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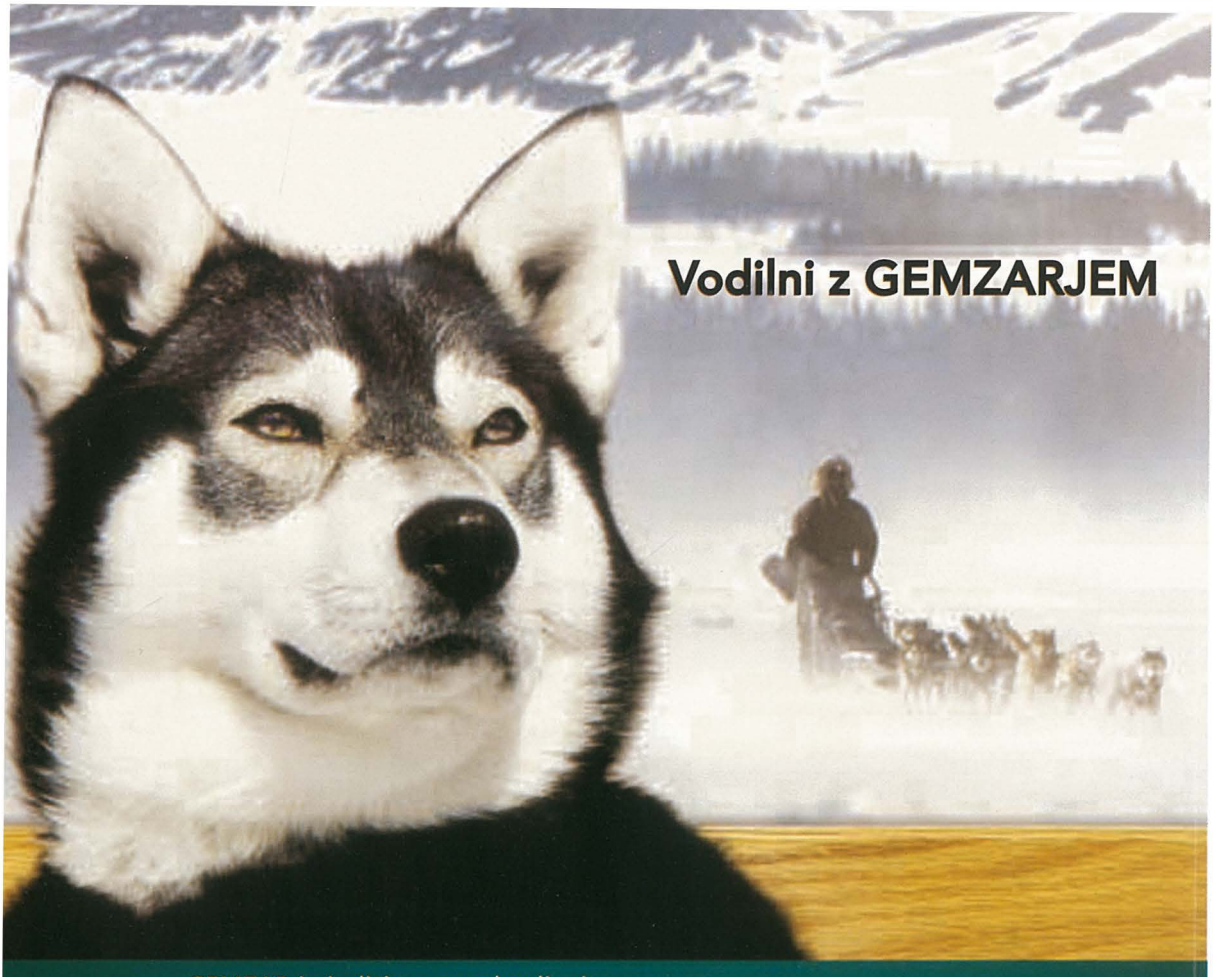

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Institute of Oncology
Zaloška 2
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Slovenia
Phone: +386 1 5879 369
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E-mail: gsersa@onko-i.si

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Imaging of small amounts of pleural fluid. Part one – small pleural effusions

Igor Kocijančič

Department of Radiology, Institute of Oncology, Ljubljana, Slovenia

Background. *Small pleural effusions are not readily identified on conventional radiographic views of the chest, but may be an important finding, sometimes leading, via thoracocentesis, to a definitive diagnosis of pleural carcinomatosis, infection or transudate.*

A small meniscus sign and a medial displacement of the costophrenic angle are the only subtle signs of small accumulations of fluid on posteroanterior chest X-rays. On lateral views the finding of a small meniscus sign in the posterior costophrenic angle is the sign of small pleural effusion.

Conclusions. *Lateral decubitus chest radiographs were used for many years for the diagnosis of small pleural effusions. In last decades ultrasonography of pleural space becomes a leading real-time method for demonstrating small pleural effusions.*

Key words: pleural effusion; thoracic radiography

Introduction

Small amount of fluid (5-20 ml) is often present in the pleural space of healthy individuals.[1] The data on the smallest amount of pleural fluid detectable by imaging methods vary considerably, but they are essentially within the same broad range whether computed tomography, sonography or X-ray examination are used.[2-10] With the advent of sonography it was shown that very small amounts of pleural fluid can be demonstrated on this way.[3-8]

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Correspondence to: Assist. Prof. Igor Kocijančič, MD, PhD, Department of Radiology, Institute of Oncology, Zaloška 2, SI - 1000 Ljubljana; Slovenia; Phone: + 386 1 587 9 505; Fax.: + 386 1 587 9 400; E-mail: ikocijancic(onko-i.si

In the literature there are only a few articles comparing the thickness of the pleural effusion as seen on sonography with X-ray and the amount of aspirated fluid. In addition, there is no clear consensus definition of a small pleural effusion on sonography. So, our term of small pleural effusions includes clinically silent effusions, which are usually unexpected findings on x-ray and/or ultrasonographic (US) examinations undertaken for different reasons.

Conventional chest radiography

a) Erect posteroanteror (PA) views

The term small pleural effusion cannot be used for the pleural fluid clearly visible on PA chest films, since it is known that the amo-

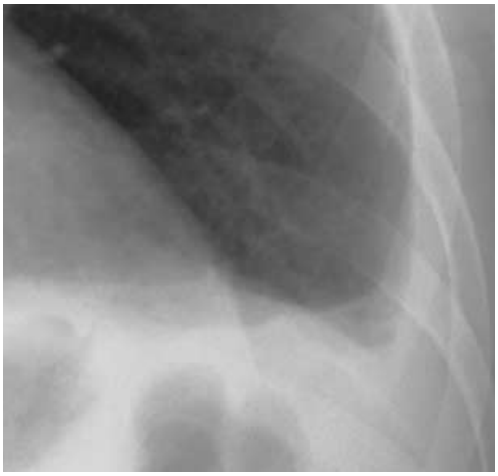

Figure 1a. Erect chet x-ray: a small meniscus sign in the left phrenicocostal sinus.

unts of 175 to 500 ml could be hidden in the pleural space on such views.[11] In the early stage with the patient in the upright position, the fluid tends to accumulate in the infrapulmonary position if the pleural space is free of adhesions and the lung is healthy, forming subpulmonary effusion. In general, it is agreed that gravity is probably the main factor of the location of fluid although some investigators implicated the elasticity of the lung, basal atelectasis and surface tensions as well.[12,13] Nearly simultaneously with the infrapulmonic accumulation, the pleural fluid will appear in the costophrenic sulcuses and can be seen as a medial displacement of the costophrenic angle first and with blunting of the diaphragm afterwards.[12]

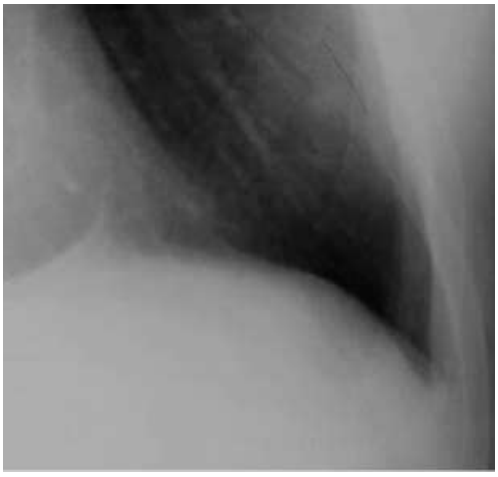

Figure 2a. Only medial displacement of costophrenic angle on erect chest X-ray.

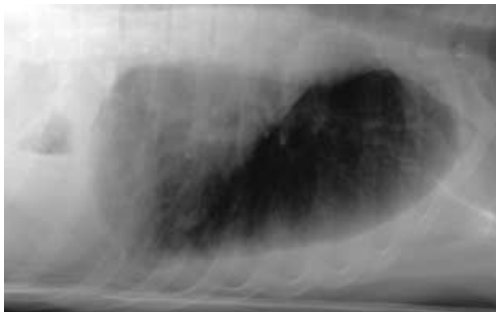

Figure 1b. Left lateral decubitus view: more than 1.5 cm thick fluid layer (approximatley 300 ml of pleural fluid).

Davis *et al*.[14] has shown that the upper limit of a free pleural effusion is horizontal and is located about the level of the apex of the meniscus shaped density. The x-ray beam traverses a greater depth of the fluid in the periphery of the thorax where the fluid is tangential to the beam.[15]

We proposed that *a small meniscus sign* (Figures 1a, 1b) and a *medial displacement of the costophrenic angle* (Figures 2a, 2b) are the only subtle signs of small accumulations of fluid on PA views. In these cases 200-300 ml of fluid can be evacuated from the pleural space[13,16] and that there is probably some residual fluid after thoracocentesis as well. We disagree with the authors who claim that a meniscus sign with blunting of one half of the he-

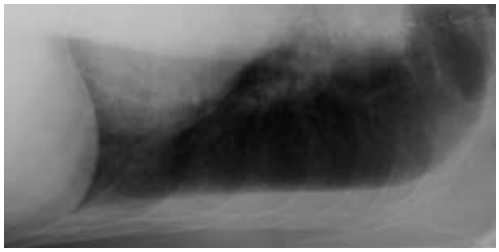

Figure 2b. About one cm thick fluid layer (approximatley 200 mm of fluid) on the the left lateral decubitus view.

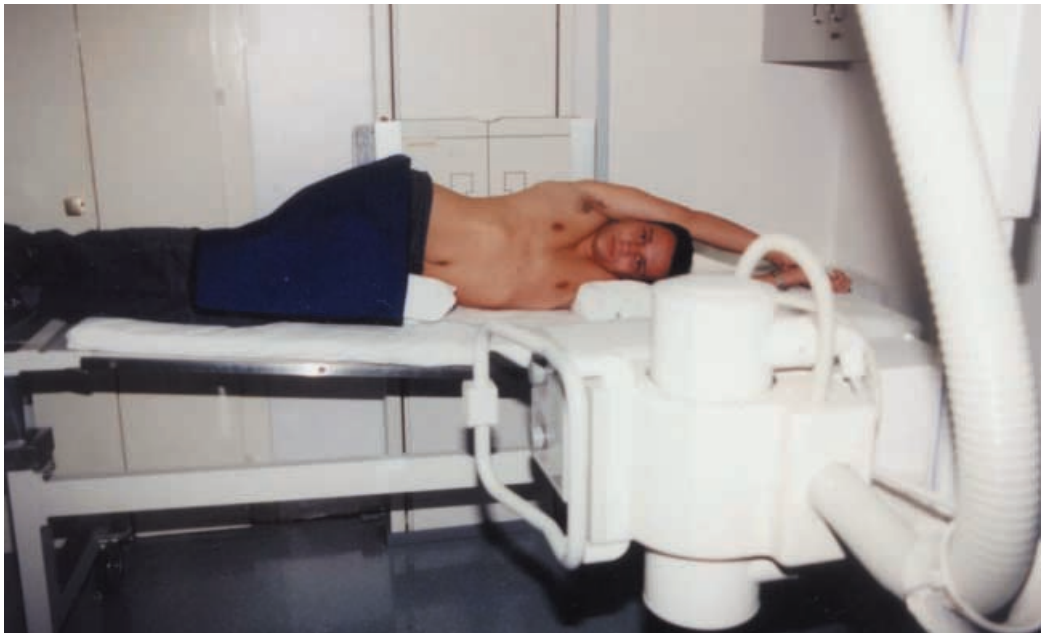

Figure 3. The patient's position during chest X-ray examination in left lateral decubitus position. After leaning 5 min with elevated hip in slight Trendellenburg position, the exposure with central X-ray beam aimed to the lateral thoracic wall was done.

mi diaphragm is the sign of small pleural effusion.[17,18]

b) Erect lateral views

In the study on roentgen pathology models Collins[11] showed that as little as 25 ml of pleural fluid (injected saline) on lateral erect chest radiograms could be detected as a subpulmonic accumulation of fluid in posterior costophrenic sulcus, but only with the presence of coexisting pneumoperitoneum. This is less reliable in practice, so we proposed the finding of a *small meniscus sign* in the posterior costophrenic angle as the sign of small pleural effusion on lateral views.

Some authors[13,19] also suggested that the junction of the major fissure with the diaphragm may commonly be the site of small amounts of small pleural effusions on lateral erect chest radiograms. The sign is described as a straight triangular shadow at the anterior diaphragmatic contour. We claim that it is difficult to interpret the sign without previous lateral chest x-rays and in the cases of superimposing fat in anterior mediastinum.

c) Lateral decubitus views

Lateral decubitus chest radiographs were used for many years for the diagnosis of small pleural effusions. This position was first mentioned in the work of Rigler.[20] Other investigators[19,21] have developed the technique and, using cadaveric studies,[2] have shown that volumes of pleural fluid as little as 5 ml may be detected. Rigler[20] did not use exposure in expiration, however, nor did he expose with central beam aimed at the lateral chest wall, parallel to the expected fluid level. The latter technical improvement was introduced by Hessen[19] together with the elevation of the patient's hip (Figure 3), while the exposure in expiration is mentioned in the work of Müller and Löfstedt,[21] but apparently without gaining wider acceptance.

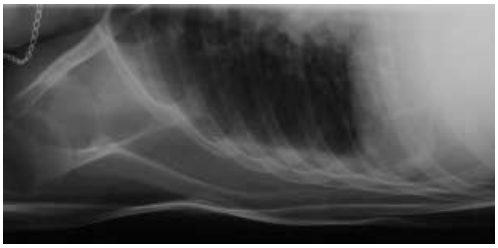

Figure 4. One cm thick fluid layer of pleural fluid in the right lateal decubitus position in a patient with systemic connective disease.

The amounts of pleural fluid detected this way have been assessed in cadaveric experiments[2] to as little as 5 ml in experimental conditions. This is probably less reliable in practice, due to the inexact results of thoracocentesis.

In the lateral decubitus position, the criteria for small pleural effusions *is a density of at least 3 mm (but not exceeding 15 mm) thick, with horizontal level* at lateral dependent chest wall (Figure 4).

The study of Kocijančič *et al.*[22] showed that lateral decubitus views taken in expiration contributed essentially to the diagnostic sensitivity of radiological examination as the fluid layer thickness changed during inspiration-expiration. The improved technique tends to facilitate the diagnosis of small pleural effusions (Figures 5a, 5b) and increased the ability to recognize artefacts such as skin folds, sheets and subcutaneous fat.

Chest ultrasonography

In last decades ultrasonography (US) of pleural space becomes a leading real-time method for demonstrating small pleural effusions.[23-26] US criteria determining pleural effusions are: *at least 3 mm thick anechogenic zone* between the parietal and the visceral pleura and/or *changing of fluid layer thickness between expiration and inspiration as well* as *changing with different positions* of the patient.[23-26] As US is a real-time method it is very important that all sonographic measurements with the probe perpendicular to the thoracic wall should be done.

Comparing chest US with expiratory lateral decubitus radiography, Kocijančič *et al.*[27] showed that both seem to be efficient methods for demonstrating small pleural effusions but US appears to assess the thickness of fluid layer more accurately than radiography does. It is interesting that in this study the main sign, allowing the demonstration of the smallest effusions, was similar in both modalities: the changing of the fluid layer during inspiration – expiration (Figure 6). The fluid layer thickness between 3-15 mm was found with both examination modalities. On erect chest radiograms only a medial displacement of costophrenic angle and a small meniscus sign were detected in 40% of patients.

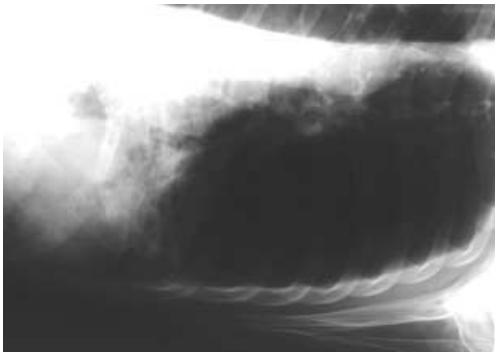

Figure 5a. Left lateral decubitus chest x-ray radiograph in a patient with left lower lobe lung cancer. The exposure taken during inspiration showed no pleural effusion.

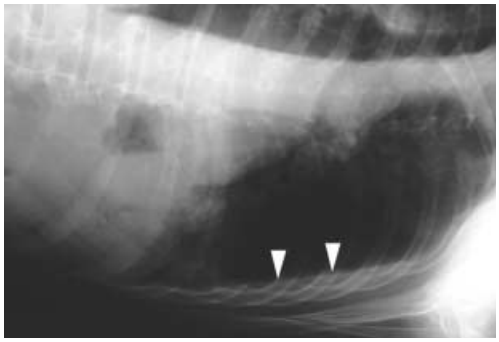

Figure 5b. Left lateral decubitus chest x-ray radiograph in a patient with left lower lobe lung cancer. The exposure taken during expiration clearly revealed approximatly 5 mm thick fluid layer.

