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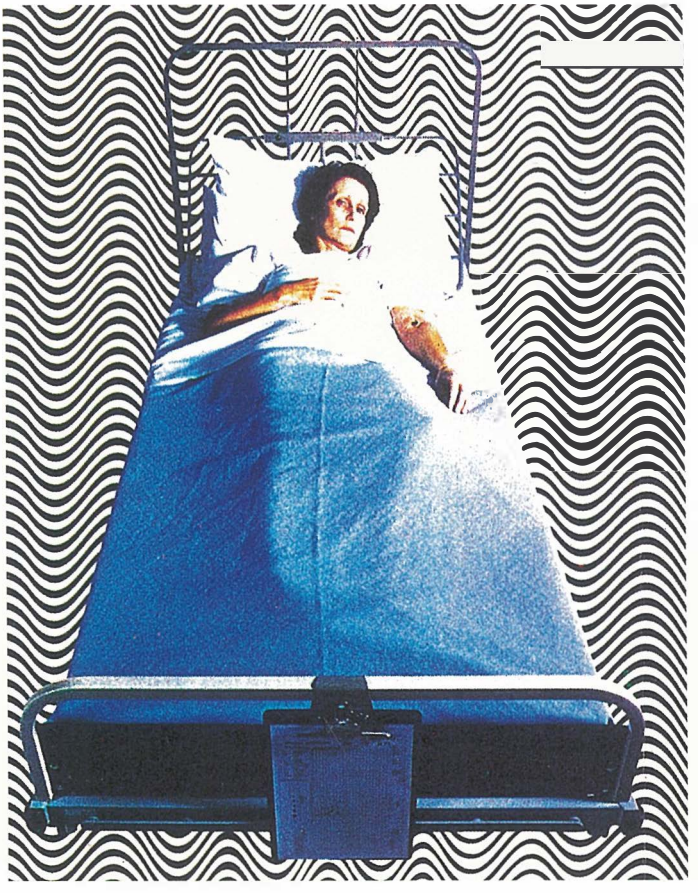

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Pseudotumor of the mediastinum – Case report

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Varices of the azygos and hemiazygos veins secondary to portal hypertension should be considered in differential diagnosis in every mediastinal pseudotumor.
We are presenting a very rare case of a female patient with a pseudotumor of the upper mediastinum resulting from thrombosis of the portal vein and portal hypertension. After a splenorenal shunt, chest X-ray showed that the shadow of the mediastinal pseudotumor almost disappeared.

Key words: mediastinal neoplasms; hypertension, portal

Introduction

Portal hypertension represents a group of pathologic clinical manifestations associated with hypertension in the portal venous system or, more precisely, with increased portocaval gradient. Increased portal vein pressure is caused by an obstruction of normal intrahepatic or prehepatic blood flow. The only exception is the so-called idiopathic portal hypertension, where the obstruction can be detected neither within the liver nor extrahepatically, with all the signs of portal hypertension. Splenomegaly, collateral circulation, bleeding, etc., can develop as a consequence of portal hypertension. Varices are most commonly localized in the esophagus or the stomach (esophagogastric varices) and other localizations are quite uncommon. Rarely, varices of the azygos and the hemiazygos veins, imitating a tumor of the mediastinum, may develop.[1–7] We are presenting the case of a young female patient with a portal vein thrombosis, portal hypertension and a pseudotumor of the mediastinum.

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Case report

Patient C. N., female, born in 1964, a tourist from Lecco (Italy), was admitted to the Intensive Care Unit, Department of Internal Medicine, Clinical Hospital Center Rijeka, on August 17, 1989 because of melena. She had no reported illnesses until 1986 when she was hospitalized for an artificial septic abortion, with probable pylephlebitis (inflamation of portal vein) and portal vein thrombosis that remained undiscovered. Several months later, due to persistent abdominal pain she underwent gastroenterological examination and varices of the esophagus and portal vein thrombosis were detected. In 1987, one year after the detection of the portal vein thrombosis and esophageal varices, bleeding from the esophageal varices was evident

for the first time, and she was hospitalized in Milan. During the next two years she experienced four bleedings from the esophageal varices. The last bleeding was evidenced in March of 1989, i.e., five months before she was admitted to our Department. During her last hospitalization in March of 1989, a chest X-ray revealed a lung shadow in the superior right mediastinum, which was considered to be a tumor of the superior mediastinum. Already then, surgical derivation of the portal circulatory system was considered. This possibility was then rejected due to the tumor of the upper mediastinum.

As stated earlier, the patient was admitted to our Department on August 17, 1989, due to melena initially not profuse. One day before being admitted to our Department, she passed three profuse, fluid, tarry stools. On admission the patient was pale, tachycardic 110/min., blood pressure 15/8 kPa. The hepar was not papable and the spleen was enlarged by 3 cm below the left costal margin. Laboratory findings showed severe anemia, decreased fibrinogen and thrombocits, decreased total proteins and serum albumin. An urgent esophagogastroduodenoscopy was immediately performed revealing very large non bleeding esophagogastric varices. Bleeding started the same night, and a Sangstaken-Blackmore tube was placed, vasopressin and ranitidine were administered parenterally. She also received a blood transfusion. After several tarry stools, it appeared that the bleeding stooped. Unfortunately not for long. Melena and hematemesis persisted and the patient almost expired due to exsanguination. This bleeding was caused by rupture of the esophageal balloon due to maximal air insufflation necessary to maintain the hemostasis effective. The Sangstaken-Blackmore tube was reintroduced, and with new transfusions applied to both hands, resuscitation was achived with great difficulty.

At this stage it was clear that conservative treatment is not effective. Even the smallest displacement of the tube would provoke new bleedings. An emergency shunt was the only solution that could save the patient. Ultrasonography of the abdomen and a chest X-ray were performed.

Ultrasonography of the abdomen showed a normal sized liver, with no dilatation of intrahepatic biliary ducts. In the body and tale of the pancreas the lienal vein 11 mm in diameter, was visualized. Only the initial part of the portal vein was demonstrated. The spleen was enlarged, $18 \times 5 \times 8$ cm. There was ascites in the abdomen.

Chest X-ray revealed a polycyclic shadow in the upper right mediastinum. Our explanation for this finding would be that it represents a vascular tumor, i.e., dilatation-varices of the azygos and hemiazygos veins (Figure 1).

As the bleeding persisted and the patient's showed no improvement, we decided to perform an emergency shunt. The patient underwent surgery on August 19, 1989, when a splenorenal shunt and a splenectomy were effected.

Surgical treatment. We performed extend left subcostal laparotomy. Over 1 liter of ascitic fluid was evacuated from the abdominal cavity. The stomach was very distended, filled with coagulated blood. Lavage was effected by a nasogastric tube. Numerous varices were found along the lesser and greater curvature of the stomach and the abdominal esophagus, with many peritoneal collaterals. The lymph stasis was pronounced. Inspection and palpation of the liver revealed normal findings. Spleen was merkedly enlarged, cyanotic, hard in palpation.

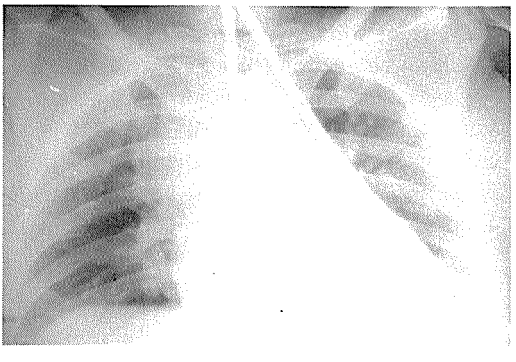

Figure 1. Polycyclic shadow in the upper right mediastinum.

Other abdominal organs were unremarkable. After preparation of the vascular hylum of the spleen, splenectomy was performed. The lienal vein was mobilized away from the pancreas. The left renal vein was accessed and partially clampeb; venotomy was effected, and an anastomosis between the lienal and renal veins was performed. After clamps were removed, a stable functional shunt was obtained. Hemostasis was evaluated, drainage of the spleen cavity effected, and the abdominal wall closed by layers.

The postoperative course was complicated by repeated hematemesis. Esophagogastroduodenoscopy revealed considerably smaller esophageal varices, and the bleeding was caused by erosions of the gastric mucosa. After treatment with blood transfusion and parenterally administered ranitidine, the bleeding eventually stopped, with considerable improvement in red cell count. The patient stayed at the Department of Surgery during August 19–31, 1989, when she was returned to the Department of Internal Medicine.

To confirm our hypothesis that this was a case of a vascular pseudotumor and not a true tumor of the upper mediastinum, we performed another chest X-ray before the patient was discharged, i.e., nearly a month after the operation. It revealed that the polycyclic shadow in the upper right mediastinum considerable reduced (Figure 2). The patient was released from hospital on September 18, 1989 in a good general condition, with a satisfactory blood count and with normal values of fibrinogen and thrombocytes. During the follow-up, esophagogastroduodenoscopy evidenced no varices of the esophagus. The shunt was well functioning. The chest X-ray was clear with no traces of polycyclic shadows in the right upper mediastinum. Four years since the operation the patient feels well, has experienced no difficulties and continued to work.

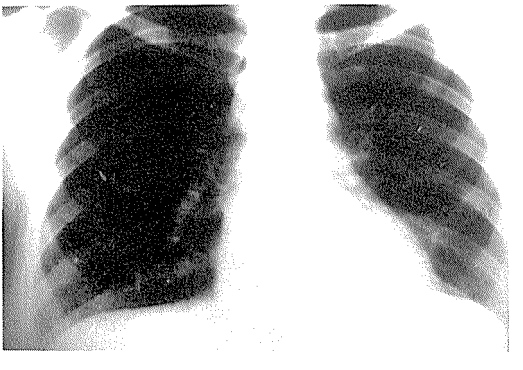

Figure 2. Polycyclic shadow in the upper right mediastinum almost disappeared after a splenorenal shunt.

Discussion

In a portal vein thrombosis there is a total obstruction of the portal venous blood flow through the liver. This causes the reverse of blood flow toward natural anastomoses that connect the portal system with the inferior and superior vena cava system. In a portal vein obstruction due to thrombosis, portal hypertension rapidly develops. In liver cirrhosis, portal hypertension develops slowly allowing natural anastomoses to gradually redirect the additional blood from the portal system toward the vena cava, and they gradually dilate. The hepatic portal circulation in cirrhosis is never completely interrupted. It is partially preserved depending on the extent of the cirrhotic process.

In portal vein thrombosis, natural anastomoses must adapt quickly to the newly developed conditions, i.e., they must be able to transport the complete portal blood flow in the opposite direction of its natural flow. In this case, we shall mention only two important natural anastomoses. One is through the esophageal veins, connected on one side to the left gastric vein pertaining to the portal system, and on the other side to the azygos and hemiazygos veins that enter the superior vena cava. The other, less known routes, are the small retroperitoneal veins, the so-called Retzius veins, that represent the anastomoses between the superior and inferior mesenteric vein on one side and the azygos and hemiazygos vein on the other.

In portal thrombosis, these anastomoses transport a large volume of blood to the azygos and hemiazygos vein, which are located in the

retroperitoneal space, extending from the abdominal to the thoracic cavity each on one side of the vertebral column, and joining before entering the superior vena cava. Their wall is thin, unresisting to pressure and dilates unevenly forming varices. They become tortuous and elongated, folding in places and developing rope-like vascular masses, giving the impression of a tumor. Such vascular tumors can be located in the abdomen or more often in the thorax. In the abdomen they are placed retroperitoneally, and in the thorax in the posterior mediastinum more often in the inferior that the superior part. According to French authors they are called pseudotumorous varices of the mediastinum.[8]

We have presented an interesting case of a female patient whose chest X-ray revealed a polycyclic shadow in the superior mediastinum, which could easily be misdiagnosed. If the patient had not bled from the varices, she could have been subjected to an invasive diagnostic procedure for the pseudotumor, with eventual catastrophic consequences. This case represents a good example why, in differential diagnosis of a mediastinal tumor, the possibility of a pseudotumor should be considered when portal hypertension is present.

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Transcatheter occlusion of patent ductus arteriosus in adults

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Patent ductus arteriosus (PDA) may escape clinical detection and persist into adulthood. For the purpose of PDA occlusion a new transcatheter technique with double Rashkind umbrella was developed. We present two successful closures of PDA in adult patients with this device. The two years follow-up findings testify to the favourable clinical course, with disappearance of the continous murmur and a normal colour flow echocardiographic examination.

Key words: ductus arteriosus, patent therapy; catheterisation

Introduction

Isolated patent ductus arteriosus (PDA) occurs in 1 in 2000 live, fullterm birth, accounting for approximately 5% to 10% of all types of congenital heart defects. PDA is very common in premature infants, and infants born at high altitude have an increase incidence. There is female preponderance with a ration ranging from 2:1 to 3:1.[1]

The clinical significance of the PDA is determined by magnitude of the shunting through the PDA. The direction of the flow will depend upon relative pulmonary and systemic vascular resistance. Normally, the pulmonary vascular resistance drops quickly after birth. Therefore, blood flows from the aorta into pulmonary arteries.[2] PDA is closed either surgically[3] or by percutaneous transcatheter technique. First successful percutanous closure of PDA was reported by Postman in 1963.[4] Since then numerous techniques have been developed over the years, but only the Rashkind double umbrella closure of PDA has been generally accepted (Figure 1).[5,6]

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Case reports

Case 1

A 18-year-old woman presented with dyspnea on effort. A clinical diagnosis of PDA had been made at the age of 5, however, her parents refused surgical correction at that time. Chest radiograph showed increased pulmonary vascularity and a prominent aortic arch. Cardiac catheterisation showed conical shaped PDA 10 mm long and 5 mm in diameter without pulmonary hypertension. After diagnostic catheterisation, a 0.035 inch, 300 cm long Amplatz

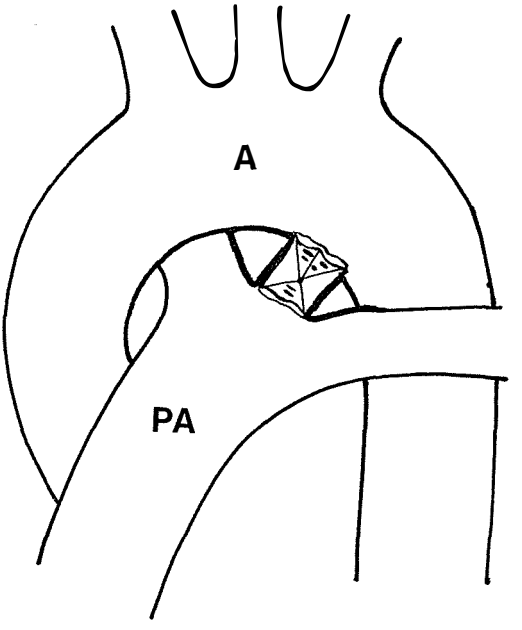

Figure 1. Rashkind PDA occluder system in position in the ductus arteriosus. PA = pulmonary artery, A = aorta.

