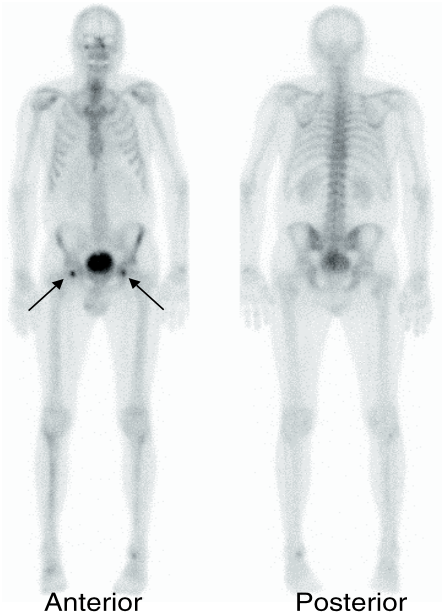

Figure 13. Bilateral focal hot spots at the head of the femora caused by inguinal punction using Seldinger technique during coronary angiography. Note, that both foci are seen in the anterior view only.

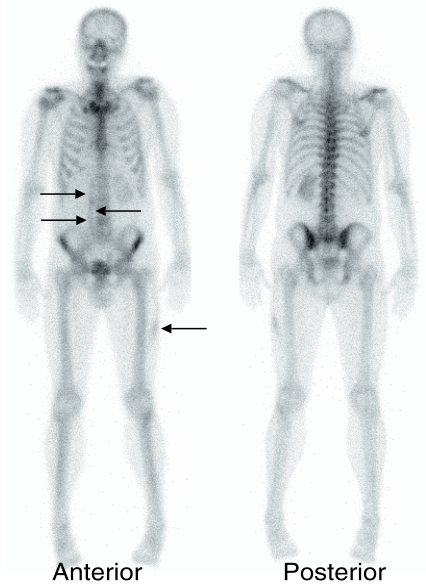

Figure 14. Longitudinal tracer uptake in the right abdomen caused by a calcifying scar following nephrektomy. Note, this tracer uptake is seen in the anterior view only. In addition soft tissue accumulation of ^{99m}Tc-HDP can be seen in the left lateral thigh, which is caused by repeated subcutaneous injection of heparine.

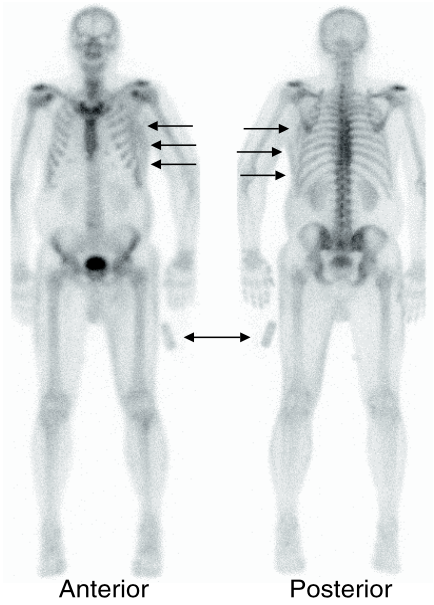

Figure 15. Soft tissue accumulation in the left axilla caused by an organizing hematoma following lymphonodectomy in a patient with malign melanoma of the left arm. Note a contaminated handkerchief in the left pocket of the trousers.

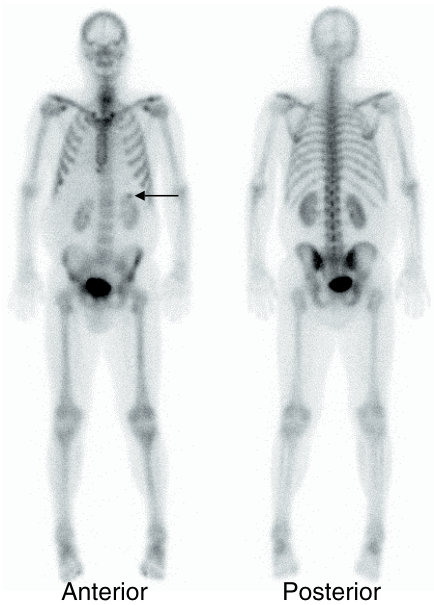

Figure 17. Focal soft tissue accumulation in the left upper abdomen caused by the insertion site of a percutaneous gastric tube in a patient suffering from esophageal cancer.

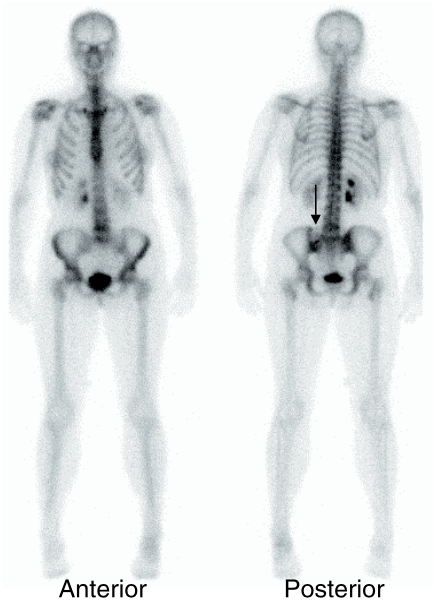

Figure 16. Cold lesion in the left sacro-iliacal joint caused by removal of bone graft.

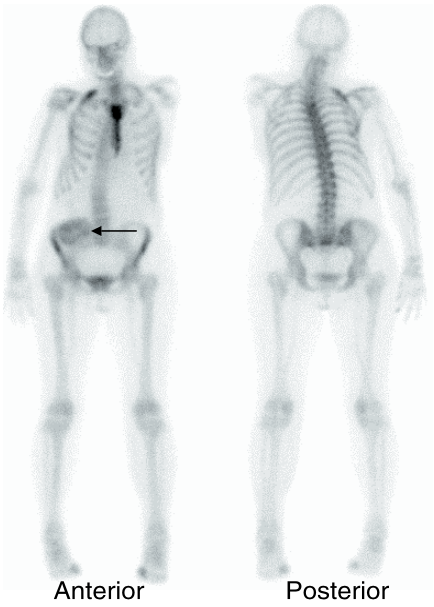

Figure 18. Absent tracer accumulation in both kidneys in *loco typico* and tracer accumulation in a kidney grafted into the right pelvis following bilateral nephrectomy due to renal cancer. Note, tracer uptake in the upper right ribs and in the sternum caused by thoracotomy due to aortocoronary bypass-grafting. Also, note the loss of the left arm in an accident 40 years ago.

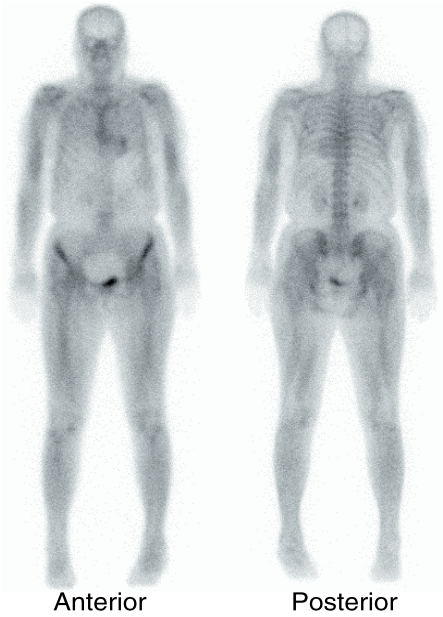

Figure 19. Reduced bone uptake and high background activity due to renal failure.

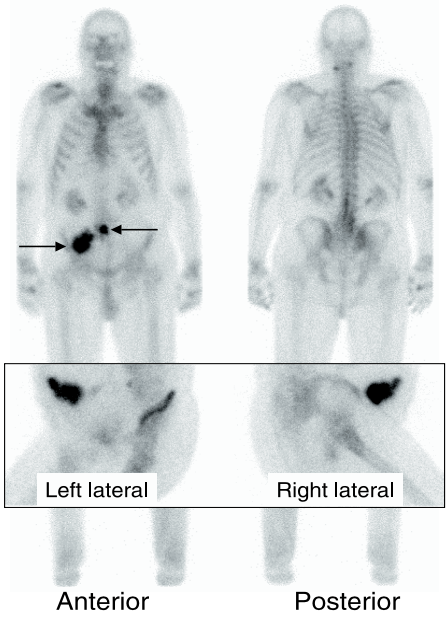

Figure 20. Urinary accumulation in the right lower abdomen due to an appendix-pouch constructed after bladder excision in a patient suffering from prostate and urothelium cancer.

Conclusions

One should be aware, that in individual patients, "artifacts" in bone scintigraphy lead to formerly unknown diagnoses, and the diagnostic and therapeutic procedure may be influenced decisively.

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Cell electropermeabilization to small molecules *in vitro*: control by pulse parameters

Alenka Maček Lebar and Damijan Miklavčič

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

A systematic study concerning the role of the different electric field parameters (pulse number, duration and amplitude) on electropermeabilization of DC3F cells to small molecules (propidium iodide) and on cell viability is presented. Cell permeabilization and viability dependence on the pulse amplitude was determined by twenty different sets of electrical parameters. The number of pulses varied between 1 and 64 and pulse duration between 20 μs and 1 ms. The most important parameter was the pulse amplitude because it triggered the electropermeabilization process and the process of cell death. Either in the case of electropermeabilization as well as in the case of cell viability experiments, the parameter U_{50} (the pulse amplitude leading to permeabilization or to the death of 50% of cell population) was not changed if the set of electrical parameters consisted of more than 16 pulses. This was independent of the pulse duration. The efficiency of permeabilization was enhanced by using of longer pulses. Such a systematic study of the influence of different electric field parameters on electropermeabilization and cell viability may serve as a base for optimization of the electropermeabilization conditions for different applications.

Key words: electroporation; electromagnetic fields; cell survival; propidium iodide

Introduction

The phenomenon of cell membrane electropermeabilization can be described as a dramatic increase in the transmembrane permeability induced by an externally applied electric field. This transient state has important practical applications like the fusion of cells [1] and the introduction of the biologically active substances like drugs [2] and genetic material [3] into cells. Electropermeabilization is nowadays widely used to manipulate biological cells, organelles, cell aggregates and tissue. The clinical applications gain increasing importance, particularly in oncology.[4,5] Electropermeabilization can be achieved with different sets of pulse parameters, *i.e.* the strength of applied field (voltage of applied pulses), the number and the shape of pulses, their duration and repetition frequency. An identical set of electrical parameters is not necessarily efficient for all applications. While eight

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Correspondence to: Damijan Miklavčič, Faculty of Electrical Engineering, University of Ljubljana, Tržaška 25, 1000 Ljubljana, Slovenia. Phone: +386 1 4768 456; Fax: +386 1 4264 658; E-mail: damijan@svarun.fe.uni-lj.si

square-wave electric pulses of 100 μs delivered at 1 Hz is the most common used set of electrical parameters in electrochemotherapy, the pulses must be much longer (eight square-wave electric pulses of 20 ms delivered at 1 Hz or 2 Hz) for DNA electrotransfer *in vivo*.[5] Likewise, it has been shown in vitro that only pulse duration equal to or longer than 1 ms was associated with the detection of macromolecules in pulsed and viable cells, while the permeabilization of cells to small molecules was already detected for the microsecond range.[6] Therefore it is important to know how the pulse parameters affect the electropermeabilization process.

The role of pulse parameters in obtaining a higher efficiency of electropermeabilization *in vitro* was investigated in a number of studies.[6-9] The permeabilization as a function of the parameters of applied electric field was quantified in two ways. Either the fraction of electropermeabilized cells in suspension was measured using fluorescence optical microscopy [10], or the permeability of each cell was integrated over the whole cell population by measuring radioactive incorporation [11] or ATP leakage.[6] Both types of information were gathered in some studies using flow cytometry.[12] Independently of the method, it was shown that the electric field intensity is the crucial parameter for inducing membrane permeabilization. The permeabilization occurs only if the electric field intensity is higher than a certain threshold value. This threshold value is a function of the pulse number and the pulse duration. It decreases by increasing either the pulse duration or the number of pulses until it reaches a "real" threshold value below which no permeabilization occurs even if using longer pulses or higher number of them. The "real" threshold value was obtained using 10 pulses or more with the duration longer than 100 μs.[13] The permeability threshold depends on the molecular size of the probe used for its measurement: the larger the test molecule, the higher the apparent threshold.[14] It also depends on the cell line because of their differences in size [15] and membrane properties.[16,17]

In almost all studies, the effect of pulse duration was studied at a given field intensity and pulse number. Similarly, the effect of pulse number was studied at a given field intensity and pulse duration, and vice-versa the effect of field intensity was studied at a given pulse duration and pulse number.[6-8] However, the fraction of electropermeabilized cells as a function of pulse duration at a given number of pulses is strongly dependent on selected field intensity (Fig. 1). Namely, a plateau is reached at shorter pulses if higher field intensity is used.

In this paper, we present a systematic study concerning the role of different electric field parameters (field intensity, pulse number, and duration) on electropermeabilization of DC3F cells to small molecules and on cell viability. DC3F cells have been chosen because considerable information about the electropermeabilization of that strain is available.[2,18,19] The fraction of electropermeabilized cells was quantified by the penetration of propidium iodide and the viability of the cells by their cloning efficiency. The cells were pulsed with twenty different sets of electrical parameters (Table 1). The number of pulses varied between 1 and 64, the pulse duration between 20 μs and 1 ms and the pulse amplitude from 40 to 600 V.

Materials and methods

Chemicals

Eagle's minimal essential medium (EMEM), trypsin and propidium iodide (PI) were purchased from Sigma Chemical Co. (St. Louis, MO). Fetal calf serum (FCS) and L-glutamine were obtained from Gibco BRL (Galthersburg, MD), penicillin, streptomycin, gentamicin from Lek (Ljubljana, Slovenia), and Crystal violet from Kemika (Zagreb, Croatia).

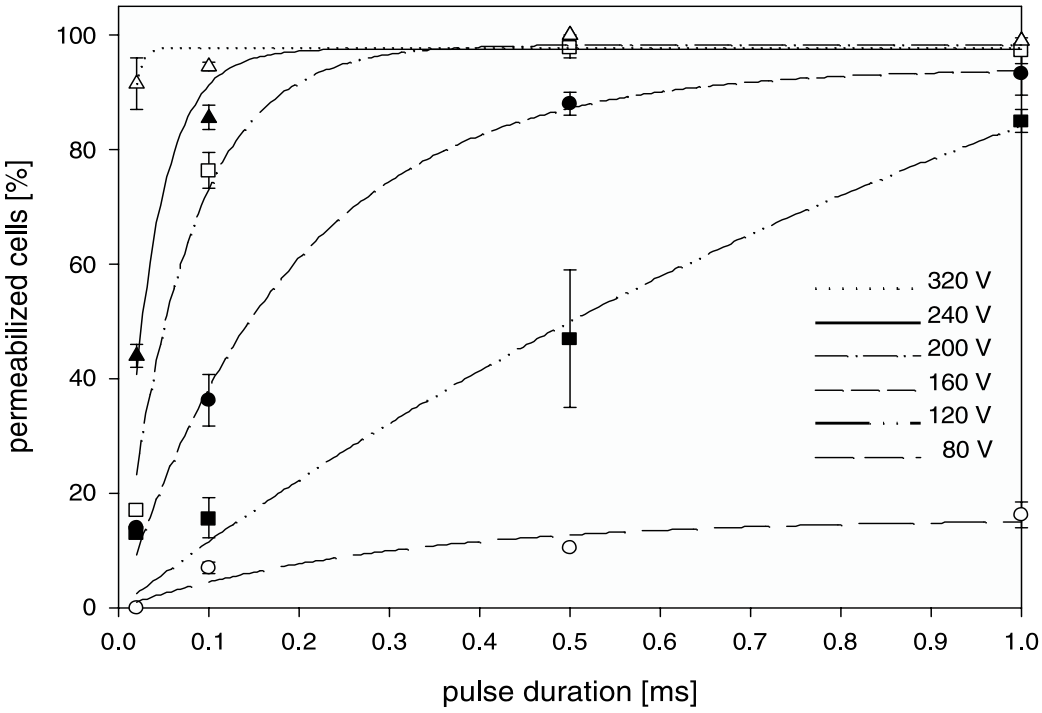

Figure 1. Fraction of electropermeabilized cells as a function of pulse duration. Cells were pulsed eight times at different pulse amplitudes. The error bars in the fraction of electropermeabilized cells represent standard deviations of the data.