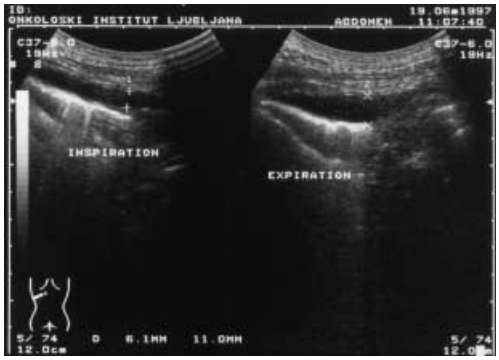

Figure 6. Sonograms in a patient with right upper lobe lung cancer. Images show a thin (6 mm; calipers) fluid collection during inspiration (left image) that was more conspicious (11 mm; calipers) during expiration (right image).

They have introduced a method of the US examination in the so called "elbow position".[27] The examination begins with the patient placed in the lateral decubitus position for 5 minutes first (similar to lateral decubitus chest radiography) and than the US examination performed with the patient leaning on the elbow (Figure 7). This manoeuvre allows the detection of small subpulmonic effusions, since the fluid tends to accumulate within the diaphragmatic pleurae in the erect position.

In the work of Wu *et al.*[28] so-called "fluid colour" sign was described as a useful indicator for discrimination between pleural thickenings and pleural effusion and a diagnostic aid to grey scale US for minimal or loculated pleural effusions. Our opinion is that this sign is not a useful diagnostic marker when the amount of fluid is very small.

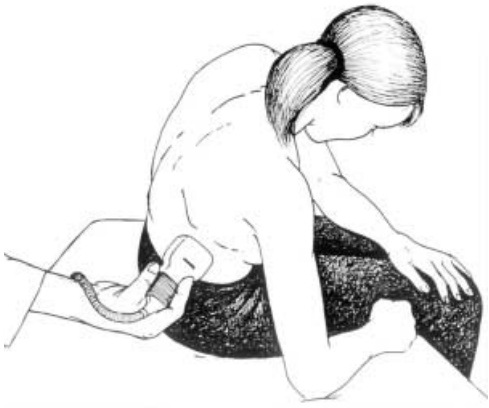

Figure 7. Diagram shows the "elbow position" with the placement of the transducer during examination of the right pleural space.

Conclusions

Both US and "classical" radiography seem to be efficient methods for demonstrating small pleural effusions. For satisfactory results, the meticulous adherence to the techniques described (exact position of the patient during lateral decubitus radiography and during chest US examinations, perpendicular US measurements) is probably the most important.

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Radiol Oncol 2005; 39(4): 243-7.

Ultrasonography of gallbladder in surgical patients with a prolonged stay (> 14 days) in the intensive care unit

Alan Šustić[1], Damir Miletić[2], Tedi Cicvarić[3]

[1]*Department of Anesthesiology and ICU, University Hospital Rijeka, Rijeka, Croatia*
[2]*Department of Radiology, University Hospital Rijeka, Rijeka, Croatia*
[3]*Department of Surgery, University Hospital Rijeka, Rijeka, Croatia*

Background. *The aim of this study was to establish the incidence of abnormal ultrasonographic (US) findings of gallbladder (GB) in surgical patients with the prolonged stay in the intensive care unit (ICU) and to correlate these findings with the severity of illness.*

Methods. *In the prospective study fifty-seven (57) adult surgical patients (male 66%; age 49±18 yr.) with the prolonged stay in ICU (>14 days) were analyzed. In all patients the US examination was performed on the 15th day of their stay in ICU. The presence of the following US findings was analyzed: GB wall thickening (?4 mm), biliary sludge, GB hydrops, striated GB wall and pericholecystitic fluid. The severity of illness was also evaluated on the 15th day of the stay in ICU using Simplified Acute Physiology Score (SAPS II).*

Results. *At least one abnormal US finding was found in 36 (63%), patients with GB wall thickening in 32 (56%), biliary sludge in 23 (40%), pericholecystitic fluid in 9 (16%), hydrops of GB in 7 (12%), and striated GB wall in 4 (7%) cases, respectively. Two to five US findings were found in 20 (35%) patients, three to five in 12 (21%), four to five in 10 (18%), while all five US findings were present in 4 (7%) cases. The patients with one and more US findings had significantly higher SAPS II than the patients who presented regular US findings of the GB (36±9 vs. 28±7; $p < 0.01$). The patients with two and more US findings had higher SAPS II than those with one or none US criteria (40±8 vs. 29±6; $p < 0.001$), while the patients with three and more had higher SAPS II than those with two, one or none (41±8 vs. 31±9; $p < 0.001$). The patients with four or five US findings had higher SAPS II than those with three or less (42±11 vs. 31±6; $p < 0.001$) while the patients with all five had higher SAPS II than all others (45±10 vs. 32±9; $p < 0.001$). A significant positive correlation between the number of US findings and SAPS II was present ($r = 0.57$; $p < 0.001$).*

Conclusions. *More than half of all surgical patients with the prolonged stay in ICU have GB abnormalities seen by ultrasonography; and it is in direct correlation with the severity of illness.*

Key words: gallbladder diseases ; ultrasonography; postoperative complications; intensive care units

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Correspondence to: Alan Šustić, MD, PhD, Dept. of Anesthesiology and ICU, University Hospital Rijeka, T.Strièića 3, Rijeka 51 000, Croatia, Europe; Phone/fax: +385 51 21 84 07; E-mail: alan.sustic@medri.hr

Introduction

Acute acalculous cholecystitis (AAC) is a serious complication in the treatment of critically ill patients. The incidence, published up to now varies from 0.2% up to 18% in patients

treated in intensive care unit (ICU).[1-3] Such variability is due to different incidences of AAC in different patients but also due to dissimilar criteria for the diagnosis of AAC. Ultrasonography (US) remains the method of choice for the diagnosis of AAC because it is a non-invasive, relatively inexpensive and transportable technique.[1-4] However, some recently published studies suggest that in critically ill patients it is not an optimal diagnostic method due to the low sensitivity and the high percentage of abnormal US findings of gallbladder (GB).[5,6]

The patients with the prolonged stay (>14 days) in ICU represent a specific group of critically ill patients with much higher incidence of complications and worse outcome but with a significantly more expensive treatment vs. other ICU patients.[7,8] On the other hand, those patients have many risk factors for abnormal US findings of GB as for example: total parentereal nutrition, hypoalbuminaemia, splanchnic ischemia/reperfusion injury, analgosedation, mechanical ventilation, infection, shock, sepsis, multiorgan failure, etc.[1,2]

The main aim of this study was to establish the incidence of abnormal US findings of GB in surgical patients with the prolonged stay (> 14 days) in ICU. The second point of this study is to correlate US findings of GB with the severity of illness in these patients at the time of the US examination.

Patients and methods

In the prospective study 60 consecutive adult surgical patients who stayed more than two weeks (14 days) in ICU were included. All were admitted in ICU after the urgent (36 cases) or elective major surgery (24 cases). Before we started the study we excluded patients under 18 years of age, neurosurgery or cardiac surgery patients, these with earlier cholecystectomy as well as the patients who at present hospitalization underwent pancreatobiliary surgery. During the study three cases of gallbladder calculosis, verified by the US examination were excluded. Finally, 57 surgical ICU patients (male 66%; age 49±18 yr.) were analyzed. Gallbladder US was performed in all patients on the 15th day of the stay in ICU. The patients were estimated by real-time ultrasound scan using a 3.5-5 MHz curved transducer (Hitachi 515 EUB; Tokyo, Japan). All examinations were performed by the same investigator (A.Š.). Standard subcostal cross-section was used, and the following US abnormality (*i.e.* criteria for AAC) were evaluated:[2-6, 9-12]

1. *Gallbladder wall thickening.* GB wall thickening was defined as thickening of GB wall in transverse diameter ≥ 4 mm.

2. *Increase of GB volume (hydrops)*. Increase of GB volume was defined as distension of GB in the longest diameter of ≥ 10 cm or when measured volume of GB was ≥ 100 ccm (ellipsoid formula was used for GB volume measurement).13

3. *Biliary sludge.* Biliary sludge was defined as an echogenic intraluminal sedimentation and gravity-dependence formation in GB.

4. *Layering or target phenomenon of GB.* Layering or target phenomenon of GB was defined as a linear hypoechogenic »halo« within the wall structure (»striated GB wall«).

5. *Pericholecystitic fluid.* Pericholecystitic fluid was defined as an anechogenic layer around the GB.

The severity of illness was estimated in all patients on the 15th day of their stay in ICU, using Simplified Acute Physiology Score II (SAPS II).[14] The mean simplified acute physiology score II (SAPS II) was 33±10.

Statistical analysis

All values are presented as number and percentages or mean value ± standard deviation. A statistical analysis was done with software Statistica 6.0 (StatSoft. inc.), using Mann-Whitney U for comparisons of quantitative

Table 1. The incidence of ultrasonography (US) criteria of acute acalculous cholecystitis (AAC) in 57 surgical ICU patients

	Yes	No
At least 1 US criteria for AAC	36 (63%)	21 (37%)
At least 2 US criteria for AAC	20 (35%)	37 (65%)
At least 3 US criteria for AAC	12 (21%)	45 (79%)
At least 4 US criteria for AAC	10 (18%)	47 (82%)
5 US criteria for AAC	4 (7%)	53 (93%)

Table 2. The frequency of abnormal ultrasonographic (US) findings of gallbladder (GB) in 57 surgical ICU patients

Abnormal US findings	Yes	No
GB wall thickening (%)	32 (56%)	25 (44%)
Biliary sludge (%)	23 (40%)	34 (60%)
Hydrops (distension) of GB (%)	7 (12%)	50 (88%)
Pericholecistitic fluid (%)	9 (16%)	48 (84%)
Striated GB wall (%)	4 (7%)	53 (93%)

variables of unpaired samples and Pearsonís moment for the estimation of correlation coefficients.

Results

The results are presented on tables 1, 2 and 3. At least one abnormal US finding of the GB was found in 36 (63%) patients, with GB wall thickening in 32 (56%), biliary sludge in 23 (40%), pericholecystitic fluid/oedema in 9 (16%), hydrops of GB in 7 (12%), and striated GB wall in 4 (7%) patients, respectively. Two to five US criteria of AAC were found in 20 (35%) patients, three to five in 12 (21%), four to five in 10 (18%), while all five US criteria were present in 4 (7%) cases. The patients with one and more US findings of AAC have had significantly higher SAPS II than the patients who presented regular US findings of the GB (36±9 vs. 28±7; $p < 0.01$). The patients with two and more US findings of AAC have had higher SAPS II than those with one or none criteria of AAC (40±8 vs. 29±6; $p < 0.001$), while the patients with three and more had higher SAPS II than those with two, one or none criteria (41±8 vs. 31±9; $p < 0.001$). The patients with four or five US criteria had higher SAPS II than those with three or less criteria (42±11 vs. 31±6; $p < 0.001$) while the patients with five had higher SAPS II than all others (45±10 vs. 32±9; $p < 0.001$). A significant positive correlation between the number of US criteria of AAC and SAPS II was present ($r = 0.57$; $p < 0.001$) (Figure 1).

Discussion

Acute acalculous cholecystitis is highly dangerous, often lethal complication during the intensive treatment of critically ill surgical patients.[1-3] Ultrasonography, as a noninvasive, simple, inexpensive and transportable method represents initial and most often used diagnostic option in the evaluation of critically ill patients with suspect AAC in ICU setting.[2-4] In many publications during the last 25 years US criteria for AAC are clearly defined: GB thickening, hydrops of GB, biliary sludge, striated GB wall and pericholecystitic fluid.[2-6, 9-12] Examining those criteria, one by one or summing them up, the majority of authors stressed the overall security of US. Hence, this bedside imaging method is nowadays accepted in many ICU as a basic diagnostic modality when evaluating critically ill patients with suspect AAC. [2-4, 9-12] Nevertheless, in majority of this studies the incidence of one or more abnormal US findings of GB were much higher than the expected incidence

Table 3. The relationship between ultrasonography criteria (US) of acute acalculous cholecystitis (AAC) and severity of illness (SAPS II) in 57 surgical ICU patients

	SAPS II		P value
	No	Yes	
≥ 1 US criteria for AAC	28±7	36±9	< 0.01
≥ 2 US criteria for AAC	29±6	40±8	< 0.001
≥ 3 US criteria for AAC	31±9	41±8	< 0.001
≥ 4 US criteria for AAC	31±6	42±11	< 0.001
≥ 5 US criteria for AAC	32±9	45±10	< 0.001

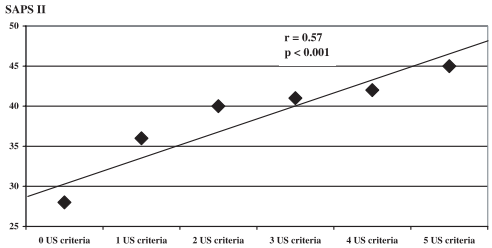

Figure 1. The correlation between the number of positive ultrasonography criteria (US) of acute acalculous cholecystitis (AAC) and severity of illnes (SAPS II) in 57 surgical ICU patients.

of AAC. For example, in the study of Molenat *et al* 50% of patients presented at least one from three major US criteria for AAC (GB thickening, hydrops of GB and biliary sludge was considered as major criteria); in the study of Imhof *et al* 80% of patients, while Helbich *et al* registered 90% of patients who had at least one US criteria for AAC.[9-11] Recently published investigations of Boland *et al* and Puc *et al* completely confirmed those results but with somewhat different conclusions.[5,6] Boland *et al* found in a heterogenic group of 44 critically ill patients 84% with one positive US criteria for AAC, 57% with three positive and 14% with all five positive US criteria.[5] The authors assume that many of findings are false positive and because of that conclude that US has a very limited value in diagnosing AAC in ICU patients.[5] On the other hand, Puc *et al* in the retrospective analysis of 62 critically ill injured patients found the very low sensitivity of US diagnostics of solely 30% (6/20) concluding that US has insufficient sensitivity to justify its use in diagnosing AAC in ICU patients.[6]

The patients with the prolonged stay (> 14 days) in ICU have many risk factors for abnormal US findings of gallbladder (GB), for example total parentereal nutrition, hypoalbuminaemia, splanchnic ischemia/reperfusion injury, analgosedation, mechanical ventilation, infection, shock, sepsis, multiorgan failure, etc.[5,9-12] To our knowledge this is the first study which analyses the incidence of abnormal US findings of GB in critically ill surgical patients with the prolonged ICU stay (> 14 days). Our results are similar to previously mentioned results. We also had over 60% of patients with at least one US criteria for AAC, 21% with three criteria and 5% with all five criteria. If we correlate these results with the expected incidence of AAC, which is approximately 1%,[1,2] we can conclude that on one patient with a true positive result (*i.e.* all five criteria) there are minimally four patients with false positive US findings. Thus, our research on a group of surgical patients with a prolonged ICU stay, confirms the conclusions of Puc *et al* that US has the unsatisfactory sensitivity in diagnosing AAC in ICU setting.[6]

In the presented study we have correlated US findings with the severity of the illness at time of the US examination (*i.e.* 15th day of stay in surgical ICU). The mean value of SAPS II in our patients was 33±10 with 63% of abnormal US findings. In the previously mentioned study published by Molenat *et al* the average SAPS I of patients was 13±3 (which corresponds to the approximate value of SAPS II about 35)[14] with 50% abnormal US findings.[9] In recent investigations of Mariot *et al* SAPS II of patients included in study was 36±11 (the criteria for inclusion in the study were positive clinical and laboratory symptoms of AAC) with even 85% of cases with abnormal US findings of GB while the sensitivity of US diagnostics was about 50%, respectively.[15] Summing up the results of our and these studies we may assume that at least 50% of patients staying in ICU with SAPS II over 35 will have abnormal US findings of GB independently of real AAC incidence. Moreover, in our study we found a statistically relevant positive correlation between positive US findings and the severity of illness, meaning that the patients with more positive US criteria for AAC had on average higher values of SAPS II. This result shows that the severity of illness is one of predictors for abnormal US findings of GB. Similar results were previo-

usly published by Pelinka *et al* who found the severity of illness as an independent predictor for abnormal US findings of GB in selective group of trauma patients.[16] Therefore, we can assume that abnormal US findings of GB will be more often possible, indeed more probable in patients who are more severely ill. However, this thesis should be adequately investigated and confirmed on various groups of ICU patients.

Summing up, more than half of all surgical patients with the prolonged stay in ICU have GB abnormalities seen by US, and these abnormalities are in a direct correlation with the severity of illness. At the end we can conclude that due to a great number of false positive results and low sensitivity US is generally of little benefit for the diagnosis of AAC in critically ill patients with the prolonged stay (>14 days) in ICU.