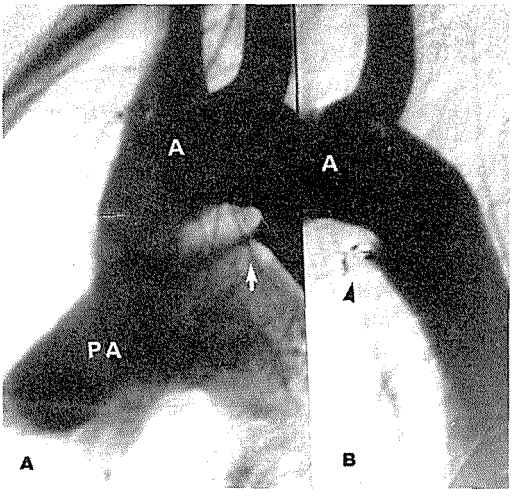

Figure 2A. Lateral angiogram showing typical funnel shaped ductus (arrow). PA = pulmonary artery, A = aorta.

Figure 2B. Lateral angiogram showing device in position (arrow) with hinge point at the pulmonary artery (PA) end, distal limbs nicely flexed and no residual shunting. A = aorta.

guide wire was introduced from the pulmonary aftery through the PDA into the thoracic aorta. The delivery catheter was advanced into the thoracic aorta and PDA was closed by 12 mm Rashkind double umbrela (Figure 2). During the 2-years follow-up, patient has been free of symptoms.

Case 2

A 30-years old woman was admitted to the hospital due to accidentally diagnosed PDA. She was symptoms free. On auscultation, a continous machinery murmur in the 2nd in 3rd left interspace was heard. She had no signs of heart failure. The 12-lead ECG showed normal sinus rhythm. The chest x-ray film showed no abnormalities. However, the transthoracic echocardiogram in modified parasternal short axis view of the base of the heart revealed colour flow Doppler signal streaming into the main pulmonary artery. A continuous flow into the pulmonary artery was recorded by continuous were Doppler examination. Both findings are characteristic for PDA without pulmonary hypertension. The diagnostic catheterisation was performed and 1 cm long conical shaped PDA was shown by aortography. At the pulmonary end it was 5 mm wide and at the aortic end 10 mm. The pressures in the right heart and in the pulmonary circulation were in normal limits. After diagnostic catheterisation the PDA was closed by double Rashkind umbrella by transcatheter technique. The typical murmur of PDA disappeared immediately after the procedure. Three days after transcatheter closure of the PDA the control echocardiogram was performed and there was no colour flow Doppler signal of PDA. The patient was examined two years after the procedure in six months intervals clinically and by echocardiography. She has been free of symptoms and echocardiogram has been in normal limits.

Discussion

PDA may escape clinical detection and persist into adulthood. If adults do not develop signi-

ficant pulmonary vascular disease, they tend to have an unpredictable progression of left ventricular failure.[7] Surgical or transcatheter occlusion of PDA is indicated in adulthood, too.

Although mortality of surgical correction of PDA is low, significant morbidity, including excessive bleeding, injury to the recurrent laryngeal or phrenic nerves, and complications of general anaesthesia, can occur in a small percentage of patients. To circumvent these problems, transcatheter closure of PDA was developed. Transcatheter occlusion is a successful mode of treatment for PDA with a low incidence of complications. Overal success rate is cca 96 % including reoclussions.[8]

Results reported by McManus (2) and McNamara (3), as well our data suggest that transcatheter closure of PDA with Rishkind double umbrella is a efficacious procedure in providing complete permanent ductus closure even in adult patients. The clinical course suggests that PDA closure is complete immediately after the procedure. After two years follow-up our patients have been free of symptoms and colour flow Doppler studies have been in normal limits.

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Simple quantitative analysis of Ga-67 lung scintigraphy - normal values

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Quantitative analysis of Gallium-67 lung scintigraphy was performed in 54 patients undergoing routine Ga-67 scintigraphy for suspected infection of the lower limb. All patients were without evidence of lung disease and without pathological Ga-67 accumulation in the lungs. Regions of interest were positioned over the soft tissue of the neck (STN), pulmonary hilus (PH), pulmonary parenchyma (PP), and liver (L).

The obtained average counts and count rations were different in males and females, probably caused by breast tissue and liver functional status. Variability was lower in STN indices in comparison to L indices.

Our quantitative approach could be of considerable value in assessing the course of disease and response to therapy in various lung diseases.

Key words: lung diseases-radionuclide imaging; Gallium radioisotopes; lung scintigrapy, Gallium-67

Introduction

Gallium-67 can concentrate in almost all pulmonary infections and granulomatous diseases, including pneumoconiosis, sarcoidosis, idiopathic pulmonary fibrosis, cytomegalovirus infections etc.[1–5] In some pulmonary diseases Ga-67 scintigraphy can be more sensitive than conventional radiography.[6–9]

Monitoring the course of disease and response to therapy is a significant contribution of Ga-67 scintigraphy in patients with lung disease.[3,10.11]

We describe a simple method for quantitative analysis of Ga-67 lung scintigraphy, which, after validation, might be a more accurate parameter for disease course and response to therapy.

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Patients and methods

We examined 54 patients (27 females, mean age 50 ± 15; 27 males, mean age 54 ± 18) referred to our department for routine Ga-67 scintigraphy. Forty five of them were referred for suspected infected hip prostheses, four for suspected infected knee prostheses and five for possible osteomyelitis of the lower limb.

All patients were without evidence of lung disease and without pathological Ga-67 accumulation in the lungs.

Static images of the lungs were recorded (500 000 counts per view) 48 hours after injection of 74 MBq of Ga-67 citrate. Data were stored on a digital computer for analysis. Regions of interest (ROI-s), all of the same size, were placed over the soft tissue of the neck (STN), the upper margin of the liver (L), pulmonary hilus (PH) and pulmonary parenchyma (PP) (Figure 1). The average counts per pixel value in each ROI was used for further calculation. PH and PP values were expressed as mean values of the right and left lung and divided by STN and L values. The final results were expressed as a mean value ± standard deviation.

Statistical analysis was performed with a t-test for unpaired data.

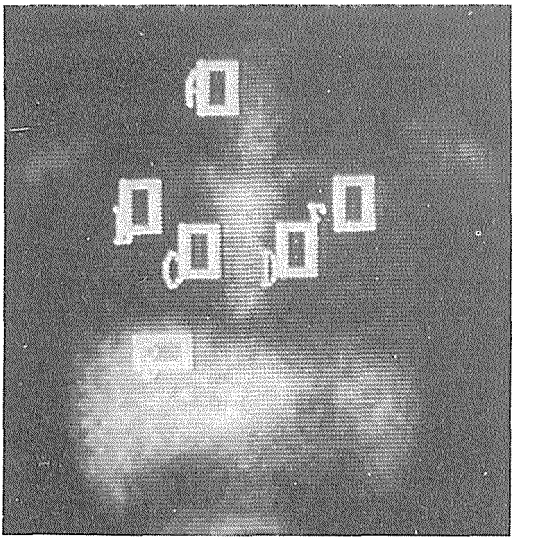

Figure 1. Regions of interest over STN (A), L (B), right PH (C), left PH (D), right PP (E) and left PP (F).

Results

Fifty four Ga-67 lung scintigrams were analysed (Table 1). The highest and most variable uptake was in the liver. The uptake in STN and PH regions was similar. The difference between the left and right lung was negligible.

Statistically significant differences in counts between females and males were obtained for all ROI-s, except STN. The L-ROI was higher in females, the other was lower than in males.

The PH/STN and PP/STN values were similar for both genders, but the indices connected with lung and liver counts were significantly higher in men than in women (Table 2).

Table 1. The average counts per pixel per ROI and per gender.

		STN	L	right PH	left PH	right PP	left PP
Male	X	170	311*	174*	173**	140*	141*
	s. d.	19	53	14	13	15	13
Female	X	165	339*	166*	163**	133*	134*
	s. d.	26	65	18	18	15	14

X – mean value, s.d. – standard deviation, * – $p < 0.05$, ** – $p < 0.025$.

Table 2. The values for four indices and per gender.

		PH/STN	PP/STN	PH/L	PP/L
Male	X	1.03	0.84	0.57*	0.47**
	s. d.	0.15	0.13	0.10	0.09
female	X	1.01	0.82	0.51*	0.41**
	s. d.	0.14	0.13	0.12	0.10

X – mean value, s.d. – standard deviation, * – $p < 0.05$, ** – $p < 0.025$.

Discussion

The concentration of Ga-67 in the lung normally is low 48 hours after administration.[7] Pathological accumulation in pulmonary hilus and parenchyma is almost always prominent, but sometimes the uptake is faint, bilateral and symmetrical with pulmonary parenchyma infiltration only, and without lymphadenopathy.[57] Normal values as obtained in our investigation could be of help in unclear situations.

Attenuation by breast tissue might be the reason for lower PH and PP values in women. Female patients in average were at the end of or beyond the fertile age. A more prominent uptake may occur in younger women.[12] Marked Ga-67 uptake in the liver is customary. However, according to our results, it is very variable, probably depending on liver functional status. The high variability may make PH/L and PP/L indices less reliable.

The liver ROI is placed over the upper margin of the liver in spite of the possibility of local lung activity superposition. A position in the middle of the liver, however, might interfere with gallbladder and bile duct activity.

We have no explanation for the higher female values. A possible reason could be different liver functional status or Ga-67 accumulation in and around the mamilla.

We believe that our simple method could be used for the assessment of disease course and response to therapy. At present, we are studying patients with sarcoidosis.

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Residual carcinoma of the uterine cervix after low-dose preoperative intracavitary irradiation

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There is an increasing evidence questioning the efficacy of post-operative irradiation in the cases of cervical carcinoma with metastatic pelvic lymph nodes or in the cases where more than a half of the cervix has been invaded by carcinoma. As there is no objective test for the evaluation of radiosensibility of cervical carcinoma, preoperative brachytherapy should be an aid of response of carcinoma of irradiation. With analysing the survival of malignant cells after preoperative brachytherapy we have tried to asses the radiosensibility of the tumor as a prognostic factor in order to make a decision on further treatment.

From 1979 to 1991 a non-randomized group of 109 patients with cervical cancer of stages IB, IIA and IIB underwent radical surgery, 71 of them after preoperative intracavitary brachytherapy. Forty Gy were delivered to point A with 226-Ra or 137-Cs sources. The time interval from irradiation to surgery ranged from 5 to 46 days.

The histopathological examination of surgical specimen revealed no residual disease in 14 patients; most of them underwent surgery between the 20th and the 30th day after brachytherapy. All stages and histopathological types were included. Only in one case positive pelvic lymphatic metastases were present. This patient was post-operatively irradiated. All survived three and more years.

Fifty-seven patients have had residual disease with positive (33,3 %) or negative (66,6 %) pelvic lymph nodes. Patients with positive lymph nodes were postoperatively irradiated. In this group 9 patients died (47,3 %), in the group with negative nodes 7 died (18,4 %) within the first three years.

In all, in patients with residual disease the prognosis is worse regardless the stage, whether operable or of histopathologic type.

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Key words: cervix neoplasms-surgery; brachytherapy; prognosis

Introduction

Several attempts were made to find prognostic factors in cervical carcinoma. The first studies were published already in 1912, and in 1959 Wentz and Reagan proposed pathohistological classification with the aim to help clinicians.[1,2] Cytomorphologic and cytophotometric studies, and also studies of irradiation with test doses did not give an adequate answer.[3,4] Most of these studies were on patients with advanced cervical carcinoma who underwent radiotherapy.[5–7]

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Retrospective and also prospective studies of cervical carcinoma showed an increasing evidence questioning the efficacy of post-operative irradiation in patients with lower stages of cervical cancer; they are treated surgically. There are no objective factors to determine the responsiveness of cancer cells to irradiation.

Therefore, we analysed the survived malignant cells after preoperative brachytherapy in order to asses the radiosensibility of the tumor as a prognostic factor and in order to make a decision on further treatment.

Material and methods

From 1979 to 1991 a non-randomized group of 109 patients underwent radical surgery for the cervical carcinoma at the Institute of Oncology, Department of Gynaecology. Seventy-one of them were preoperatively intracavitary irradiated. Patients with stages IB, IIA, and IIB with initial parametrial infiltration, but primary cancer more than 5 cm in the greatest dimension with huge necrosis, haemorrhage, and superimposed infection were preoperatively irradiated. Brachytherapy with radium (226-Ra) or cesium (137-Cs) sources was performed. Tumor dose 40 Gy was delivered to point A.

Seventy-one patients with early cervical carcinoma (median age 44, range 23 to 70 years) underwent pretreatment evaluation. As it has several objectives, the most important of these is to establish the clinical extent of the disease, both in pelvis and elsewhere in the body if possible. Complicating disease, anaemia, infection, impaired function of kidney, and performance status of the patient have also been taken into consideration, although the size and the extent of tumor growth, its histopathological type, and necrosis, haemorrhage or infection of the tumor were taken in account.

All cases were histologically confirmed. Planocellular carcinomas were classified according to the WHO classification, but planocellullar carcinomas, undetermined, were added. This was a retrospective study; the patients were treated at our Institute and after initial diagnostic workup in other centres. That is why the histopathological differentation did not correspond to WHO classification in all cases or was less precisely. A group of patients with adenocarcinoma was added to the patients who underwent both preoperative brachytherapy and than radical surgery.

The time interval from brachytherapy to surgery varied and ranged from 5 to 43 days. There were no objectives for such a different time interval. To determine the optimal time for surgery, we analysed the survival of malignant cells of different histopathological types or the disappearance of cervical cancer after initial brachytherapy. As there are no objective tests to determine the radiosensibility of cancer cells, the local responsiveness could give an information of it.

As surgical treatment, radical hysterectomy and pelvic lymph nodes dissection were performed.