PI was dissolved in sterile H_2O at a concentration of 100 μM.

Cell culture

DC3F cells, a line of spontaneously transformed Chinese hamster lung fibroblasts, were grown in monolayers in the culture medium consisting of EMEM supplemented with 10% heat-inactivated FCS, 10mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 11 μg/ml gentamicin. The cells were incubated at 37°C in a humidified atmosphere with 5% CO_2, and were routinely subcultured every 4 days.

Cell exposure to electric field

Cells from the exponential growth phase were trypsinized and centrifuged for 5 min at 4°C and 1500 rpm in the culture medium. They were then resuspended in the serum-free medium supplemented with 0.5 mM $CaCl_2$ at a concentration of 2.2 x 10^7 cells/ml. 90 μl cell suspension was mixed with 10 μl PI for the determination of electropermeabilization, or with medium supplemented with 0.5 mM $CaCl_2$ for determination of electropulsed cell viability. A 50 μl droplet of the cell suspension was placed between two flat, parallel, stainless steel electrodes (length = 6 mm, width = 6mm, interelectrode distance = 2 mm). The electrodes were connected to a voltage generator (Jouan GHT 1287 B, France) generating monophasic square-wave electric pulses with independently adjustable electric parameters (voltage, number of pulses and duration). The cells were pulsed at 1 Hz frequency. The pulse parameters were monitored by an oscilloscope (Hameg HM 205-3,

Table 1. Sets of electrical parameters. Repeated pulses were delivered at 1 Hz frequency

Set of electric field parameters	Number of pulses	Pulse duration [μs]
1.20	1	20
1.100	1	100
1.500	1	500
1.1000	1	1000
4.20	4	20
4.100	4	100
4.500	4	500
4.1000	4	1000
8.20	8	20
8.100	8	100
8.500	8	500
8.1000	8	1000
16.20	16	20
16.100	16	100
16.500	16	500
16.1000	16	1000
64.20	64	20
64.100	64	100
64.500	64	500
64.1000	64	1000

Germany). All experiments were performed under sterile conditions in a laminar flow hood at room temperature.

Determination of electropermeabilization

Electropermeabilization of cells was quantified by the penetration of impermeant dye PI. When the membrane is permeable, PI binds to nucleic acids and becomes highly fluorescent. Therefore, it is not necessary to wash the cells to eliminate nonincorporated PI as in case of other fluorescent dyes. A selected evaluation method avoids the negative consequences of pipetting and centrifuging the cells that have been already pulsed.[20] The cells were pulsed and incubated 5 min at room temperature. Thereafter, 25 μl of cell suspension was resuspended in 1 ml of 0.01 M phosphate-buffered saline (PBS, pH 7.4) and kept at 4°C till being analyzed by flow cytometry (FACSort, Becton Dickinson, CA). The flow cytometer was used to measure the number of fluorescent and therefore permeabilized cells. Excitation was set at the wavelength 488 nm and emission was detected at 640 nm. Fluorescence was recorded for 5000 particles. Only particles large enough to qualify as cells were taken into consideration. The number of stained cells was determined and normalized to the number of all cells to get the percentage of permeabilized cells.

Determination of electropulsed cell viability

Cell viability was determined by means of colony-forming assay. After the exposure to electric pulses, the cells were incubated for 5 min at room temperature. They were then diluted in the culture medium and seeded in triplicate (300 cells per 60 mm diameter petri dish). After five days, the colonies were fixed with 96% ethanol, stained with Crystal violet and counted. The survival of the cells treated with electric pulses was calculated as the percentage of the colonies obtained from the untreated control cells.

Statistical analysis

All experiments were repeated at least three times on different days. For each experimental point, mean and standard deviation were calculated. Using nonlinear regression, a two-parameter sigmoid curve was fitted to the data

$$f(U) = \frac{100}{1 + \exp\left[(U_{50} - U)/b\right]}$$

where U is the pulse amplitude, f is the percentage of permeabilized or alive cells, and U_{50} and b are the two parameters of the sigmoid curve. Parameter U_{50} is the pulse amplitude leading to permeabilization of 50% of cell population in the case of electropermeabilization and the pulse amplitude leading to the death of 50% of cell population in the case

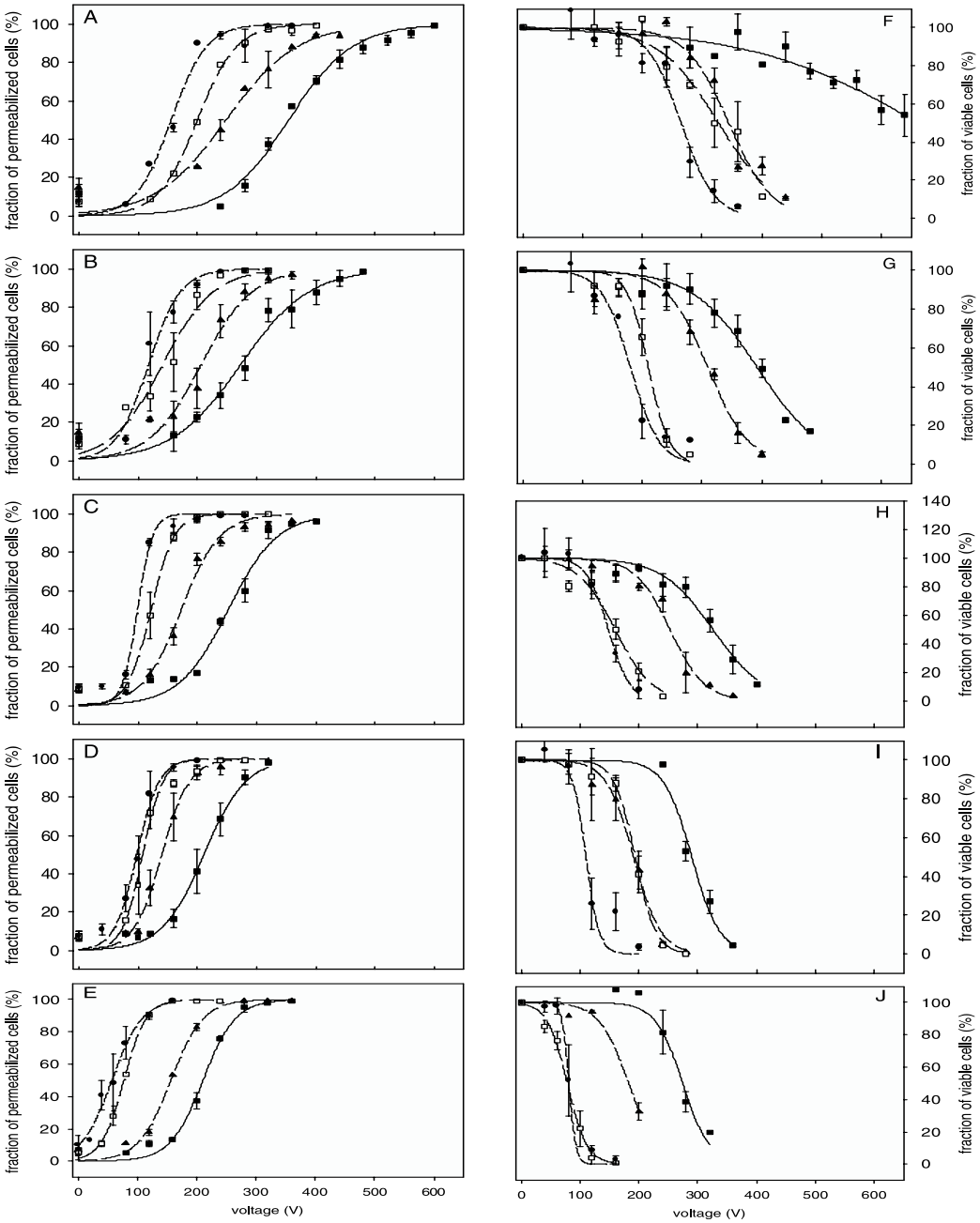

Figure 2. Effect of pulse amplitude on electropermeabilization of cells and cell viability. DC3F cells were pulsed at 1 Hz one time (A - electropermeabilization, F - cell viability), four times (B - electropermeabilization, G - cell viability), eight times (C - electropermeabilization, H - cell viability), sixteen times (D - electropermeabilization, I - cell viability) and sixtyfour times (E - electropermeabilization, J - cell viability). Pulse duration was equal to (■) 20 μs, (▲) 100 μs, (□) 500 μs and (●) 1000 μs. The symbols denote the means and the error bars are the standard deviations.

of cell viability experiments. Parameter b governs the inclination of the sigmoid curve. Smaller absolute value of parameter b means that the process reaches its plateau at smaller interval of pulse amplitudes, while its larger value means that the process reaches its plateau at larger interval of pulse amplitudes. In the literature, parameter b was paralleled with the efficiency of permeabilization.[13]

Results

In this study we focused on the effect of pulse amplitude, number of pulses and pulse duration on the cell permeabilization and viability. Repeated pulses were delivered at 1 Hz frequency. The cell permeabilization and viability dependence on the pulse amplitude was determined using twenty different sets of electrical parameters (Table 1). Figure 2 shows the results of these measurements. The symbols denote the means and the error bars are standard deviations. A two-parameter sigmoid curve is fitted to the data of each set of electrical parameters. For easier comparison of different sets of electrical parameters, the parameter U_{50} and b of all electropermeabilization curves and curves presenting cell viability are collected in Table 2 and presented in Figures 3 and 4.

Electropermeabilization

The fraction of electropermeabilized cells in population is under control of the field intensity, the pulse duration and the number of pulses. Electropermeabilization occurs only for pulse amplitudes higher than a certain threshold value. This value is lower if longer pulses are used, or if the number of pulses is higher. Permeabilization curves are completely shifted to the lower pulse amplitudes (Fig. 2A-E). If the set of electrical parameters consisting of 16 and 64 pulses, 0.5 ms electropermeabilization curve more or less coincides with 1 ms electropermeabilization curve (Fig. 2D, E). It means that, in the sets of electrical parameters that consist of 16 pulses or more, the usage of the pulses longer than 0.5 ms does not change the fraction of electropermeabilized cells in population at certain pulse amplitude.

Parameter U_{50} is not changed if the set of electrical parameters consists of more than 16 pulses (Fig. 3A). This is independent of the pulse duration. The relation between the pulse duration and the number of pulses at a given parameter U_{50} is linear on logarithmic scale, if less than 16 pulses are used.

In the literature, parameter b was paralleled with the efficiency of permeabiliza-

Table 2. Parameters U_{50} and b at different sets of electrical parameters. Parameters are determined by sigmoid curves which are fitted to the experimental data of electropermeabilization and cell viability using nonlinear regression.

Set of electric field parameters	Electropermeabilization		Cell viability	
	U_{50} [V]	$\lvert b \rvert$ [V]	U_{50} [V]	$\lvert b \rvert$ [V]
1.20	354	53	664	143
1.100	249	59	345	36
1.500	200	33	328	50
1.1000	156	29	265	28
4.20	273	58	392	54
4.100	206	43	309	34
4.500	141	43	210	18
4.1000	118	27	180	24
8.20	252	41	326	43
8.100	173	32	253	30
8.500	122	20	159	32
8.1000	99	13	148	18
16.20	211	35	290	24
16.100	140	23	191	23
16.500	108	17	193	17
16.1000	98	19	110	11
64.20	211	27	274	23
64.100	157	27	182	25
64.500	77	19	77	17
64.1000	57	25	80	6

tion.[13] Parameter b of the sigmoid curve is smaller, if the duration of the pulses is longer (Fig. 3B). Therefore, the efficiency of permeabilization is enhanced by the usage of longer pulses. The enhancement is less pronounced if the set of electrical parameters consists of higher number of pulses.

Cell viability

Also the fraction of viable cells in population is under control by the field intensity, the pulse duration and the number of pulses as well. Cell death occurs at the field intensities higher than a certain threshold value. This value is lower if the pulses are longer or their number is higher. In that case cell viability curves are completely shifted to the lower pulse amplitudes (Fig. 2 F-J).

Likewise, in the case of electropermeabilization, the relation between the pulse duration and the number of pulses at a given parameter U_{50} is linear on logarithmic scale if less than 16 pulses are used. Parameter U_{50} is not changed if the set of electrical parameters consists of more than 16 pulses (Fig. 4A). This is evident for the pulses shorter than 500 μs.

Absolute value of the parameter b of the sigmoid curve is smaller if the duration of the pulses is longer (Fig. 4B). The viability of cells is changed on smaller interval of pulse amplitudes if longer pulses are used.

Discussion

The application of electric field pulses to DC3F cells results in the permeabilization of their plasma membrane. Electrical parameters, *i.e.* pulse amplitude, pulse duration and the number of pulses, have an important role in electropermeabilization process as well as an effect on cell viability. The most important parameter is the pulse amplitude because it triggers the electropermeabilization process and the process of cell death. Both processes have their characteristic threshold values. Either pulse duration or the number of pulses can modulate these threshold values.

In our study, we quantified the fraction of

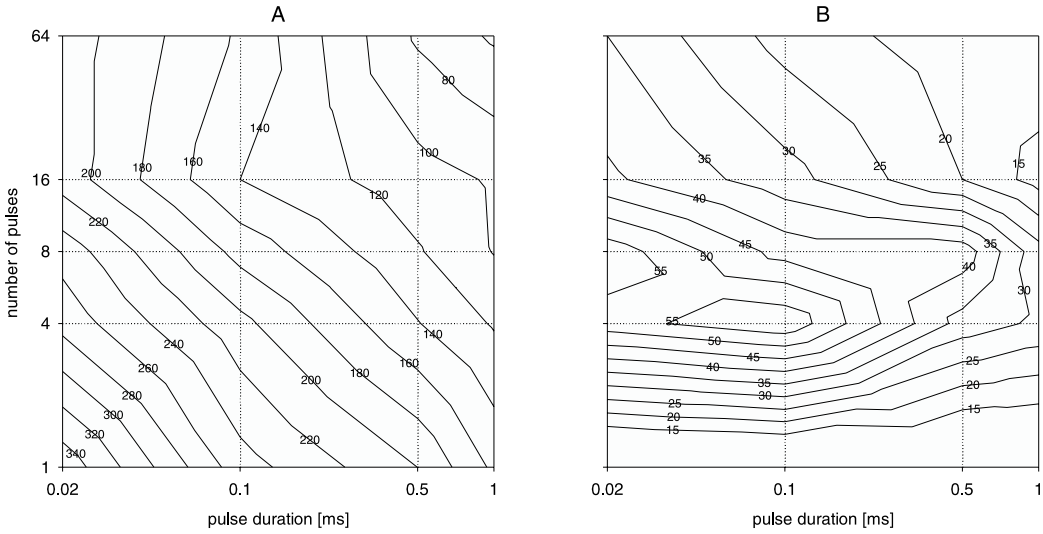

Figure 3. Values of parameter U_{50} and b in the field of pulse duration and number of pulses in the case of electropermeabilization experiments. Each curve represents one value of the parameter U_{50} (A) or b (B). The value is written on the curve. Scales are logarithmic.