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case report

The usefulness of transrectal endosonography in differentiating an anal abscess from a rectal carcinoma. A case report

Malgorzata Kolodziejczak[1], Iwona Sudol - Szopinska[2]

[1]*Proctology, Sub-Department of General Surgery, City Traumatic Hospital, Warsaw, Poland*
[2]*Central Institute for Labour Protection – National Research Institute, Warsaw and Department of Imaging Diagnostics, Second Faculty, Medical School, Warsaw, Poland*

Background. *The high anal abscess might have not a typical, chronic clinical course, and its diagnosis may be difficult.*
Case report. *The authors describe a case of a patient with the initial diagnosis of rectal cancer. Because of non-specific clinical symptoms suggesting a high anal abscess with atypical, chronic course of the disease, additional investigations were suggested. The final diagnosis was high, submucous-intersphincteric abscess.*
Conclusions. *In the described case the most important ones turned out to be an exact finger per rectum examination, clinical proctologic assessment, and the transrectal ultrasound.*

Key words: anus diseases; abscess; rectal neoplasms; endosonography; diagnosis, differential

Introduction

The high anal abscess might have not a typical, chronic clinical course, and its diagnosis may be difficult. In the diagnostics of anal abscesses one of the most crucial things is early diagnosis, followed by its incision, without waiting for evident clinical symptoms. The longer the process last the greater is the risk of creation of the complex fistula. The treatment for such a fistula carries the risk of anal sphincters trauma. Another important thing is an exact definition of the type of anal abscess in order to plan the surgical approach.[1,2]

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Correspondence to: Assist. Prof. Iwona Sudol-Szopinska, MD, PhD, CIOP-PIB, ul. Czerniakowska 16, 00-701 Warsaw, Poland; Fax +48 2232 65991; E-mail: iwsud@ciop.pl

Case report

Half a year ago, a 46-year old woman was hospitalized at the Neurosurgical Department due to the dystaesthesia and numbness sensation in the lower limbs. The contrast examination led to the suspicion of a tumour in the right curvature of the colon. Colonoscopy did not, however, confirmed that diagnosis and it did not find any pathology.

A few months ago she was again admitted to the hospital, to the Oncological Department by her gynaecologist who on palpation confirmed the presence of a rectal mass. The contrast examination and colonoscopy were

repeated and have again not found any signs of rectal carcinoma.

Because she also had discrete symptoms of periodical discharge of puss from the anus, feeling of pressure against the walls of the anal canal, without neither pain nor fever, she was admitted to the Proctologic Department with the suspicion of a high abscess of the anal canal. The general examination of the patient did not show any abnormalities.

During a proctologic assessment, a hard mass covered by mobile mucous was palpated, about 3 cm from the anal verge, on the right side of the rectum. The lower margin of the mass was reaching level of the dentate line. Little discomfort was felt by the patient during palpation.

In the place corresponding to the painful area rectoscopy showed a bulg of mucosa, but the mucosa itself looked normal. No other abnormalities were found. Rectoscopy did not show any lesion within 10 cm of the colon, and a normal mucosa was seen covering all walls of the colon.

Biopsy specimens were taken from the painful and palpated lesion. The histopatological assessment confirmed the presence of normal fragments of the mucous membrane of the colon with small infiltrations of lymphocytes into the mucosa and submucosa.

Fiberosigmoidoscopy was performed next and the rectum and distal sigmoid colon were assessed. Bulbing of the rectal wall was again seen 5-6 cm from the anal verge, covered by swollen and congested mucous membrane. Biopsy specimens were taken. During the examination a leakage of puss content from the outlet was visualized 3 cm above sphincters. No other changes within the colon were found.

Computer tomography (layers 10 mm thick) confirmed the presence of a tumour localized just above the anal sphincters. Images were, however, not conclusive.

Transrectal ultrasound (TRUS) was performed on the BK Medical unit 3535 with 7 MHz endorectal mechanical probe. It showed the submucosal-intersphincteric abscess with diameters reaching 41x17x25 mm on the anterior-right anal wall 5-6 cm from anal verge (Figure 1a).

From its distal part a channel of an anal fistula crossing the internal anal sphincter at the level of puborectal muscle was originating. The internal fistulous opening was located between high and middle parts of the anal canal, on the right wall (Figure 1b). The ima-

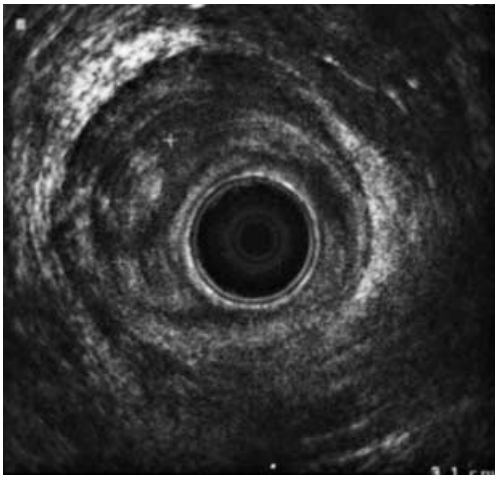

Figure 1a. Submucosal component of the abscess located on the right wall of the rectum, above the anal sphincters (crosses).

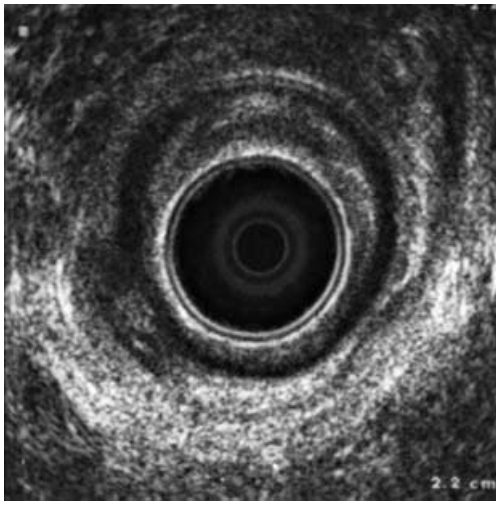

Figure 1b. Intersphincteric part with an anal fistula on the right wall.

ge was typical for high submucosal-intersphincteric abscess and fistula, and there were no suspected signs of rectal cancer.

The patient was classified for a surgical intervention. During surgery the submucosal compartment of the abscess was opened and its intersphincteric part was then visible. The incision was prolonged in the direction of the anal verge and the intersphincteric space was opened. Open wound was left to healing. The solid, hard fragments of circumferential tissues were taken to the histopathologic investigation.

The result of histopathologic investigation was following: fragments of mucous membrane of large intestine with signs of unspecific inflammation.

The postsurgical period was not complicated. The patient was sent home the third day after the operation.

Discussion

The presented case exemplifies a rare case of high abscess of anal canal about chronic, many months' course. The chronic inflammatory state caused the swelling and the induration of the circumferential tissues which were responsible for diagnostic difficulties to differentiate it from neoplastic tumour. The abscess of anus is in majority of the cases a disease about sharp course with main symptoms such as pain and temperature. In the presented case the clinical presentation was not, however, characteristic because the abscess was located above the dentate line. In this area there are no nerves responsible for pain sensation so the patient did not complain on pain, and only on periodical feeling of »dilating« in rectum.[3] The lack of other typical symptoms like fluctuation and redness of the perianal skin were other reasons which made the diagnosis more difficult. Although it should also be bear in mind that patients with Crohn's diseases have asymptomatic abscesses in 62% of the cases,[4] this patient did not, however, suffer from non-specific inflammatory bowel disease.

In many cases an anal fistula is the first symptom of an anal abscess. According to Choen *et al*[5] and Deen *et al*[6] these two diseases coexist in 50% and 45% of the patients, respectively. At the time of surgery for the anal abscess such a fistula remains unrecognized in the clinical examination in 18-95% of the cases, which leads to the recurrence of the abscess or fistula in 48-62% of the cases.[7]

The presence of discharge from anus might have helped here because it is often the first symptom of an intersphincteric abscess, but only under the condition that it spontaneously pierces through the anal crypt. In the presented case only occasionally the abscess emptied itself to the anal crypt, which was noted by the patient as the periodical leakage of pus from the anus, but it has never been accompanied by a high fever.

Anoscopy revealed an internal opening with puss sipping from it. TRUS is currently the most commonly used for the diagnostics of the anal canal diseases.[8-10] However, in the presented case, the detailed history of the disease and the exact proctologic assessment pointed to the inflammatory disease, atypical symptoms suggested initially a rectal cancer. TRUS immediately and easily helped with the differentiation of these two diseases, saving time and costs of further diagnostics. It also showed an excellent agreement with surgery in regard to defining anatomy of abscess and anal fistula, and helped planning the surgical approach. Simple drainage of the diagnosed abscess would be mostly insufficient and that is why the surgeons, relying on TRUS, broadened the cryptal outlet.

Conclusions

Transrectal ultrasound is an useful examination enabling the differentiation of rectal carcinoma from an abscess of the anal canal.

Aknowledgement

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review

New marker of angiogenesis CD105 (endoglin): diagnostic, prognostic and therapeutic role

Mateja Legan

Institute of Histology and Embryology, Medical Faculty University of Ljubljana, Ljubljana, Slovenia

Background. *The well established notion that malignant tumours depend on angiogenesis to grow and metastasize focused the investigators' 'interest on tumour vasculature' into visualization and validation. Pan-endothelial markers (CD31, CD34, F8) and CD105 are differentially expressed in angiogenic and normal vessel endothelial cells. Since the former are excellent markers for the normal vasculature, CD105 (endoglin) is more suitable for identifying tumour angiogenesis. Endoglin is a transforming growth factor (TGF) - beta binding receptor, preferentially expressed on endothelial cells of angiogenic tissues, essential for angiogenesis and vascular development.*

Conclusions. *Tumour microvessel density expressed by CD105 immunohistochemical staining in paraffin-embedded tissue sections correlates significantly with tumour aggressiveness and prognosis in many solid tumours. Also, targeting of tumour neovasculature specific antigens offers the possibility of future therapeutic approaches.*

Key words: neoplasms – blood supply; neovascularisation, pathologic; angiogenesis factor; prognosis

Importance of tumour angiogenesis

In 1971, Folkman proposed that tumour growth is dependent on angiogenesis.[1] Angiogenesis is an essential process in the progression of malignant tumours because solid tumours cannot grow beyond 1-2 mm in diameter without angiogenesis.[2] Tumour neovascularization promotes growth because the new vessels allow the exchange of nutrients, oxygen and waste products by a crowded cell population for which the simple diffusion is no longer adequate.[3] Next to perfusion effect, endothelial cells of vessels release important paracrine growth factors for tumour cells (like insulin growth factor-2, basic fibroblast growth factor, platelet-derived growth factors). By releasing collagenases, urokinases and plasminogen activators they facilitate spread of tumour into the adjacent fibrin-gel matrix and connective tissue stroma.[4,5]

Tumour neovasculature has structural and functional abnormalities, increasing the opportunity for tumour cells to enter the circu-

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Correspondence to: Mateja Legan, MD, PhD, Institute of Histology & Embryology, Medical Faculty, University of Ljubljana, Korytkova 2, SI-1000 Ljubljana, Slovenia; Phone: +386 1 543 7375; Fax: +386 1 543 7361; E-mail: mateja.legan@mf.uni-lj.si

lation.[6] They have the abnormal vessel wall: incomplete or missing endothelial lining, interrupted or absent basement membrane, lack of pericytes, pharmacological and physiological receptors. Abnormal vascular architecture with contour irregularities, tortuosity, elongation of vessels, as well as loss of hierarchy is found in tumour vasculature, plenty of arteriovenous shunts and abnormal vascular density (chaotic network). Altered morphology results in functional abnormalities: shunt perfusion, absence of vasomotion, unstable blood circulation, obstruction of microvessels by leucocytes and tumour cells. Changed tumour perfusion results in platelet aggregation, micro- and macrothrombosis and in the increase of viscous resistance. Consequences of increased vascular permeability are hemoconcentration, interstitial bulk flow, extravasation of blood cells and hemorrhages.[6]

Neoangiogenesis is often a significant independent prognostic indicator for both the overall and the disease-free survival. Intratumoural microvessel density (MVD) - commonly measured with a histomorphometric method on tissue sections - is a widely regarded predictor of tumour growth, metastasis and patient's survival. Many studies have shown that MVD correlates with tumour aggressiveness of many different tumour types.[4,7,8] Meta-analysis by Uzzan and coworkers[9] of 88 published studies on MVD as a prognostic factor in women with breast cancer showed that high MVD significantly predicted the poor survival.

Validating tumour angiogenesis

The most important question in validating tumour angiogenesis is what proportion of tumour vascular network is due to pre-existing parent tissue vessels or newly formed vessels. We know plenty of pan-endothelial markers, such as CD34 - a cell surface sialomucin-like glycoprotein expressed by endothelial cells, CD31 - platelet-endothelial cell adhesion molecule and von Willebrand factor - also known as F8.[10] These markers detect both, tumour and parenteral vessels, but the former not to the same degree. Assessing tumour microvessel density with immunohistochemistry by antibodies against CD31, CD34 and von Willebrand factor may not be accurate, since these markers are expressed also in normal vessels, and on the other hand, they are not always expressed in all tumour vessels.[11,12] Besides, they are generally better expressed in larger vessels than in microvessels.[13]

In summary, because endothelial cells are heterogeneous, the markers of normal endothelial cells are apparently unfit for the studies of angiogenesis in tumour tissues. The growth of tumours includes not only the increase of blood vessels in number, but also the change of protein molecules in structure of endothelial cells. An ideal marker for angiogenesis should detect the newborn vessel quality as well as its quantity.[14]

In last years, imaging of tumour neovasculature by targeting a proliferation-associated endothelial marker CD105, called also endoglin, gave fruitful results.[15] CD105 is a new kind of cell adhesion molecules, first found in a human pre-B cell line.[16] It is a receptor that is strongly up-regulated in proliferating endothelial cells, and - as such - an optimal indicator of proliferation of endothelial cells also in tumour neovasculature. In contrast to pan-endothelial markers, CD105 is preferentially expressed on endothelial cells of all angiogenic tissues, including tumours, but weakly or not at all with those of normal tissues,[17-20] giving the superiority of CD105 as a marker of tumour angiogenesis.

CD105 (endoglin) is a disulfide-linked homodimeric cell membrane glycoprotein of 180 kDa. It is a transmembrane phosphorylated glycoprotein, a component of the receptor complex of transforming growth factor (TGF)-β, which is a pleiotropic cytokine that modulates

angiogenesis by the regulation of different cellular functions, including proliferation, differentiation and migration.[19] CD105 binds several components of the TGF-β superfamily, in particular TGF-β1 and TGF-β2. The overexpression of CD105 antagonizes several cellular responses to TGF-β1, while down-regulation of CD105 potentiates cellular responses to TGF-β1.18 Endoglin is essential for angiogenesis and vascular development.[21-23] The inhibition of CD105 expression on human umbilical vein endothelial cells (HUVEC) by an antisense approach, enhanced the ability of TGF-β1 to suppress their growth, migration and capacity to form capillary tubes. Much evidence supports an important role of endoglin in cardiovascular development and vascular remodelling in humans and chicken.[24,25] Endoglin is highly expressed at the endocardial cushion during heart septation by mesenchimal cells[24] and it is up-regulated in response to tissue injury and atherosclerosis.[26-28] The gene of CD105 is located on 9q34.[20] The loss of function in the human endoglin gene causes hereditary hemorrhagic teleangiectasia Type 1.[23,29]

The detection of CD105 with immunohistochemical staining using anti-endoglin monoclonal antibody shows that CD 105 is almost exclusively expressed on endothelial cells of both peri- and intratumoural blood vessels.[18] Staining is very selective for the blood vessel endothelium and reacts specifically with endothelial cells without the significant cross-reactivity of inflammatory or stromal cells within the neoplasm.[30,31] Endoglin staining reduces false-positive staining of blood vessels compared with other commonly used panendothelial markers. It can be readily performed on formalin-fixed, paraffin-embedded tissues. It is a good illuminator of tumour vasculature in solid malignancies (Figure 1).[17-19] CD105 is also expressed on non-endothelial cells including haemopoetic progenitor cells, fibroblasts, follicular dendritic cells, melanocytes, vascular smooth muscle cells, macrophages and mesangial cells, however, this expression is very weak.[18,19] Since CD105 is expressed on the most immature cellular subtypes in acute leukaemias, it can also be used in diagnozing haemopoietic tumours.[16,19]

The experience shows that, in many types of cancer, MVD counted by CD105 is a better estimator of tumour prognosis and survival than MDV counted by pan-endothelial markers. In colorectal cancer CD105 demonstrated significantly more proliferating neoplastic microvessels than CD31 and was a more specific and sensitive marker for tumour angiogenesis than commonly used panendothelial markers.[31] Also CD105, but not other markers, correlated significantly with liver metastases and lymph node invasion. Akagi *et al*[32] quantified MVD detected using monoclonal antibodies CD34 and CD105 in 54 cases of colorectal adenoma and in 20 cases of carcinomas. A significant increment of MVD detected by anti CD105 was found from low-grade to high-grade dysplasia and from high-grade dysplasia to carcinoma. In contrast, no significant difference of MVD assessed by anti CD34 was observed in the colorectal adenoma-carcinoma sequence. Microvessels positive for CD105 were preferentially observed on the surface area of adenomas (whereas CD34 staining was distributed uniformly in the sections), suggesting that angiogenesis mainly took place in this area.