Results

Results of brachytherapy were divided into three groups: no residual disease (NRD) with positive or negative pelvic lymph nodes, residual disease (RD) with positive lymph nodes and residual disease with negative pelvic lymph nodes (Table 1).

Table 1. The outcome of preoperative intracavitary brachytherapy according to the histopathological types of cervical carcinoma.

Histopathological type	Pathological status of specimen		
	NRD/L–	RD/L–	RD/L+
Planocellular cornescens	2	5	4
Planocellular undifferen.	4	19	9
Macrocellular	3	7	4
Microcellular	3	1	2
Adenocarcinoma	1	5	1
	13 (+1 NRD/L+)	37	20

NRD no residual disease, RD residual disease, L+ positive lymph nodes, L– negative lymph nodes. In the group of no residual disease is only one patient with positive lymph nodes.

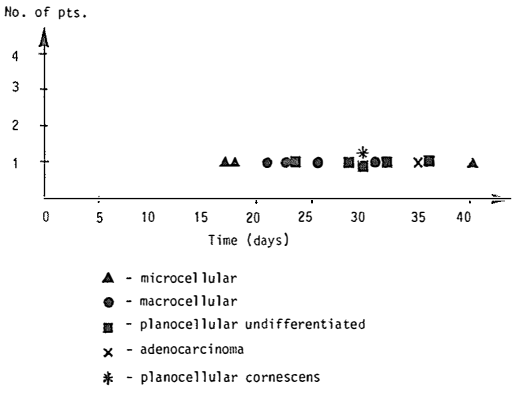

Figure 1. Disaperance of malignant cells after preoperative intracavitary brachytherapy.

As the time interval between brachytherapy and surgery was not determined, it was different. The first negative surgical specimen appeared after 17 days, most of them between 20 and 30 days after brachytherapy (Figure 1). Negative surgical specimen were in all histopathological types.

Comparing with stages, positive and negative surgical specimen were in all (Table 2).

There is a minimum follow-up of three years for all patients. Thirteen patients with NRD and negative lymph nodes survived three years and more, with no evidence of recurrent disease or distant metastases. Only one patient with NRD and positive lymph node was post-operatively irradiated, but no follow-up was possible, because she was from another country.

In the group of patients with RD and negative lymph nodes 7 (18,4 %) of 38 died, in the group of patients with RD and positive lymph nodes 9 (47,3 %) of 19 died within three years.

Table 2. Stages and residual disease after brachytherapy.

Stage of disease	Pathological status of specimen NRD/L–	RD/L–	RD/L +
Stage IB	7	27	8
Stage IIA	2	7	0
Stage IIB	4	4	11
	13 (+1 NRD/L +)	38	19

NRD no residual disease, RD residual disease, L + positive lymph nodes, L– negative lymph nodes.

Discussion

There is little data of the time interval after preoperative irradiation to surgery. As preoperative irradiation consists either of teletherapy or brachytherapy, we collected in our group only the patients intracavitary irradiated to get information about radiosensibility of the tumor. Several studies show that the therapeutic results obtained with radiation or surgery alone or with the combination of both in patients with stage IB and IIA carcinoma of the cervix are approximately the same.[8]

A prospective surgical-pathological study of disease free interval in patients with stage IB of planocellular carcinoma of the cervix published by Delgado identified independing prognostic factors. These factors were: clinical size of the primary tumor, capillary/lymphatic space (CLS) involvement, and depth of tumor invasion.[9]

Post-operatively in the surgical specimen CLS involvement and depth of invasion could not be measured in patients with preoperative radiation because of necrosis and partial disappearance of the primary tumor.

As the time interval was very different, althought not exactly determined, most of the patients underwent surgery between two and six weeks.[8,10] In the group of patients studied, no residual tumor was found between the 17th and the 40th day after brachytherapy.

In the same time-interval most of the tumor disappeared in other cases, although vital malignant cells were still present and huge necrosis of the tumour persisted (groups with residual diseases). One would expect that the combination of preoperative irradiation followed by hysterectomy to remove the residual tumor should improve pelvic control or survival. There are two groups with residual tumors: one with positive and another with negative pelvic lymph nodes. Our results are more or less the same as the ones discussed above, although the mini-

mum follow-up was three years. Three years survival for patients with residual desease and positive lymph nodes is 52,7%, and with residual disease and negative lymph nodes 81,6% and it is consistent with other reports in the literature.[11,12]

Only in the group of patients with no residual disease regardless the clinical stage and the histopathologic types of tumors, a better prognosis can be expected.

Preoperative brachytherapy as a mode of combined therapy for cervical carcinoma can only be accounted for on the basis that preoperative irradiation consistently sterilizes the tumor and reduces its extension into adjoining lymphatics. The only problem is what to do when residual disease is present and pelvic lymph nodes are positive. In our group, patients with positive lymph nodes were post-operatively irradiated, but the survival is at least as disappointing as by the patients who were not irradiated. All patients were irradiated only to the true pelvis, but extended-field irradiation gives better survival as reported.[13]

As most of primary tumor disappeared between the 20th and the 30th day after brachytherapy, delayed surgery is of no choice to get information about radiosensibility of the tumor. As the group with residual carcinoma and positive lymph nodes had a bad prognosis although they are irradiated post-operatively, another mode of treatment should be carried on.

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Outcome of pneumonectomy for primary non-small cell lung cancer – A ten-year experience

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Between 1982 and 1992, 105 patients with non-small cell lung cancer underwent pneumonectomy and mediastinal node dissection. Thirteen patients (12%) were categorized as pathological stage I, 18 (17%) were categorized as pathological stage II, 63 (60%) were categorized as pathological stage IIIa, and 11 (11%) were categorized as pathological stage IIIb. The overall actuarial 3-year survival rate was 27%. Postpneumonectomy complications occurred in 20 patients. Operative mortality occurred only in 8 pathological stage IIIa male patients (7.6%). No significant differences in operative mortality were noted for the following criteria: age, sex, side of resection, pathological stage, histologic classification, preoperative forced expiratory volume in 1 second (FEVI) of 2.0 L or less, and predicted postoperative FEVI of 1.0 L or less. Of the surviving 97 patients, 3 patients were lost during follow-up, 13 patients (13.8%) died due to pneumonia and/or respiratory failure, and 40 patients (42.6%) died due to distant metastases, with bone as the most common metastatic site, during the mean follow-up time of 39 months. Pathological stage I and stage II patients showed actuarial 3-year survival rates, of 60.0% and 31.3% respectively. The survival rates of stage I and stage II patients were higher than for stage IIIa patients.

Key words: carcinoma non-small-cell lung, lung neoplasms- surgery, penumonectomy, survival rate

Introduction

Surgical resection remains the most curative modality for non-small cell lung carcinoma.[1-3] With improvements in preoperative preparation and postoperative fluid and respiratory therapy, the mortality and morbidity rates for pulmonary surgery have dramatically decreased over the past decade.[4]

In 1933, Graham and Singer reported a successful one-stage pneumonectomy for carcinoma of the lung.[5] In 1950, Churchill and associates reported improved results with lobectomy, which remains the procedure of choice for the majority of patients with carcinoma of the lung.[6]

What is the most appropriate resection for cancer of the lung? That most appropriate procedure is the one that excises all of the carcinoma and preserves as much of the normal tissue as possible. In previous studies, operative

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mortality after pneumonectomy has ranged from 6.2% to 12.4%[7-11] and the 5-year survival rate from 8% to 25% for all stages of carcinoma of the lung,[7, 9, 12]

We retrospectively reviewed 105 patients with non-small cell lung cancer who had undergone pneumonectomy, to identify the postoperative morbidity and mortality, factors associated with postoperative mortality, the survival times for various stages, and the outcomes.

Materials and methods

Between 1982 and 1992, 105 patients with non-small cell lung cancer underwent pneumonectomy and mediastinal lymph node dissection in our institute. There were 93 men and 12 women with a mean age of 62.0 years (range, 42 to 72 years).

The patients were categorized as operable with a clinical stage of IIIa or less. Clinical staging was performed using TNM classification.[13] Extensive preoperative evaluations were conducted including plain chest roentgenograms and computed tomographic scans of the chest and abdomen. Clinical N2 disease was defined by the presence of an enlarged mediastinal lymph node greater than 1 cm in diameter. A bone scan and liver sonography were routinely performed for each patient. A computed tomographic scan of the brain was performed if symptoms were present. Mediastinoscopy was performed to rule out N3 disease. All patients received preoperative pulmonary function tests including spirometry, regional pulmonary ventilation and perfusion scan using Xenon-133 gas and Tc-99m MAA, and arterial blood-gas determination.

Pneumonectomy was performed in patients whose tumor was considered to be completely resectable, and was determined by tumor size and local extension. The necessity for pneumonectomy was determined, at the time of operation, by the surgeon. Patients with a completion pneumonectomy were excluded from this study.

Operative mortality was defined as death within 30 days of pneumonectomy or death during hospitalization after pneumonectomy. Risk factors of pneumonectomy were examined, including age, sex, side of resection, pathological stage, histological classification, preoperative FEV1 of 2.0L or less, and predicted postoperative FEV1 of 1.0L or less (using percent perfusion to the uninvolved lung from the Tc-99m MAA perfusion scan study).

All patients were followed until death. There were 3 patients who were lost during follow-up. The mean follow-up time was 39 months (range, 12 to 118 months). The actuarial survival rates of the remaining 94 patients were analyzed by pathological stage.

Statistical analysis

Survival distributions were calculated using the methods of Kaplan and Meier. Differences in operative mortality, based on variable criteria, were calculated using the chi-square test.

Results

Eighty-four patients (80%) presented with squamous cell carcinoma; 16 patients (15%) presented with adenocarcinoma, 4 patients (4%) presented with adenosquamous carcinoma and 1 patient presented with (1%) large cell carcinoma.

Sixty-nine patients (66%) underwent a left pneumonectomy and 36 patients (34%) underwent a right pneumonectomy. After operation, patients were staged based on pathological examination of the tumor and lymph node mapping. There were 13 patients (12%) categorized as pathological stage I, 18 patients (17%) categorized as stage II, 63 patients (60%) categorized as stage IIIa, and 11 patients (11%) categorized as stage IIIb.

Table 1 shows the complications that occurred in 20 patients and their association with operative mortality.

Table 2 shows the results of various criteria associated with operative mortality. Although all 8 operative deaths (7.6%) occurred in male patients with a pathological stage of IIIa, there

Table 1. Incidence of complications and their association with operative mortality.

Complications (n = 34)	No. of patients (n = 20)	Deaths	
		No.	%
Respiratory causes (35.3%)			
pneumonia	6	4*	67
prolonged ventilation (>24 hrs)	3	0	0
pulmonary edema	2	0	0
bronchopleural fistula	1	1	100
Cardiac causes (29.4%)			
arrhythmia	7	0	0
heart herniation	2	0	0
myocardial infarction	1	1	100
Others (35.3%)			
hemorrhage	2	0	0
chylothorax	2	0	0
stroke	2	1	50
pulmonary embolus	1	1	100
renal failure	1	1*	100
wound infection	2	0	0
recurrent nerve palsy	2	0	0

* one of these four patients had renal failure.

Table 2. Differences in operative mortality for various criteria.

Criteria	No. of deaths	No. of patients	%	P value
Age (years)				
<70	6/90		6.7	NS
≥70	2/15		13.3	
Sex				
Male	8/93		8.6	NS
Female	0/12		0.0	
Side of resection				
Right	4/36		11.1	NS
Left	4/69		5.8	
Staging				
I + II	0/31		0.0	NS
IIIa	8/63		12.7	
Pathology				
Squamous cell ca.	7/84		8.3	NS
Adenocarcinoma	1/16		6.3	
Preop. FEV1				
≥2.0 L	5/44		11.4	NS
<2.0 L	3/23		13.0	
Predicted postop. FEV1				
≥1.0 L	8/65		12.3	NS
<1.0 L	0/2		0.0	

FEV1: forced expiratory volume in 1 second. NS: no significance.

were no significant differences in operative mortality for gender or pathological stage (using the chi-square test).

Actuarial survival was determined for all patients (Figure 1), as well as by pathological stage (Figure 2). The overall survival rate was 27% after 3 years. Patients with pathological stage I, stage II, stage IIIa, and stage IIIb diseases showed 3-year survival rates of 60.0%, 31.3%, 18.2%, and 0%, respectively. The median survival times of patients with pathological stage I, stage II, stage IIIa, and stage IIIb diseases were 3.82 years, 2.66 years, 1.46 years and 0.93 years, respectively.

Among the surviving 94 patients, 13 patients (13.8%) died due to pneumonia without evidence of disease before death, and 40 patients (42.6%) died due to distant metastasis, with bone as the most common metastatic site during the follow-up period.

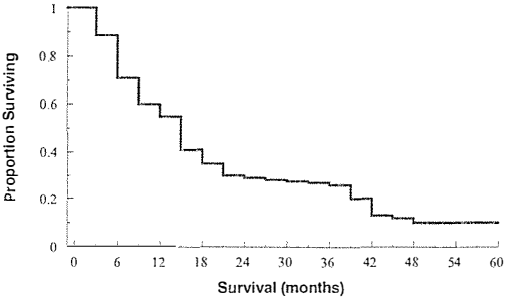

Figure 1. Overall actuarial survival od 94 patients who underwent pneumonectomy for primary non-small cell lung cancer.

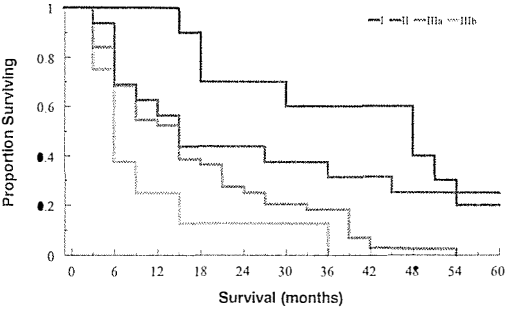

Figure 2. Actuarial survival of 94 patients who underwent pneumonectomy for primary non-small cell lung cancer by pathological stage.