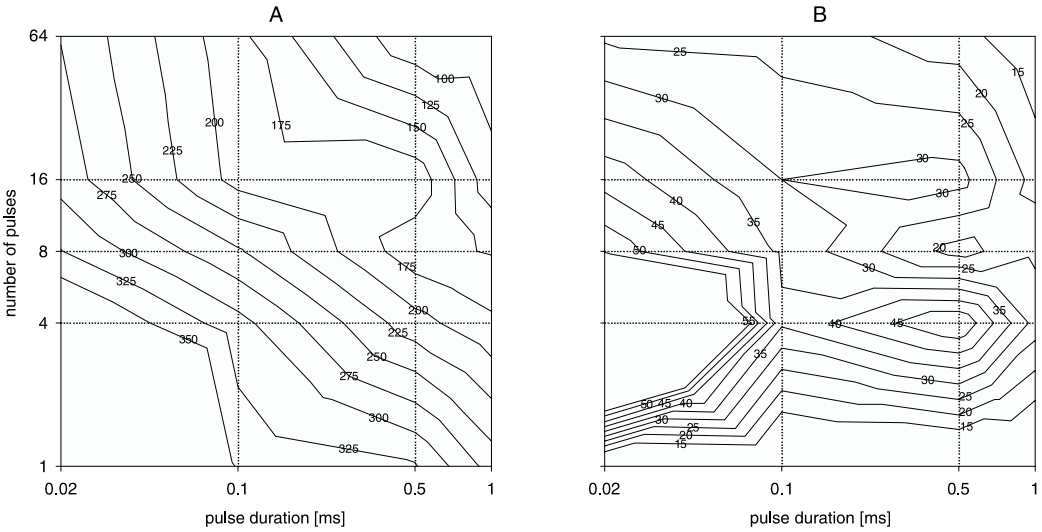

Figure 4. Values of parameter U_{50} and b in the field of pulse duration and number of pulses in the case of cell viability experiments. Each curve represents one value of the parameter U_{50} (A) or b (B). The value is written on the curve. Scales are logarithmic.

electropermeabilized cells by the penetration of PI. Because PI also leaks into healthy cells over time [21], we noticed some fluorescence cells also in the untreated control cells. Therefore, it was difficult to determine the pulse amplitude, which triggers electropermeabilization process, *i.e.* threshold value. That is why our conclusions were made by observing electropermeabilization curves, their parameters U_{50}, *i.e.* the pulse amplitude leading to permeabilization of 50% of cell population in the case of electropermeabilization and the pulse amplitude leading to the death of 50% of cell population in the case of cell viability experiments, and parameters b, *i.e.* parameter which governs the inclination of the sigmoid curve. Our conclusions can be summarized as follows: (1) In the sets of electrical parameters consisting of 16 or more pulses, the usage of pulses longer than 0.5 ms does not change the fraction of electropermeabilized cells in the population at a selected pulse amplitude. (2) Both in the electropermeabilization as well as in the cell viability experiments, parameter U_{50} is not changed if the set of electrical parameters consists of more than 16 pulses, which is independent of the pulse duration. (3) The efficiency of permeabilization is enhanced by the usage of longer pulses. (4) The fraction of electropermabilized cells and viability of cells vary at a smaller interval of pulse amplitudes if longer pulses are used.

In spite of a variety of studies investigating the role of pulse parameters in the electropermeabilization efficiency, the studies analyzing the control of the cell viability by pulse parameters are rare.[9,18] This is probably due to the effects of pulsing media which can contain a variety of undesirable and even toxic substances. In our study, these undesirable substances are Ca2+ ions. We performed electropermeabilization experiments in EMEM medium, which is a culture medium of DC3F cells. Because of the compatibility of results the pulsation of the cells in case of the cell viability, experiments were made in EMEM like in electropermeabilization experiments. Although EMEM Ca^{2+} concentration (1.8 mM) is in the range of approximate harmless limits of the extracellular fluids concentrations for short periods (0.5 - 2.0 mM) [22],

it can be toxic due to the impairment of Ca^{2+} cellular transports during cell injury. During and some time after the cells are exposed to electrical pulses, Ca^{2+} easily diffuses through the transiently permeable membrane due to very low cytosolic Ca^{2+} free concentration (~0.1 μM). An increase in cytosolic Ca^{2+} concentration can directly lead to cell lysis by causing disruption of the cytoskeleton, DNA fragmentation or extensive damage to other cell components.[23] Therefore, the cell viability is affected at lower pulse amplitudes as in the experiments prepared in media without Ca^{2+}. However, the effects of electrical parameters have the same trends as if performed in Ca^{2+} free medium (*i. e.* SMEM) (data not shown).

Our results were obtained using DC3F cell line and small test molecules. In one of our previous studies, we showed that electropermeabilization curves, obtained by PI for a given set of electrical parameters, are comparable with the electropermeabilization curves obtained by using anticancer drug bleomycin as a marker of cell permeabilization.[19] So, we can conclude that our observations are valid also for bleomycin, which is the drug of choice for electrochemotherapy.[4,5]

A systematic study of the influence of different electric field parameters (field intensity, pulse number, and duration) on electropermeabilization and cell viability may serve as a base for the optimisation of the electropermeabilization conditions for different applications.

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Electroporator for *in vitro* cell permeabilization

Marko Puc, Karel Flisar, Stanislav Reberšek and Damijan Miklavčič

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

The use of high voltage electric pulse technology, electroporation, in cell biology, biotechnology and medicine has attracted an enormous interest. Electroporation is a transient phenomenon that increases the permeability of cell plasma membrane. In the state of high permeability, the plasma membrane allows small and large molecules to be introduced into the cytoplasm, although the cell plasma membrane represents a considerable barrier for them in its normal state. The effectiveness of electroporation depends on many parameters that can be divided into the parameters of the electric field and the parameters that define the state of cells and their surrounding, i.e. temperature, osmotic pressure, etc. In this article, we present a prototype electroporator ***GT-1*** *for in vitro electropermeabilization that we have developed. Our electroporator offers a vast flexibility of parameters and can generate high and low voltage pulses, of which the latter ones are used for electrophoretic transfer of charged molecules through permeabilized cell plasma membrane.*

Key words: electroporation - instrumentation - methods; cell membrane permeabilization

Introduction

Viability of a cell depends on the integrity of its plasma membrane. The plasma membrane prevents the exchange of substances between intracellular and extracellular spaces. Exposing the cell to the electric field can increase the permeability of the cell plasma membrane. The increased permeability of the plasma membrane allows small and large molecules to be introduced into the cytoplasm. This phenomenon is transient and is termed electropermeabilization and often also referred to as electroporation. The electroporation is widely used in different medical and biological applications *i.e.* electrochemotherapy, transdermal drug delivery and gene transfection. The effectiveness of all these applications depends on many parameters that can be divided into the parameters of the electric field and the parameters that define the state of cell and its surrounding, *i.e.* temperature, osmotic pressure, conductivity of cytoplasm and extracellular fluids etc. With respect to this, optimal parameters of electroporation have to be used to achieve best efficiency of the method.[1]

For *in vitro* experiments, where most commonly used electrodes are parallel plates with a 2 mm inner distance, the threshold voltages typically range from 120 V to 300 V[1,2], with

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Correspondence to: dr. Damijan Miklavčič, University of Ljubljana, Faculty of Electrical Engineering, Tržaška 25, SI-1000 Ljubljana, Slovenia. Phone: +386 1 4768456; Fax: +386 1 4264658; E-mail: damijan@svarun.fe.uni-lj.si

the pulse durations from several microseconds to several milliseconds.[1,3] Beside this, it is often necessary to deliver more then one pulse to increase efficiency of permeabilization. In that case, pulses must be delivered in a certain period requiring repetition frequencies from 1 Hz to several hundred Hz.

All these demands are fulfilled by special devices, often referred to as electroporators. Nowadays, there are a lot of commercially available electroporators that are designed for *in vitro* experiments. The problem of most of these electroporators is that the flexibility of the parameters of electric pulses is not sufficient, especially if we want to study the effects of different pulse parameters on the cell permeabilization, survival or average uptake of different molecules.

In this paper, we present the design of electroporator for *in vitro* cell plasma membrane permeabilization. The device is operated by an internal computer that allows the user to choose the parameters of electric field. The computer drives the pulse generator that is composed of a digital pulse generator generating the signal, high voltage amplifier amplifying the signal, and current amplifier that provides the signal with sufficient energy to prevent the voltage amplitude from dropping during the pulse delivery. Beside this, the last version of the developed electroporator also includes a special unit that generates low voltage pulses usually used for the electrophoretic transfer of the charged molecules through the permeabilized cell plasma membrane.

System design

Figure 1 shows the basic system design of the electroporator. It consists of a computer, pulse generator, voltage amplifier, current amplifier and low voltage pulse generator. Besides this, the device comprises two high voltage power supplies and several low voltage devices that are necessary for normal operation but are not drawn on the figure.

The internal computer of the device is in the first place used for selecting pulse parameters. Therefore, the computer includes a user interface composed of a display and a keyboard. The second task of the computer is to control pulse generation after activation has

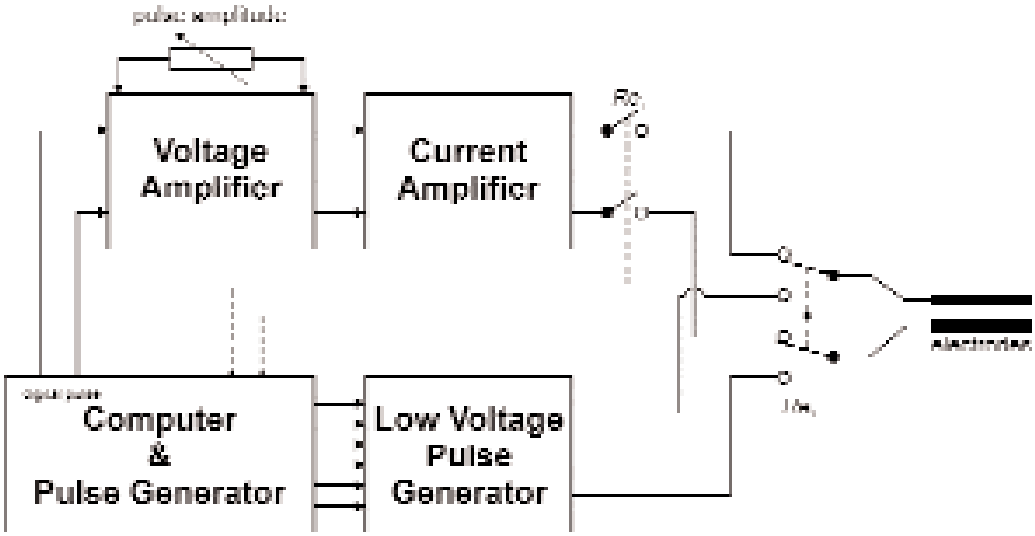

Figure 1. Block diagram of *in vitro* electroporator **GT-1**.

been triggered. All pulse parameters, except pulse amplitude, are then transferred to the pulse generator. This subunit generates digital signal that is then amplified in the voltage amplifier to the value that we set by external potentiometer. The amplified signal is then intensified by the energy from the current amplifier because we have to fulfill the energy requirement as defined by the load between electrodes.[4] At this point, the generation of the high voltage signal that is used for permeabilization of cell plasma membrane is finished.

The low voltage pulse generator, which we have constructed just recently, is used for the electrophoretic transfer of charged molecules through permeabilized cell plasma membrane. The structure of this subunit, controlled by the internal computer, is similar to that of the DC power supply. This version allows to change the amplitude in 10 V steps in a range from 0 V to 50 V.

Performance and experimental results

The developed *in vitro* electroporator, which is still a prototype, has been used in our laboratory for more than two years. During that time, we found and repaired some deficiencies in design and we also made several other improvements that reflect on greater flexibility of the parameters. The current prototype **GT-1** allows the user to set high and low voltage pulses in a range that is given in Table 1. The pulse repetition frequency f is calculated by using the following equation:

$$f = \frac{1}{T \cdot (1 + D_R)}, \qquad (1)$$

where T is pulse duration and D_R is value of the parameter Delay/Pulse ratio.

Furthermore, to demonstrate the performance of the device we designed an experiment where we measured voltage and current on the output (Fig. 2). In the experiment, we exposed 50 µl of pure SMEM, which was placed between 2mm plate electrodes, to five consecutive 100 µs pulses of 500 V at a repetition frequency of 2.5 kHz (D_R = 3).

Table 1. Output parameters of the developed in vitro electroporator

	Parameter	Value		
		Min.	Max.	Increment
HIGH VOLTAGE PULSES	Pulse duration	5µs	5000µs	1µs & 50µs
	Delay/Pulse ratio*	3	65535	1
	Pulse amplitude	25V	500V	linear
	Number of pulses	1	128	1
	Pulse current		30A	
LOW VOLTAGE PULSES	Pulse duration	5ms	9999ms	1ms
	Delay duration	0ms	9999ms	1ms
	Pulse amplitude	0V	50V	10V
	Number of pulses	1	1000	1
	Pulse current		1A	

* - parameter defines pulse repetition frequency that is calculated by the equation (1).

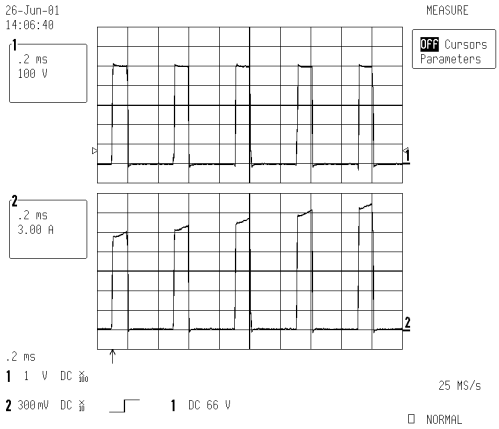

Figure 2. Performance of electroporator loaded with 50 µl drop of SMEM that was placed between 2 mm plate electrodes. Electroporator was programmed to generate five consecutive 100 µs pulses of 500 V and repetition frequency of 2.5 kHz (D_R = 3). Top trace (signal 1) presents voltage and bottom trace (signal 2) presents current flowing through the system. The measurements were performed using LeCroy LT9310C digital oscilloscope, a LeCroy AP015 current probe, and a Tektronix P5100 1:100 voltage probe. The voltage was 500 V, while current increased from 14 A to 19 A.

It is evident from the Figure 2 that the instrument was able to deliver all the pulses without any distortion. Even more, it could easily increase the current between two consecutive pulses that is usually necessary due to the polarization of the sample between the electrodes.

To compare the performance of the prototype **GT-1** with that of Jouan GHT 1287B, we evaluated cell permeabilization, using a bleomycin method[5], with both devices. The results of experiments that are shown on Figure 3 are comparable. T-test showed no statistically significant difference between both experiments.

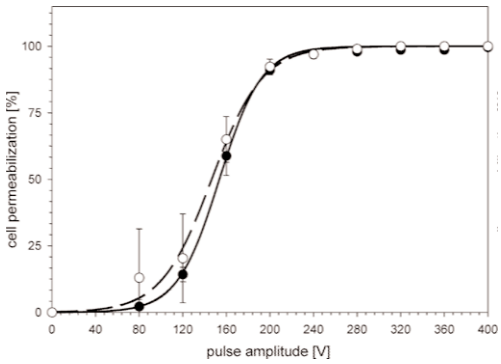

Figure 3. Comparison of cell permeabilization as a function of pulse amplitude obtained with electropulsator Jouan GHT 1287B (●), and *in vitro* electroporator **GT-1** (○).

Besides the performance experiments that we carried out, our colleagues already published the results of the experiments using the developed electroporator.[6,7] The two experiments were performed to study the influence of the parameters of electroporation (medium conductivity [6] and pulse repetition frequency [7]) on the cell permeabilization, survival and average uptake.

Conclusions

The comparison of the developed prototype **GT-1** with the commercially available device Jouan showed that **GT-1** fulfils all demands of the *in vitro* investigations. Furthermore, it offers a vast flexibility of the parameters and has the ability to generate high and low voltage pulses, where low voltage pulses can be used for the electrophoretic transfer of charged molecules through permeabilized cell plasma membrane.

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Bone metastases from malignant melanoma: a retrospective review and analysis of 28 cases

Elias Brountzos[1], Irene Panagiotou[2], Dimitrios Bafaloukos[2], Dimitrios Kelekis[1]

[1]Second Department of Radiology, Medical School, Athens University, Eugenidion Hospital, Athens, Greece, [2]Second Department of Oncology, Metaxa Cancer Hospital, Piraeus, Greece

Background. *The aim of the study was to evaluate the clinical characteristics, the radiological findings, and the treatment effect on the patients with bone metastases from malignant melanoma.*

Patients and methods. *Retrospective review of 293 stage IV melanoma patients during a 15-year period was made.*

Results. *Twenty-eight patients (9.5%) with bone metastases were identified; all patients had a thick or intermediate primary melanoma (Breslow 2.7-9.9). Most of the patients presented with multiple (95.6%), symptomatic (92.6%) skeletal lesions. Imaging depicted 90 bone lesions. Axial metastases were more common (86%); 54% of them were located at the spine. Skeletal radionuclide scintigraphy was non-specific; radiographij and computed tomography was diagnostic. Typical bone metastases were osteolytic (92.5%). Sixty-six lesions were treated with radiotherapy; in 79%there was a palliative response. There was no correlation between total dose or fraction size and effective palliation. The skeletal lesions did not respond to concurrent chemotherapy and/or biphosphonates. Median response duration to treatment was estimated to 2.6 months and median survival to 4.7 months.*

Conclusions. *Osseous metastases from malignant melanoma occur in the patients with more advanced primary lesions. They are most frequently osteolytic and located in the axial skeleton. Radiographij and computed tomography is diagnostic. Radiotherapy still remains the treatment of choice.*

Key words: bone neoplasms; malignant melanoma, bone metastases; imaging; radiotherapy

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Correspondence to: Elias Brountzos, MD, Second Department of Radiology, Medical School, Athens University, Eugenidion Hospital, 20 Papadiamantopoulou st, Athens 11528, Greece; Phone: +301 7227488; Fax: +301 729 2280; E-mail: ebrountz@cc.uoa.gr

Introduction

In most clinical series bone metastases from malignant melanoma are less frequent, ranging from 11%-17%.[1] Nevertheless, the autopsy series have revealed that skeletal involvement is more common (23-49%).[2-4]

Skeletal metastases generally occur in patients with widespread metastatic disease and usually represent a late site of recur-

rence.[5] Median survival is estimated to 2-6 months.[1-5]

The aim of our study was to present our experience concerning this uncommon site of melanoma dissemination. We retrospectively evaluated the clinical course, the radiological abnormalities and the response to treatment in this subgroup of melanoma patients.