The similar findings were shown in patients with head and neck squamous cell carcinomas,[33] where patients with high CD105-MVD had a significantly shorter disease-free interval and overall survival; but CD34-MVD was not associated with the survival. The evaluation of angiogenesis in non-small cell lung cancer,[34] determined with CD105 as well as CD34 immunostaining, also proved CD105 expression superior in the evaluation of angiogenesis. Five- year survival rate was significantly lower in patients with high CD105 expression regarding patients with a low CD105 expression. The difference in the longevity of

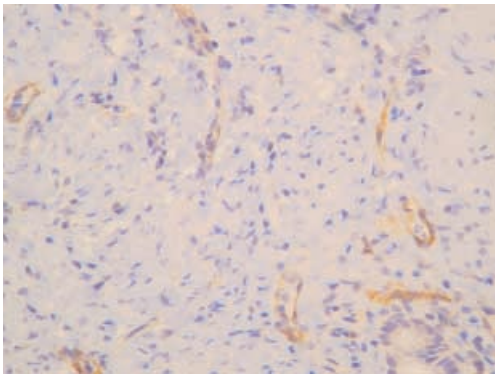

Figure 1. Expression of CD105 on endothelial cells of tumour vessels in gallbladder carcinoma (immunohistochemical staining using anti-endoglin monoclonal antibody on paraffin-embedded tissue section), magnification: x400.

survival between patients with high CD34 expression and low CD34 expression was, however, the same, but statistically insignificant. The hypothesis that the use of CD105 antibody should reduce the incidence of false-positive staining of normal blood vessels entrapped within a tumour and those located within the close vicinity of a cancerous mass was confirmed in the study of Kumar *et al*[35], who reported that vascular density determined using CD105 antibody correlates with the tumour prognosis in breast carcinoma.

However, not in all types of tumours MVD correlated with the prognosis. There was also a lot of discrepancies between different studies due to the diversity of technical approaches, variation in tissue pre-treatment protocols and non-standardized counting methods.[35,36] There is a trend to standardise the procedures so that results from different studies would be comparable. In clear cell renal carcinoma,[37] there was the inverse relationship between MVD and patient's survival: tumours with higher vascular density were associated with a greater post-operative 5-year survival rate than tumours with lower vascular density. Decreased MVD was associated with tumour fibrosis (which has morphological effect of decreasing MVD in a given tumour) and the development of large diameter vascular channels. It was concluded that the association between tumour microvessel density and the prognosis is not identical for all forms of malignancy but may be modified by architectural remodelling during tumour evolution. Besides, lower scores of MVD-CD105 were found in larger sized and more aggressive hepatocellular carcinomas,[38] however, the study did not provide significant any prognostic information. But active angiogenesis as highlighted by diffuse CD105 staining microvessels in the adjacent non-tumorous liver tissues was predictive for the early recurrence in this study.

Clinical potential of CD105 in human malignancies

As angiogenesis is crucial for tumour development and progression, the antiangiogenic therapy represents a promising approach for the cancer treatment. CD105 therapeutic targeting was investigated *in vitro*[39] and in animal models.[14,40,41]

She *et al*[42] investigated the mechanisms by which anti-endoglin monoclonal antibodies (mAbs) - termed SN6 series mAbs, suppress the growth of proliferating endothelial cells. They found that four SN6 series mAbs suppressed the growth of human umbilical vein endothelial cells (HUVECs) in a dose-dependent manner. Matsuno and co-workers[39] induced a long-lasting complete regression of distinct solid tumours in immunodeficient mice with the intravenous administration of antiendoglin conjugates, but not with the control conjugate. The same *in vivo* evidence was shown in a canine mammary carcinoma model.[40] In fact this study[40] was the first *in vivo* evidence that targeting of CD105 could represent an effective strategy to image solid malignancies. The antiangiogenic therapy of the mouse chimeras bearing established human skin tumours using various anti-endoglin monoclonal antibodies SN6, was effecti-

ve in the suppression of tumours. The efficacy was enhanced by combining a chemotherapeutic drug (cyclophosphamide).[43] Nowadays, next to systemic intravenous drug approach, a transcriptional targeting of conditionally replicating adenovirus with drug-substance to dividing endothelial cells is possible. Savontaus *et al*[41] utilized the regulatory elements of endoglin genes to construct two conditionally replicating adenoviruses (CRAD). *In vitro* studies it was demonstrated that both CRADs controlling the endoglin promoter, inhibited by 83-91% the capillary network formation in an *in vitro* angiogenesis assay in HUVECs, compared with the non-replicating control virus. This principle may be incorporated into novel therapeutic agents to develop anti-angiogenic treatment for cancer.

Endoglin has been detected also in the circulation of cancer patients, next to some other angiogenic growth factors. Increased CD105 in the circulation of patients with cancer results from angiogenesis both within and the immediate vicinity of the tumour mass. The main question was whether soluble CD105 levels associate with the disease progression. Li *et al*[44] demonstrated in 92 breast cancer patients that serum CD105 might be a valuable novel angiogenetic marker for identifying high risk breast cancer patients, since plasma levels of soluble CD105 (measured with indirect ELISA assay) correlated with metastasis. In 2001, Takahashi *et al*[45] reported about the association of serum endoglin with metastasis in patients with colorectal, breast and other solid tumours. In addition, they showed that chemotherapy exerts a suppressive effect on the serum endoglin. They suggested that serum endoglin may be a useful marker for monitoring early signs of metastasis and cancer relapse in a long-term follow-up of solid tumour patients. In 2003, Li and his group[36] compared the expression of CD105 in vasculature of resected colorectal cancer by MVD assessment and CD105 levels in the blood of the patients. CD105-MVD was an independent prognostic parameter for the survival of patients with colorectal cancer, and the plasma levels of CD105 were useful parameter for assessing the disease progression (serum-CD105 positively correlated with Dukes' stage).

Conclusions

It is likely that evaluating tumour angiogenesis will become an integral part of more consistent tumour staging system and routine prognostic evaluation. CD105 (endoglin) is proliferation-associated endothelial cell adhesion molecule, showed as an optimal indicator of tumour neovasculature. Moreover, targeting of tumour neovasculature specific antigens (like CD105) offers the possibility of future therapeutic approaches.

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review

MHC class II molecules and tumour immunotherapy

Irena Oven

University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Domžale, Slovenia

Background. *Tumour immunotherapy attempts to use the specificity and capability of the immune system to kill malignant cells with a minimum damage to normal tissue. Increasing knowledge of the identity of tumour antigens should help us design more effective therapeutic vaccines. Increasing evidence has demonstrated that MHC class II molecules and $CD4^+$ T cells play important roles in generating and maintaining antitumour immune responses in animal models. These data suggest that it may be necessary to involve both $CD4^+$ and $CD8^+$ T cells for more effective antitumour therapy. Novel strategies have been developed for enhancing T cell responses against cancer by prolonging antigen presentation of dendritic cells to T cells, by the inclusion of MHC class II-restricted tumour antigens and by genetically modifying tumour cells to present antigen to T lymphocytes directly.*

Conclusions. *Vaccines against cancers aim to induce tumour-specific effector T cells that can reduce tumour mass and induce development of tumour-specific T cell memory, that can control tumour relapse.*

Key words: neoplasms; immunotherapy, adoptive; CD4 – positive T – lymphocytes; T – lymphocytes, helper – inducer; cancer vaccines

Introduction

Immunotherapy denotes a strategy for manipulating a patient's immune response. In cancer or infectious disease the approach is designed to boost the patient's response to tumour antigens or pathogens.[1] Many strategies for enhancement of the immune response to autologous tumours have recently been developed. These strategies use tumour cells transfected with genes encoding molecules that enhance immune responses.[2] Tumour specific immunity is mediated by T lymphocytes. T cells play a major role in the antitumour immune response and surveillance and represent an important basis for the development of cancer immunotherapy.[3] Identification of immunogenic tumour antigens has significantly advanced our understanding of tumour immunity and provides opportunity for the development of effective antigen-specific cancer therapy.[4] Since most cancers do not express major histocompatibility complex (MHC) class II molecules on their surface and $CD8^+$ cytotoxic T lymphocytes (CTLs) are able to induce lysis of tumour cells upon recogni-

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Correspondence to: Irena Oven, University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Groblje 3, SI-1230 Domzale, Slovenia; Phone: +386 1 7217 916; E-mail address: irena.oven@bfro.uni-lj.si

tion of tumour antigen derived peptides, presented by the tumour's MHC class I molecules, the research has been focused mainly on modulation and use of MHC class I antigen presenting pathway for tumour immunotherapy. However, clinical trials using MHC class I restricted antigens have elicited only modest and transient immune responses in most immunized patients. A possible reason for this failure is the lack of tumour specific $CD4^+$ T cell responses[5], so recently a lot of progress has been made in acknowledging the importance of MHC class II molecules in mediating antitumour immune response.[6-8]

MHC class I and MHC class II antigen presentation pathways

The MHC is a large multigene family that encodes cell surface glycoproteins involved in binding and presentation of antigenic peptides to T lymphocytes. MHC class I molecules, which are expressed on most nucleated cells, present peptides to $CD8^+$ cytolytic T lymphocytes (CTLs). In contrast, the constitutive expression of MHC class II molecules, which are essential for antigen presentation to $CD4^+$ T helper (T_H) cells, is restricted to antigen presenting cells, such as dendritic cells, B cells, monocytes, macrophages and thymic epithelial cells. Expression of MHC class II molecules can however be induced by interferon-γ (IFN-γ) on most other cell types.[9] Class II molecules usually present exogenously synthesized peptides, which are acquired in the cellular compartment for peptide loading, whereas class I molecules usually present endogenously synthesized self-peptides.[10] Endogenous antigens are degraded by proteasome into short peptides. These peptides are transported into the endoplasmic reticulum (ER) by TAP complex. Here the newly synthesized MHC class I heavy chains assemble with the light chain and peptide and this complex is transported to the cell surface for presentation to $CD8^+$ CTLs. MHC class II molecule is usually unable to bind endogenous peptides, because the peptide antigen binding groove is occupied by invariant chain (Ii) molecules in the ER. This assembly stabilizes the MHC II complexes and its CLIP region prevents the binding of endogenous antigen peptides present in the ER. Ii also contains two sorting signals in its cytoplasmic tail, which are responsible for the transport of the MHC/Ii complexes into endosomal and lysosomal compartments, where Ii is degraded by cathepsins and only CLIP peptide is left in the binding groove. HLA-DM then catalyses the release of CLIP, allowing the groove to bind the antigen-derived peptides, which come from the lysosome (Figure 1).[5,11,12]

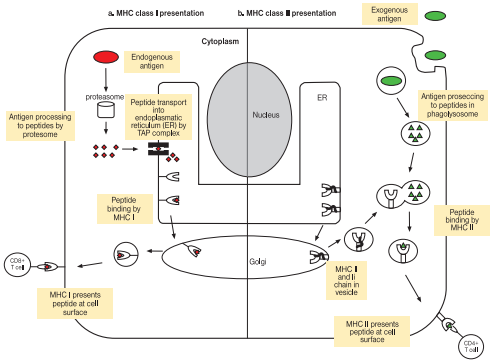

Figure 1. MHC class I and class II antigen processing and presentation pathways. (a) Proteasome degrades endogenous antigens into peptides, which are transported into the ER by TAP complex. Here the newly synthesized MHC class I molecules assemble with peptide and the MHC-peptide complex is transported through the Golgi to the cell surface for presentation to $CD8^+$ T cells. (b) Exogenous antigens are taken in by endocytosis and processed by proteases in an endosome into short peptides. The alpha and beta chains of MHC class II, along with an invariant chain, are synthesized, assembled in the endoplasmic reticulum, and transported through the Golgi apparatus to reach the endosome, where the invariant chain is digested, and the peptide fragments from the exogenous protein are able to associate with the MHC class II molecules, which are finally transported to the cell surface for presentation to $CD4^+$ T cells.

The role of CD4+ T cells in immunity

$CD4^+$ T lymphocytes play a central role in the onset and maintenance of adaptive immunity. $CD4^+$ T cells help antibody responses and also help the activation and expansion of $CD8^+$ T cells and are essential in maintaining the $CD8^+$ T cell memory and long-lasting anti-tumour immune response (Figure 2).[5,13]

$CD4^+$ T cells can be divided into two main subsets: T_H1 and T_H2, depending on the cytokines they produce in response to antigen activation. T_H2 produce IL-4 and IL-5. IL-4 activates B cells to become antibody secreting plasma cells. IL-5 is a growth and activation factor for eosinophils. It has been reported that a significant cytotoxicity against tumour cells can be mediated by eosinophils after IL-5-mediated *in vivo* activation and that eosinophils may be involved in the antitumour response *in vivo*.[14] T_H1 cells produce IL-2, IL-12 and IFN-γ, which are important for cellular immunity. IL-2 has been used in several studies in which its administration facilitates tumour eradication.[13,15] IL-12 plays an essential role in the interaction between the innate and adaptive immunity. IL-12 acts on T cells and NK cells by inducing proliferation and production of cytokines, especially IFN-γ. IL-12 is also the major cytokine responsible for the differentiation of T_H1 cells, which are potent producers of IFN-γ. In experimental tumour models, recombinant IL-12 treatment has a dramatic anti-tumour effect on transplantable tumours, on chemically induced tumours, and in tumours arising spontaneously in genetically modified mice.[16] IFN-γ also plays an important role in tumour rejection. IFN-γ could have direct effects on tumour cells by (a) cytotoxic activity on tumour cells, mediated by production of oxygen derivatives and nitric oxide, (b) up-regulation of MHC class II molecules expression, thus increasing tumour cell recognition and elimination, (c) alteration of the endogenous antigen-processing machinery, and (d) induction of inhibitors of angiogenesis in the cells.[13,17]

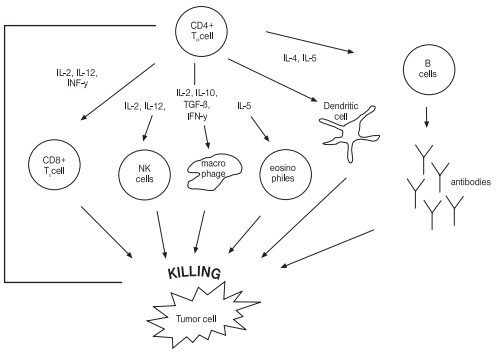

Figure 2. The role of $CD4^+$ T cells in immune response against cancer. T_H cells play a crucial role in regulating the host immune response. They help B cells to produce antibodies, they release cytokines, which stimulate other varieties of immune cells to kill the invading tumour cell. They provide help in stimulating $CD8^+$ CTL, which directly kill tumour cells. Finally, T_H cells are also involved in the inhibition of tumour growth in the absence of $CD8^+$ T cells.

The importance of $CD4^+$ T cells in response to tumours and protection against tumour growth is now widely recognized. Strategies have evolved to generate tumour cells that can directly present tumour peptides and specifically activate tumour-specific $CD4^+$ T_H cells. This approach is based on the assumption that the effectiveness of $CD8^+$ T cells is dependent on sufficient help from tumour-activated $CD4^+$ T cells, and that optimal immunological memory can be generated if both $CD4^+$ and $CD8^+$ T cells are stimulated.[18]

Tumour immunotherapy by modulating MHC class II gene expression in tumour cells

Down regulation of MHC class I or class II expression is one way for tumours to escape immunosurveillance. Whereas some tumours do express variable levels of MHC class II molecules, they often up-regulate expression of the Ii protein and thus prevent MHC class II presentation of endogenous tumour antigens.

Tumour cells that co-express class II and Ii molecules, such as B-lymphomas, are not capable of directly presenting tumour peptides and are thus no more immunogenic than class II negative tumour cells.[19] Melanoma tumours also express MHC class II molecules, which can present tumour antigens. However, as these tumours lack co-stimulatory molecules that are necessary to activate naïve $CD4^+$ T cells, such as B7 ligand, this may result in tumour antigen presentation and the induction of tumour antigen-specific $CD4^+$ T lymphocyte anergy. Through these mechanisms the MHC class II molecules may participate in melanoma progression and immune escape.[20,21] In contrast, high levels of MHC class II expression in gastrointestinal and breast cancers are often associated with better prognosis, showing the involvement of $CD4^+$ T cells in protective immune response against the tumour.[22,23] In fact several groups have published to successfully treat MHC class II negative tumours by converting them into MHC class II positive and thus making them APCs.[7,24]

MHC class II gene expression is regulated mainly on the transcriptional level. One of the most important factors is the class II transactivator (CIITA), which acts as coactivator by virtue of its ability to interact with other components of the MHC class II enhanceosome, which are present on MHC class II promoters. CIITA is a non-DNA binding protein and controls constitutive and inducible MHC class II gene activation. Coinciding with MHC class II expression, the constitutive expression of CIITA is confined to APCs only, and CIITA expression can be induced by IFN-γ in various other cell types. The transcriptional regulation of human CIITA is controlled by at least three independent promoter units (CIITA-PI, -PIII and -PIV), each transcribing a unique first exon. These isoforms of the protein are cell type specific. CIITA-PI and CIITA-PIII are used for constitutive expression in dendritic cells and B cells, respectively. CIITA-PIV has been the promoter shown to be predominantly IFN-γ inducible.[25]

The first demonstration that lack of IFN-γ mediated induction of MHC class II antigens was caused by the absence of expression of CIITA was made in foetal trophoblast-derived tumour cell lines. Expression of CIITA following gene transfer resulted in the induction and subsequent cell surface expression of all isotypes of MHC class II molecules.[9,26]

A variety of mouse tumours have been transfected with syngeneic MHC class II genes, and the resulting transfectants are very effective vaccines against subsequent challenge with the wild type class II-negative tumors.[10,27]

Interestingly, the expression of other genes whose products are involved in the MHC class II antigen presentation pathway, such as invariant chain and HLA-DM molecules, although not absolutely depending upon CIITA, is strongly increased in the presence of CIITA. Some studies show that coexpression of Ii is required for expression of functional MHC class II molecules[28], while others show, that class II are functional in the absence of Ii[29,30] and that coexpression of MHC class II and Ii correlates with poor tumour prognosis.[31]

Meazza *et al* show, that by modifying the murine mammary adenocarcinoma TS/A cell line by CIITA gene transfer, $CIITA^+$ tumour cells express surface MHC class II molecules. Even though these cells also up-regulate the invariant chain mRNA and corresponding protein, $CIITA^+$ tumour cells were rejected in syngeneic recipients and the capacity to be rejected correlated with the amount of CIITA-mediated MHC class II expression. Tumour rejecting mice also became resistant to the rechallenge with the wild type tumour. This rejection required both $CD4^+$ and $CD8^+$ cells.[7] Other groups however show, that up-regulation of Ii chain expression converts an immunogenic tumour to non-immunogenic, that is highly malignant in autologous mice.[32,33]

In vivo studies demonstrate that MHC class II^+ /Ii^- tumour cells, and not host derived cells, were the predominant antigen-presenting cells for MHC class II-restricted nuclear antigens.[34] Due to allele heterogenicity, the transfection of genes for autologous MHC class II molecules is not practical clinically. Alternative approaches inducing expression of MHC class II molecules with transfection of CIITA or IFN-γ stimulation of CIITA expression and suppression of Ii protein by antisense methods using short oligonucleotides have been used successfully in several types of tumours. The cytotoxic effect can be enhanced by co-injecting the cells with IL-2 gene expressing plasmid, since IL-2 promotes T cell infiltration and activation against tumour antigens.[35] Intra-tumoural gene therapy can also be aided by radiation of tumours to enhance the therapeutic efficacy of intra-tumoural gene therapy for in situ induction of tumour-specific immune response.[36] There are several possible mechanisms for radiation enhancement of gene therapy, which include (a) slowing of the tumour growth, so that immunotherapy has time to develop, (b) radiation induced tissue damage mobilizes inflammatory cells in the tumour vicinity, (c) radiation limits suppressive immunoregulatory T cells and (d) radiation increases gene transduction efficiency and duration of expression of surviving tumour cells.