Discussion

Most surgeons agree that pneumonectomy, for patients with non-small cell lung cancer, should be used only for extensive tumors that cannot be removed by lobectomy, or sleeve lobectomy.[14, 15] In this study, the patients who underwent pneumonectomy showed a predicted postoperative FEV1 equal to or greater than o.8 L (the preoperative FEV1 multiplied by the percent of perfusion of the uninvolved lung). In an experimental clinical study, Adams et al showed the importance of cardiopulmonary reserve as a determinant of risk.[16] Ventilation perfusion studies, using Xenon-133 gas and Tc-99 m MAA showed good reliability for calculating predicted lung function.[17, 18] These predictors are very useful clinically because regional and overall pulmonary functions remain stable after pneumonectomy.[19]

At the time of operation, if a (bi-) lobectomy or a sleeve resection was determined to be insufficient for removing the local disease, a pneumonectomy was performed. We traced the medical records of the patients who had undergone pneumonectomy and found that the procedure was performed if the following criteria were present: (1) tumor encroaching to the pulmonary aftery, (2) tumor invasion into the hilar area, (3) tumor invasion into the main bronchus, and (4) tumor invasion into the interlobar fissure.

Our study showed an operative mortality rate of 7.6%, which is in accordance with the results of Ginsberg et al (6.2%),[7] Patel et al (8.6%),[11], and Putnam et al (8%).[20] Various risk factors of pneumonectomy have been studied. Didolkar et al found that advanced age was associated with an increased mortality rate in pulmonary resection for lung cancer,[21] however, this factor was not suggested in the study by Patel et al[11] or in our study. This may be due to a patient group with a limited number subjects aged 70 or above; 14.3% in our study and 16% in the study of Patel et al.[11] Our report, unlike that of Weiss,[10] did not find that women fare better after resection. This may be due to the fact that the number of male patients (93 patients) greatly surpassed the number of female patients (12 patients) in our study. The side of resection was not related to mortality, although Higgins et al[22] and Harmon et al[23] suggested that right pneumonectomy is more hazardous in terms of bronchopleural fistula and empyema. In our study, although all eight operative mortalities occurred in pathological stage IIIa, there was no statistical significance when compared to stages I and II. This may be due to a greater percentage of patients (60%) in pathological stage IIIa. We found that no single variable enables the clinician to predict operative moratlity after pneumonectomy, and patients should not be excluded from pneumonectomy on the basis of any single criterion.

The complications after pneumonectomy were variable, and some were associated with mortality. In our study, although all patients received prophylactic antibiotics, pneumonia developed in 6 patients. Among them, four patients died due to respiratory failure. Cardiac arrhythmia was the most common complication, occurring in 7 patients (35%), but was not associated with mortality. Wahi et al reported that patients who developed atrial arrhythmia had a longer stay in the intensive care unit and a longer postoperative hospital stay.[4] The cause of the abnormal rhythm is unknown. Mediastinal shift, hypoxia, abnormal pH of the blood, as well as other factors, have been implicated but none have been proven. Shields suggested prophylactic digitalization for older patients undergoing pneumonectomy.[24]

We found that the most significant factor for determining long term survival of patients undergoing pneumonectomy is the pathological stage. Patients categorized as pathological stage I or stage II showed a better 3-year survival rate than those with stage IIIa (60% or 31.3% vs 18.2%). We did not stratify the survival time of the various subtypes of pathological stage IIIa by T and N status. Wilkins and co-workers found a significant correlation for survival time and lymph node status in pneumonectomy-treated patients. Patients with uninvolved nodes had a better 5-year survival rate than those with

involved nodes (42.1 % vs 16.3 %).[3] Putnam et al found that patients classified as pathological stage I, stage II, stage IIIa showed 3-year survival rates of 45 %, 47 %, and 24 %, respectively. In addition, no patient in pathological stage III with a percent predicted FVC of 64 % or less survived more than 1.3 years, compared with a 26 % survival rate after 3 years for other pathological stage III patients ($P = 0.009$).[20] Further multivariate studies may be needed to clarify the significant factors that determine the survival of the pneumonectomy-treated patient.

The fate o patients after pneumonectomy has seldom been reported. Ogilvie and coworkers studied patients with lung cancer 10 years after pneumonectomy, and found that patients had a poorer ventilatory capacity and a higher incidence of cor pulmonale than those patients with lung cancer 1 year after pneumonectomy.[25] In our study, we found that 13.6 % of the patients died due to pneumonia and/or respiratory failure during a mean follow-up time of 39 months. A management strategy that includes prevention of pulmonary infection, respiratory exercise, prohibition of smoking and regular expectoration should be considered during follow-up. The benefit of adjuvant therapy for the various stages of patients after pneumonectomy remains to be verified.

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Tumor necrosis factor-α (TNF-α): Biological activities and mechanisms of action

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Tumor necrosis factor-α/cachectin (in further text TNF-α) was originally defined for its ability to cause hemorrhagic necrosis of different types of tumors. In the meantime, it has become clear that TNF-α is a multifunctional immunoregulatory cytokine with a broad spectrum of activities upon hematopoetic and nonhematopoetic cells. Some of the pleiotropic activities of TNF-α are growth inhibition of some tumor cells; stimulation of human fibroblast, B cell and thymocyte proliferation; activation of phagocytic and endothelial cells; induction of prostaglandin synthesis as well as regulation of oncogenes, transcription factors and major histocompatibility complex antigen expression. The effect of TNF-α (antiproliferative or stimulative) depends upon the type of target cells, presence of TNF receptors, TNF-α concentration in tissues or upon presence of other mediators capable of affecting the activities of this cytokine. In this paper we are reviewing biological as well as physico-chemical properties of the cytokine, its production and some mechanisms of TNF-α action.

Key words: tumor necrosis factor

TNF history

More than 200 years ago some physicians noticed tumor reduction in patients with bacterial inflammations; a logical conclusion was that bacteria or their products somehow retard the growth of tumors.[1] This finding encouraged the physicians of the 19th century to treat patients with solid tumors by means of a direct introduction of microorganisms into the tumors. Such a therapy gave different outcomes ranging from complete disappearance of tumors or partial reduction of tumor burden to complete failure of the therapy.[2] The high infectiveness of microorganisms (*Streptococcus pyogenes*) and consequently a serious risk for bacterial infection in patients was the motive that forced dr. William B. Coley to treat his patients with the toxins from bacterial cultures instead of using microorganisms themselves. For the preparation of bacterial toxins he chose *Streptococcus pyogenes* and *Bacillus prodigious* (now called *Serratia marcescens*). With the above stated therapy Coley achieved noteworthy results (disappearance or partial reduction of tumors) that are described in his articles from 1894, 1896 and 1898.[3, 4, 5] On the account of higher efficacy and safety of Coley's toxins (as they were called

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later) for the treatment of cancer patients this method found acceptance and became commercially attractive. Coley's toxins were produced and used for treatment until the end of 1920 when they gave up their place to radiotherapy.

However, the Coley's toxins were not forgotten. In the years 1931,[6] 1932,[7] 1935[8, 9] and 1936[10] various researchers executed quite a few experiments on tumors in mice using filtrates of Gram negative bacterial cultures as antitumor agents. All these experiments share a common fact: tumors with good vascular supply became centrally necrotic at the beginning of therapy and afterwards, necrosis spread out to the external parts of tumors. Bacterial toxins were injected intratumorally - i.e. locally in all cited experiments. In the year 1943 Shear[11] reported about experiments with tumor model Sarcoma-37 (Sa-37) where he injected tumor-bearing mice intraperitoneally (i.p.) with polysaccharide isolated from *S. marcescens*. Such kind of systemic treatment caused necrosis in the centre of tumors and even more, systemically treated tumors, in which necrosis spread out to periphery, later on completely disappeared (like in the case when they were treated locally).

All the above stated experiments were a good starting point for Carswell and co-workers. In the year 1975 they published an article in which they, for the first time, used the appellation "tumor necrosis factor" and speculated about its production and its effects.[12] Namely, the very same researchers established that animals, infected with *Bacillus Calmette Guerin* (BCG) and afterwards treated intravenously with endotoxin, produce "with endotoxin induced serum factor" which causes hemorrhagical necrosis of tumors. This serum factor responsible for necrosis of tumors was named simply "tumor necrosis factor" (TNF). TNF displayed cytotoxic activity against numerous tumor cells, yet not against normal mouse fibroblasts (later it turned out to be growth factor for normal fibroblasts). On the basis of these findings Carswell and co-workers described TNF as an agent with selective antiproliferative effect only on tumor cells. In the same article authors also presumed that macrophages are the main producers and bacterial endotoxins the main inducers of TNF. Both presumptions later turned out to be correct. It is well known today, that TNF-α (i.e. the agent which Carswell and co-workers named TNF) can be produced not only by macrophages but also by other types of cells, and that there are other factors beside endotoxins which can serve as inducers of TNF-α production.

TNF-α is not the only tumor necrosis factor known nowadays, since another substance with similar activities, was discovered already in 1968. Ruddle and Waksman[13] and also Granger and Wiliams,[14] independently one from another, described a substance, produced by lymphocytes, with powerful cytotoxic effect on syngenic embrional fibroblasts or on L929 cells. Ruddle and Waksman named this substance "cytotoxic factor", while Granger and Wiliams called it "lymphotoxin". Because of the similarity in the amino acid sequence (35% homology)[15] and in their activities (ranging from the cytotoxicity against L929 cells to the ability to produce necrosis of some sorts of sarcomas *in vivo),*[16] lymphotoxin was classified among tumor necrosis factors and called TNF-β, while Carswell's TNF was renamed into TNF-α.

Today, it is well known that TNF-α can be synthesised not only by macrophages but also by other cells while TNF-β is produced exclusively by T lymphocytes. Yet, the mechanisms of stimulation of TNF-α production in macrophages are completely different from the mechanisms of stimulation in other cells.[17]

Production of TNF-α

TNF-α production is a multistep process which includes the induction of gene transcription and the amplification of TNF-α mRNA, the synthesis of prohormone (233 aminoacids-AA), the activation of prohormone with cleavage of the molecule to 157 AA (molecular weight approximately about 17 kDa), and the secretion of TNF-α.[18]

Inducers of TNF-α production

Substances that trigger TNF-α production can be divided according to their source into extracellular (exogenous) and intracellular (endogenous) inducers. One of well known and most often used extracellular inducers of TNF-α production is bacterial lipopolysaccharide (LPS), with its active part - lipid A, which is responsible for most of the biological properties of LPS.[19, 20] The second (by frequence of its use) extracellular inducer is muramyldipeptide - MDP (or its structural analogues) that can be employed, either alone or in combination with other agents (LPS, IFN-γ), to stimulate the production of TNF-α in macrophages. Besides, a higher degree of transcription of TNF-α gene *in vitro* was ascertained in the cells exposed to radiation, viruses, bacteria, parasites, as well as to their products.[21-26] Some tumor cells[27] and plant polysaccharides[28] are also known to act as extracellular inducers.

On the other hand, interferons (IFN-α and IFN-γ), growth factors (GM-CSF), interleukin-2 (IL-2) and TNF-α itself are the most frequently applied intracellular inducers.[29-35]

Inhibitors of TNF-α

Excessive production of TNF-α can be very harmful for the organism. As a consequence - the production and activities of TNF-α as well as of other cytokines in the organism are strictly controlled. The control is realised mostly through the supervision of gene transcription and translation, or through the production of substances that block cytokine's effects (by binding either to the cytokine molecule or to cytokine's specific membrane receptors).

Mechanisms of action of the inhibitors, which directly influence transcription and translation of TNF-α, are still quite unclear. However, the researchers are already acquainted with the fact that individual cytokines, as for example interleukins (IL-4, IL-6), transforming growth factor β (TGF-β), prostaglandins and corticosteroids, can operate as inhibitors in these processes.[36-40]

The second group of TNF-α inhibitors, which block the activities of the very cytokine, are proteins that were discovered in urine samples of febrile patients. One of these proteins is called uromodulin; i.e. a glycoprotein with molecular weight of 85 kDa. It operates by binding to TNF-α molecules and thus preventing the attachment of these molecules to receptors.[41, 42] The second specific protein, isolated from urine samples, has molecular weight of 40-60 kDa; this one also inhibits activities of TNF-α by means of binding to its molecule and blocking of its attachment to receptors.[43, 44]

Beside the inhibitors that were isolated from urine another group of proteins with similar effects was described by Scuderi and co-workers[45] in serum. The authors assumed that these proteins were plasma α-globulins. They supposed that the blockade of activities of secreted TNF-α was carried out through the binding of α-globulins to TNF-α molecule which prevented the binding of TNF-α to cell receptors.

A more thorough biochemical analysis of TNF receptors demonstrated that proteins (isolated from urine and serum) which inhibit activities of TNF are nothing but the soluble form of TNF receptors. In this light, uromodulin was connected with TNF R75 (TNF receptor with molecular weight of 75 kDa), while the other inhibitory protein isolated from urine was identified (according to its molecular weight) as a substance related to TNF R55 (TNF receptor with molecular weight of 55 kDa).

Moreover, there is some evidence of existence of substances capable of blocking the activities of TNF cytotoxic products. Among the most thoroughly examined substances of this character belong manganese superoxide dismutase (MnSOD) and metaloproteins (e.g. metalothionein).[46, 47] MnSOD prevents the production of oxygen free radicals in mitochondria while metaloproteins bind these free radicals and thus neutralise their effect on cell structures.

The best known synthetic TNF inhibitors originate from the group of serine protease inhibitors. Serine proteases play an important

role in cleavage of prohormone molecules and production of biologically active form of TNF-α. The inhibitors of these proteases interfere with serine protease activity and consequently block the secretion of biologically active form of TNF-α.[48]

The control of TNF-α production and its activities is a complex process that most likely includes (beside above cited mechanisms of regulation of transcription, translation and secretion, as well as beside the direct effect on the very cytokine) other manners of supervision. All these mechanisms and factors interweave and work together as an entirety.

TNF-α producers

Quite a few years ago Beutler and co-workers[40] established that monocytes and macrophages are the basic producers of TNF-α. Today, it is known that also other cells are capable of TNF-α production. Among the most important producers we classify NK cells,[49] T lymphocytes,[50] some non-hematogenous cells like for example muscle cells, endothelial cells and microglia,[21, 51-53] as well as some tumor cells: cells of colorectal adenocarcinoma, Hodgkin's lymphoma, ovarian carcinoma, breast carcinoma.[54-56]

Production of TNF-α in monocytes/macrophages

As it was already mentioned before LPS, i.e. the endotoxin isolated from cell walls of Gram negative bacteria, is the best known extracellular inducer of transcription of TNF-α gene and of TNF-α synthesis in leukocytes.[40] That explains why the LPS's effect on monocyte/macrophage population and its stimulation of lymphocytes to produce TNF-α are most thoroughly examined. Despite of that, exact molecular mechanisms of signal transduction and of initiation of TNF-α gene transcription remain uncleared. The researchers presume, that the process of lymphocyte stimulation (to produce TNF-α) is somehow connected to the activity of phospholipase A_2 and afterwards to the metabolism of arachidonic acid and cAMP activity.[22, 31] Namely, Motrri and co-workers[31] ascertained that LPS statistically significantly increases the activity of membrane-bound phospholipase A_2. On the other hand, the inhibitors of phospholipase A_2 statistically significantly reduce TNF-α mRNA production in monocytes stimulated with LPS. Unfortunately, all these findings are not sufficient for exact conclusions about molecular mechanisms involved in regulation of transcription, translation and secretion of TNF-α. Not until these mechanism are understood, the possibility to influence effectively the quantity of cytokine produced and its cooperation in different activities in organism will be given.