Patients and methods

We reviewed the records of all patients with stage IV malignant melanoma treated in the 2nd Department of Medical Oncology in Metaxa Cancer Hospital from 1985 to 2000. We recorded the patients with disseminated bone disease. The inclusion data were the following: age, sex, location and thickness of primary tumor, time interval between primary tumor and bone metastases, distribution of metastases, location of the metastatic lesion, and presenting signs and symptoms.

Table 1. Characteristics of our patients' population

Age at AJCC stage IV diagnosis, years (Median, range)	50, 26 - 86
Male (%)	59
Female (%)	41
Site of primary melanoma (%)	
Lower extremity	14.1
Upper extremity	11.1
Trunk	33.3
Head & neck	37.8
Unknown origin	3.7
Breslow's thickness, mm (Median, range)	4.1, 2.7 - 9.9
Stage III diagnosed prior to stage IV (%)	70
Adjuvant treatment before stage IV (%)	22.2
Disease free interval before stage IV, months (median, range)	37, 0 - 267
Bone metastases as sole & initial site (%)	3.7
Multiple bone metastases (%)	95.6

X-ray, computed tomography, magnetic resonance and bone scintigraphy images, detecting all radiological abnormalities, were carefully reviewed. The type and response to treatment were clinically and radiologically evaluated. Effective palliation was clinically defined as significant relief from symptoms for at least 1 month. Palliative responses to radiotherapy were correlated with total radiation dose and fraction size. Survival was defined as the number of months between the diagnosis of bone metastases and death or last follow-up.

Statistical analysis was performed using the chi-squared test for the correlation between primary melanoma's thickness and the development of bone metastases. In the comparison, statistical significance was determined using a p-value level of 0.001.

Results

From a total of 293 patients with stage IV malignant melanoma, 28 patients (9.5%) with skeletal metastases were identified. The disease dissemination was diagnosed in all 293 patients using computed tomography of the chest and/or abdomen: all patients were submitted to computed tomography during regular follow-up or were evaluated with computed tomography when they became symptomatic. Table 1 shows the 28 patients' characteristics.

All our patients' primary melanomas were thick or intermediate (Breslow's thickness ranging from 2.7-9.9). From the 293 stage IV patients, 165 had a thick or intermediate primary melanoma. When correlated, statistical significance was found ($p<0.001$). Additionally, most patients (70%) had AJCC stage III disease before the diagnosis of bone metastases.

Twenty-six patients presented with symptoms of bone metastases at the time of the diagnosis: bone pain (85.1%), pathologic frac-

tures (14.8%) and/or bone marrow infiltration (7.4%) were the most common findings. Four patients (14.8%) presented with hypercalcaemia. There were no compression fractures or neurological symptoms. Only 2 patients (7.4%) were asymptomatic; in these patients, skeletal involvement was found during routine abdominal computed tomography.

In all 28 patients, thoracic and/or abdominal computed tomography was initially performed, followed by skeletal radionuclide scintigraphy in the 26 symptomatic patients, and skeletal survey radiographs in 14. Computed tomography of the appendicular skeleton was performed in 9 symptomatic patients, and MRI of the painful skeletal site in 5 patients. All studies yielded positive results.

A total of 90 metastatic bone lesions were depicted.

Table 2 shows the distribution of the lesions: axial metastases were more common (86%) than appendicular bone metastases; the lesions were located more commonly at the spine, particularly at the thoracic and lumbar vertebrae.

Table 3 demonstrates the radiological features found in radiographs and computed tomography. More than 90% of the lesions were osteolytic. A mixed osteolytic-osteoblastic pattern was uncommon (Figure 1). We found

Table 2. Skeletal distribution of the lesions

Location	Percentage of lesions (%)
Spine	54
Pelvis	21
Ribs	11
Femur & tibia	7
Humeral, ulna & clavicle	4
Temporal, maxilla & mandible	3

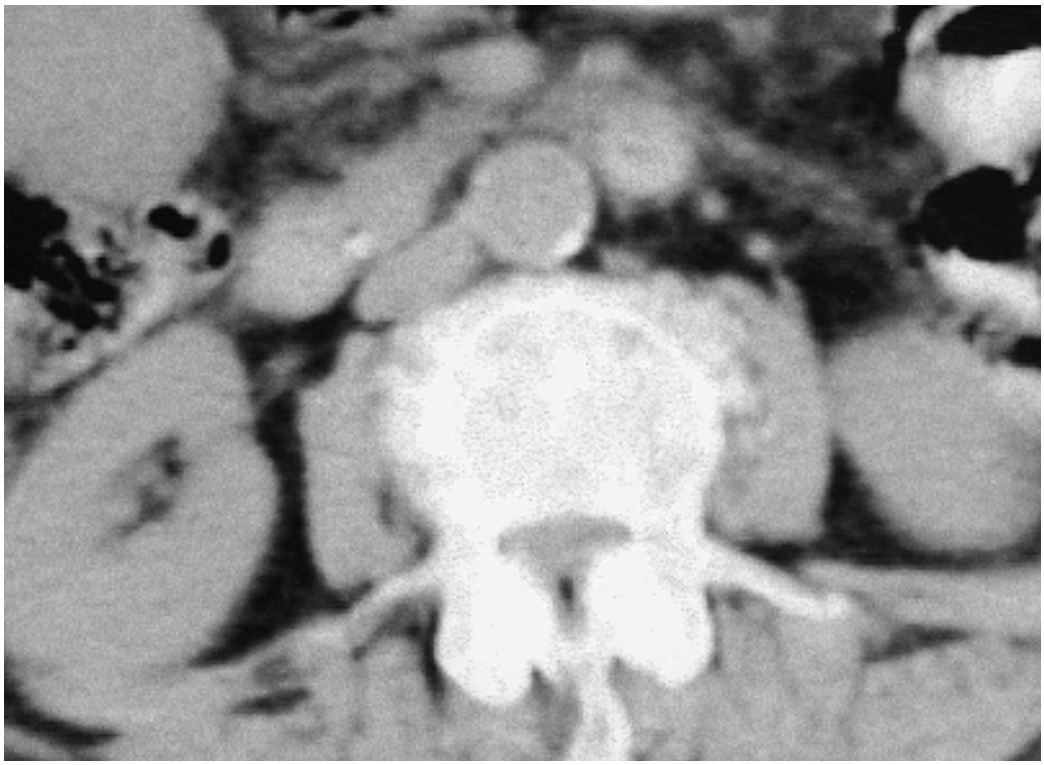

Figure 1. An unusual mixed osteolytic-osteoblastic metastatic pattern located at the lumbar vertebrae.

Table 3. Imaging features of the metastatic skeletal lesions (x-ray, CT)

Imaging features	Percentage of lesions (%)
Osteolytic	92.5
Cortical erosion & destruction	36.6
Soft tissue involvement	10.5
Mixed osteolytic-osteoblastic pattern (Fig.1)	8.2
Completely osteoblastic	2.3

Table 4. Palliation depending on total dose and fraction size

Total dose (cGy)	No palliated lesions / No lesions treated	(%)
< 3000	13/16	81.25
3000-4000	30/37	81.08
> 4000	9/13	69.23
Dose per fraction (cGy)	No palliated lesions / No lesions treated	(%)
< 200	2/3	67.00
201-300	21/26	80.76
301-400	8/9	88.88
401-500	10/15	66.66
> 500	11/13	84.61

that 91.1% of the lesions had a medullary origin. Scintigraphy uniformly depicted metastatic lesions as sites of increased uptake of Tc-99m. Magnetic resonance imaging was more specific in depicting the soft-tissue involvement.

Whenever imaging follow-up was performed to evaluate the response to treatment, certain radiological response patterns were identified. Recalcification, sclerotic rim and periostal reaction were the most common findings.

The 66 bone lesions -found in the 26 symptomatic patients - were treated with irradiation, and showed an effective palliative response rate of 79% (52/66). Table 4 demonstrates that the palliation of bone metastases was not related to total dose or fraction size.

In 18 (of the 26) symptomatic patients, 31 symptomatic bone lesions were treated with radiotherapy and concurrent chemotherapy and/or biphosphonates, while 15 asymptomatic lesions were treated only with chemotherapy and/or biphosphonates. Systemic chemotherapy was also given to the two asymptomatic patients with the metastatic lesions at the lumbar vertebrae. In all 20 patients, the disease progressed in extraskeletal sites. As shown in imaging follow-up, 61% of the symptomatic lesions responded to therapy; all of them were clinically palliated. All asymptomatic lesions treated with chemotherapy and/or biphosphonates did not respond radiologically or clinically, but progressed.

Median response duration to treatment was estimated to 2.6 months and median overall survival was estimated to 4.7 months.

Discussion

In our study, only 9.5% of the stage IV melanoma patients had skeletal involvement. A similar low percentage of antemortem diagnosis of bone metastases from malignant melanoma is reported in the literature.[1-4] Since clinical diagnosis of skeletal involvement is infrequent, little has been published describing the pattern and natural history of melanoma metastatic to the bone.

The limitation of our study is that we did not perform routine screening, so we do not know the exact incidence of skeletal metastases. Based on melanoma surveillance guidelines,[6,7] our patients were not routinely submitted to bone radionuclide scintigraphy. Nevertheless, we evaluated our patients during the follow-up with chest and abdominal computed tomography, so we were able to detect the majority of skeletal metastases. Melanoma bone metastases occur more frequently in the axial skeleton, and are therefore easily diagnosed by computed tomography.[8,9] Nevertheless, the real incidence of

osseous disease might be slightly higher than 9.5%, since appendicular bone metastases could not be depicted by thoracic and abdominal computed tomography.

Only 3.7% of our patients had bone metastases as the first and only site of recurrence; the rest of the patients had widespread disease in multiple metastatic sites. This is also in agreement with the literature.[5]

In our series, skeletal metastases were more frequent in patients with primary melanomas of the trunk and the head and neck area.

We also found that all patients with bone metastases had a thick or intermediate primary melanoma ($p<0.001$). Additionally, most patients had stage III disease prior to the development of skeletal metastases. Our findings may suggest that the patients with more advanced primary lesions are more likely to develop bone metastases, and should be more closely monitored.

Only 7.4% of our patients were asymptomatic and the skeletal involvement was found during routine imaging follow-up. The metastatic disease was located at the lumbar vertebrae and was detected by an abdominal computed tomography. In our series, axial metastases (86%) were more common than appendicular bone metastases and 54% of them were located in the spine. Our findings are similar to those previously reported, and suggest that whenever a computed tomography is performed to evaluate metastatic melanoma, the axial skeleton should be carefully examined.[8,9]

Bone radionuclide scintigraphy was nonspecific; all metastatic lesions were depicted as sites of increased uptake of Tc-99m. Plain radiographs and computed tomography images were diagnostic. In the literature, there are few reports of the imaging findings of skeletal melanoma metastases.[9-11] In our series, melanoma bone metastases were osteolytic with medullary origin. Lesion growth caused cortical erosion and destruction, pathologic fractures and soft-tissue involvement. Atypical skeletal metastases exhibited a mixed osteolytic-osteoblastic pattern or, even more infrequently, a completely osteoblastic pattern. Magnetic resonance imaging better depicted the extent of soft-tissue involvement.

The response assessment of bone metastases to therapy is difficult; in most cases, decisions about the efficacy of treatment are based on symptomatic response or change in extraskeletal metastatic disease. In our study, radiographs and computed tomography were useful in evaluating the tumor's response to treatment.

One could argue that since the real incidence of bone metastases in clinical series is low, the role of computed tomography or other imaging studies is only complementary. Nevertheless, we showed that serial radiographs or computed tomography are essential in establishing the diagnosis, guiding the treatment planning and assessing the tumor's response to treatment.

In our series, radiotherapy offered an effective palliation rate of 79%. We believe that it still represents the treatment of choice in this subgroup of patients; the treatment with chemotherapy and/or biphosphonates did not appear as effective. We agree with Rate and al. that concurrent chemotherapy has no influence on palliation.[12] We found that in palliative treatment of bone lesions from melanoma, the application of high total dose or high fraction size was not advantageous at all. High doses should be avoided since they do not offer more effective palliation and can create greater complications. Similar results were reported by Konefal *et al.*[13] in the analysis of dose fractionation in the palliation of bone and brain metastases from malignant melanoma.

Despite the palliation offered with radiotherapy, our patients' prognosis was poor. Median survival was similar to the one previously reported in the literature[1,2,5] and esti-

mated to 4.7 months. Even if patients with thick or intermediate primary cutaneous melanomas could be more closely monitored in order to detect asymptomatic bone metastases, no change in palliation or survival would be achieved.

In conclusion, we recommend a skeletal evaluation with radiographs or computed tomography - whenever symptoms develop - and a careful examination of the axial skeleton in the patients with advanced primary melanomas. The life expectancy of these patients is short, but conventional fractionation radiotherapy can offer effective palliation to most of these patients. It is therefore worthwhile to pursue the diagnosis.

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Ranking radiotherapy treatment plans: physical or biological objectives?

Martin Ebert

Department of Radiation Oncology, Sir Charles Gairdner Hospital, Western Australia, Australia

Background. *The ranking of treatment plans in radiotherapy is of importance when there are alternative approaches to treating an individual patient, in assessment of dose information collected during clinical trials and in formulation of objectives for optimization routines.*
Methods. *Several physically and radiobiologically-based dose indices were calculated for a series of model dose-volume histograms (DVHs). The ranking of these DVHs according to each dose index was examined. Variation in the ranking of the radiobiological indices with parameters used in the models was also examined. Ranking according to the indices was also examined for DVHs of planning target volumes (PTVs) for a series of 18 patients treated with external beam radiotherapy for prostate carcinoma.*
Results. *It was found for both the model and real DVHs that treatment plan ranking depends explicitly on the model used for ranking target-volume doses (i.e., the dose index used). For the radiobiological models, there is a strong dependence of DVH ranking on the radiobiological parameters used in the models (specifically, the 'alpha' value from the linear-quadratic model).*
Conclusion. *When ranking radiotherapy treatment plans during planning or in evaluation of clinical trials, attention should be paid to the models used in dose evaluation.*

Key words: radiotherapy planning, computer assisted; radiobiology

Introduction

Many situations arise in radiotherapy treatment planning where multiple treatment plans need to be compared in order to evaluate the optimal plan, or in order to provide a direct comparison of the relative merits of each of the plans. Such situations arise during:

- The treatment planning process for an individual patient. If two or more irradiation strategies are available, it will be necessary to decide which is the best strategy.
- During evaluation of dosimetric data collected during clinical trials. In order to correlate treatment outcome with delivered dose, a method of describing dose distributions to target volumes must be used.

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Correspondence to: Dr Martin A. Ebert, Director of Physics Research, Department of Radiation Oncology, Sir Charles Gairdner Hospital, Hospital Avenue, Nedlands, Western Australia, 6009, Australia, Tel: +61 8 9346 4900; Fax: +61 8 9346 3402; E-mail: Martin.Ebert@health.wa.gov.au

- During inverse planning optimization procedures. Inverse planning requires specification of an objective function which describes the optimality of a given dose distribution. Successive optimization iterations require a comparison of treatment plans on the basis of that objective function.[1-3]

In order to perform the plan comparison in terms of the dose distribution delivered to the target volume (the PTV encompassing the tumor volume), indices need to be stated which reduce the complex distribution of dose/volume throughout the PTV to a single scalar value. The dose distribution is frequently presented in the form of a dose-volume histogram (DVH), which can be easily reduced to a single index (a 'dose index') by computational methods.