The advantage of the methods that include converting tumour cells into antigen presenting cells is not only killing of the cells directly contacted by tumour therapy, but also eliciting of an immune response which in turn eradicates tumour cells and deposits at both locoregional and distant sites.[36]

Dendritic cells as tumour-antigen presenting cells

Dendritic cells (DCs) are the most potent antigen-presenting cells. They can present tumour antigens to immunologic effector cells. MHC II molecules on DC surfaces play an important role in priming effector cells against tumour cells and their antigens, so they may be used to overcome tumour escape. DCs capture and process antigens in periphery, express lymphocyte co-stimulatory molecules, migrate to lymphoid organs, and secrete mediators to initiate immune responses.[37] DCs present peptides to naïve T cells and induce a cellular immune response that involves both $CD4^+$ T_H1 cells and cytolytic $CD8^+$ T cells. They can also stimulate humoral immunity by activating naïve and memory B cells.[38] Effective cancer vaccines will need to elicit both $CD4^+$ IFN-γ producing and $CD8^+$ cytotoxic T cell responses. Successful antitumour immunity will therefore depend on receipt by DC of maturation signals, which drive differentiation of naïve $CD4^+$ and $CD8^+$ T cells into T_H1/T_C1 effector cells.[1] Thus DCs represent a powerful tool for vaccination against tumour cells,[38,39] but one has to consider, that immature DCs can induce tolerance and only mature DCs, which express co-stimulatory molecules on surface and produce inflammatory cytokines, induce effective antitumour immunity.[1]

Marten *et al* demonstrate the transfection of CIITA gene into DCs, which strongly increases MHC class II expression. Transfection of the DCs with CIITA leads to an increase in antitumoural immunostimulatory capacity and therefore suggests the use of DCs in treatment of cancer cells.[39]

DCs pulsed with tumour antigens have also been used in several studies. Such DCs have been successfully used in raising specific $CD8^+$ T cells. Similarly this approach is also used to raise specific $CD4^+$ T cells by loading them with MHC class II restricted antigens.[38,40] Even though more and more tumour-restricted antigens are being identified, unfortunately most tumours still have no defined tumour antigens,[39] so this method has only limited applicability in clinical therapy.

Zhao *et al* show that short incubation of mRNA-transfected DCs with antisense oligonucleotides directed against the Ii chain enhances the presentation of mRNA-encoded class II epitopes and activation of $CD4^+$ T-cell responses *in vitro* and *in vivo*. Immunization of mice with the antisense oligonucleotide-treated DCs stimulates a more potent and longer lasting $CD8^+$ CTL response and enhances the antitumour efficacy of DC-based tumour vaccination protocols. Since vaccination with tumour mRNA-transfected DCs does not require the identification of the effective tumour antigens in each patient with cancer and is not limited by tumour tissue availability, this approach could represent a broadly useful method to augment antitumour T cell immunity alongside $CD8^+$ T-cell immunity.[31]

Conclusions

Our current understanding of molecular mechanisms of cancer and tumour-specific immune responses has greatly benefited from the advances in molecular genetics and immunology. At the same time, the advances in recombinant DNA technologies have been made, that enable development of immunotherapy for the disease. Different immunotherapy strategies have proven to be very effective in animal models; however patients in most clinical trials conducted so far have elicited only weak and transient immune response. Therefore combination of different treatment strategies, such as gene therapy combined with cytokine treatment, radiation and/or chemotherapy will have to be considered.

The identification of MHC class I and class II-restricted tumour antigens has enabled development of methods for the targeting of either defined epitopes or whole antigens into the MHC pathway. Tumour cells can be genetically engineered to function as APCs, thereby facilitating the generation of tumour-specific immunity. The advantage of this method is that prior identification of tumour antigens is not necessary. By inducing a potent antitumour immune response, tumour cells throughout the body that are left behind after surgery or radiotherapy could be eradicated. By enabling induction of a potent $CD4^+$ and $CD8^+$ T cell antitumour immune response, the clinical outcomes in patients with cancer should be greatly improved.

DCs are also an attractive target for therapeutic manipulation of the immune system in cancer. By loading them with combined MHC class I and class II peptides, they can be used to immunize patients.

The combined use of MHC class I and class II-restricted tumour antigens, co-stimulatory molecules and cytokines that can be used to enhance immune responses represent an unprecedented opportunity for the development of new generation of effective cancer vaccines.

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Quantitative analysis of fine needle aspiration biopsy samples

Mirjana Rajer[1], Marko Kmet[2]

[1]Department of Radiotherapy, Institute of Oncology Ljubljana, Slovenia,
[2]Department of Internal Medicine, General Hospital Novo mesto, Slovenia

Background. *The fine needle aspiration biopsy (FNAB) is one of the methods used in tumour evaluation. Since a certain number of tumour cells are needed for a complete diagnostic algorithm, we wanted to test how many cells remain in the needle and syringe after routine stains have been made and which factors influence this number. The remaining cells are used in ancillary diagnostic procedures.*

Materials and methods. *One hundred fifty two FNAB samples of tumours of the breast, thyroid and lymph nodes were included in our study. We counted the cells which were left in the needle and the syringe after the standard smears had been made. Buerker-Tuerk's chamber was used for this purpose.*

Results. *The number of cells depended on the organ from which the cells had been aspirated, on the type of tumour and, in the case of breast cancer, also on the level of experience of the FNAB performer. The percentage of samples with too few cells for all modern diagnostic methods (<5x105) is lowest in FNAB of lymph nodes (4.9%), followed by breast (16.7%) and thyroid (18%).*

Conclusions. *We concluded that FNAB in the majority of cases grants a sufficient number of cells for the standard microscopic evaluation and also ancillary diagnostic procedures.*

Key words: neoplasms – pathology; biopsy, needle; cytodiagnosis; cell count

Introduction

The fine needle aspiration biopsy (FNAB) is a quick, simple, safe, painless and inexpensive method. It is of the utmost importance in the preoperative diagnostics of tumours.[1] The diagnostic reliability of the method is good. It enables us to classify tumours as malignant or benignant in almost 100% cases and to further specify the type of the tumour in 80-98% of cases.[2] Serious side effects (pneumothorax, severe bleeding, infection, pain, vomiting etc.) are rare.[3] In the process of aspiration the cells are seldom extensively damaged since the small diameter of the needle enables it to push aside the tissue rather than tearing it.[1,4]

New, highly specialized methods of treatment require the specification of the tumour lesion to the highest extent. Any additional information about the morphology and cell structure, which determines the prognosis and helps to choose an appropriate treat-

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Correspondence to: Mirjana Rajer, MD, Department of Radiotherapy, Institute of Oncology Ljubljana, Zaloška 2, 1000 Ljubljana, Slovenia; Phone: + 386 41 26 99 46; Fax.: + 386 1 587 9 400; E-mail: mrajer@onko-i.si

ment, is a welcome addition to the standard morphologic analysis. Such analyses are used for example in determining the hormonal receptor status in breast cancer.[5] The number of cells in the sample is, however, limited and determines how many additional diagnostic procedures can be made, besides the standard two smears.[6]

The purpose of our study was two-fold. First of all, to determine the number of cells that remain in the needle and syringe after FNAB had been performed and the standard two smears had been made and secondly, to establish eventual impact of tumour characteristics and performer's previous experience on the results. For our study, we chose tumours of the breast, thyroid and lymph nodes as the number of ancillary cytological methods is the greatest in these types of lesions.

Materials and methods

Data from 152 samples (54 tumours of the breast, 33 of the thyroid and 65 of the lymph nodes) were included in our study. We analysed the cells which were left in the needle and syringe after FNAB and the standard two smears have been made. We rinsed the needle and syringe with a so called »rinsing solution«, composed of 4.5% of bovine serum albumin and 0.45% EDTA in phosphate buffer with 100 I.U. of penicillin in 100 ml of the solution. We processed the sample according to the Buerker-Tuerk's protocol for cell counts; this was done in Buerker-Tuerk's chamber. We counted the cells by using a 100x magnification of the standard light microscope. Cells in four squares of the chamber were counted and the average number was calculated.

Next, we calculated the number of remaining cells. The equation used for this purpose was:

$$x = c \times V$$

where x stands for the total number of cells, c for density and V for volume of the remainder of the sample with the volume of the rinsing fluid included.

The density of the cells was calculated by using the following equation:

$$c = n \times 20 \times 10^4$$

where n stands for the number of counted cells. We had to multiply this number by 20 as the dilution ratio of the cell suspension to Buerker Tuerk's solution had been 10 μl: 190 μl. The volume of the chamber is 104 ml, hence the last multiplicator.[7]

Eight cytologists performed the biopsies. They were divided in two groups, based on their previous experience. In the first group there were three cytologists with more than five years of experience each. The rest, with less than one year of experience each, were in group number two. A two-sided t-test was used to calculate the level of the statistical significance of the between-group comparison.

Results

The percentage of samples with 500.000 cells in the syringe after the two standard smears were made was 95% in lymph node biopsy, 82% in breast cancer biopsy and 81% in thyroid cancer biopsy.

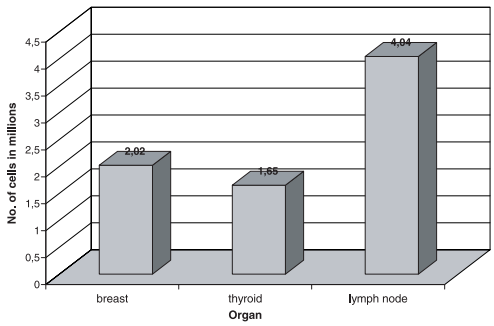

Figure 1. Average number of cells regarding the target organ on which FNAB was performed.

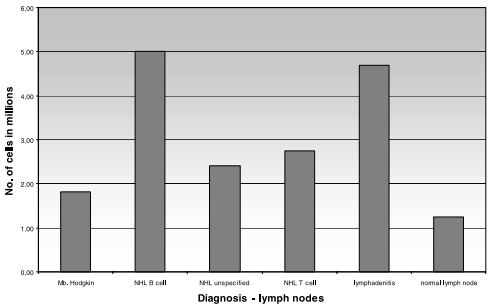

Figure 2. Average cell number according to the type of lymph node pathology.

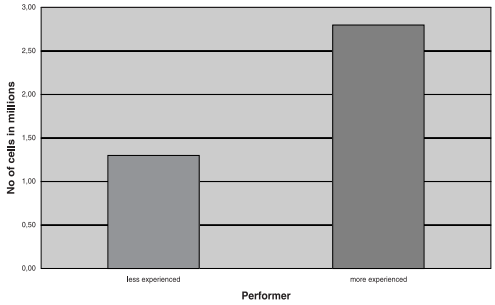

Figure 3. Average number of cells regarding the experience of the FNAB performer in breast samples.

Average number of cells regarding the target organ

We found that the average number of cells was the highest in samples acquired from lymph nodes, followed by breast samples. The average number was the lowest for thyroid samples. The difference was statistically significant when comparing averages of lymph node to breast ($p = 0.0003$) and lymph node to thyroid samples ($p = 0.00006$). The difference between breast and thyroid samples was not statistically significant (Figure 1).

Average number of cells regarding the type and size of the tumour

There was a statistically significant difference when comparing different types of tumours. For example, in invasive ductal carcinoma of the breast the number of cells was significantly higher than in invasive lobular carcinoma ($p = 0.01$). The results for different lymph node tumours were similar (p values ranging from 0.0002 to 0.05) while there was no statistically significant difference in different types of tumours of the thyroid (Figure 2).

In all three organs there was no significant difference in the number of acquired cells regarding the tumour size.

Average number of cells regarding the performer of FNAB

The only statistically significant difference in the number of cells between younger and older performers was present in FNAB samples of the breast ($p = 0.03$), while samples of the thyroid and lymph node did not show any significant difference (Figure 3).

Discussion

The percentage of samples containing enough cells to perform ancillary diagnostic methods (more than 500.000 cells in the syringe after the two standard smears)[1] was different according to the organ from which the sample was taken. Most cells were present in lymph nodes samples, on average 4 millions, followed by breast samples with 2 million on average and thyroid samples with 1.65 million. This result is not surprising if we consider that the tissues have a different structure.

We expected to get more cells from bigger tumours, but this was not the case in our study. The possible explanation for this fact could be that in bigger tumours, there is more tumour regression and necrosis which lowers the number of aspirated cells.

Different types of tumours have a different structure and the number of cells obtained from them was different. Cells of malignant lymphoma are connected by fragile nests of stroma and surrounded by a gentle capsule.8 This explains why we can obtain a large number of cells with ABTI by applying only a low pressure to the needle.

Even though the absolute average number

of aspirated cells was lower in all three organs in the second, younger group of cytologists, we found that the only statistically significant difference was present in FNAB of the breast. While there is some difference between the two groups it is not so important and proves that FNAB is easy to learn and to perform. All the FNAB-s in our study were done in the same centre and it would be interesting to compare different centres.

In most of the cases FNAB provides enough cells for basic and advanced diagnostic procedures. The number of necessary cells is especially high in breast cancer, because of the number of available diagnostic tests. In the future we expect more new and accurate diagnostic procedures that will enable us to make a clearer picture of the nature of the tumour and will thus lead to better treatment decisions.

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Correlation of clinical target volume and the margins to define planning target volume with beam arrangements for three-dimensional conformal radiation therapy delivery for prostate cancer

Haldun Şükrü Erkal,[1,2] Meltem Serin[1,2]

[1]*Department of Radiation Oncology, Ankara University Faculty of Medicine, Ankara, Turkey*
[2]*Department of Radiation Oncology, Inönü University Faculty of Medicine, Malatya, Turkey*

Background*. A conceptual study was undertaken to correlate the clinical target volume and the margins to define the planning target volume with the beam arrangements for a three-dimensional conformal radiation therapy delivery on two patients with prostate cancer having considerably different prostate shapes and volumes.*

Material and methods. *The clinical target volume was defined as prostate and seminal vesicles. Uniform margins of 0.4, 0.8 and 1.2 cm were added around the clinical target volume to define three planning target volumes. Three well-established coplanar beam arrangements were simulated for all planning target volumes. Dose-volume histograms were calculated and quantitatively compared.*

Results. *The mean dose (Dm) for PTVs ranged from 98.7 to 99.9%, with standard deviations ranging from 1.5 to 1.7%. Plan I appeared to be the best considering the Dm for the rectum, whereas Plan II appeared to be the best considering V95 (fraction of volume receiving a dose higher than 95% of the isocenter dose for the rectum). Plan III appeared to be the best considering the Dm and V95 for the bladder and also considering the Dm and V50 for the femur.*

Conclusions. *This conceptual study suggested that the differences in shapes and volumes of planning target volume might be taken into consideration in an attempt to individually establish the optimum beam arrangements for three-dimensional conformal radiation therapy delivery in prostate cancer.*

Key words: prostatic neoplasms radiotherapy; radiotherapy, conformal

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Correspondence to: Haldun Şükrü Erkal, M.D., Department of Radiation Oncology, Inönü University Faculty of Medicine, TR-44280, Malatya, Turkey; Phone: + 90 422 341 00 45; Fax: + 90 422 341 00 36; E-mail: hserkal@inonu.edu.tr

Introduction

A three-dimensional conformal radiation therapy is characterized by the conformation of the radiation dose to the target volume besides the reduction of the radiation dose to the nor-

mal tissues at risk.[1] The three-dimensional conformal radiation therapy requires the accurate delineation of gross tumor volume (GTV) and meticulous identification of the margins to define the clinical target volume (CTV) and the planning target volume (PTV).[2] The design of the beam arrangements for the three-dimensional conformal radiation therapy delivery could be hampered by the variations in the shape and the volume of GTV as well as the variations in the margins to define CTV and PTV.