Physical and chemical properties of TNF-α

TNF-α is a glycoprotein whose sequence consists of 156-157 amino acid residues (156 AA mouse TNF-α, 157 human and rabbit TNF-α); its molecular weight is 16-18 kDa (16-18 kDa mouse, 17 kDa human, and 18 kDa rabbit TNF-α). These molecular weights are the ones of TNF-α after electrophoresis in sodium-dodecylsulfate-polyacryl-amide gel (SDS-PAGE), when the active form decomposes into monomers. Molecular weight of unaltered protein (as for example after gel filtration) ranges from 34 kDa (rabbit) to 45 kDa (human).[57] The above stated facts lead us toward conclusion that the active form of TNF-α is its trimeric form, which has also been proved by means of ultracentrifugation and crystallography.[58, 59]

Trimeric form of TNF-α is susceptible to temperature changes, since the increase of temperature causes decomposition of trimmer form. The isoelectric point (pI) of human TNF-α is somewhere between 5 and 6, of mouse TNF-α between 3 and 5 and of rabbit TNF-α about 5.[57] Besides, TNF-α is susceptible to trypsin and chimotrypsin action, as well as to the action of some other proteases (V8),[57] for it looses its biological activities when being exposed to these proteases. On the other hand, TNF-α retains its biological activities when exposed to low pH or organic solvents.

TNF-α exists in two different forms, i.e. as a free (soluble) cytokine or bound to cellular membranes (of monocytes for example). Membrane-bound TNF-α was at first supposed to be the excreted cytokine that reversely attached itself to its own membrane receptors.[60] Hafsli and co-workers[61] proved that this statement is incorrect. Namely, they ascertained by means of immunofluorescent microscopy that the number of membrane-bound TNF-α molecules remains unaltered regardless of the presence of added soluble TNF-β. As a matter of fact, if membrane-bound TNF-α was the soluble form reversely attached to its own receptors, then, in the presence of TNF-β, there would be less TNF-α molecules bound to membrane, because of the competition for free receptors. Membrane-bound TNF-α acts cytotoxically as a transmembrane protein; the exact mechanism of action is still unclear, yet it is known to kill target cells in direct cell-to-cell contact.

Biological properties and actions of TNF-α

TNF-α is classified together with TNF-β, interferons, interleukins and growth factors into the group of hormone-like substances called cytokines. Its name "tumor necrosis factor" originates from its first known biological property, i.e. from its ability to cause necrosis of tumors.[12]

In the year 1984 Pennica and co-workers[62] succeeded in cloning of TNF-α cDNA, thus enabling the synthesis of recombinant TNF-α (rTNF-α). Since physical and chemical properties, as well as biological activities of rTNF-α are identical to the ones of native TNF-α and because of low cost production of rTNF-α this accelerated experimental work with the cytokine. Today it is known that biological activities of this very cytokine are not confined to cytostatic/cytotoxic effect upon tumor cells only, but that TNF-α co-operates in many other processes (Table 1).

TNF-α has (beside the above cited activities) also quite a few side-effects (just like the other cytokines do). The most frequent side-effects are fever, anorexia, diarrhea, nausea and hypotension.[63-68]

The cited activities rank among the most important ones of this cytokine, yet the list of TNF-α effects is by no means at the end. A complete register of processes, in which TNF-α takes part directly or indirectly, is very difficult to elaborate, since this pluripotent cytokine is secreted by different cells (blood, liver, spleen, kidney, muscle cells and cells of central nervous system) and participates in a large number of processes in the organism. Also, the pathogenesis of cerebral malaria and the destruction of tissues in inflammation are connected to hyperproduction of TNF-α.[69, 70]

Table 1. Short review of biological activities of TNF-α.

Antitumor activities
• modulates MHC I and II antigenic expression (thus it operates as an immunomodulator);[71, 72]
• induces transcription of enzyme inhibitors;[73]
• induces differentiation of tumor cells;[74]
• participates in cytotoxic activities of monocytes and macrophages (membrane-bound or soluble TNF-α);[75, 76]
• stimulates IL-1, IL-6 and IL-8 production in macrophages/monocytes;[77-79]
• stimulates prostaglandine E_2 (PGE2) production in macrophages/monocytes;[77]
• induces synthesis of IL-2 receptors on T lymphocytes;[80]
• activates NK cells;[81]
• induces synthesis and expression of transforming growth factor (TGF-α) and of receptors for epidermal growth factor (EGFR) on human pancreatic cancer cell line.[82]
Other activities
• operates as a mediator of inflammation and cellular immune response;[83-85]
• participates in regulation of cellular and physiological processes: in differentiation of cells, in regulation of sleep (i.e. inducer of sleep);[67, 68, 86-88]
• influences cellular metabolism;[89]
• operates as a growth factor (stimulates fibroblasts);[90-92]
• has antiviral, antibacterial and antiparasitic effects;[93]
• operates as a modulator for neurons in hypothalamus (regulates growth and their functions);[94]
• has radioprotective effect (most probably by means of stimulating the stromal cells in bone marrow to produce growth factors CSF, that are known to stimulate hematopoiesis).[95, 96]

Antitumor activities of TNF-α

Carswell discovered TNF-α as a factor capable of causing hemorrhagical necrosis of tumors in mice.[12] Later, Carswell and different researchers found out that TNF-α acted cytostatically and cytotoxically on various tumor cells, while having no effect on growth of normal human cell lines.[97, 98] On the basis of these facts they concluded that TNF-α acts directly cytostatically/cytotoxically on tumor cells, and that this very cytokine possesses a distinctively selective antiproliferative potential only against tumors cells. Further studies disproved the above conclusion, since TNF-α acts either antiproliferatively on tumor and normal cells or stimulates the growth of both types of cells. The mode of its action depends upon growth conditions in the cell culture and upon the quantity of cytokine added.[82, 99, 100] As an example we would like to mention the study of Palombella and Vilček,[100] where rTNF-α had a cytotoxic effect on 3T3 fibroblasts in non-confluent layers, while promoting growth of the very same fibroblasts in confluent layers. In the same study researchers noticed, that the higher the dose of TNF-α the more intensive was the DNA synthesis.

The above mentioned *in vitro* studies[82,99,100] thus do not support the presumption about selective antiproliferative function of TNF-α (only against tumor cells), but prove that the mode of its activities *in vitro* depends upon external conditions, as for example confluency of cell layer, composition of growth medium or presence of other cytokines.

Activities of TNF-α in *in vivo* systems are even more complicated. Carswell and co-workers[12] observed a pronounced *in vivo* antitumor effect of TNF-α (then only TNF) on transplanted sarcomas in mice. But they were surprised to see that TNF-α was completely inefficacious as an cytostatic/cytotoxic agent on Meth-A sarcoma cell line *in vitro*. This fact led towards conclusion that TNF-α in vivo performs its antitumor activities also indirectly and that this indirect action depends upon the treated organism to a great extent. Experiments with tumor models in nude mice confirmed that the antitumor effect of TNF-α virtually depended upon the treated organism (i.e. upon host), actually upon its immune system. Namely, the treatment of experimental tumors in nude mice with TNF-α was a complete failure and even application of high doses of the cytokine did not result in hemorrhagical necrosis of tumors.[101] Indirect antitumor action of TNF-α is brought about mostly by activation of host immune system. The cytokine activates macrophages so that they become tumoricidal (in less than 20 minutes triggers the synthesis of mRNA for TNF and IL-1 in these cells). At the very same time TNF-α activates T lymphocytes, NK cells and neutrophils,[81, 102-104] exerts an effect upon stromal cells of the host organism and triggers the synthesis of growth factors,[95, 96] as well as the production of other cytokines that participate in antitumor activities (IL-1, IL-6 and TGF)[77-79] (see Table 1).

Besides, TNF-α exerts an effect upon endothelial cells where it induces the synthesis of several adhesion molecules (which are located on the cell surface) and modulates the coagulation properties of cell surface, thereby increasing vascular permeability. The effect upon endothelial cells of tumor blood vessels is potentiated if the vessels are newly formed. Most probably these changes result from the direct and indirect actions of TNF-α, which lead to intravascular thrombosis and complete destruction of tumor blood vessels.[105-107]

Obstruction of blood vessels represents an important mode of TNF-α antitumor activities, especially against non-immunogenic tumors. Poorly immunogenic tumors are quite resistant to the action of TNF-α and destruction of tumor tissue is brought about mostly by means of destroying of blood vessels, which leads to reduced tumor blood supply and afterwards to the phenomenon of central necrosis.[101, 108]

Immunogenic tumors (e.g. with methylcholanthrene induced sarcomas) are more susceptible to TNF-α activities, since such tumors can be completely destroyed with very low doses of this cytokine (injected intratumorally). High susceptibility of immunogenic tumors to TNF-α activities suggests that the immunogenicity of a

tumor is an important factor in antitumor action of this cytokine and that there are also other mechanisms (above stated), beside the effect on tumor blood vessels, which participate in affecting of immunogenic tumors.

Mechanisms of cytotoxic activity of TNF-α

For most of the cytokines it is true that their effect upon target cells is conditioned with the presence of specific receptors on target cell membranes, with binding of the cytokine to these receptors and with transduction of the signal into the interior of the cells.

Certain authors assume that TNF-α - receptor complex is transferred into the interior of the cell (by endocytosis), where it disintegrates in lysosomes, excessive material is then excreted, while TNF-α participates in different cellular processes.[109, 110]

Besides, Hasegawa and Bonavida[111] noticed that the cytocidal activity of TNF-α depended upon the presence of substances capable of inducing formation of pores in cell membrane, i.e. perforins. This led towards conclusion that TNF-α enters cells directly through membrane pores without binding to receptors (nonspecifically). The mechanisms of action of TNF-α after its direct entrance into the cytoplasm differ from the mechanisms of action after its specific entrance (which is brought about by binding of TNF-α to receptors). Direct entrance of TNF-α into the cell generally results in a strong cytotoxic effect in contrast to a broader spectrum of activities of TNF-α after specific binding to cell receptors. Also, Smith and co-workers[112] proved that the activities of this cytokine in the cell interior depend upon the mode of cytokine's entrance and that the cell membrane represents some kind of selective barrier of TNF-α activities. When TNF-α was introduced into the cytoplasm of normal macrophages directly, the researchers expected these cells to become stimulated (as in the feedback loop of TNF-α effect upon macrophages), yet the only outcome was a strong cytotoxic activity.

On the other hand, the latest studies report that internalisation of TNF is not obligatory for the activation of intracellular processes. Namely, binding of TNF to receptors causes segregation of receptors and trimerization of intracellular parts of receptors, which is sufficient for the activation of signaling pathways.[113-118]

The cytotoxic action of TNF-α results in "programmed cell death" - apoptosis or/and necrosis of target cells. The "nucleus dependent" mechanism of action is supposed to be brought about by means of stimulation of gene transcription and of synthesis of different proteins. It is well known that TNF-α activates protooncogenes *c-fos* and *c-jun* and that the products of these protooncogenes operate as activation factors for promotors of numerous other genes: e.g. for synthesis of various endonucleases, MHC I antigenes, TGF-α, and EGFR.[119]

Apoptosis is a consequence of TNF-α action upon microfilaments (TNF-α causes the disintegration of microfilaments) and/or upon the activation of endonucleases which "cut" (cleave) the cell chromatin (DNA) to short fragments of approximately 200 base pairs.[75, 98] This chromatin cleavage proceeds nonspecifically at internucleosomal loci. Cells, damaged this way, are no longer capable of repairing the damage and instead of entering the S phase of cell cycle they pass over to apoptosis (Figure 1).

Necrosis is the result of production of free radicals, which have an effect on mitochondria and on cell structures different form mitochondria (i.e. cytoskeleton), as well as a result of inhibition of mitochondrial functions.[101, 120] This theory is relatively old and it is supported by the fact that antioxidants, as for example mitochondrial enzyme MnSOD, are capable of protecting the cells from TNF-α effects.[121, 122] Another evidence that speaks for the above mentioned theory are experiments of Yamauchi and co-workers.[123] Namely, they quantified the cellular production of OH- and found out that the amount of this powerful oxidant depends upon the duration of cell exposure to TNF-α. Such kind of dependence was observed only with cells that were susceptible to TNF-α, while

there were no signs of increased production of OH- in cells resistant to TNF-α action.

On the other hand, Wong and co-workers[121] and later on Okamoto with co-workers[122] proved that the group of proteins induced by TNF-α includes also MnSOD. They observed a statistically significant increase of MnSOD concentrations following the transplantation of TNF-α gene into tumorigenic mouse fibroblasts which are originally susceptible to TNF-α. Cells, changed genetically in such a way, were now able to produce both endogenous TNF-α, as well as MnSOD and thus became resistant to TNF-α. This means that TNF-α operates autoregulatively and that the mechanisms of its action interrelate and integrate with each other.

Thus, the basic effect of TNF-α in process of necrosis is the stimulation of production of free oxygen radicals that later on affect cellular structures. Additional proofs speaking for this theory came from experimental results which indicated that the inhibition of cell respiration is a consequence of generation and activities of free radicals (after TNF-α treatment of cells). Further confirmations are results of studies where previous treatment with antioxidants protected cells from cytotoxic activity of TNF-α.[120, 121, 123, 124]

TNF-α as a growth factor

With regard to all biological activities of TNF-α it is difficult to define which one is primary: its antitumor activity, its role in inflammation processes or its activity as a growth factor. Sugarman and co-workers[97] were among the first to describe TNF-α effects upon different normal and malignant cell lines. They established that TNF-α statistically significantly reduced the cell number of only seven out of twenty-two malignant cell lines (after exposure time of 72 h) while having a stimulative effect on growth of some normal cell lines (different human fibroblast cell lines).