Several alternatives exist for dose indices. In purely physical terms, the delivered radiation doses can be treated as quantities, which directly relate to treatment outcome. In this case, 'physical objectives' are used to describe the optimality of a treatment plan. An alternative is to attempt to relate the physical dose distribution more directly to some actual indication of probable response. In this case, we are using a 'radiobiological objective', which will be based upon some hypothesized (possibly validated) model for cellular response.

The usefulness of either physical or radiobiological dose indices depends very much on the correlation of those indices with treatment outcome. Such validation requires evaluation of data from large-scale clinical trials. In relating dose indices to each other, it is important to consider whether individual dose indices will rank alternative treatment plans differently, and whether that ranking will depend on the specifics of the models themselves. This study aimed at examining those differences and dependencies.

Methods

Dose indices considered

A series of physical dose indices were used. These were:

- mean dose;
- minimum dose;
- maximum dose;
- dose standard deviation; and
- least-squares deviation from prescription dose.

The radiobiolgically-based indices (DVH reduction values) considered were:

- tumour control probability (TCP); [4,5] and
- equivalent uniform dose (EUD). [6-8]

Both of these models were based on the linear-quadratic approach to describing cell kill, ignoring time and fractionation effects and assuming independence of all tumor cells. Thus, for a DVH described by a distribution of N doses, d_i, at discrete volumes, v_i, for a tumor with uniform cell density ρ, the equations used for TCP are:

$$TCP = \frac{1}{K}\sum_{m=1}^{K}\exp\left[-N_s\right], \qquad (1)$$

with

$$N_s = \prod_{i=1}^{N}\rho v_i \exp\left[-\alpha_i d_i\right]. \qquad (2)$$

Equation (1) provides population averaging by sampling TCP over a large range (K - typically 10^4) described by a normal distribution of alpha-values defined by a mean alpha value, α_m, and a standard deviation, α_σ. For EUD, population sampling is not necessary (Ebert, 2000) and the equation for EUD is:

$$EUD = \frac{-1}{\alpha}\sum_{i=1}^{N}\exp\left[v_i \exp\left(-\alpha_m d_i\right)\right], \qquad (3)$$

where N is the number of bins in the DVH. In all calculations, parameter values of α_σ = 0.1 Gy^{-1} and $\rho = 10^8$ cells/cm^3 were used. Values of α_m in the range 0.05 Gy^{-1} to 0.8 Gy^{-1} were considered.

The physical and radiobiological indices listed above were calculated for a series of model and real DVHs in order to examine how those DVHs were ranked according to each index.

Model DVHs

A series of artificial DVHs were considered (Figure 1) which represented a large range of possible dose-volume conditions in a PTV. These distributions are:

1. A normal (Gaussian) distribution with a standard deviation of 5% of the prescription dose.
2. A normal distribution with a standard deviation of 10% of the prescription dose.
3. A single-sided normal distribution with a standard deviation of 10% of the prescription dose.
4. Uniform dose delivery except for a hot spot of 150% over a volume of 5%.
5. Uniform dose delivery except for a cold spot of 50% over a volume of 5%.

For all model DVHs a tumor volume of 100 cm^3 and a mean dose of 60 Gy was used.

DVHs for prostate treatments

Figure 2 shows DVHs for PTV for 18 patients treated with external beam radiotherapy for prostate carcinoma (follow-up information pending).

Results

Model DVHs

For the model DVHs, data has been summarised in Figure 3. For each dose-index, the value has been shown for each DVH. The ranking of the DVHs according to each index is also shown. Figure 4 shows the variation in TCP and EUD for each of the model DVHs with variation in the value of α_m.

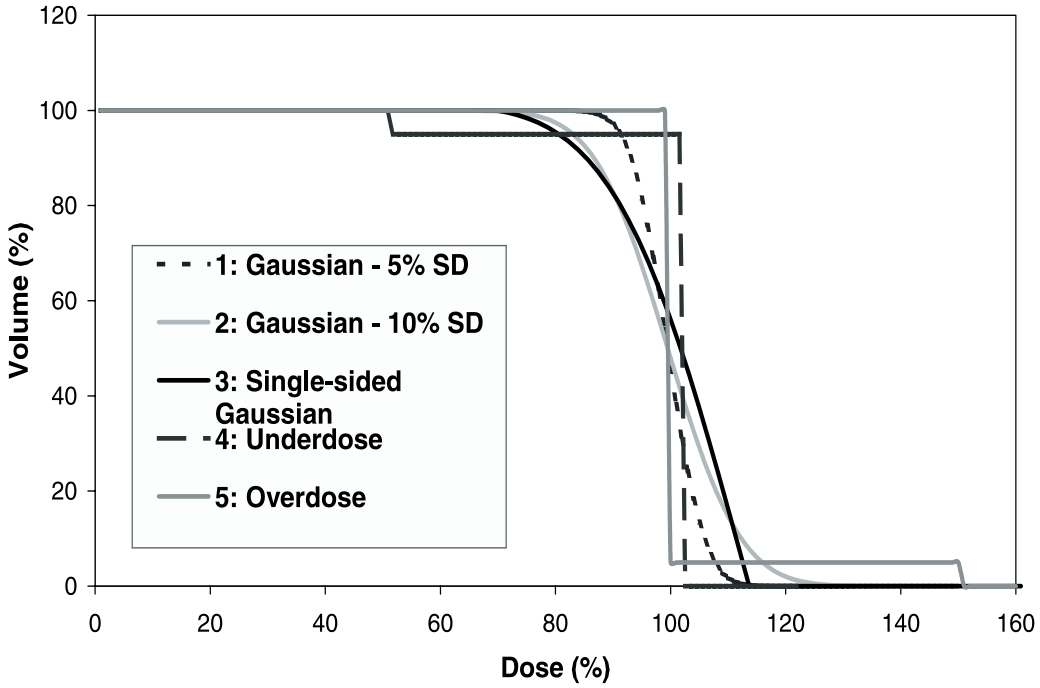

Figure 1. Model DVHs used to represent a broad range of feasible dose distributions.

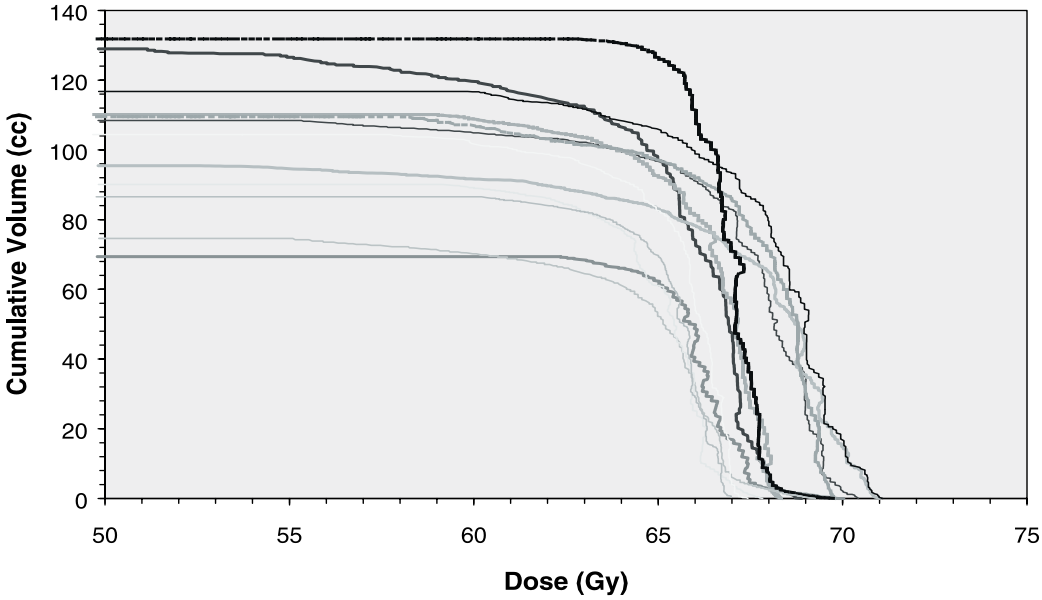

Figure 2. DVHs for PTV for 18 patients treated with external beam radiotherapy for prostate carcinoma. Prescription dose was 66 Gy (100 % level) for all cases.

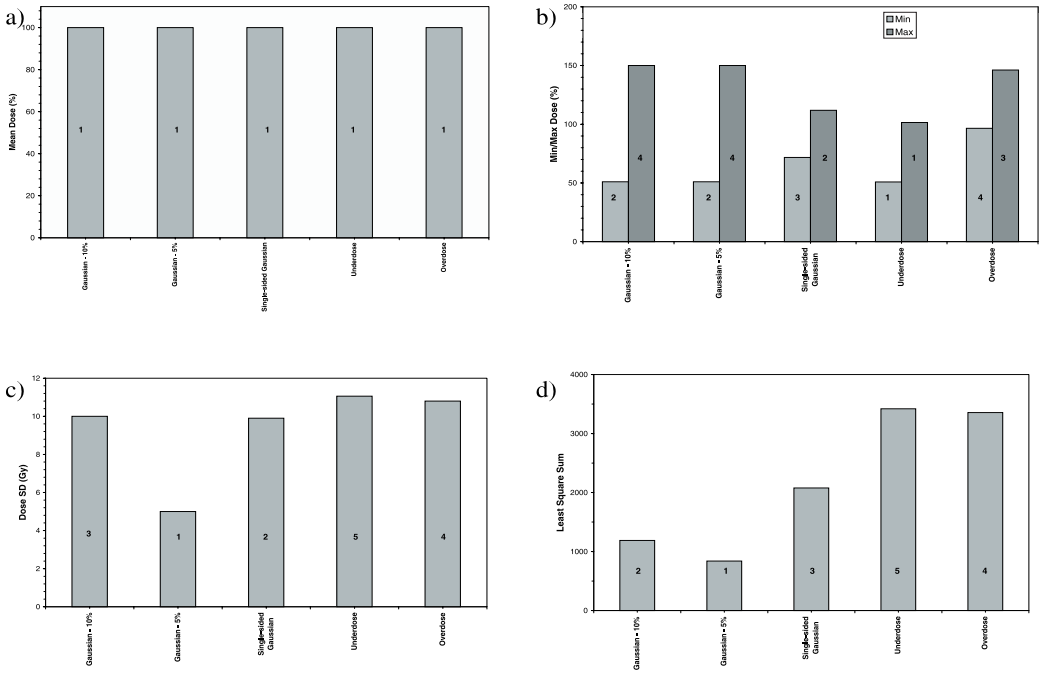

Figure 3. Summarized results for physical dose-indices for the five model DVHs. a) Mean dose, b) Maximum/Minimum dose, c) Dose standard deviation, d) Sum of least-squares. The numerals show the order of DVH ranking according to each dose-index.

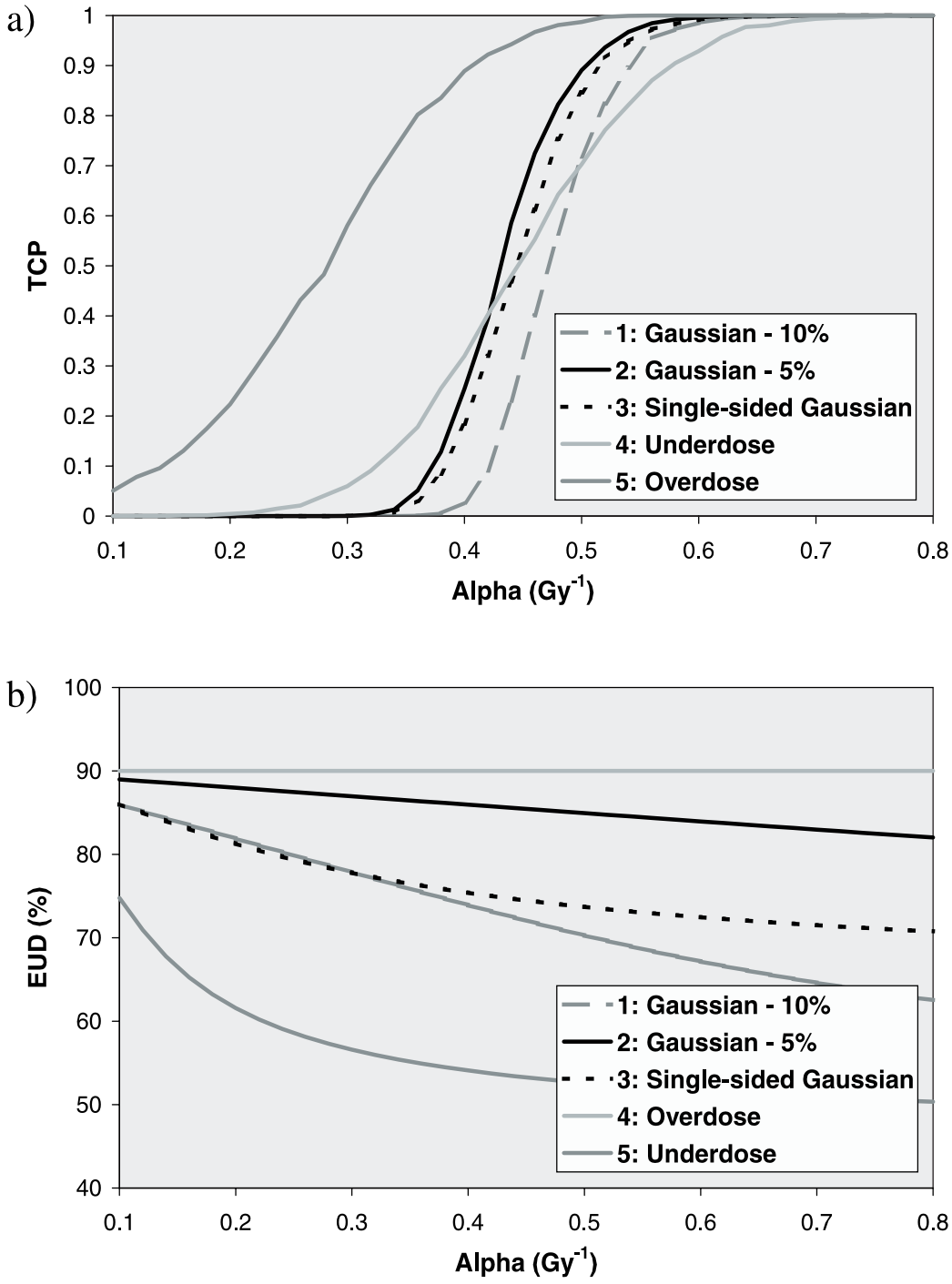

Figure 4. Variation in values of a) TCP and b) EUD with alpha (Gy^{-1}) value, showing some overlap in the order of DVH ranking.