The aim of this conceptual study was to correlate CTV and the margins to define PTV with the beam arrangements for the three-dimensional conformal radiation therapy delivery on two descriptive patients with prostate cancer having considerably different prostate shapes and volumes.

Material and methods

Of two patients with localized prostate cancer investigated in this study, Patient I had a concave shaped prostate with a comparatively small CTV of 48.3 cm^3 and Patient II had a non-concave shaped prostate with a comparatively large CTV of 82.1 cm^3. The patients were positioned supine with a full bladder and immobilized in a molded foam cradle (Redifoam, Med-Tec Inc., Orange City, United States of America). Following the administration of the contrast material into the bladder and the rectum, transverse computed tomography images of the pelvis were obtained on a dedicated scanner (IQ-TC, Picker International, Cleveland, United States of America) with a slice thickness of 0.2 cm (at 0.2 cm steps) throughout the region containing the target volume (from the bottom of the sacroiliac joints to the penile urethra) and a slice thickness of 0.5 cm (at 0.5 cm steps) throughout the regions above and below the region containing the target volume. The prostate, the seminal vesicles, the bladder (from the apex to the dome), the rectum (from the anus at the level of the ischial tuberosities for a length of 15 cm) and right femur (to the level of the ischial tuberosities) were outlined on a virtual simulation workstation (Acqsim, Picker International, Cleveland, United States of America).

With respect to the International Commission on Radiation Units and Measurements (ICRU) Report 50,[2] GTV was defined as the prostate and CTV was defined as the prostate and the seminal vesicles. Uniform margins of 0.4 cm (Margin I), 0.8 cm (Margin II) and 1.2 cm (Margin III) were added around CTV through the automatic volume expansion to take into account the variations in the shape and the volume of CTV as well as to take into account the uncertainties in patient positioning. Margin I, Margin II and Margin III defined PTV I, PTV II and PTV III, respectively. To define the block edges, a margin of 0.7 cm was added around PTVs to account for the effect of the penumbra.

Three well-established coplanar beam arrangements were simulated for PTV I, PTV II and PTV III. Plan I had an anteroposterior field and two lateral 30° wedged fields, Plan II had an anteroposterior field, a posteroanterior field and two lateral fields and Plan III had an anteroposterior field, a posteroanterior field, two anterior oblique fields and two posterior oblique fields. Dose distributions for equally weighted fields were calculated for 18 MV photons and normalized at the isocenter on a three-dimensional treatment planning system (Cadplan, Varian-Dosetek Oy, Finland). The reference dose was considered as 95% of the isocenter dose. Dose-volume histograms (DVHs) for PTV, the bladder, the rectum and the femur were calculated. For the quantitative comparison of DVHs, the mean dose (Dm) and the fraction of volume receiving a dose higher than 95% of the isocenter dose (V95) were considered for the rectum and the bladder and Dm and the fraction of volume receiving a dose higher than 50% of the isocenter dose (V50) was considered for the femur.

Results

The Dm for PTVs ranged from 98.7 to 99.9%, with standard deviations ranging from 1.5 to 1.7%.

Considering Dm for the rectum, Plan I appeared to be the best and Plan III appeared to be the worst beam arrangement regardless of the shape and the volume of the prostate and regardless of the margin added around CTV. Considering V95 for the rectum, Plan II appeared to be the best and Plan III appeared to be the worst beam arrangement for Margin I and Margin II while Plan III appeared to be the best and Plan I appeared to be the worst beam arrangement for Margin III for Patient I, whereas Plan I appeared to be the best and Plan III appeared to be the worst beam arrangement regardless of the margin added around CTV for Patient II.

Considering Dm for the bladder, Plan III appeared to be the best and Plan II appeared to be the worst beam arrangement regardless of the shape and the volume of the prostate and regardless of the margin added around CTV. Considering V95 for the bladder, Plan III appeared to be the best beam arrangement for Margin I and Plan I appeared to be the best beam arrangement for Margin II and Margin III while Plan II appeared to be the worst beam arrangement regardless of the margin added around CTV for Patient I, whereas Plan I appeared to be the best and Plan II appeared to be the worst beam arrangement regardless of the margin added around CTV for Patient II.

Considering both Dm and V50 for the femur, Plan III appeared to be the best and Plan I appeared to be the worst beam arrangement regardless of the shape and the volume of the prostate and regardless of the margin added around CTV.

Dm and V95 values for the rectum and the bladder and Dm and V50 values for the femur are shown in Table 1 and Table 2, respectively, for Patient I and Patient II.

Discussion

For patients with prostate cancer treated with the three-dimensional conformal radiation therapy, a wide range of variation has been reported for CTV as dictated by the volume of the prostate. Forman *et al*. have reported the volume of the prostate to range from 10 to

Table 1. Comparisons of Dm and V95 values for the rectum and the bladder and Dm and V50 values for the femur for different PTVs with different beam arrangements for Patient I.

	Plan I			Plan II			Plan III		
	PTV* I	PTV II	PTV III	PTV I	PTV II	PTV III	PTV I	PTV II	PTV III
Bladder									
Dm**	29.39	34.92	37.92	34.41	39.36	42.31	26.21	31.85	37.08
V95***	11.09	19.46	30.33	14.57	22.29	35.01	10.76	19.79	32.18
Rectum									
Dm	25.05	30.74	33.69	28.27	33.19	35.51	29.33	34.75	36.92
V95	18.35	32.12	43.48	18.24	30.48	42.61	20.54	35.72	42.19
Femur									
Dm	30.25	34.10	37.41	22.68	26.29	37.80	19.48	23.59	26.64
V50****	76.60	90.21	100.42	59.72	75.47	86.56	18.65	32.00	40.70

*PTV: Planning target volume, **Dm: The mean dose, ***V95: The fraction of volume receiving a dose higher than 95% of the isocenter dose, ****V50: The fraction of volume receiving a dose higher than 50% of the isocenter dose.

Table 2. Comparisons of Dm and V95 values for the rectum and the bladder and Dm and V50 values for the femur for different PTVs with different beam arrangements for Patient II.

	Plan I			Plan II			Plan III		
	PTV* I	PTV II	PTV III	PTV I	PTV II	PTV III	PTV I	PTV II	PTV III
Bladder									
Dm**	37.36	42.21	46.56	42.04	46.77	50.40	34.76	39.98	45.54
V95***	13.18	20.38	36.08	18.42	29.35	44.00	13.37	23.00	39.54
Rectum									
Dm	42.43	50.35	57.00	50.71	57.03	61.36	51.05	58.32	63.67
V95	2.96	9.68	16.75	5.04	11.97	19.64	5.31	12.04	20.92
Femur									
Dm	42.65	45.05	47.81	32.21	34.02	36.42	23.45	25.89	28.73
V50****	130.36	138.55	146.86	120.43	129.22	138.97	6.56	16.20	46.85

*PTV: Planning target volume, **Dm: The mean dose, ***V95: The fraction of volume receiving a dose higher than 95% of the isocenter dose, ****V50: The fraction of volume receiving a dose higher than 50% of the isocenter dose.

155 cm^3 (median, 52 cm^3) in patients with prostate cancer treated with three-dimensional conformal radiation therapy.[3] In the intervening years since the publication of the ICRU Report 50 in 1993, the acceleration in the clinical application of three-dimensional conformal radiation therapy has necessitated a more accurate definition of PTV. In 1999, the ICRU Report 62 has been published as a supplement to Report 50, addressing the different sources of uncertainties to be taken into account in delineating PTV.[4]

The ICRU Report 62 has defined an internal margin (IM) to take into account the uncertainties in the shape and the volume of CTV and a set-up margin (SM) to take into account the uncertainties in patient positioning. While IM has mainly been related to the physiological variations that have been difficult or impossible to control, SM has mainly been related to the technical factors that could have been reduced by the more accurate immobilization and the set-up of the patient, as well as the improved mechanical stability of the treatment machine.[4] Tinger *et al*. have reported margins ranging from 0.7 to 1.1 cm to be added around CTV to encompass the overall uncertainties with a 95% probability and margins ranging from 1.0 to 1.6 cm to be added around CTV to encompass the overall uncertainties with a 99% probability.[5]

The three-dimensional conformal radiation therapy for prostate cancer has traditionally been delivered through well-established coplanar three-field, four-field or six-field beam arrangements.[6-8] Although these beam arrangements have been compared in terms of dose distributions to the normal tissues at risk through dose-volume histograms, the different sources of uncertainties to be taken into account in delineating PTV have generally not been appreciated.[9,10] Therefore, the contributions of the shape and the volume of the prostate and the magnitude of the margin defining PTV to the selection of the beam arrangements for the three-dimensional conformal radiation therapy delivery have not been independently described.[11,12]

In this conceptual study, the differences in the shapes and the volumes of PTVs for the investigated patients underlined the establishment of different beam arrangements as the optimum beam arrangement for different patients. However, the same beam arrangements were established as the optimum beam arrangement for a given patient, regardless of the increases in the magnitudes of the margins defining PTVs. These findings suggest

that the inherent characteristics of the patients, such as the shape and the volume of the prostate, might lead to more critical contributions for the establishment of the optimum beam arrangements when compared to the margins typically added around the target volumes based on the established policies of the institutions. Further studies of a larger scale are warranted to confirm that the selection of the beam arrangements for three-dimensional conformal radiation therapy delivery in patients with prostate cancer having considerably different prostate shapes and volumes should call for individual rather than class solutions.

Acknowledgement

This study has been presented at the "3rd Takahashi Memorial International Workshop on Three Dimensional Conformal Radiotherapy" in Nagoya, Japan in 2001.

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Radiol Oncol 2005; 39(4): 237-42.

Majhna količina proste plevralne tekočine. Prvi del – majhen plevralni izliv

Kocijančič I

Izhodišča. Majhne plevralne izlive običajni pregledni posnetki prsnih organov redko odkrijejo. Tak izvid pa je pomemben, saj lahko skupaj s probatorno punkcijo vodi do končne diagnoze karcinomatoze, vnetja ali transudata. Majhen rentgentski znak meniskusa in medialni pomik frenikokostalnega recesusa sta edina, ki nakazujeta možnost manjše količine plevralne tekočine na posnetku prsnih organov stoje. Na stranskem posnetku stoje je majhen meniskus v posteriornem frenikokostalnem recesusu znak majhnega plevralnega izliva.

Zaključki. Že vrsto let uporabljamo za dokazovanje majhnih količin proste plevralne tekočine posnetke prsnih organov leže na boku. V zadnjem desetletju pa je vodilna metoda prikazovanja majhnih plevralnih izlivov ultrazvočni pregled plevralnega prostora.

Radiol Oncol 2005; 39(4): 243-7.

Ultrazvočna preiskava žolčnika pri bolnikih s podaljšanim bivanjem (>14 dni) v enoti intenzivnega zdravljenja

Šustić A, Miletić D, Cicvarić T

Izhodišča. Namen pričujoče raziskave je bil ugotoviti pogostnost nenormalnega ultrazvočnega (UZ) izvida žolčnika pri kirurških bolnikih, ki so bivali v enoti intenzivnega zdravljenja več kot 14 dni. Prav tako smo želeli primerjati te UZ izvide z resnostjo obolenja.

Metode. V prospektivno raziskavo smo zajeli 57 odraslih kirurških bolnikov (66% moških; starost 49±18 let) s podaljšanim bivanjem v enoti intenzivnega zdravljenja. Pri vseh bolnikih smo UZ preiskavo naredili 15. dan bivanja v intenzivni enoti. Ultrazvočno smo ugotavljali: debelino stene žolčnika (≥4 mm), vsebino žolčnika, brazgotinjenje žolčnikove stene in periholecistitično tekočino. Isti dan smo ocenjevali tudi resnost obolenja po SAPS II lestvici.

Rezultati. Vsaj en UZ znak obolenja žolčnika je imelo 36 (63%) bolnikov, med njimi zadebeljeno steno žolčnika 32 (56%), žolčno usedlino 23 (40%), periholecistitično tekočino 9 (16%), hidropičen žolčnik 7 (12%) in zabrazgotinjeno steno žolčnika 4 (7%) bolniki. Od naštetih 5 UZ znakov obolenja žolčnika smo dva in več ugotovili pri 20 (35%) bolnikih, tri in več pri 12 (21%), štiri in več pri 10 (18%) in vseh pet pri 4 (7%) bolnikih. Bolniki z enim ali več UZ znakom obolenja žolčnika so imeli statistično značilno resnejše obolenje po SAPS II lestvici glede na bolnike z normalnim UZ izvidom (36±9 vs. 28±7; p<0,01). Statistično značilno resnejša obolenja po SAPS II lestvici smo tudi ugotovili, ko smo primerjali bolnike z dvema UZ znakoma obolenja žolčnika in bolnike z nobenim ali enim (40±8 vs. 29±6; p<0,001), bolnike s tremi znaki in bolniki z dvema ali manj znakoma (41±8 vs. 31±9; p<0,001), bolnike s štirimi in bolnike s tremi ali manj znaki (42±11 vs. 31±6; p<0,001) ter bolnike s petimi zanki in bolnike z manj UZ znaki obolenja žolčnika (45±10 vs. 32±9; p<0,001). Tako smo ugotovili statistično značilno povezavo med številom UZ znakov obolenja žolčnika in resnostjo obolenja po SAPS II lestvici (r = 0.57; p <0,001).

Zaključki. Več kot polovica kirurških bolnikov, ki smo jih več kot 14 dni obravnavali v enoti intenzivnega zdravljenja, je imelo nenormalen UZ izvid, ki je bil v neposredni povezavi z resnostjo obolenja.

Radiol Oncol 2005; 39(4): 249-52.

Koristnost transrektalne endoluminalne ultrazvočne preiskave pri ločevanju analnega abscesa in rektalnega karcinoma. Prikaz primera

Kolodziejczak M, Sudol – Szopinska I

Izhodišča. Visoko ležeči analni abscesi imajo lahko neznačilen, kronični klinični potek, zato jih je težje diagnosticirati.
Prikaz primera. Avtorici opisujeta primer bolnice, pri kateri so sprva sumili, da ima rektalni karcinom. Ker so neznačilni klinični simptomi kazali na možnost visokoležečega abscesa s kroničnim potekom bolezni, so naredili dodatne preiskave. Potrdili so, da ima bolnica visokoležeči, submukozni, intersfinkterski absces.
Zaključki. Zgoraj opisani primer bolnice kaže, da sta bili pri ugotavljanju bolezni najvažnejša natančna digitalna interrektalna priskava ter transrektalni endoluminalni ultrazvok.

Radiol Oncol 2005; 39(4): 253-9

Novi označevalec angiogeneze CD105 (endoglin): diagnostični, napovedni in terapevtski pomen

Legan M

Izhodišča. Angiogeneza je ključna za napredovanje in metastaziranje malignih tumorjev, zato so številne raziskave usmerjene v prikaz in vrednotenje tumorskega žilja ter ugotavljanje povezanosti le-tega z napovedjo poteka bolezni. Panendotelijski označevalci (CD31, CD34 in F8) ter novejši označevalec CD105 so različno izraženi v endotelijskih celicah nastajajočih (predvsem tumorskih) žil in endotelijskih celicah normalnega žilja. Prvi so idealni označevalci normalnega žilja, CD105 (imenovan tudi endoglin) pa je primernejši za prikaz tumorske angiogeneze. Endoglin je receptor za tumorski rastni dejavnik (tumor growth factor -TGF) beta, ki ga najdemo na endotelijskih celicah angiogenih tkiv. Potreben je pri aktivni angiogenezi v tumorju kot tudi pri razvoju žil pri zarodku.

Zaključki. Gostota tumorskega žilja – prikazana z imunohistokemičnim označevanjem CD105 v tkivnih rezinah tumorja in izmerjena s histomorfometričnimi metodami – je značilno povezana z agresivnostjo tumorja in napovedjo poteka bolezni pri številnih solidnih tumorjih. Učinkovanje na endoglin, ki je specifični antigen v tumorskem žilju, ima tudi velike terapevtske možnosti.

Radiol Oncol 2005; 39(4): 261-8.

Pomen molekul MHC II pri imunoterapiji tumorjev

Oven I

Izhodišča. Imunoterapija tumorjev izrablja sposobnost imunskega sistema, da specifično ubija tumorske celice, pri tem pa minimalno poškoduje normalno tkivo. Vedno večje poznavanje tumorskih antigenov prispeva k načrtovanju bolj učinkovitih terapevtskih cepiv. Študije narejene na živalskih modelih so pokazale, da so poleg molekul MHC I in celic CD8+ T pri nastanku in vzdrževanju imunskega odziva proti tumorjem pomembne tudi molekule MHC II in celice CD4+ T. Rezultati nakazujejo, da bo za učinkovito protitumorsko cepivo potrebno aktivirati tako celice CD4+ kot tudi CD8+ T. V zadnjem času so se razvile nove strategije za okrepitev T celičnega odgovora proti raku, ki izrabljajo sposobnost dendritičnih celic, da delujejo kot antigeni. Z vključevanjem tumorskih antigenov specifičnih za molekule MHC II in z genetičnim spreminjanem tumorskih celic, da delujejo kot antigeni (antigen-predstavitvene celice), lahko podaljšamo predstavljanje antigenov celicam T preko dendritičnih celic.

Zaključki. Z združitvijo različnih pristopov bi lahko naredili učinkovito protitumorsko cepivo, ki bi vzpodbudilo delovanje za tumor specifičnih celic T, te pa bi ubile tumorske celice. S tem bi zmanjšale obseg tumorja, obenem pa bi vzpodbudile tudi nastanek za tumor specifičnega celičnega spomina T, ki bi omejil ali preprečil ponovni nastanek tumorja.