On the other hand, Piacibello and co-workers[125] ascertained that TNF-α not only has a stimulative effect on growth of normal cell lines, but also promotes growth of some malignant cell lines. Namely, they found out that low doses of TNF-α in the presence of GM-CSF (granulocyte-macrophage colony stimulating factor) stimulate the growth of human normal, as well as leukemic stem cells whereas higher doses of TNF-α in the presence of G-CSF (granulocyte colony stimulating factor) inhibit the growth of only normal stem cells. These observations were confirmed by other authors who demonstrated that low doses of TNF-α stimulate growth of, while high doses have an antiproliferative effect on, certain tumor cell lines.[101] In normal human fibroblast cell lines TNF-α operates as growth factor and in most of the cases there is a dose dependence: the higher concentrations the better the cell growth.[97] It is important to stress again that beside the dose there are different external factors like confluence of cell cultures, composition of growth mediums, phases of cell cycle and presence of growth factors or cytokines, which influence the mode of TNF-α action.[100, 126]

The TNF-α activity as a growth factor is, just like its antitumor activity, direct and indirect. The direct action comprises activation of genes responsible for synthesis of proteins that direct the cell from G0 to G1 phase of cell cycle thus increasing the number of cell divisions and accelerating the proliferation of cells.[127] The indirect action includes stimulation of cells to produce other growth factors and specific receptors for growth factors. Direct and indirect mode of TNF-α action intertwine and it is impossible to fix their limits.

TNF-α as an immunomodulatory agent

TNF-α in its immunomodulatory role has an effect upon T and B lymphocytes, affects the expression of MHC class I and II antigens, and stimulates macrophages as well as other cells to produce cytokines.[71, 128]

Effect on T and B lymphocytes

TNF-α affects T and B lymphocytes predominantly as a mitogenic factor. Vine and co-wor-

kers[127] established that this cytokine accelerates transition of T lymphocytes from G0 to G1 phase of cell cycle and thus stimulates multiplification of these cells while not stimulating the production of IL-2 (i.e. one of the elementary products of T lymphocytes) at the same time. The effect upon immune system executed by stimulation of B lymphocytes is even more indirect, since TNF-α is capable of promoting the proliferation of these cells, yet only in the presence of IL-2. Because it is well known that TNF-α affects predominantly the division of T and B lymphocytes and co-operates only indirectly in the induction of cytokine synthesis in lymphocytes, there still remains a question about its immunomodulatory role in activation of T and B lymphocytes. It is quite interesting that all lymphocytes after treatment with TNF-α demonstrate a statistically significant increase of receptors for TNF-α.

Effect on NK cells

The effect of TNF-α on NK cells represents a special pattern of autocrine stimulation. Bancroft and co-workers[129] treated immunodeficient mice (suppressed B and T lymphocytes) with dead bacteria of *Listeria monocytogenes species,* isolated spleen cells of these animals and measured their production of IFN-γ. They ascertained that IFN-γ production depended upon the dose of injected bacteria or upon the number of activated macrophages, respectively. Since the macrophages do not produce IFN-γ (or at least this has not been demonstrated yet), the researchers concluded that NK cells are being stimulated with macrophages or their products, respectively. Because IFN-γ is known to be a powerful stimulator of TNF-α production in macrophages Bancroft and co-workers assumed that it is TNF-α that stimulates NK cells to produce IFN-γ: 1. bacteria activate macrophages and trigger synthesis of TNF-α in these cells; 2. TNF-α acts upon NK cells and IFN-γ production; 3. IFN-γ in a feedback loop triggers additional synthesis of TNF-α and the cycle repeats.

Effect on macrophages

The effect of TNF-α on macrophages represents a classical example of autocrine activity. TNF-α, which is itself a product of macrophages, binds in a feedback loop to specific cell receptors and stimulates these cells.[130] It is well known that activated macrophages act cytostatically/cytotoxically on tumor cells that are susceptible to TNF-α. This fact leads towards conclusion that the antitumor activity of macrophages is brought about by producing TNF-α which then affects tumor cells. However, this is not the only form of cytotoxic activity of activated macrophages. These cells can act cytotoxically also by means of other products as for example IL-1, hydrogen peroxide (H_2O_2) and nitric oxide (NO). The combined treatment with TNF-α and NO has a synergistic cytotoxic effect on tumor cells.[131, 132] Especially effective production of NO and TNF-α in macrophages is achieved after stimulation with IFN-γ and IL-2, whereas the stimulation of macrophages with IFN-γ and MDP or its structural analogues primarily increases the TNF-α production.[132, 133]

Receptors for TNF-α

In the year 1990 different researchers cloned the cDNA for two types of cell surface receptors for TNF (TNF-α and TNF-β).[70, 134] Both types of receptors are present on the cell surface of most of the cell lines, yet in a different mutual percent relation.[135,136] These two types of receptors, named TNF R-1 (55 kDa) and TNF R-2 (75 kDa), consist of an extracellular and an intracellular part. The extracellular part of TNF R-1 comprises 182 amino acids and the extracellular part of R-2 235 amino acids. The intracellular parts of receptors are larger - the one of R-1 includes 221 amino acids and the one of R-2 439 amino acids.

Since the cloning of two distinct receptors for TNF (each of which binds TNF-α and TNF-β), the past few years have witnessed the rapid emergence of two superfamilies, of which TNFs and their receptors are only representatives. To

date 12 receptors have been identified with which we can associate eight TNF-related proteins.[137]

According to the amino acid sequence the two types of receptors differ from each other to a great extent. Human TNF receptors demonstrate an amino acid homology of only 27% and most of the homologous amino acids (70%) are placed in the extracellular parts of receptors. Thus the structure of intracellular parts differs considerably between the two types of receptors, which indicates that TNF is connected to different functions or different processes, respectively (according to the type of receptor to which TNF binds). It is interesting that there is a higher resemblance in the structure of human TNF R-1 and mouse TNF R-1 (homology of 64%), which is also true for human TNF R-2 and mouse TNF R-2 (homology of 62%). The resemblance between human and mouse receptors appears mostly in the intracellular parts of receptors (homology of 73% between human and mouse TNF R-2).[136, 138] On the basis of cited facts we can conclude that the activity of TNF is relatively species unspecific and that the intracellular processes in which TNF co-operates (after binding to receptors) are quite similar in different animal species.

Human TNF R-1 and TNF R-2 thus demonstrate only a slight resemblance in the amino acid sequence of their extracellular parts and differ almost completely in the structure of their intracellular parts. Besides, the intracellular parts (according to their amino acid sequence) are not even similar to any of the known proteins.[138] The recapitulation of the above statements is that TNF binds to the two types of receptors, induces different processes in the cell (in dependence upon the type of receptor to which it binds) and that the details of TNF activities inside the cell remain to be explained.

Thoma and co-workers[139] blocked TNF R-1 with antagonistic monoclonal antibodies and in this way prevented the cytostatic/cytotoxic effect of TNF-α on different cell lines. From these results they made an inference that TNF acts cytotoxically trought binding to TNF R-1 receptors.

Brouckaert and co-workers[140] demonstrated a good antitumor effect of recombinant mouse TNF-α (rMuTNF-α) on B16 BL6 melanoma in C57Bl/6 mice, whereas the therapy with recombinant human TNF-α (rHuTNF-α) turned out to be unsuccessful. They also ascertained that 50% more experimental animals died owing to toxic side effects of rMuTNF-α when compared to the number of lethal outcomes after rHuTNF-α therapy. On the other hand, when the mice were pretreated with galactosamine, also rHuTNF-α demonstrated some degree of antitumor activity, but also the toxic side effects were more pronounced. These results lead towards conclusion that the species specific activity of TNF-α (if it is present) can be neutralised and that such activity does not depend only upon the binding to receptors but also upon other factors.

In January 1993 Nature published an article about TNF-α activities after its binding to different receptors.[141] Authors of this article cited some results which are identical to the ones mentioned before. Namely, they confirmed that the cytotoxic effect on tumor cells results from binding of TNF-α to TNF R-1 and described also an interesting example of species specific activity of human and mouse TNF-α; human TNF-α demonstrated an antitumor effect in experimental mice but caused no toxic side effects. The presence of antitumor effect and the absence of toxic side effects of human TNF-α in mice were explained with its ability to bind only to TNF R-1 but not to TNF R-2. Mouse TNF-α, on the other hand, bound to both types of receptors on human cell lines and did not act species specifically.[141]

Contrary to the above stated results, from which could be concluded that the antitumor activity of TNF-α is a consequence of its binding to and operating by means of TNF R-1, other authors established that mouse fibroblasts (TA 1 cells) are insensitive to human TNF-α but very sensitive to mouse TNF-α. Since human TNF-α binds only to TNF R-1 on mouse cells, while mouse TNF-α binds to both types of receptors, there is a logical inference that the cytotoxic effect upon these cells was mediated

by TNF R-2 receptors. The second confirmation, that the cytotoxic effect of TNF-α can also be mediated by TNF R-2, is the fact that HeLa cells, which are otherwise completely insensitive to the antiproliferative activity of human TNF-α and have only TNF R-1, become very sensitive after insertion of a gene coding for TNF R-2 into their DNA.[119] The existence of two types of receptors for TNF-α is supposed to be important also for its activity as a growth factor. In the year 1991 Tartaglia and co-workers[142] ascertained that mouse TNF-α accelerated cell division of mouse thymus cell line and of mouse T lymphocyte cell line (CT-6) while human TNF-α had no effect on the proliferation of cells employed in the experiment. Since human TNF-α binds only to TNF R-1 on mouse cells, the authors substituted TNF-α with agonistic polyclonal antibodies against TNF R-2 and TNF R-1. Agonistic antibodies against TNF R-2 stimulated cell division of both cell lines whereas agonistic antibodies against TNF R-1 were inefficacious. A logical conclusion is that TNF R-2 most probably mediate the stimulation of cell division.

The researchers of Hoffman-La Roche[135] incubated human monocytes from peripheral blood with TNF-α and various inhibitors of TNF-α binding to receptors: i.e. with antibodies against TNF-α, or with recombinant receptor proteins, or with specific neutralising antibodies against TNF R-1 and TNF R-2. In contrast to the above cited data they established that the stimulation of cell division by TNF-α is mediated through both types of receptors, yet the mechanisms of stimulation differ and depend upon the type of receptor.

The antiviral effect of TNF-α is supposed to be mediated by TNF R-1. Namely, Wong, Tartaglia and co-workers[143] stimulated the antiviral activity by means of using agonistic antibodies against TNF R-1. The addition of antibodies against TNF R-2 had no antiviral effect.

Kalthoff and co-workers[144] demonstrated that TNF-α actions, mediated through binding to different receptors, are not completely explained yet. They ascertained that binding of TNF-α or agonistic antibodies to TNF R-1 of human malignant pancreatic cell lines (HPAF, Capan 2) causes a rapid transcription of TNF R-2 gene and that TNF R-2 operates as a specific receptor through which TNF-α mediates transcription of TGF-α gene. In the very same study the authors state that binding of agonistic antibodies to TNF R-1 triggered transcription of EGF receptor gene.

On the basis of cited data it is quite difficult to draw universal conclusions about activities of TNF-α mediated either by one or by the other type of receptors. Common to all known reports are the facts that the species specific activity (if such activity exists) is expressed by means of both types of receptors and that the effect of TNF-α can be substituted by binding of specific agonistic antibodies to receptors. We can also conclude that TNF R-1 and TNF R-2 (after binding of TNF-α to either of the receptors) mediate or stimulate, respectively, different processes in cells. However, quite often the transduction of signals for intracellular processes is realised through simultaneous binding of TNF-α to both types of receptors.

Biochemical mechanisms of TNF-α actions

Biochemical processes that follow the entrance of TNF-α into the cell or the segregation of receptors are still not known: neither the proteins which bind complementarily to the complex TNF-α - TNF-R after internalization, nor the exact procedure of its further action on cellular organelles or cell processes, respectively.[138] Variety of its activities inside the cell (Table 1) indicates that TNF-α is invovlved in different chemical processes which represent additional complications at creating a general sheme of biochemical mechanisms of TNF-α action.

The cytotoxic effect of TNF-α is one of its most thoroughly examined activities. Since such an effect reflects either as apoptosis or as necrosis of cells there are at least two different biochemical mechanisms of cytotoxic activity. The first one is orientated directly to cell nucleus, whereas the other mechanism affects

cellular organelles or cytoskeleton, respectively. However, regardless of the fact that there are different mechanisms by which TNF-α acts upon the cells, it is known that during its intracellular action this cytokine activates protooncogenes *c-fos* and *c-jun* (i.e. immediate early genes) and stimulates the synthesis of various proteins which direct the cell towards either apoptosis or necrosis.

Most probably also the growth factor-like activity of TNF-α is realised through activation of protooncogenes and stimulation of synthesis of proteins responsible for transition of cells into S phase of cell cycle. The activation of protooncogenes *c-fos* and *c-jun* is mediated through nuclear factor kappa B (NF ϰB), which is itself supposed to be activated by protein kinase C.

Biochemical mechanisms of TNF-α action can also be triggered through activation of phospholipase A_2 (Figure 1). Namely, the cells following the addition of TNF-α produce arachidonic acid and prostaglandins,[145-147] which indicates that activation of phospholipase A_2 is also involved in signal transduction. Besides, Palombella and Vilček[100] suceeded in blocking both the cytotoxic and the mitogenic activity of TNF-α by means of inhibiting phospholipase A_2 with dexamethasone.

Undoubtedly TNF-α is a cytokine with a very broad spectrum of effects, owing to which we could hardly expect a simple explanation for its biochemical mechanisms of intracellular activities. Figure 1 represents the authors' global idea of TNF-α activities based on more or less known data from the literature.