DVHs for prostate treatments

In order to visualize the ranking of the 18 prostate-patient DVHs according to the physical dose-indices, the DVHs were ordered according to one of the indices, and all indices plotted together. Thus in Figure 5a, the DVHs have been ordered according to their mean dose. The figure then shows how the DVHs

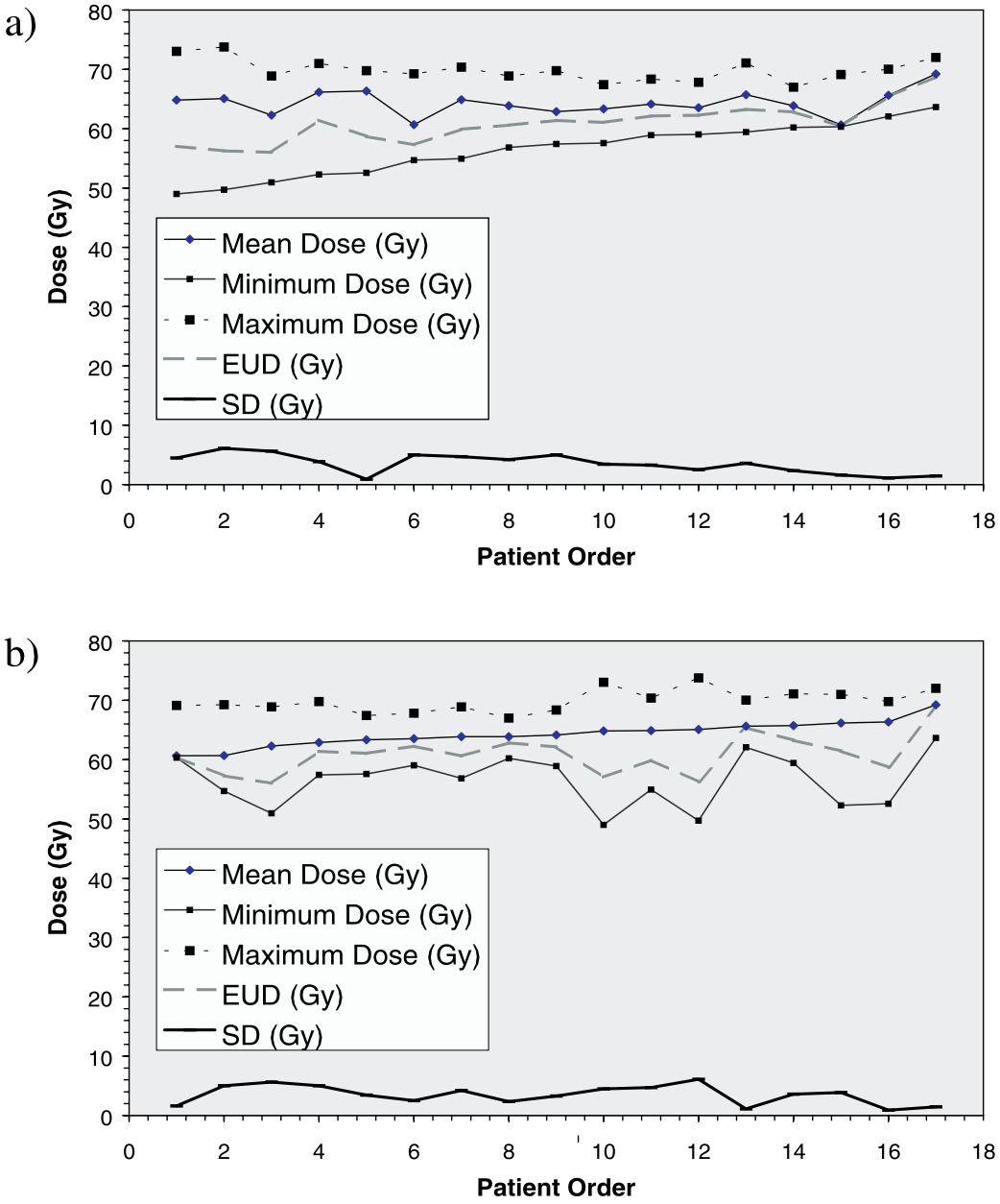

Figure 5. Variation in physical dose-indices across all 18 patients DVHs. a) Data sorted by increasing mean dose, b) data sorted by increasing minimum dose. EUD values calculated using α_m = 0.35 Gy^{-1}.

compared according to the other dose-indices. Figure 5 b shows the same information with DVHs ranked according to minimum dose.

Figure 6 shows variation TCP and EUD values for all 18 DVHs as they vary with mean alpha value in the respective radiobiological models.

In Figure 7, the ranking of the 18 DVHs according to TCP or EUD has been shown using an intensity scale at each value of α_m.

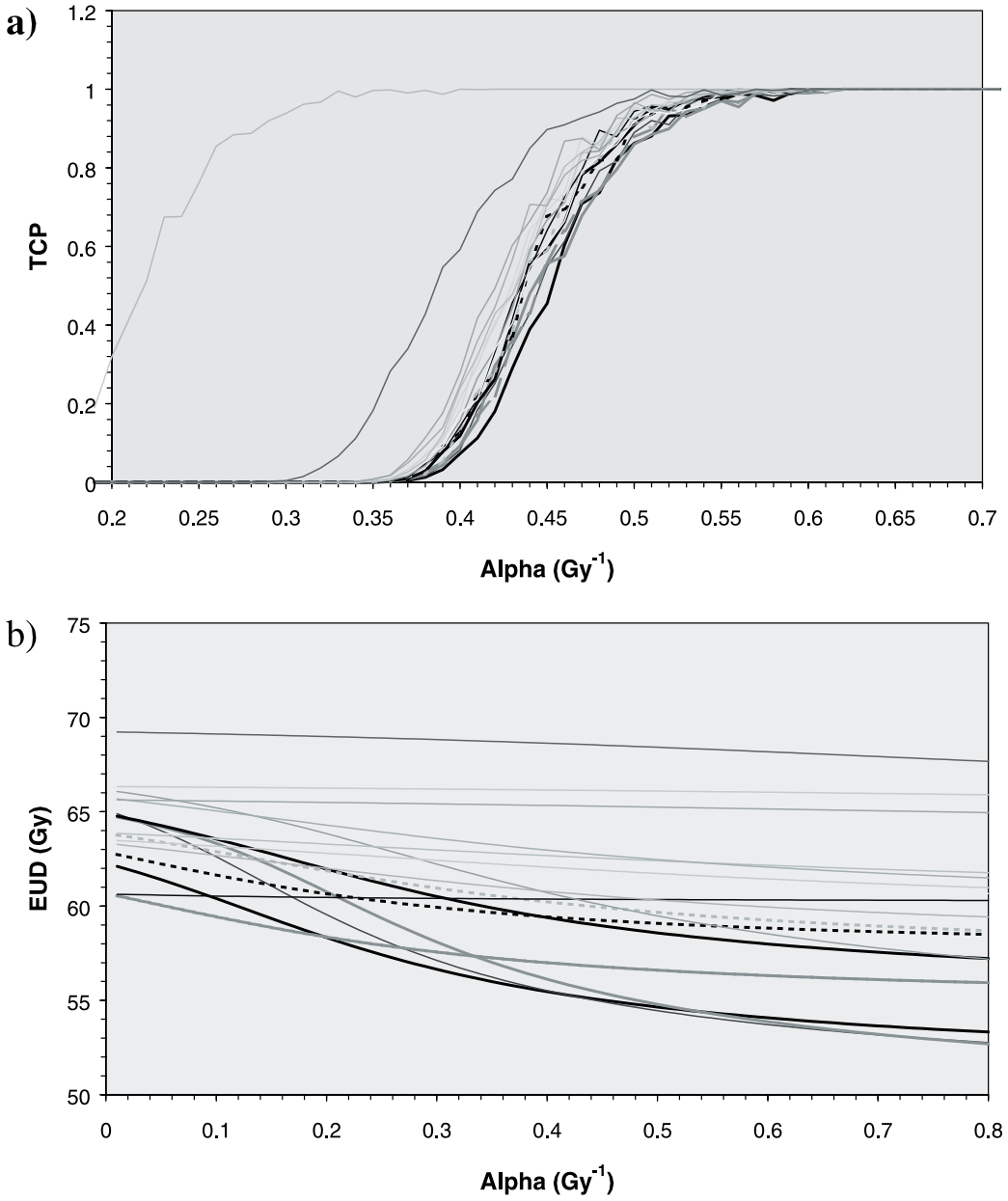

Figure 6. Variation in values of a) TCP and b) EUD with alpha (Gy-1) value.

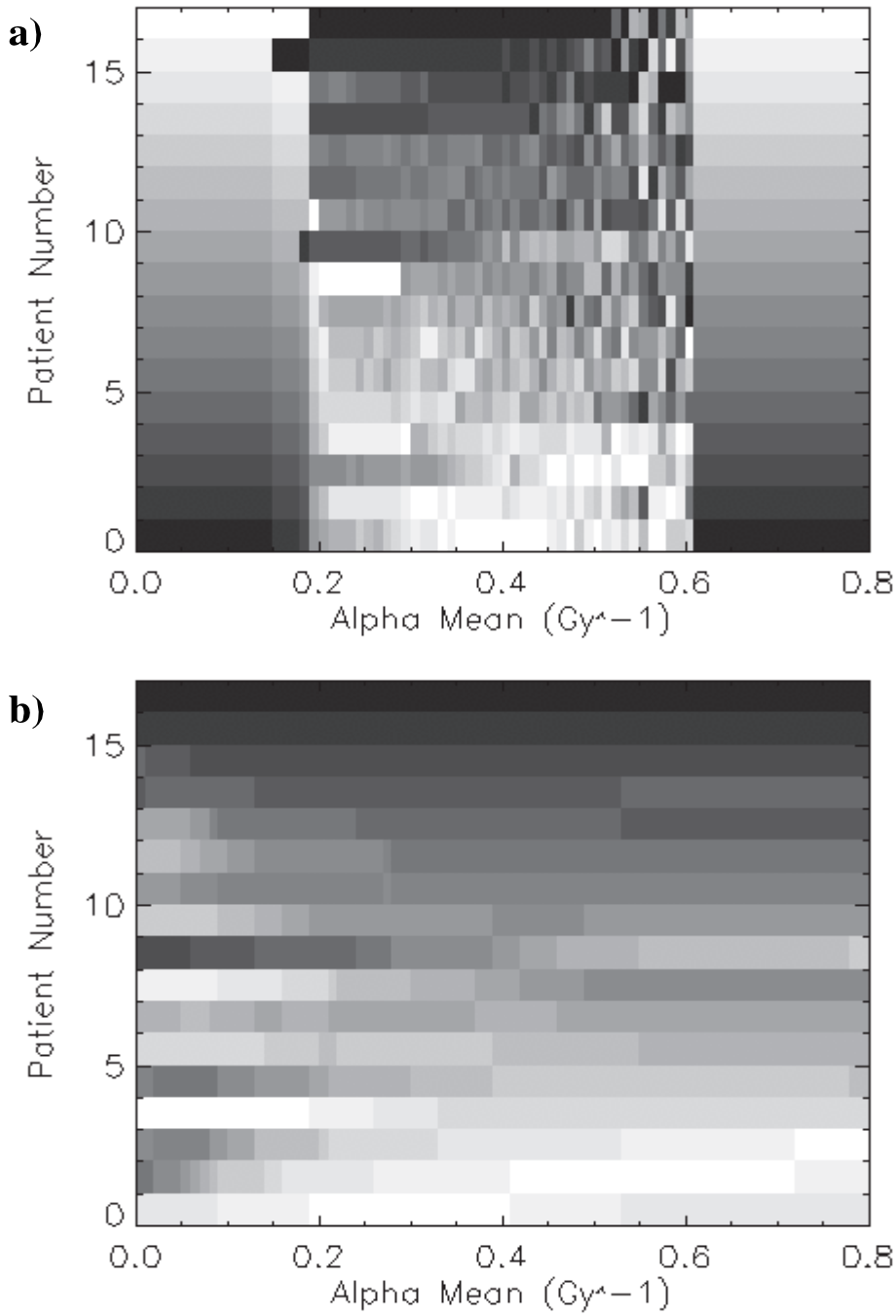

Figure 7. Variation in ranking of the 18 patient DVHs according to a) TCP and b) EUD, and according to the alpha (Gy^{-1}) value used in the TCP/EUD models. Patients have been ranked according to TCP/EUD at $\alpha = 0.4\ Gy^{-1}$. The intensity of the image at each alpha value indicates the ranking of each of the 18 DVHs from lowest TCP/EUD (black) to highest TCP/EUD (white).

Discussion

The results presented above show that if dose-indices are going to be used to rank rival treatment plans, then the resulting ranking is going to depend explicitly on the particular dose-index used. The model DVHs were considered on the basis of the significant differences between them and, as such, it is not surprising that the physical dose-indices give different DVH rankings as shown in Figure 3. What is more surprising is the subtle change in ranking according to EUD as the alpha-parameter was varied (Figure 4b), and the more dramatic change in ranking with TCP as the alpha-parameter was varied (Figure 4a). The change in ranking is also not consistent between TCP and EUD. In Figure 4a the 'underdose' DVH is seen to jump ranking order quite rapidly with change in alpha-value, whereas in Figure 4b it is the 'single-sided Gaussian' distribution which changes ranking most quickly. The strong dependence of TCP on alpha for the underdose DVH is not unexpected as TCP has been shown to be very sensitive to the presence of regions of low dose.[8]

For the data taken from patient PTV dose distributions, there are relatively smaller differences between the 18 DVHs. As a result, smaller but more frequent changes in ranking would be expected. In Figure 5 it is seen that, when the 18 DVHs are ordered according to one of the physical dose-indices, there is considerable variation in the order of the other dose-indices. In Figure 5b, some correlation is seen between minimum and maximum dose and dose standard deviation as may be expected.

In terms of DVH ranking according to the radiobiological dose-indices, Figure 6 shows that there is considerable overlap both for EUD and TCP. This overlap is reflected in Figure 7 as the ranking of individual DVHs (indicated by the intensity of the plot at each combination of patient number and alpha value) changes rapidly with alpha-value indicating a strong sensitivity not only to the radiobiological models, but this parameter of the radiobiological models.

Close examination of Figure 6a a shows some 'noise' in the TCP vs alpha-value curves. This is due to the statistical sampling methods used to incorporate population sampling in the TCP model. Using large values of K in equation (1) leads to significantly long calculation times for TCP and only minimal smoothing of these curves (due to strong effects of low alpha values on TCP). The result is that there will be some overlap of DVH rankings as a result of the sampling routines used and this will lead to some of the rapid variations in ranking displayed in Figure 7.

Conclusions

This study has shown that for a variety of DVH conditions, the ranking of DVHs is dependent on the model used for both physical and radiobiological dose-indices. In addition, the ranking of the DVHs also depends on the particular characteristics of the model being used (in this case, the alpha-value in TCP and EUD models based on the linear-quadratic equation). Variations in the ranking result from non-linear transformations between the indices. This must be considered whenever scalar indices are being used to present dosimetric information in treatment planning, plan optimization or in analysis of dosimetric data from clinical trials.

The usefulness of the variety of available indices for describing non-uniform dose distributions will depend on the correlation of each index with treatment outcome. This information will only become available following detailed assessment of data from large-scale clinical trials.

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Zadrževanje diha pri bolnikih, ki so radiološko preiskovani: primerjava časov po izdihu in po vdihu z ali brez hiperventilacije

Groell R, Schaffler GJ, Schloffer S

Izhodišča. Pri slikovnih preiskavah kot so računalniška tomografija, magnetna resonanca ali ultrazvok je potrebno, da bolnik zadrži dihanje. Namen pričujoče študije je bil primerjati čase zadrževanja diha po vdihu in izdihu in ocenti učinek hiperventilacije.

Bolniki in metode. V študiji smo obravnavali 30 bolnikov in 19 zdravih prostovoljcev, vsi so podpisali pismeno privolenje. Čas zadrževaja diha je bil merjen po izdihu in izdihu, pred in po hiperventilaciji.

Rezultati. Srednji čas zadržanega diha po izdihu (bolniki 24±9 sekunde, prostovoljci 27±7 sekunde) je bil značilno krajši kot po vdihu (bolniki 41±20 sekunde, $p<0.001$; prostovoljci 62±18 sekunde, $p<0.001$). Razlika se je značilno povečala s hiperventilacijo (40-60%, $p\leq0.005$) po izdihu in vdihu, tako pri bolnikih kot pri prostovoljcih.

Zaključki. Čeprav je zadrževanje diha po izdihu priporočlivo pri različnih slikovnih preiskavah, zlasti še pri preiskavah trupa in trebuha, pa zadrževanje dihanja po vdihu omogoča, da bolniki zadržujejo zrak znatno dlje.

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Ultrazvočno vodena perkutana drenaža trebušne tekočine namesto laparotomije ali relaparotomije

Miletić D, Uravić M, Fučkar Ž, Glavaš R, Topljak-Polić D

Izhodišča. Namen študije je bil ugotoviti učinkovitost in zanesljivost perkutane trebušne drenaže pri kirurških bolnikih in ovrednotiti medreberno drenažo pri kopičenju tekočine pod trebušno prepono.

Material in metode. 87 bolnikov, starih od 29 do 84 let (srednja starost 55,5 let) smo perkutano drenirali pod kontrolo ultrazvoka zaradi kopičenja tekočine v trebuhu po operaciji ali pri še neoperiranih bolnikih, kjer bi bila sicer potrebna laparotomija. Za odstranitev tekočine smo uporabljali katetre števila 8 do 14, ki smo jih uvedli med rebra ali pod njimi z lateralne ali sprednje strani trebuha.

Rezultati. Z medrebernim pristopom smo drenirali 31 (60,8%) od 51 kopičenj tekočine pod trebušno prepono. Trajanje drenaže ni bilo odvisno od vrste pristopa, ali smo drenirali med rebri ali pod njimi. Značilno pa se je podalšalo trajanje drenaže ($p<0.05$, Mann-Whitney U test) pri gnojnih tekočinah (srednja vrednost 18 dni; razpon 7-73 dni) v primerjavi s hematomi, bilomi in drugimi negnojnimi kopičenji tekočine (srednja vrednost 11 in 6 dni). Ultrazvočno vodena perkutana drenaža je bila uspešna pri 92% bolnikov, manjših zapletov pa je bilo le 9,2%. Uspešnost posega je bila večja pri drenaži kopičene tekočine pod diafragmo (96%).