Radiol Oncol 2005; 39(4): 269-72.

Kvantitativna analiza vzorcev aspiracijske biopsije

Rajer M, Kmet M

Izhodišča. Aspiracijska biopsija s tanko iglo (ABTI) je varna, hitra, enostavna, neboleča in poceni metoda v preoperativni diagnostiki tumorjev. Za postavitev diagnoze je potrebno z ABTI pridobiti določeno število celic za izdelavo rutinskih celičnih razmazov ter za dodatne, novejše preiskave, ki so pomembne za natančnejšo opredelitev prognostičnih dejavnikov in določitev ustreznega zdravljenja. Zanimalo nas je, koliko celic ostane v igli in brizgalki po pripravi rutinskih preparatov in kaj vpliva na to število, kajti ravno na tem delu vzorca opravljamo te dodatne preiskave.

Material in metode. V prospektivno raziskavo smo vključili 152 vzorcev ABTI tumorjev dojke, ščitnice in bezgavke. S pomočjo Buerker-Tuerkove komore smo šteli celice, ki ostanejo v brizgalki in igli po pripravi rutinskih preparatov.

Rezultati. Po ocenah sodelavcev Onkološkega inštituta je za dodatne preiskave potrebno z ABTI pridobiti vsaj 500.000 celic poleg tistih, ki smo jih uporabili za dva rutinska razmaza. To smo dosegli pri vzorcih 95% tumorjev bezgavk, 82% tumorjev ščitnice in 81% tumorjev dojk. Ugotovili smo, da je število celic odvisno od organa, ki ga punktiramo. Pri tumorjih dojk in bezgavk je število odvisno tudi od vrste tumorja, velikost tumorja pa na število celic ne vpliva. Ko smo primerjali število celic, ki so jih pridobili izkušeni citologi s številom pri manj izkušenih, smo pri ABTI tumorjev dojk dobili statistično značilno razliko ($p = 0,03$), pri ostalih dveh pa razlika ni dosegla statistične značilnosti.

Zaključki. ABTI je metoda, ki v večini primerov zagotovi zadostno število celic za standardno mikroskopsko preiskavo in dodatne analize tumorskih celic. Število celic je odvisno od organa, ki ga punktiramo, lastnosti tumorja in pri ABTI dojke tudi od izvajalca.

Radiol Oncol 2005; 39(4): 273-8.

Primerjava med kliničnim tarčnim volumnom in varnostnim robom, ki določata planirni tarčni volumen ter obliko polj pri tridimenzionalni konformni radioterapiji raka prostate

Erkal HS, Serin M

Izhodišča. Primerjalna študija je bila opravljena na dveh bolnikih z rakom prostate različnih oblik in volumnov. Želeli smo ugotoviti razmerje med kliničnim tarčnim volumnom in varnostnim robom, ki določata planirni tarčni volumen (PTV) ter obliko polj pri tridimenzionalni konformni radioterapiji.

Material in metode. Klinični tarčni volumen je vključeval prostato in seminalne vezikule. Varnostne robove dolžin 0,4 cm, 0,8 cm in 1,2 cm smo enakomerno dodali na klinični tarčni volumen in tako določili tri PTV. Pri vseh PTV smo simulirali tri dobro osnovana istoravninska (koplanarna) polja. Naredili smo histograme, ki so kazali razmerje med dozo in volumnom ter jih med seboj kvantitativno primerjali.

Rezultati. Povprečna doza za PTV je bila v razponu od 98,7 do 99,9%, s standardno deviacijo med 1,5 in 1,7%.

Plan I je bil najboljši glede na povprečno dozo sevanja, ki ga je prejel rektum, medtem ko je bil Plan II najboljši glede na V95 za rektum (delež volumna, ki prejme dozo višjo od 95% doze določene v izocentru).

Plan III pa je bil najboljši tako glede na povprečno dozo sevanja in V95 za mehur kot tudi glede na povprečno dozo sevanja in V50 za glavice kolkov.

Zaključki. Primerjalna študija kaže, da moramo upoštevati razlike v oblikah in volumnih planirnih tarčnih volumnov. Takšen pristop nam omogoča individualno določevanje optimalnih polj potrebnih za tridimenzionalno konformno radioterapijo v primeru raka prostate.

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.*

Thoracic oncology

January 13-15, 2006

The "5th Annual UCSF Clinical Cancer Update" will be held in Lake Tahoe, California, USA.

Contact University of California, San Francisco, Office of Continuing Medical Education, Box 0742, 3333 California St., San Francisco, CA 94143; or e-mail CME@ocme.ucsf.edu; or http://www.ucsfcme.com

Cellular biology

January 25-28, 2006

The cellular biology meeting "Cell Signaling World. Signal Transduction Pathways as therapeutic targets." will take place in Luxembourg.

See http://www. transduction-meeting.lu

Oncology

January 25-28, 2006

The IASLC workshops of British Thoracic Oncology Group will be offered in Dublin, Ireland.

E-mail obyrne@st.james.ie

Prostate cancer

February 12-16, 2006

The ESTRO teaching multisdisplinary course about prostate cancer will take place in Gent, Belgium.

Contact ESTRO office, Avenue E. Mounierlaan 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Radiotherapy

February 17-19, 2006

The IASLC workshops "Development of a Japanese-North American Cooperative Clinical Trial Comparing Stereotactic Radiation Therapy with Surgery for Stage I non-small cell lung cancer" will be offered in Maui, Hawaii.

Contact Dr. R. Komaki, e-mail rkomaki@mdanderson.org;

Prostate cancer

February 24-26, 2006

The ASCO symposium will be held in San Francisco, California, USA.

Contact E mail: enews@asco.org; or see http:// www/asco.org

Molecular Oncology

March 4, April 30, 2006

The ESTRO teaching course "Molecular Oncology for the Radiation Oncologists" will take place in Granada, Spain.

Contact ESTRO office, Avenue E. Mounierlaan 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Oncology

March 8-12, 2006

The 11th NCCN Annual Conference will be held in Hollywood, Florida, USA.

See http:// www.nccn.org

Oncology

March 9-12, 2006

The ESO advanced course "Modifying Cancer Response to Therapy: From Molecular signalling to Cancer Care" will be offered in Lugano, Switzerland.

Contact: Chatrina Melcher, European School of Oncology, ESO Bellinzona Office, IOSI, Ospedale Regionale Bellinzona e Valli, CH-6500 Bellinzona, Switzerland; or cal +41 91 8111 8050; or fax +41 91 811 8051; or e-mail eso2@esoncology.org

Oncology

March 12-15, 2006

ICTR 2006, the "3rd International Conference on Translation Research and Pre-Clinical Strategies in Radiation Oncology" will be offered in Lugano, Switzerland.

Contact: ICTR 2006 Secretariat, Department of Radio-Oncology, Oncology Institute of Southern Switzerland, CH-6504 Bellinzona, Switzerland; or fax +41 91 811 8678; or http://www.iosi.ch/ictr2006.html

Oncology

March 15-18, 2006

The 3rd International Croatia Conference in Oncology will be offered in Zagreb, Croatia.

Contact: Penta d.o.o., Ms. Danijela Ćurčić, A Hebranga 20, 1000 Zagreb, or call +385 1 4553 290; or fax +385 1 4553 284; or e-mail danijela@penta-zagreb.hr; or http://www.penta-zagreb.hr

Breast cancer

March 21, 2006

The ESTRO pre-meeting workshop on radiotherapy in early breast cancer will take place in Nice, France.

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Experimental and translational oncology

March 22-26,2006

The "4th Conference on Experimental and Translational Oncology" will take place in Kranjska gora, Slovenia.

Contact conference secretariat: Helena Končar, National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia, or call +386 1 241 29 70, fax +386 1 241 29 80, E-mail ceto@nib.si, or see http://www.onko-i.si/ceto

Radiotherapy

March 26-30, 2006

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

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Brachytherapy

March 26-30, 2006

The ESTRO teaching course "Modern Brachytherapy Techniques" will take place in Prague, Czech Republic.

Contact ESTRO office, Avenue E. Mounierlaan 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Lung cancer

April 19-26, 2006

The "2nd Latin American Conference on Lung Cancer" will be offered in Cancun, Mexico.

Contact E-mail: LungCancerLA@meet-ics.com: or see http:// www.LCLA2006.com

Molecular oncology

April 30 - May 4, 2006

The ESTRO teaching course "Molecular Oncology for the Radiation Oncologist" will take place in Granada, Spain.

Contact ESTRO office, Avenue E. Mounierlaan, 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Radiotherapy

May 7-11, 2006

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

Contact ESTRO office, Avenue E. Mounierlaan, 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Radiology

May 15-17, 2006

The UK radiological congress will be held in Birmingham, UK.

Contact UKRC 2006 Organisers, PO Box 2895, London W1A 5RS, UK; or call + 44(0) 207 307 1410/20; or fax +44(0) 207 307 1414; or e mail conference@ukrc.org.uk / exhibition@ukrc.org.uk; or see http://www.ukrc.org.uk

Ethics in oncology

May 18-20, 2006

The ESO course on "Ethics in Oncology" will be held in Bled, Slovenia.

Contact course secretariat, Rita de Martini, European School of Oncology, Via del Bollo 4, 20123 Milan, Italy, or call +39 02 854 645 27, or fax +39 02 854 645 45; or e-mail rdemartini@esoncology.org

Clinical oncology

June 2-6, 2006

The 42nd ASCO Meeting will be offered in Atlanta, USA.

Contact E mail: enews@asco.org; or see http://www/asco.org

Radiotherapy

June 11-15, 2006

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

Contact ESTRO office, Avenue E. Mounierlaan, 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Cancer research

June 17-23, 2006

The 8th intensive workshop for European junior clinical oncologists "Methods in Clinical Cancer Research" will take place in Flims, Switzerland.

Contact Federation of European Cancer Societies (FECS), Avenue E. Mounier, 83, B-1200 Brussels, Belgium; or call +32 2 775 02 06; or fax +32 2 775 02 45; or e-mail workshop@fecs.be; or see http://www.fecs.be

Lung cancer

June 18-21, 2006

The "10th Central European Lung Cancer Conference" will be offered in Prague, Czech Republic.

Contact: +420-608-408-708; or e-mail celcc@conference.cz; or see http://www.conference.cz/celcc2006

Prostate cancer

June 25-27, 2006

The ESTRO teaching course "Brachytherapy for Prostate Cancer" will take place in Barcelona, Spain.

Contact ESTRO office, Avenue E. Mounierlaan 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Bronchology

June 25-28, 2006

The 14th Word Congress of Bronchology and the 14th Word Congress of Bronchoesophagology will take place in Buenos Aires, Republic Argentina.

Contact: General Secretariat, Ms. María Graziani & Asociados with phone +4394 7726 4393 3437; or Fax: +541 439 33436; or E-mail: mg@mariagraziani.com

Radiotherapy

June 25-29, 2006

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

Contact ESTRO office, Avenue E. Mounierlaan 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Clinical trial statistics

June 28-30, 2006

The EORTC (European Organisation for Research and Treatment of Cancer) course "Clinical Trial Statistics for Non Statisticians" will take place in Brussels, Belgium.

Contact Danielle Zimmermann, EORTC Education Office, Avenue E. Mounier 83 B. 11, B-1200 Brussels, Belgium; or call +32 2 774 16 02; or fax +32 2 772 62 33; or e-mail dzi@eortc.be; or see http://www.eortc.be

Lung cancer

June 30 - July 2, 2006

Inaugural IASLC Australian Lung Cancer Conference on Multidisciplinary Care will be offered in Palm Cove, North Queensland, Australia.

E-mail fongk@health.qld.gov.au

Cancer research

July 1-4, 2006

The "19th Meeting of the European Association for Cancer Research EACR 19" will take place in Budapest, Hungary.

Contact EACR-19 Secretariat, Federation of European Cancer Societies, Avenue E. Mounier, 83, B-1200 Brussels, Belgium; or call +32 2 775 02 01; or fax +32 2 775 02 00; or e-mail EACR19@fecs.be; or see http://www.fecs.be

Gynaecological malignancies

August 31 - September 1, 2006

The ESTRO teaching course "Brachytherapy for Gynaecological malignancies" will take place in Vienna, Austria.

Contact ESTRO office, Avenue E. Mounierlaan 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Radiotherapy

September 3-7, 2006

The ESTRO teaching course "Physics for Clinical Radiotherapy" will take place in Innsbruck, Austria.

Contact ESTRO office, Avenue E. Mounierlaan, 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Oncology

September 8, 2006

The EORTC (European Organisation for Research and Treatment of Cancer) course "One-Day Introduction to EORTC Trials" will take place in Brussels, Belgium.

Contact Danielle Zimmermann, EORTC Education Office, Avenue E. Mounier 83 B 11, B-1200 Brussels, Belgium; or call +32 2 774 16 02; or fax +32 2 772 62 33; or e-mail dzi@eortc.be; or see http://www.eortc.be

Radiobiology

September 17-21, 2006

The ESTRO teaching course "Basic Clinical Radiobiology" will take place in Lisbon, Portugal.

Contact ESTRO office, Avenue E. Mounierlaan, 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Lung cancer

September 25-26, 2006

The "2nd International Workshop Early Invasive Lung Cancer: New Diagnostic Tools & Treatment Strategies" will be held in Turin, Italy.

Contact E-mail: a.crippa@congressiefiere.com or see http://www.congressifiere.com

Oncology

October 8-12, 2006

The ESTRO 25 / ECCO 14 Conference will take place in Leipzig, Germany.

Contact FECS office, Av. E. Mounierlaan, 83/4, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estroweb.org

Radiation oncology

October 22-27, 2006

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

Contact ESTRO office, Avenue E. Mounierlaan, 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Lung and head & neck

October 26-28, 2006

The "4th Lung & Head and Neck Conference" will be offered in Chicago, Illinois.

Contact: Taryn Klocke; call +1 770-984-5113; or e-mail evokes@medicine.bsd.uchicago.edu

Lung cancer

November 8-12, 2006

The "3rd IASLC/ASCO/ESMO International Conference on Targeted Therapies in Lung Cancer" will be held in Taormina, Sicily, Italy.

Contact E-mail: fred.hirsch@UCHSC.edu

Radiotherapy

November 19-23, 2006

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

Contact ESTRO office, Avenue E. Mounierlaan 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Surgical oncology

November 30 – December 2, 2006

The "13th Congress of the European Society of Surgical Oncology ESSO 2006" will take place in Venice, Italy.

Contact Conference Secretariat, ESSO 2006, Federation of European Cancer Societies, Avenue E. Mounier, 83, B-1200 Brussels, Belgium; or call +32 2 775 02 01; or fax +32 2 775 02 00; or e-mail ESSO2006@fecs.be; or see http://www.fecs.be

Radiotherapy

December 3-7, 2006

The ESTRO teaching course "Image-guided Radiotherapy (IGRT)" will take place in Brussels, Belgium.

Contact ESTRO office, Avenue E. Mounierlaan 83/12, B-1200 Brussels, Belgium; or call +32 2 775 93 40; or fax +32 2 779 54 94; or e-mail info@estro.be; or see http://www.estro.be

Toxicology

July 15-19, 2007

The "11th International Congress of Toxicology" will be offered in Montreal, Canada.

Contact Congress Secretariat, e-mail: ict2007@nrc-cnrc.gc.ca; or see http://www.ict2007.org

Lung cancer

September 2-6, 2007

The "12th World Conference on Lung Cancer" will be offered in Seoul, Korea.

Contact Conference Secretariat; e-mail WCLC 2007@ncc.re.kr; or see http://www.iaslc.orgIumages/12worldconfannounce.pdf

Oncology

September 23-27, 2007

The "14th European Cancer Conference ECCO 14" will take place in Barcelona, Spain.

Contact Conference Secretariat, ECCO 14, The European Cancer Conference, European Cancer Societies (FECS), Avenue E. Mounier, 83, B-1200 Brussels, Belgium; or call +32 2 775 02 01; or fax +32 2 775 02 00; or e-mail ECCO14@fecs.be; or see http://www.fecs.be

Lung cancer

August 21-24, 2009

The "13th World Conference on Lung Cancer" will be offered in San Francisco, USA.

Contact Conference Secretariat; e-mail WCLC 2007@ncc.re.kr; or see http://www.iaslc.orgIumages/12worldconfannounce.pdf

As a service to our readers, notices of meetings or courses will be inserted free of charge.
Please send information to the Editorial office, Radiology and Oncology, Zaloška 2, SI-1000 Ljubljana, Slovenia.