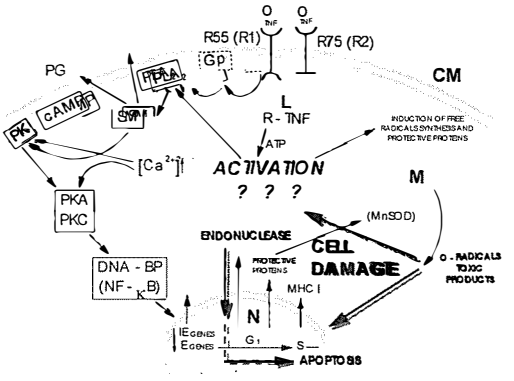

Figure 1. Biochemical mechanisms of TNF-α (TNF) activities at the cellular level. Binding of TNF to receptors [R55 (R1), R75 (R2)]; putative passage of TNF through the cell membrane (CM) into the interior of the cell (endocytosis); degradation of TNF-receptor - TNF complex (R-TNF) with lysosomal enzymes (L); generation of adenosine-triphosphate (ATP); activation of G proteins (Gp); activation of phospholipase A_2 (PLA_2); production of second messengers (SM) as for example cyclic adenosine-monophosphate (cAMP), arachidonic acid, diacylglycerol and inositolphosphate; activation of protein kinases (PK); arachidonic acid is either excreted from the cell or represents a substrate for PLA_2 - products of which are prostaglandins (PG); activation of protein kinases A and C, which results with generation of "DNA binding proteins" (DNA-Bp) like for example "nuclear factor ϰB" (NF-ϰB); NF-ϰB operates in the nucleus (N) and activates "immediate early genes" (IE genes) as *c-myc, c-fos* and *c-jun,* which are further responsible for activation of early genes (E genes); the next step is transcription of genes that are normally being transcribed in G1 phase of cell cycle; products of these genes determine whether the cell continues to S phase or is directed into apoptosis (which is supposed to be a consequence of endonucleases' activities); in the synthesis of endonucleases and "DNA binding proteins" as well as in most of energy-dependent cell processes participate Ca^{2+} ions; TNF affects cell organelles as mitochondria (M) where it stimulates synthesis of oxygen free radicals and other toxic products; TNF also triggers production of protective proteins (which protect the cell from its activities) e.g. manganese superoxide dismutase (MnS●D).

TNF-α and other cytokines

The role of TNF-α in the defence of organism against foreign or own antigens and the mode of TNF-α action quite often depend upon the presence and activity of other cytokines. When interacting in such a way the cytokines either stimulate or inhibit mutually their activities. The stimulative activity includes stimulation of cytokine as well as of cytokine receptor synthesis. On the other hand, the inhibitory action (of cytokines upon other cytokines) is realised through inhibition of transcription, translation and/or secretion of cytokines or their specific receptors. Besides, the cytokines can compete for the very same receptors on the cellular

membrane (e.g. TNF-α and TNF-β) and in respect to the efficiency of their binding to receptors direct cellular processes. Cytokines thus represent some sort of polypeptide hormones at the cellular level which through interreactions transduce different signals to the cells.

The best known functional dependence of TNF-α upon other cytokines is its cytostatic/cytotoxic activity against tumor cells in combination with interleukins and interferons. Namely, in the year 1985 Sugarman and co-workers[97] ascertained that the antiproliferative effect of TNF-α on tumor cells in vitro can be potentiated with IFN-γ. Similar results were obtained by Serša and co-workers[148, 149] on human adenocarcinoma and human malignant melanoma cell lines where IFN-α enhanced the antitumor effect of TNF-α. Enhanced in vitro antitumor effect was also observed by other authors after treatment of tumor cells with TNF-α and IL-1.[150]

In vivo experiments demonstrated that combined treatment with TNF-α and IFN-γ not only has a direct antitumor effect but also the indirect one which is carried out through affecting of the immune system (synthesis and activities of other cytokines, potentiation of cytokine's own antiproliferative activity). Young and Wright[151] established that low doses of IFN-γ and TNF-α reduce the number of suppressor T lymphocytes and decrease the concentration of growth factors. Thus these two cytokines are capable of affecting the growth of primary or residual tumors and the development of metastases. The antiproliferative effect of TNF-α and IFN-α on leukemic cells in patients with chronic myelogenous leukemia (CML) represents a special pattern of co-operation between the two cytokines. Namely, Moritz and co-workers[152] ascertained that IFN-α treatment of leukemic patients gives very promising results but only until the development of resistance to IFN-α. However, when the patiens were pretreated with TNF-α, the resistance to IFN-α did not appear and further successful therapy with IFN-α was enabled.

A complete stop of tumor growth in mice (5 different types of subcutaneous tumors) was described by Winkelhake and co-workers[153] following combined treatment with TNF-α and IL-2. It is quite interesting that in the very same experiment monotherapy with a single cytokine was inefficacious and that the efficacy of combined treatment depended predominantly upon the concentration of TNF-α. Maximal effect of the combination was achieved when maximal sublethal doses of TNF-α were used, whereas the concentration of IL-2 even 90% lower than maximal sublethal dose did not affect the efficacy of treatment. Beside the fact that combined therapy inhibited the growth of subcutaneous tumors, the very same therapy prevented completely the development of lung metastases if only it was started early enough.

The fact that efficacy of combined therapy with TNF-α and IL-2 depends also upon other factors, like for example immunogenicity of tumors, general condition of immune system and scheduling of cytokine application was demonstrated by Agah and co-workers.[154] Additive effect of combined therapy (TNF-α and IL-2) on the development of lung metastases (induced by methylcholanthrene) was observed when the researchers treated animals first with IL-2 and later with TNF-α. Reverse order of cytokine application was less effective. On the other hand, the antitumor activity of combined therapy with TNF-α and IL-2 was extremely low when the very same experiment was repeated using mice with suppressed immune system.

Beside the antiproliferative effect also the growth factor-like activity of different combinations with TNF-α and other cytokines represented an interesting challenge for the researchers. Some of them established that TNF-α together with growth factors *in vitro* effectivelly affects bone marrow cells where on one hand it stimulates the growth of stem cells by means of inducing synthesis of growth factors and, on the other hand inhibits the growth of some leukemic cell lines.[155, 156] TNF-α acts synergistically together with TGF-β on differentiation of human leukemic cells (i.e. stimulates the differentiation process) which results in a reduction of their malignant potential.[157]

When speaking about combined activity of TNF-α with other cytokines we must also em-

phasise its pluripotent role in affecting the cells. Mechanisms of common action of TNF-α with other cytokines are complicated and quite often unclear. In general, combination of TNF-α with interleukins or interferons synergistically inhibits tumor growth; with TGF-β it acts synergistically on the process of cell differentiation and has in lower doses, combined with growth factors a synergistic effect on cell proliferation.

Future perspectives

Cloning of human TNF-α gene was accepted by many researchers as a great step towards the discovery of universal medication for different malignant diseases. Such expectations were a logical consequence of numerous reports confirming the fact that TNF-α demonstrates a distinctive antitumor activity *in vitro* and in some cases an even more pronounced antitumor effect in tumor models *in vivo*. Unfortunately, the results of experiments with tumor models never gained an approval in clinical praxis. The problems which accompanied TNF-α applications in clinical conditions were not a result of its insufficient antitumor activity but derived from severe dose-limiting toxicity (elevated body temperature, anorexia, diarrhoea, nausea and hypotension). Nevertheless, ten years of experiments with TNF-α in clinics are far from a complete failure. The researchers are seeking after such a mode of TNF-α application that would retain its antitumor activity while minimizing the toxic side effects. One of the possibilities is a synthesis of new analogues of TNF-α, which according to incomplete knowledge of the role of certain molecular domains represents quite a difficult task. Namely, the cytokine molecule should be changed in the domain responsible for dose-limiting toxicity, while preserving its structure responsible for cytostatic/cytotoxic effect on tumor cells. Besides, the efforts are being made to create analogues capable of a longer retention at the tumor site, which would limit the effects of cytokine to tumor cells only. In this prospect, there is an idea of synthesising TNF-α chimeric proteins with specific affinity for certain antigens on the surface of tumor cells. The second possibility of local treatment with TNF-α is isolation perfusion with high doses of TNF-α. Problems arising from such kind of therapy derive from incompetence to control and retain completely the cytokine within the treated organ. Quite often TNF-α "escapes" from the artificial circulation loop which results in serious adverse effects. The third but most prospective point of view of clinical uses of TNF-α and its analogues is a gene therapy. Gene therapy represents a kind of systemic treatment where genetically engineered cells (i.e. with TNF-α gene transfected cells) produce a controllable amount of endogenous cytokine. However, the genetic engineering techniques to date do not allow major interventions at the level of human genome without a serious risk. They remain time consuming and expensive, and further developments will be needed before it can become a commonplace treatment.

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Determination of the breast volume after breast conservating surgery

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A mathematical description has been developed to estimate the difference in volume between the breasts after breast conservating surgery. This method can help the physician to evaluate the cosmetic results after treatment. It is a simple method for the radiotherapist who routinely uses a simulator.

Key words: mastectomy; mammography-methods; breast conservating therapy, cosmetic evaluation, breast volume

Introduction

The patient with a diagnosis of early breast cancer can now be successfully treated by conservative techniques. Studies results have demonstrated that for small tumors (T1 and small T2) lumpectomy followed by radiotherapy is a valid alternative to mastectomy.[1]

Today, the physician tries not only to cure the patient but to achieve the best possible cosmetic results.[2] In the breast the cosmetic outcome is generally evaluated by the difference in size between the two breasts, teleangiectasia and fibrosis.

Measurement of the size of the breast is somewhat subjective. We describe in this paper a more objective method of measuring breast size.

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Method

Theory

Breast is a region bounded by the chest wall on one side and by the skin on the other. This could be schematized by regarding the breast as a segment of a sphere, with the pectoral muscle being a second segment of a sphere within the first. The volume of interest is represented by the difference between the two spherical domes.

The problem can now be resolved mathematically. The common base of the two segments is a circle of radius R. The outer segment is bounded by the skin (height H); the inner segment is defined by the fascia of the pectoral muscle (height h) from the chest wall. The volume of the breast (v) can be assimilated to the difference in volume of these two spherical domes mathematically expressed as:

$$V = \frac{\pi}{6}(H^3 - h^3) + \frac{\pi}{2} R^2 (H - h)$$

However, it appears more suitable to the geometry encountered in most of the patients to consider the base of the segments as elliptical (Figure 1a, 1b).

The maximum and minimum radii need to be defined. The maximum radius is in the transverse axis (R) and the minimum in the cranio caudal axis (r).

We can now use the ratio (r/R) to modify the above equation

$$V = \frac{r}{R}\frac{\pi}{6}(H^3 - h^3) + \frac{\pi}{2}R^2(H - h)$$

V = volume of the breast.
R = half the maximum transverse "width" of the breast.
r = half the maximum cranio caudal "length" of the breast.
H = elevation of the breast.
h = elevation of the chest wall.

We have used this formula to calculate the volume of the breast in this study.

Measurement of the geometrical dimensions of the breast

With the patient in the supine position, using a conformator (or a CT-scan if available), we drew the contours of the thorax including the breast at two different levels.

The first contour C_{Med} is taken at the level of the two nipples, the second at the level of the inframammary fold. The cranio caudal diameter (2 r) of the breast is measured directly on the patient as shown on the picture. The most medial limit (A) and the most lateral limit (B) of the breast tissue are located clinically on the patient and are indicated by the conformator on the median contour (C_{Med}). The "width" (2R) of the breast is equal to the distance AB.

The height H of the external segment of a sphere (to which the breast can be assimilated) is the maximal distance (h) between the median contour C_{Med} and the AB line.

The height of the inner segment of a sphere (to which the chest wall is approximated) can only be estimated, as the conformator cannot trace its contour. We can consider that the maximal distance (h) between the contour C_{Inf} at the level of the inframammary fold and the AB line represents its best approximation and can be easily measured on the drawing.

All the measurements required for the calculation of the volume of the breast are then available.

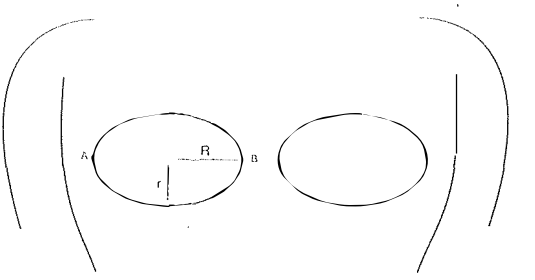

Figure 1a. Representation of the base of the breast as an ellipse of which the great radius R is half the width of the breast and small radius r is half its height.

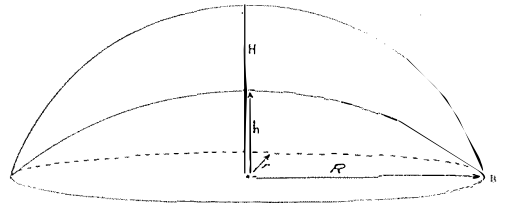

Figure 1b. Representation of the volume of the breast as being the difference between two domed segments of heights H and h and of which the base is an ellipses of radiuses R and r.

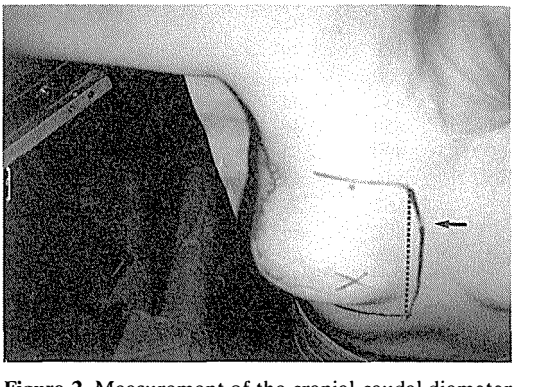

Figure 2. Measurement of the cranial caudal diameter 2 r.

Application

We have measured the volume of the breast of 22 women who had been treated by lumpectomy for breast cancer prior to radiation. Table 1 compares the volumes of the treated breast and of the other breast:

1) the volume of the other breast ranges from 169 cm^3 to 846 cm^3, with an average of 435 cm^3.

2) the volume of the treated breast ranges from 163 cm^3 to 799 cm^3, with an average of 405 cm^3.

3) the difference between the volume of the treated breast and the volume of the other breast ranges from 2% to 27%, due mainly to variation of the difference H-h between treated breast and other breast.

Table 1. Comparison of the volumes of the treated breast and of the other breast.

N° of cases	Volume (cm^3)		Difference in volume between the two breasts	
	Treated breast	Other breast	cm^3	%
1	166	169	3	1,8
2	163	190	27	14,2
3	226	244	18	7,4
4	230	251	21	8,4
5	200	275	75	27,3
6	256	280	24	8,6
7	272	288	16	5,5
8	254	304	50	16,4
9	298	319	21	6,6
10	309	348	39	11,2
11	388	397	9	2,3
12	380	411	31	7,5
13	432	448	16	3,6
14	450	463	13	2,8
15	490	540	50	9,3
16	546	572	26	4,5
17	562	578	16	2,7
18	498	608	110	18
19	604	628	24	3,8
20	647	660	13	2
21	742	763	21	2,75
22	799	846	47	5,5

The same method could be used to measure the difference in volume between the breast as it appears just after radiotherapy and as it becomes after months or years.