Zaključki. Ultrazvočno vodena perkutana drenaža je metoda izbora pri odstranjevanju trebušne tekočine, kjer bi bila sicer potrebna laparotomija. Če je mesto vboda vsaj dva medreberna prostora nižje od oboka prepone in kateter ni uveden skozi pleuralno tekočino, je medreberna drenaža enako učinkovita in enako varna kot drenaža pod rebri.

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Ultrazvočno vodeno uvajanje centralnega venskega katetra pri bolniku, ki je imel obojestransko radikalno odstranjene vratne bezgavke: prikaz primera

Šustić A, Cerović R, Juretić M

Izhodišča. Obsežne in težavne operacije v onkologiji večkrat zahtevajo uvajanje centarlnega venskega (CV) katetra. Pri nekaterih bolnikih je zaradi anatomskih sprememb, ki jih je povzročila bolezen, ovirano ali celo onemogočeno običajno "slepo" uvajanje CV katetra. V takšnih primerih je uporaba utrazvoka uspešna dodatna metoda pri uvajanju CV katetra.
Prikaz primera. Predstavljamo bolnika, ki je imel obojestransko radikalno odstranjene vratne bezgavke in pri katerem je posledično prišlo do pomembnih anatomskih sprememb ter smo mu ultrazvočno uspešno uvedli CV kateter.
Zaključki. Ultrazvok je lahko koristna dodatna metoda pri uvajanju CV katetra pri onkoloških bolnikih. V primerih, kjer je uvajanje otežkočeno, priporočamo uporabo ultrazvoka.

Radiol Oncol 2001; 35(3): 179-83.

Bifokalni primarni intrakranialni germinom pri otroku. Prikaz primera

Koren A

Izhodišča. Bifokalni primarni intrakranialni germinalni tumorji so redki, tako samo 5-10% vseh germinalnih tumorjev odkrijemo supraselarno in v področju češarike.
Prikaz primera. Predstavljamo primer bolnika z dvema primarnima intrakranialnima germinomoma v področju hipofize in češarike, ki sta bila operativno uspešno odstranjena. Opišemo tudi nekatere radiološke značilnosti germinomov in nekatere diferencialne diagnostične možnosti.
Zaključki. Čeprav končne histološke diagnoze intrakranialnih germinomov ni mogoče napovedati samo z računalniško tomografijo ali/in magnetno resonanco, lahko k dokončni diagnozi veliko pripomore natančna analiza nevroradioloških preiskav.

Radiol Oncol 2001; 35(3): 185-91.

Artefakti in kopičenje radiofarmaka izven kosti pri scintigrafiji okostja. Slikovni prikaz 20 primerov

Weiner GM, Jenicke L, Müller V, Bohuslavizki KH

Izhodišča. Številni možni artefakti lahko znatno otežijo oceno scintigrafije okostja.
Prikazi primerov. V članku smo naredili slikovni pregled običajnih in nenavadnih primerov, ki lahko zavedejo ocenjevalce scintigramov okostja. Zmotne interpretacije nastanejo ob povečanem ali zmanjšanem kopičenju radiofarmaka v mehkih tkivih ali v kosteh.
Zaključki. Posebno pozorni moramo biti na stanja, kjer bi morebitni artefakti privedli do nove (napačne) diagnoze in vplivali na nadaljnje (napačno) obravnavanje bolnika.

Radiol Oncol 2001; 35(3): 193-202.

Elektropermeabilizacija celic z majhnimi molekulami *in vitro*: vpliv električnih parametrov

Maček Lebar A, Miklavčič D

V članku predstavljamo sistematično študijo vloge različnih električnih parametrov (število, trajanje in amplituda električnih pulzov) v procesu elektropermeabilizacije celic DC3F za majhne molekule in vpliva teh parametrov na preživetje celic. Permeabilizacijo celic in njihovo preživetje smo določili za dvajset različnih naborov električnih pulzov. Število električnih pulzov smo spreminjali od 1 do 64, njihovo trajanje pa med 20 μs in 1 ms. Najpomembnejši električni parameter je amplituda električnih pulzov, saj sproži proces elektropermeabilizacije in proces umiranja celic. Tako v primeru elektropermeabilizacije celic kot tudi ob študiju celičnega preživetja smo ugotovili, da se parameter U_{50} (tj. amplituda električnih pulzov pri kateri je permeabiliziranih (ali mrtvih) 50 % celic v populaciji) ne spremeni, če uporabljeni vlak električnih pulzov sestavlja več kot 16 električnih pulzov. Ugotovitev je neodvisna od njihovega trajanja. Permeabilizacija je učinkovitejša, če uporabimo daljše električne pulze. Tako sistematično študijo vpliva električnih parametrov na elektropermeabilizacijo in preživetje celic lahko uporabimo kot osnovo za optimizacijo elektropermeabilizacijskih pogojev pri različnih aplikacijah elektropermeabilizacije.

Radiol Oncol 2001; 35(3): 225-30.

Radiol Oncol 2001; 35(3): 203-7.

Elektroporator za permeabilizacijo celičnih membran *in vitro*

Puc M, Flisar K, Reberšek S in Miklavčič D

Elektroporacija je pojav, pri katerem s kratkotrajnim visokonapetostnim električnim impulzom v membrani biološke celice povzročimo strukturne spremembe. Pore, ki nastanejo v plazmalemi, povečajo prepustnost, zato lahko snovi neposredno vstopajo v celico. Učinkovitost elektroporacije je odvisna od številnih parametrov, ki jih razdelimo na električne parametre in parametre, ki so povezani s stanjem celic in njihovo okolico npr. osmotski tlak, temperatura itd. V tem delu predstavljamo prototip elektroporatorja **GT-1** za *in vitro* elektroporacijo. Napravo smo zasnovali tako, da omogoča spreminjanje električnih parametrov na širokem področju. Poleg visokonapetostnih impulzov pa naprava proizvaja tudi nizkonapetostne impulze, ki se jih navadno uporablja za elektroforetični vnos nabitih molekul preko električno permeabilizirane plazmaleme.

Radiol Oncol 2001; 35(3): 209-14.

Zasevki malignega melanoma v kosteh. Retrospektivni pregled in analiza 28 primerov

Brountzos E, Panagiotou I, Bafaloukos D, Kelekis D

Izhodišča. Študijo smo izvedli z namenom, da bi ocenili klinične značilnosti, radiološke izvide in učinke zdravljenja bolnikov z zasevki malignega melanoma v kosteh.
Bolniki in metode. V 15-letnem obdobju smo retrospektivno pregledali 293 bolnikov z melanomom stadij IV.
Rezultati. Med pregledanimi primeri smo zasledili 28 bolnikov (9,5%) z zasevki na kosteh. Pri večini bolnikov je bil primarni melanom z veliko ali srednje veliko globinsko invazijo (Breslow 2.7-9.9), kostni zasevki so bili večinoma multipli (95,6%) in simptomatski (92,6). S slikovno diagnostiko smo odkrili 90 zasevkov v kosteh. Bolj pogosti so bili zasevki v aksialnem skeletu (86%), kar 54% smo jih odkrili na hrbtenici. Radionuklidna scintigrafija skeleta ni bila dovolj značilna, da bi lahko z njo diagnosticirali zasevke melanoma v kosteh, tako smo naredili tudi rentgensko slikanje in računalniško tomografijo kosti. Najbolj tipični zasevki v kosteh so bili osteolitični (92,5%). Z radioterapijo je bilo zdravljenih 66 zasevkov; paliativen učinek smo dosegli pri 79%. Med velikostjo celokupne doze ali doze na frakcijo obsevanja ter paliativnim učinkom nismo našli korelacije. Če smo bolnike ob obsevanju zdravili tudi s kemoterapijo in/ali z bifosfonati, nismo dobili večjega učinka zdravljenja. Srednji odgovor na zdravljenje je bil 2,6 meseca, srednje preživetje pa 4,7 meseca.
Zaključki. Zasevki malignega melanoma v kosteh se najpogosteje pojavljajo pri bolnikih z lokalno napredovalimi primarnimi tumorji. Največkrat so zasevki osteolitični in se najraje zasejejo v aksialni skelet. Diagnozo potrdimo z rentgensko preiskavo in računalniško tomografijo, najbolj priporočljivo zdravljenje pa je radioterapija.

Radiol Oncol 2001; 35(3): 225-30.

Radiol Oncol 2001; 35(3): 215-24.

Vrednotenje načrtov za obsevalno zdravljenje: fizikalni ali biološki pristop?

Ebert M

Izhodišča. Vrednotenje obsevalnih načrtov (planov) je v radioterapiji posebno pomembno, kadar so možni različni načini zdravljenja posameznega bolnika, ko ocenjujemo podatke o dozah, ki jih prejemajo bolniki v kliničnih študijah in pri določanju optimalnih načinov običajnega zdravljenja.
Metode. Naredili smo modele dozno volumskih histogramov (DVH) in pri tem upoštevali več fizikalnih in radiobioloških pokazateljev. DVH smo vrednotili glede na vsak posamezen dozni indeks. Pri vrednotenju smo spremljali tudi spreminjanje radiobioloških pokazateljev v odvisnosti od parametrov, ki smo jih v modelih uporabljali. Nadalje smo ocenjevali DVH pri načrtovanih tarčnih volumnih 18 bolnikov s karcinomom prostate, ki smo jih zdravili s teleradioterapijo.
Rezultati. Videli smo, da je vrednotenje obsevalnih načrtov neposredno odvisno od modelov, ki smo jih uporabili pri ocenjevanju doz v tarčnih volumnih oziroma od uporabljenih doznih indeksov. Pri vrednotenju DVH-ov v radiobioloških modelih opazimo močno odvisnost od radiobioloških parametrov, ki smo jih v modelih uporabili (posebno to velja za vrednosti "alfa" iz linearno kvadratnega modela).
Zaključki. Ko vrednotimo načrte za obsevalno zdravljenje, bodisi med načrtovanjem obsevanja ali pa ob ocenjevanju kliničnih študij, moramo biti posebno pozorni na modele, ki smo jih uporabili za izračun obsevalne doze.

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.*

Gastro-intestinal malignancies

October, 2001

The ESO training course will be offered in Cairo, Egypt.

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

Pediatric oncology

October, 2001

The ESO training course will be offered in Trabzon, Turkey.

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

Chest and head & neck

October 4-6, 2001

The "Second International Chicago Symposium Malihnancies of the Chest and Head & Neck" will take place in Chicago, USA.

Contact Center for Continuing Medical Education, The University of Chicago, 950 East 61st Street, Chicago, Illinois, USA; or fax +1 773 702 1736; or e-mail marlene@delphi.bsd.unchicago.edu; or see http://www.uchicago.edu/bsd/cme/

Breast cancer

October 4-6, 2001

The ESO training course will be offered in Sarajevo, Bosnia and Herzegovina.

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

Head and neck cancer

October 4-6, 2001

The "4th European workshop on Basic Biology of Head and Neck Cancer: Recent Advances in Molecular Biology" will take place in Amsterdam, the Netherlands.

Contact Marion van Zuilen; e-mail kno@nki.nl

Lung cancer

October 5-7, 2001.

The "International Conference on Non Small Cell Lung Cancer: Standards and New Trends in Diagnosis and Therapy" will be offered in Bialystok, Poland.

Contact Jacek Niklinski, MD, PhD, Dept. of Thoracic Surgery, Bialystok Medical University, M. Sklodowska-Curie Str. 15-276, Bialystoc, Poland, or call/fax +348 85 742 35 37; or e-mail niklinsj@cksr.ac.bialystok.pl

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Radiotherapy

October 7-11, 2001

The ESTRO teaching course "Evidence-Based Radiation Oncology: Principles & Methods" will take place in Cairo, Egypt.

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

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Radiotherapy

October 7-11, 2001

The ESTRO teaching course "Basic Clinical Radiobiology" 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

Renal carcinoma

October 9-11, 2001

The ESO training course "Renal Carcinoma" will be offered in Moscow, Russia.

Contact M. Vukelic, CSC Ltd., Heligenstadter Strasse 395b, 1190 Vienna, Austria; or call +43 1 369 0444; or fax +43 1 369 0444 20

Breast cancer

October 12-14, 2001

The "7th Annual Puerto Rico Breast Cancer Conference" will take place in San Juan, Puerto Rico.

Contact with e-mail genteinc@prtc.net

Malignant lymphoma

October 19-20, 2001

The ESO training course will be offered in Nicosia, Cyprus.

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

Radiation therapy

October 21-25, 2001

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

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

Lymphoma

October 26-28, 2001

The ESO training course "Non Hodgkin's Lymphoma, Patho-Biology, Classification and Clinical Relevance" will be offered in Cairo, Egypt.

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

Clinical trials

November, 2001

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

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

Psycho-oncology

November, 2001

The ESO training course will be offered in Nicosia, Cyprus.

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

Radiation therapy

November 4-7, 2001

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

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

Neck and thyroid surgery

November 5-7, 2001

The master course will be offered in Milan, Italy.

Call P. Lonati +39 (0)257 489 490; or fax +39 (0)257 489 589 491; or e-mail head&neck@ieo.it

Cancer risk

November 12-13, 2001

The ESO conference "Reducing Cancer Risk. Focus on the four big killers" will take place in New York, USA.

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

Radiation oncology

November 28-30, 2001

The ISRO teaching course "Radiation Oncology in the New Millennium: What have we learnt from evidence based medicine?" will take place in Mumbai, India.

Contact with e-mail info@isro.be; or see http://www.isro.be

Medical physics

November 30 - December 1, 2001

The "17th Symposium of the Belgian Physicists Association (BHPA)" will take place in Brussels, Belgium.

See http://www.md.ucl.ac.be/rbnt/bhpa/2001

Radiation oncology

November 30 - December 1, 2001

"Advanced Seminar on Stereotactic Radiosurgery and Radiotherapy " will take place in London, U.K..

Contact with e-mail anna.dowe@rmh.nthames.nhs.k; or see http://www. roylmarsden.org/training/courses

Lung cancer

December 6-8, 2001

The International Forum for Lung Cancer will be offered in Athens, Greece.

Contact Congress Secretariat - Organising Bureau, "MOEL" Ltd, 36, Eleon str. - GR 14564, Nea Kifissia, Greece; or call +301 6203 614; or fax +301 8078 342; or e-mail liagramo@internet.gr

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

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

Brachyherapy

March 24-26, 2002

The ESTRO teaching course "Endovascular Brachytherapy" will take place in Wien, 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

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

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

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

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 Lisboa, 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

Radiation 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

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

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

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

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.

See http://www.osg.ch/ictr2003.html

Lung cancer

August 10-14, 2003.

The "10th World Conference of the Internationač 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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Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education - A Report for the Srecond Quarter of 2001

The activity of the members of the "Dr. J Cholewa" Foundation for cancer research and education took a respite in the summer months of the year 2001, although a debate on several important issues continued without interruption. Among the topics discussed the most important were certainly the problems associated with changes in some of the donors' attitude towards the Foundation and the ways how to adapt to the new circumstances.