Author Index 2005

Subject Index 2005

Fondacija "Docent dr. J. Cholewa"
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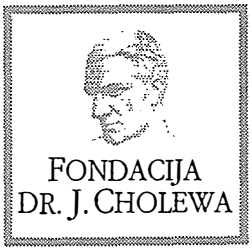


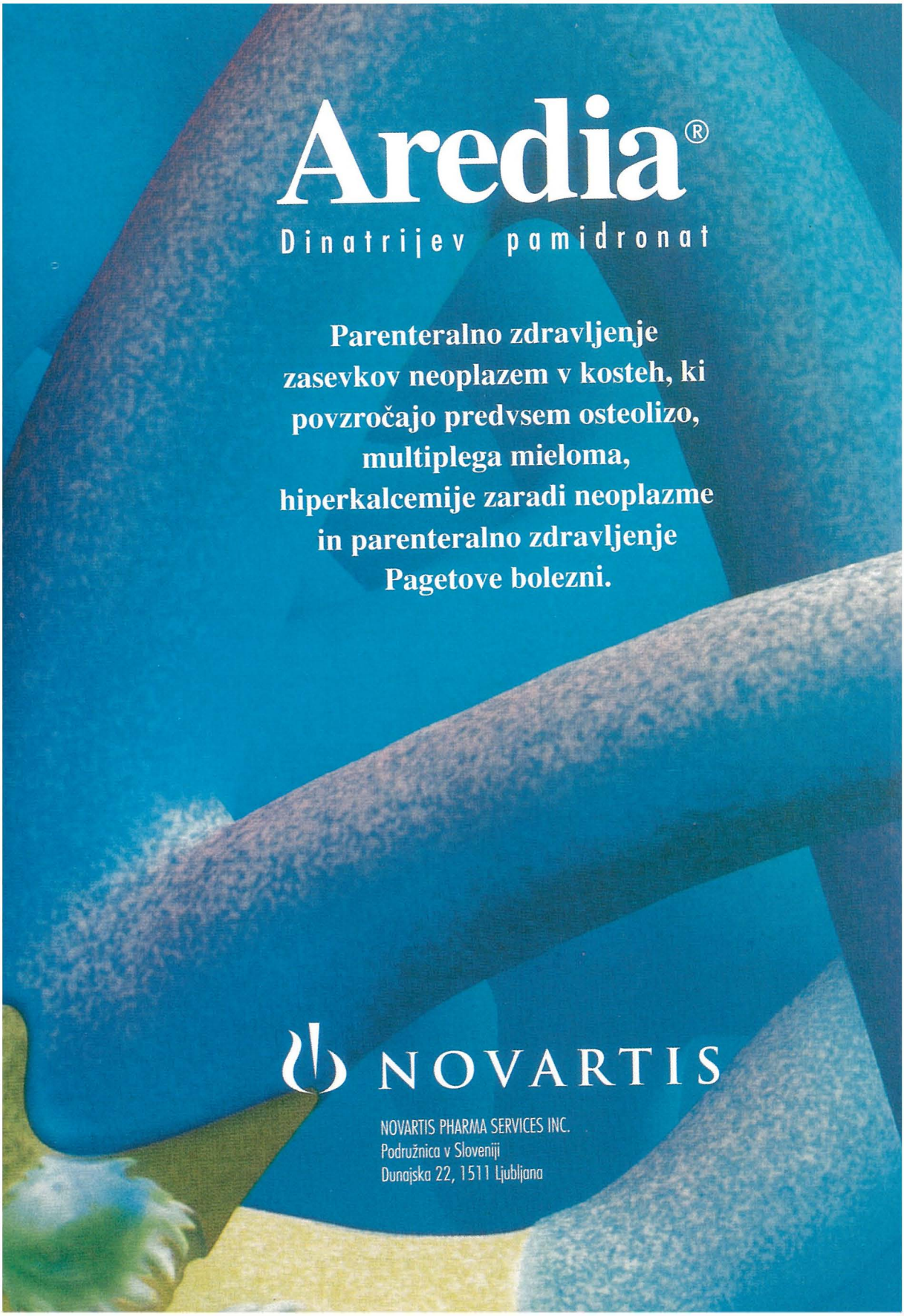
Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education - A Report for the Final Quarter of 2005

The Dr. J. Cholewa Foundation for Cancer Research and Education continues to support activities associated with cancer research and education in Slovenia with different grants and other forms of financial support. All the requests are being dealt with by the responsible bodies formed by Foundation members with clinical and cancer research experience and by members with important experience in finance.

The Dr. J. Cholewa Foundation for Cancer Research and Education continues to support the regular publication of "Radiology and Oncology" international scientific journal, which is edited, published and printed in Ljubljana, Slovenia, as it has done over the last couple of years. This support is considered to be one of its more important and permanent commitments. Among other recipients of grants and other forms of support it is possible to find several experts in the field of lung and breast cancer, Slovenian Association of Pathology and Slovenian Cancer Society.

It is the policy of the Foundation to lend support to individuals and institutions that participate in cancer research and education in Slovenia. The Dr. J. Cholewa Foundation for Cancer Research and Education therefore respectfully acknowledges the importance of the commitment of various public companies and private individuals to its cause.

Andrej Plesničar, MD
Borut Štabuc, MD, PhD
Tomaž Benulič, MD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Datum priprave besedila januar 2005

Podrobnejše informacije so na voljo pri proizvajalcu.

Krka, d. d., Novo mesto, Šmarješka cesta 6, 8501 Novo mesto, www.krka.si

Že 50 let.

KRKA

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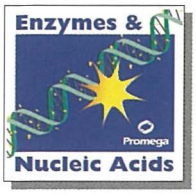
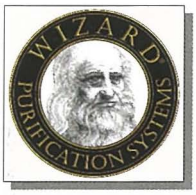
Promega



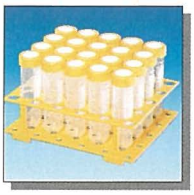


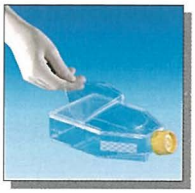
IZDELKI ZA MOLEKULARNO BIOLOGIJO

SYNGENE

DOKUMENTACIJA IN ANALIZA GELOV

TPP





PLASTIKA ZA CELIČNE KULTURE

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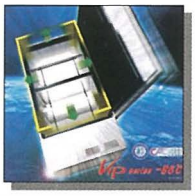

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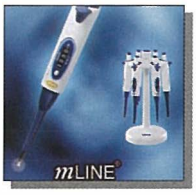
CELIČNE KULTURE, GELI IN MOLEKULARNA BIOLOGIJA

BIOHIT



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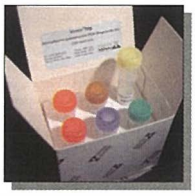

DIAGNOSTIKA MIKOPLAZEM IN LEGIONEL

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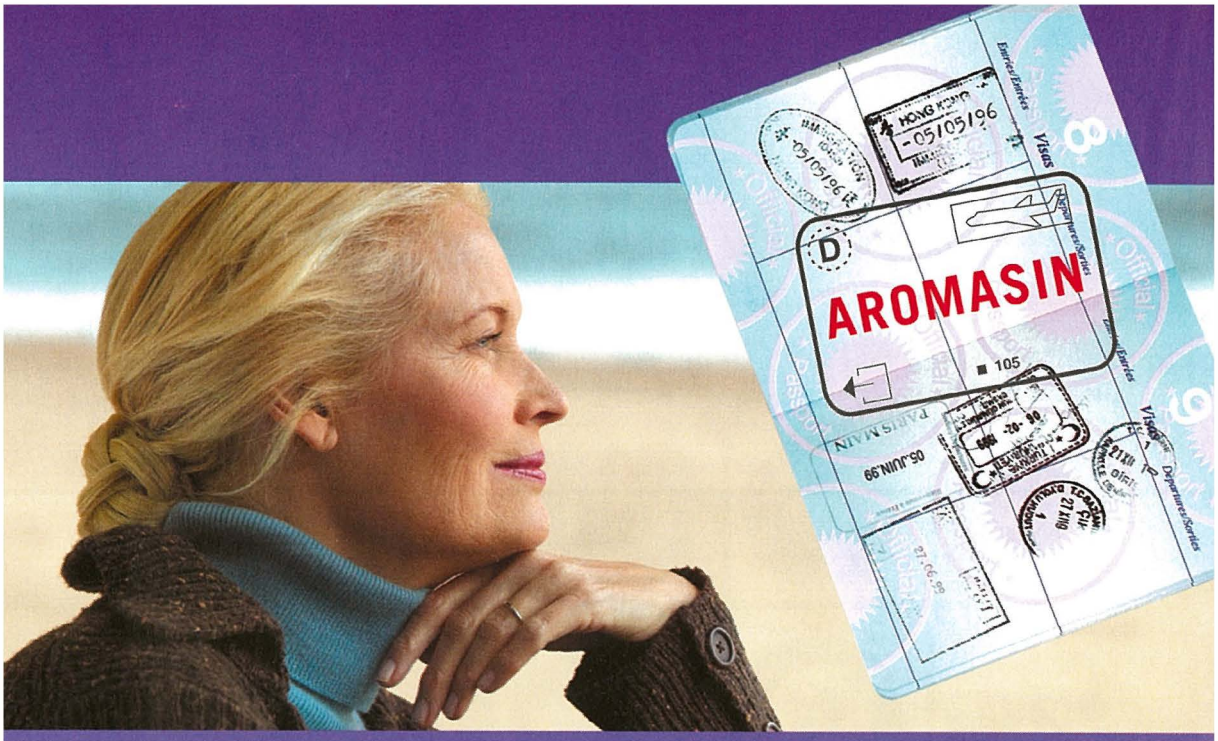
SEKVENATORJI



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Pred predpisovanjem se seznanite s celotnim povzetkom glavnih značilnosti zdravila.

Podrobnejše informacije o zdravilu so na voljo pri:
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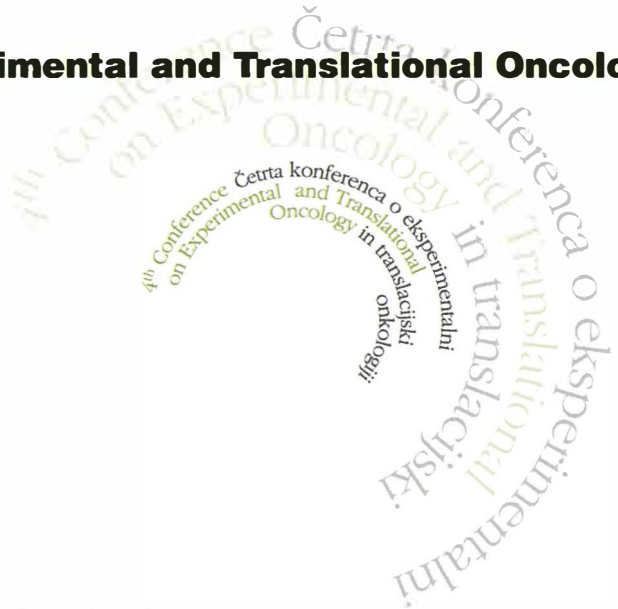
4th Conference on Experimental and Translational Oncology

Kranjska gora, Slovenia, March, 22-26, 2006



Organized by: Tamara Lah, Gregor Serša and Janko Kos

Topics:

- Mechanisms of tumour progression
- Tumour markers
- Delivery systems in cancer therapy
- New drugs and therapeutic targets
- Immune response and cancer

Location:

Hotel Lek
4280 Kranjska gora, Slovenia
http://www.hotel-lek.si

Correspondence:

Conference secretary:
Phone: +386 1 241 29 70
Fax: +386 1 241 29 80
Email: helena.koncar@nib.si
http: http://www.onko-i.si/ceto

Organized under patronage of Association of Radiology and Oncology

EUROPEAN SCHOOL OF ONCOLOGY

in cooperation with

INSTITUTE OF ONCOLOGY LJUBLJANA

organize an advanced course on

ETHICS IN ONCOLOGY

Chairs: Antonella Surbone and Matjaz Zwitter
Course Coordinator: Antonella Surbone
Date and venue: May 18 – 20, 2006, Bled, Slovenia

Course venue:
Grand Hotel Toplice
C. svobode 12
4260 Bled, Slovenia
Phone: +386 4 579 10 00
Fax: +386 4 574 18 41
E-mail: info@hotel-toplice.com
www.hotel-toplice.com/en-index.html

Secretariat:
Rita de Martini
European School of Oncology
Via del Bollo, 4
20123 Milan, Italy
Phone: +39 02 85464527
Fax: +39 02 85464545
E-mail: rdemartini@esoncology.org

On-line registration available at www.cancerworld.org/eso

ETHICS IN ONCOLOGY

The field of oncology, as a front-line specialty that involves intense and often dramatic circumstances and outcomes, magnifies the experience of the common ethical dilemmas that physicians and nurses encounter in clinical practice.

Furthermore, special issues arise in clinical oncology related as the severity and uncertainty of the cancer patient's condition and his physical and psychological suffering; the tension inherent in patient-doctor communication about cancer; the complexity of cancer diagnosis and treatment; and the toxicity of many cancer therapies, coupled with the prominent role of experimental treatments; and the fact that cancer is not only the illness of a person, but also of his entire family.

Thus, on a daily basis, the health-care workers caring for cancer patients face the ethical implications of cultural diversity vis-à-vis communication of diagnosis and prognosis and of end-of-life decisions. Most oncologists and oncology nurses are involved in the development and/or conduct of clinical trials and must deal with the moral implications of enrolling cancer patients in human experimentation. Moreover, emerging fields such as that of genetic testing and therapeutics have raised new ethical quandaries.

This course is designed to provide an in-depth exposure to theoretical and practical ethical issues in oncology, as well as to provide clinical guidelines for the solution of common ethical dilemmas. The course will be extremely interactive, with ample time for general and one-to-one discussion between participants and speakers.

FACULTY

Lea Baider
Sharett Institute of Oncology, Hadassah University Hospital, Jeruzalem, IL
Jan Foubert
Institute Jules Bordet, Bruselj, BE
Carl J. Fürst
Palliative Care Unit, Stockholms Sjukhem, Stockholm, SE
Steven Joffe
Dana-Farber Cancer Institute, Boston, US
Tore Nilstun
Medicinsk Etik, Lund, SE
Darius Razavi
Université Libre de Bruxelles, Brussels, BE
Lidia Schapira
Massachusetts General Hospital, Boston, US
Antonella Surbone
European School of Oncology, Milan, IT
Borut Telban
Slovenian Academy of Sciences and Arts , Ljubljana, SI
Joze Trontelj
Committee for Medical Ethics, Ministry of Health, Ljubljana, SI
Kirsi Vähäkangas
University of Kuopio, FI
Matjaz Zwitter
Institute of Oncology, Ljubljana, SI

Program, registration form and hotel reservation form are available on http://www.onko-i.si/en/Research and Education/Scientific Events/Meetings for Medical Doctors

Instructions for authors

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

Manuscript written in English should be submitted to the Editorial Office in triplicate (the original and two copies), including the illustrations: *Radiology and Oncology*, Institute of Oncology, Zaloška 2, SI-1000 Ljubljana, Slovenia; (Phone: +386 1 5879 369, Tel./Fax: +386 1 5879 434, E-mail: gsersa@onko-i.si). Authors are also asked to submit their manuscripts on a 3.5" 1.44 Mb formatted diskette. The type of computer and word-processing package should be specified (Word for Windows is preferred).

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

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

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

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

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

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

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

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

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

References must be numbered in the order in which they appear in the text and their corresponding numbers quoted in the text. Authors are responsible for the accuracy of their references. References to the Abstracts and Letters to the Editor must be identified as such. Citation of papers in preparation, or submitted for publication, unpublished observations, and personal communications should not be included in the reference list. If essential, such material may be incorporated in the appropriate place in the text. References follow the style of Index Medicus. All authors should be listed when their number does not exceed six; when there are seven or more authors, the first six listed are followed by "et al.". The following are some examples of references from articles, books and book chapters:

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

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

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

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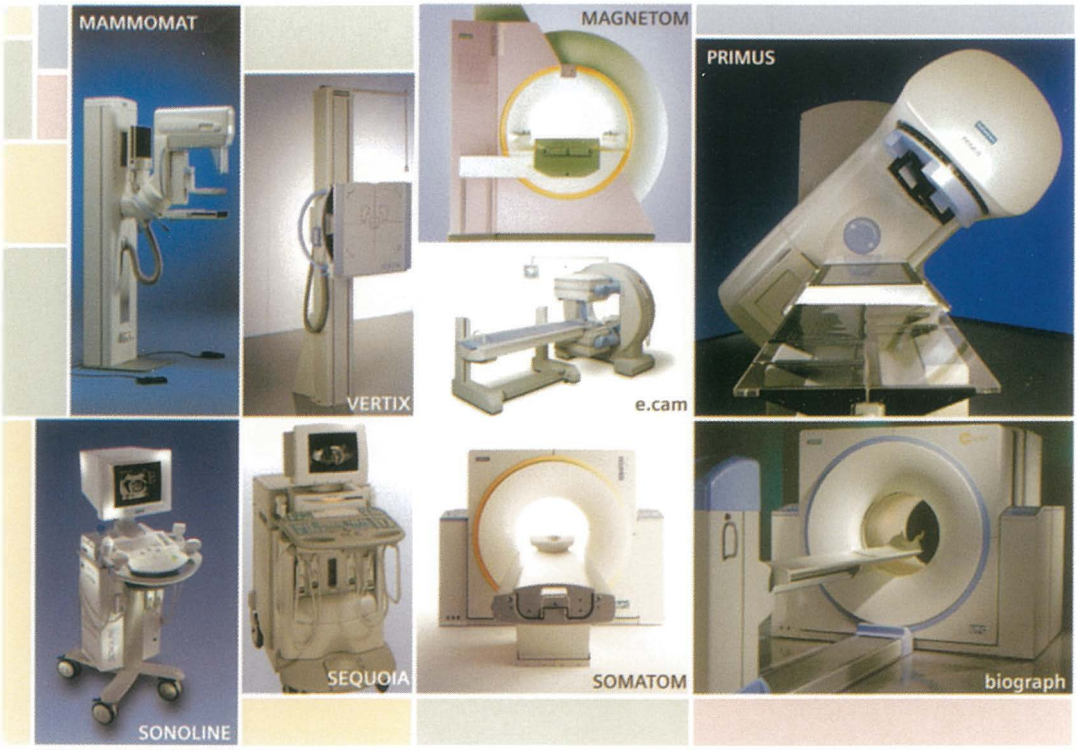

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