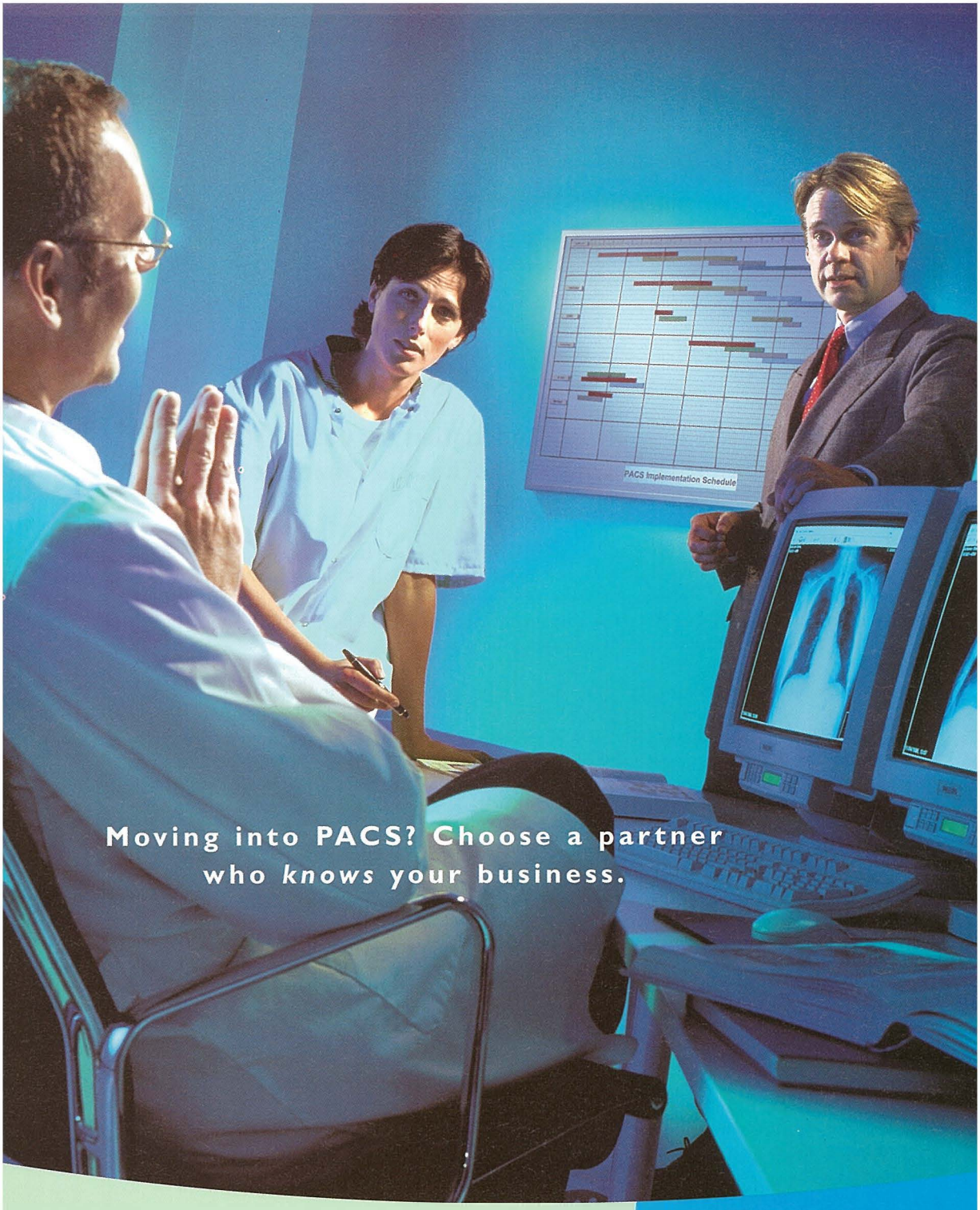
The Foundation plans to inaugurate some new forms of activity in the coming autumn of 2001 and later. Various oncology themes are to be presented in a newly formed type of scientific meeting with a day of lectures on the Medical Faculty in Ljubljana and dedicated to the memory of Dr. J. Cholewa. Lectures should are planned to be given by the recipients of the "Dr. J. Cholewa" Foundation awards and grants in order to spread their newly acquired knowledge in oncology to interested individuals from Slovenia and abroad.

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 shortly.

In the recent months the Foundation also gave financial support to the Second Central European Cancer Conference in Opatija, Croatia; International Congress on Medical Ethics in Bled, Slovenia; International Congress on Genetics in Bled, Slovenia; EASL International Symposium in Portorož, Slovenia; and to the First Slovenian-Croatian-Austrian Gastroenterology Symposium in Portorož, Slovenia, dedicated to the problems associated with pancreatic cancer. It should also be noted that a number of grants was awarded to experts from various parts of Slovenia in order to attend various oncological conferences and meetings around the world.

The Foundation moved back to its original headquarters in Mesesnelova Street No.: 9 in Ljubljana in the beginning of the year 2001. Despite these changes the Foundation will nevertheless continue to pursue its stated objectives.

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



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kriptokokni meningitis	*prvi dan 400 mg, nato od 200 do 400 mg na dan*
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Kontraindikacije: Preobčutljivost za zdravilo ali sestavine zdravila. **Interakcije:** Pri enkratnem odmerku flukonazola za zdravljenje vaginalne kandidoze klinično pomembnih interakcij ni. Pri večkratnih in večjih odmerkih so možne interakcije s terfenadinom, cisapridom, astemizolom, varfarinom, derivati sulfonilureje, hidroklorotiazidom, fenitoinom, rifampicinom, ciklosporinom, teofilinom, indinavirom in midazolamom. **Nosečnost in dojenje:** Nosečnica lahko jemlje zdravilo le, če je korist zdravljenja za mater večja od tveganja za plod. Doječe matere naj med zdravljenjem s flukonazolom ne dojijo. **Stranski učinki:** Povezani so predvsem s prebavnim traktom: slabost, napenjanje, bolečine v trebuhu, driska, zelo redko se pojavijo preobčutljivostne kožne reakcije, anafilaksija in angioedem – v tem primeru takoj prenehamo jemati zdravilo. Pri bolnikih s hudimi glivičnimi obolenji lahko pride do levkopenije in trombocitopenije in do povečane aktivnosti jetrnih encimov. **Oprema in način izdajanja:** 7 kapsul po 50 mg, 28 kapsul po 100 mg, 1 kapsula po 150 mg. Na zdravniški recept. 1/99.
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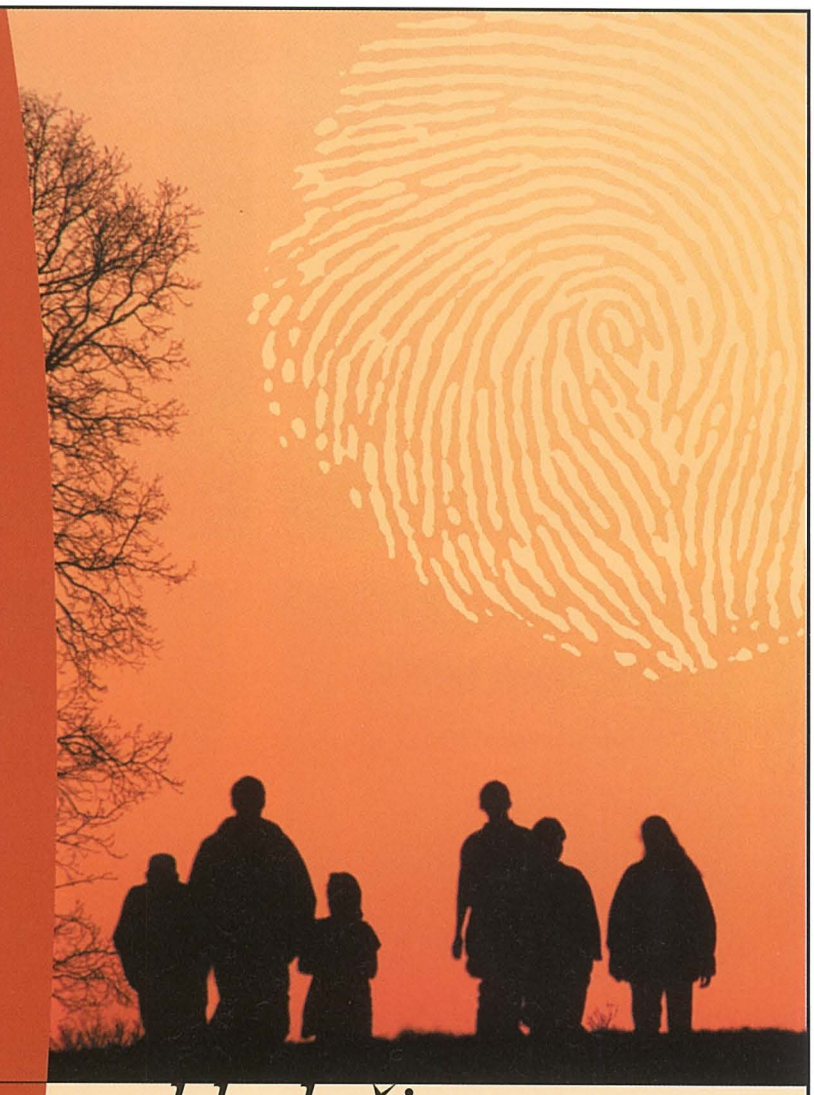

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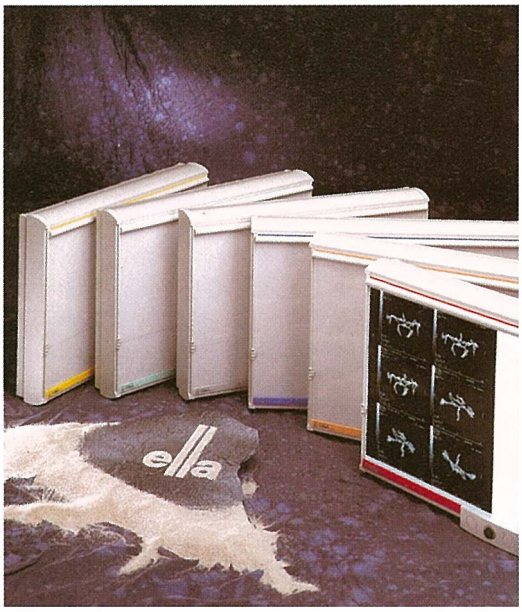

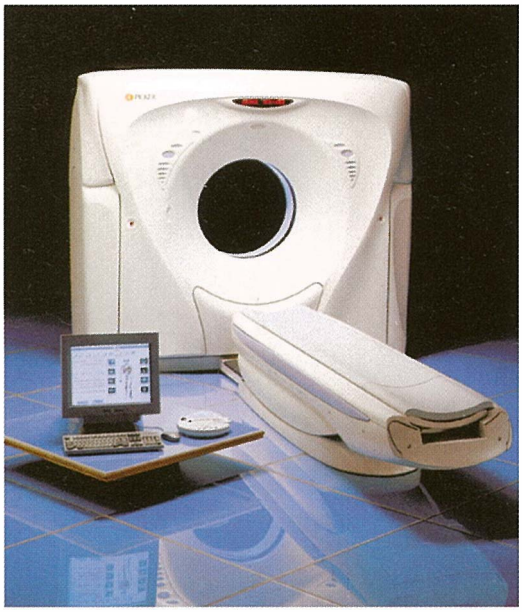

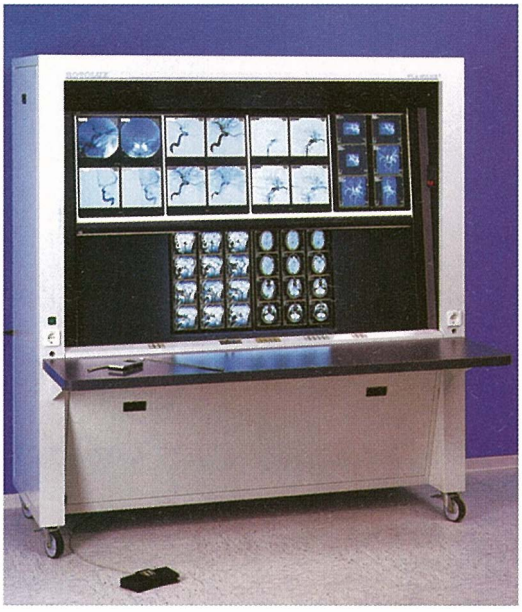

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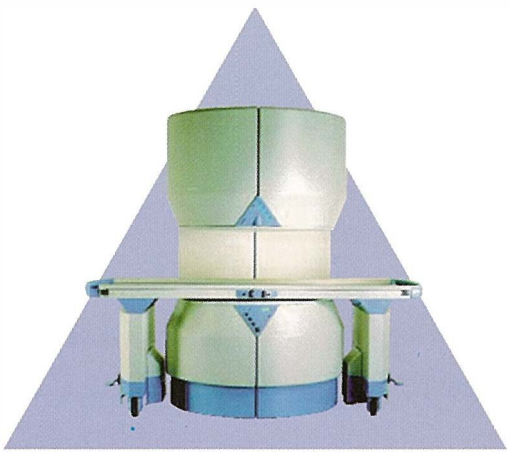

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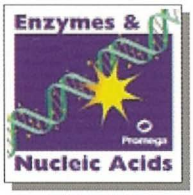

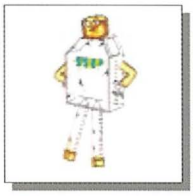

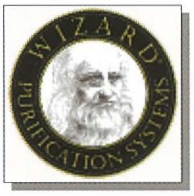

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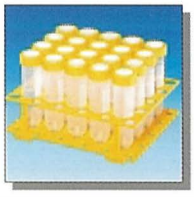

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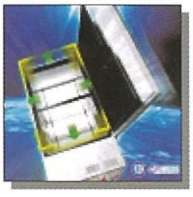

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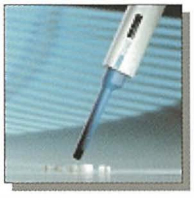

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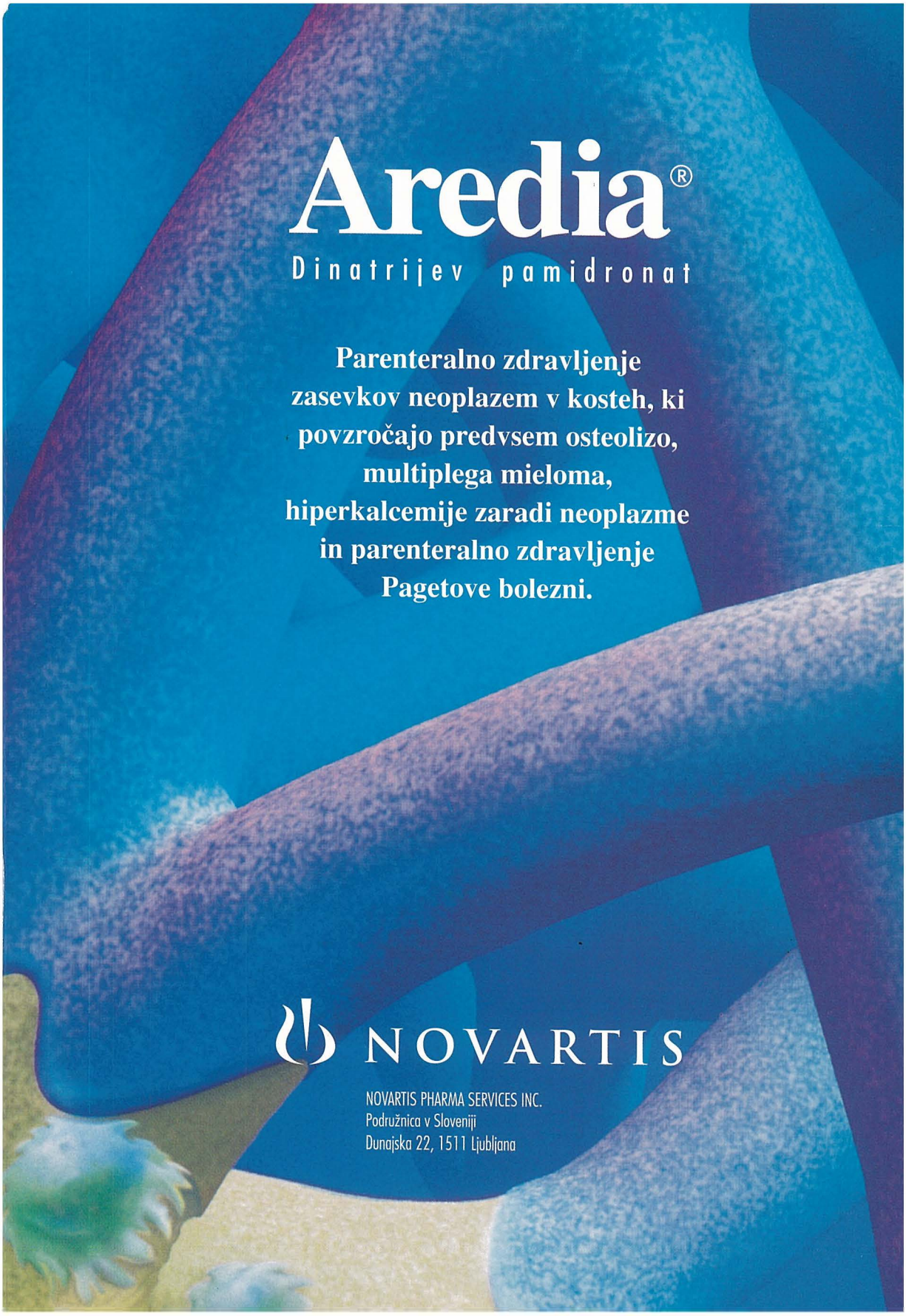
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