The study of a greater number of cases should enable us to establish a correlation between the calculated difference of volume and the qualitative assessment (minor, marked or major difference), and to derive an objective assessment of one of the factors of the aesthetic result of a conservative treatment of breast cancer.

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Terilateral retinoblastoma

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A case of trilateral retinoblastoma in a 5 year old girl is described. The patient was diagnosed at the age of 7 months as having a trilateral retinoblastoma and treated with enucleation of the right eye and radiation therapy. Fifty two months later she started vomiting, showed gait disturbances and ataxia. A computed tomography (CT) scan revealed a pineal mass and chemotherapy with radiation therapy were initiated. Despite a temporary improvement she died from progressive CNS disease 4 months later.

Key words: eye neoplasms-surgery; retinoblastoma; pinealoma

Introduction

Trilateral retinoblastoma is a syndrome that includes an intracranial tumor and bilateral retinoblastoma.[1] The location of the tumor is usually in the pineal gland. First trilateral retinoblastoma was described in 1977. The pineal neoplasm displays histologic features similar to retinoblastoma and propably represents a primary tumor rather than a metastatic focus.[2] With the recognition and location of retinoblastoma in the pineal gland new diagnostic and therapeutic procedures have been suggested but the poor prognosis has not been improved.

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Case report

The patient was seen at the age of 7 months for evaluation of an abnormal light reflex in both eyes. The family history was insignificant for bilateral retinoblastoma. A computed tomography (CT) scan showed enhancing lesions in both eyes with no evidence of orbital or intracranial extension. The patient underwent enucleation of the right eye followed by radiation to the left eye. Histology of the enucleated eye did not reveal evidence of extraocular extension and optic nerve invasion was not present. The left eye was treated with 4100 cGy in 20 fractions with 6 mV photons.

The patient remained stable until 5 years of age, when she was seen for vomiting, ataxia and gait disturbances. CT scan (Figure 1) of the head revealed 4 cm enhancing mass in the pineal region with severe hydrocephalus, finding confirmed by nuclear magnetic resonance (Figure 2) too. There was no evidence of tumor

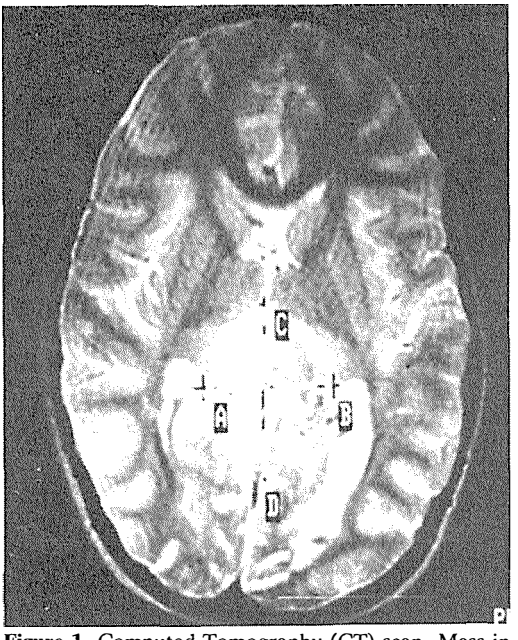

Figure 1. Computed Tomography (CT) scan. Mass in the pineal region with severe hydrocephalus.

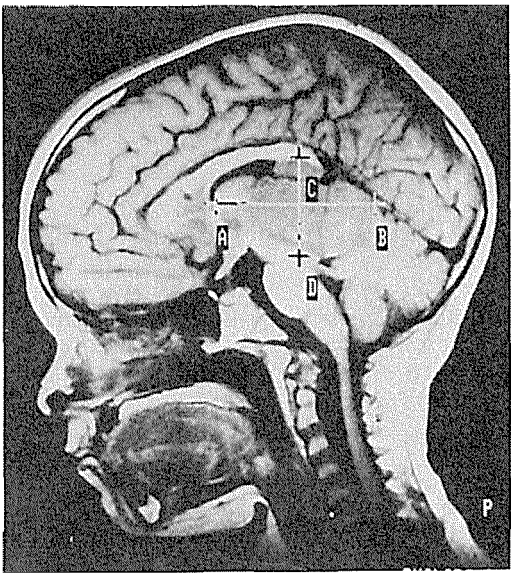

Figure 2. Nuclear magnetic resonance. Tumor in the pineal region (4 × 3,8 × 5,2 cm).

in either orbit. A trilateral retinoblastoma was diagnosed and a ventriculoperitoneal (VP) shunt was placed. Patient received craniospinal irradiation (3000 cGy) in 15 fractions and irradiation to the pineal with 4000 cGy. Following radiation therapy she was initiated on chemotherapy consisting of: Prednizone 300 mgr/m^2, Vincristine 1,5 mgr/m^2, Cis-4, diaminodichloroplatine (Cis-Platin) 60 mgr/m^2, Cis-chloreathylnitrosurea (CCNU) 75 mgr/m^2. Repeated computed tomography (CT) scan, one month later, showed decreased of the pineal mass up to 60 % and no signs of hydrocephalus.

Three weeks later the patient developed ataxia, difficulty in walking and progressive quadreplexia. Four days later she died from respiratory failure.

Discussion

Trilateral retinoblastoma denotes a solitary midline intracranial tumor in association with bilateral retinoblastoma.[1, 2] The location of the tumor is usually in the pineal gland (the third eye) and may originate from photoreceptor-like cells in ocular tumors and from vestigial photoreceptor cells of the pineal organ.[2, 3]

This clinical entity was first described in 1977[4] while in 1982 the term trilateral retinoblastoma has been establihsed.[2] No more than 40 cases of trilateral retinoblastoma have been described so far.[5–7, 11]

In almost all cases reported there, bilateral retinoblastoma appears early such as 7,2 months as in our case, with a 68 % positive family history in most of the cases described.[5] The mean age of diagnosis of trilateral tumor was reported to be long, with an interval of 33 months, and almost all patients presented with signs and symptoms of increased intracranial pressure.[8] In the case we describe, the patient started having refractory vomiting and ataxia of a short duration.

In initial reports patients did not receive any therapy and all died from CNS and spinal metastases with median of 1–5 months.[9] Since the initiation of chemotherapy in CNS tumors and especially in meduloblastomas which comprise another primitive predominately midline neuroectodermal tumors, same approach has

been applied in trilateral retinoblastomas also.[10, 12]

Despite intensive chemotherapy and radiotherapy it seems that this tumor has a propensity to seed the cerebrospinal fluid. [12, 13] Aggressive chemotherapy and radiation therapy seems to be indicated[4] with both modalities increasing the overall survival.[5–7, 14]

With the rising awareness of the trilateral retinoblastoma syndrome and increasing availability of sensitive central nervous system imaging procedures, the incidence of this entity is increasing.

Follow up of the CNS in patients with bilateral retinoblastomas as well as aggressive and uniform therapy in these tumors are necessary to improve the bad prognosis.

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Quantitative analysis of terminal blood network in human spinal cord and progressive radiation myelopathy

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A quantitative analysis of terminal blood network in human spinal cord was done. The relative area of capillars in the gray matter is higher than in the white one and it increases from posterior to anterior spinal cord horns. The capillar area of the gray matter is larger in thoracic segments than in cervical ones. In the white matter, the largest capillar area was found in the anterior columns, the lowest in the deep parts of posterior columns. Cervical white matter has the lowest relative capillar area whereas lumbar white matter is of the largest one.
The results are compared to findings in the irradiated spinal cord. The more radiosensitive parts have been shown of having the lowest values of the relative capillar area. Possible explanation is discussed.

Key words: *Spinal cord-blood supply; capillaries; myelitis; radiotherapy – adverse effects; quantitative analysis*

Introduction

Progressive radiation myelopathy (PRM) – late reaction of the spinal cord – is a very tragic sequel of radiation treatment. Unfortunatelly, it usually arises in patients with malignancy controlled long after radiotherapy was completed. PRM was described at first by Ahlbom[1] in 1941. The diagnosis of PRM can be stated when the following criteria proposed by Pallis et al.[2] are fulfiled:[1] The spinal cord must be included in the field irradiated,[2] the main neurological lesion must be within the segments of the cord exposed to radiation,[3] the cord compression from metastasis as the cause of the neurological disorder must be excluded. PRM is one of four clinical forms of cord radiation injury according to Reagan et al:[3]

- transient myelopathy
- signs of lower neurones
- acutely developing quadru-/paraplegia
- progressive radiation myelopathy.

The PRM mechanism has not been known up to now. Didactically, three theories could be described: vascular, glial, and immunological, according to the most injured cells in volume irradiated. This injury had been suggested as a crucial in PRM pathogenesis. Because of

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the mutual functional dependence of particular cell compartments and because the radiation hits all of them randomly, the resulted radiation reaction is to be surely considered as a very complex event. Up to now, the correct relationship between radiation dose, length of cord irradiated and possible cord radiation injury (which could be very clear as being radiotherapist's daily bread) is not known.

The cervical spinal cord has been found to be more radiosensitive than the thoracic one and the lumbar cord most radioresistant. Therefore, cases of human lumbar radiation myelopathy are rather rare in comparison to the cervical and thoracic ones.[4–7]

The systematic experimental study of human PRM is not possible, therefore, we can judge a course of events by results of animal experiments. The best and the most comprehensive study of radiation effects on an animal spinal cord is Kogel's careful study[8]. His experiment conclusion is, that early lesions are caused by death of oligodendroglial cells (responsible for myelinisation) and later lesions are caused by the injury of vessels. Lower radiation doses cause later damage – primarily vascular as well. After higher doses, the earlier oligodendroglial damage appears.

The vascular component is damaged with both, lower and higher doses. Therefore, we suggested the results of the vascular spinal cord network study could be a suitable substratum for an explanation of different radiosensitivity of various parts of the cord. The spinal cord vascular supply in a human is described in detail both, macro- and microscopically.[9–11]

In literature there are papers dealing with quantitative evaluation of capillary network of various brain regions, mainly in the cortex.[12, 13] Papers describing human spinal cord vascular supply do report in general much more dense capillary network in the gray matter than in the white one.[10]

The aim of the study is to find differences in size and the density of capillary in white and gray matters between various regions in one segment and between segments of the cervical, thoracic and lumbar spinal cord.

Materials and methods

The spinal cord was studied on the spinal cords of three adult men (age of 20, 25 and 26 years). The material was gained by necropsy. After the spinal channel was opened, the whole cord was taken out and according to spinal roots it was divided in particular segments.

Tissues were fixed by 10 % neutral phosphate formol and embeded in parafin. Slices of 15 μm were stained by lucid methods (hematoxylin-eosin, cresylum violet and method by Cluver-Barrery), the impregnation by the Bodian's method and the PAS histochemical reaction was carried out as well. The best method for capillary quantitave study seems to be the method where slices were stained by the PAS reaction because capillary basic membranes are best shown.

By the quantitative evaluation of blood capillary, paraffin slices have been screened with the help of the projection microscope Pictoval (Zeiss Jena) by the magnification of 200 times on a paper and outlines of thin praecapillaries and the thinnest sections of capillaries have been drawn. Arteriolae and larger vessels have not been taken in account. A quantitative analysis has been carried out on such outlines with the semiautomatic device MOP AM O3 fy Kontron. Surface, circumference, diameter and shape factor of terminal parts of blood network have been measured. The total areas of gray and white matters in the examined spinal cord regions have been measured as well. The total number of capillaries in regions has been recalculated for the relative numbers show the density of capillaries in the area unit of 1 mm^2. Three spinal cords segments – C7, Th5 and L3-were evaluated in each spinal cord in this way.

An arrangement of blood vessels network corresponds to literature data. The difference of the density of capillary network in gray and white matters is remarkable. However, without the quantitative evaluation, there is no chance to judge any other difference. For the purpose of an evaluation of various regions in white and gray matters, the white matter area has been divided into eight regions:

– region 1. – anterior columns
– region 2. – ventral parts of lateral columns
– region 3. and 4. – lateral parts of lateral columns
– region 5. – dorsolateral parts of lateral columns encompassing pyramidal tracts
– region 6. – deep perigriseal parts of lateral columns
– region 7. – fasciculus cuneatus of posterior columns
– region 8. – fasciculus gracilis of posterior columns.

Gray matter has been evaluated according to the classification Rexed's laminas, however, nine zonas would be too detailed for our purpose. Therefore, gray matter has been divided into four levels marked with letters A, B, C, D. The central gray region, responding to the Rexed's zona X, was evaluated separately and it is marked with letter E.

Blood capillaries are put in histological preparates either on transverse or longitudinal (or moreless obligue) sections. From the point of view of a quantitative analysis, there are two inhomogeneous groups, which were evaluated separately. But for the purpose of density measurement, they were evaluated together.

Results

Dimensions of blood capillaries in the gray matter

By the evaluation of dimensions and shapes of blood capillaries, the following parameters were measured: the area of capillary section, the circumstance of capillary section, the maximal diameter and the shape factor. The information value of area and circumstance parameters were too low, therefore, results of maximal diameters and shape factors are only given here. To emphasize a difference between ventral and dorsal regions of the gray matter, the results of measurement for levels A and B (they respond to Rexed's laminas I–VI) and levels C and D (Rexed's laminas VII–IX) are given together. The range of average values describes parameters which are better observed than the average values themselves; therefore, those ranges are shown in tables. The transversal sections of capillaries are somewhat larger in ventral parts of the gray matter than in white one in all examined segments (Table 1).

Table 1. Variation range of average values of diameters of blood capillaries, which were transverally cut in examined regions of the gray matter in segments given.

Segment	Area	Variation range of blood capillaries diameters (um)
C7	A + B	13.8–15.6
	C + D	14.5–19.2
Th5	A + B	15.0–17.7
	C + D	15.5–18.1
L3	A + B	14.3–19.8
	C + D	16.3–18.9

Table 2. Variation range of average values of lengths of blood capillaries, which were longitudinally cut in examined regions of the gray matter in segments given.

Segment	Area	Variation range of blood capillaries diameters (um)
C7	A + B	27.8–53.4
	C + D	34.1–44.2
Th5	A + B	36.7–52.9
	C + D	32.1–41.7
L3	A + B	35.8–56.5
	C + D	46.5–54.8

The difference of gray matter capillary dimensions between segments are minimal, however, minimal values are in cervical segment C7, and a bit higher in thoracic segment Th5 and also in the lumbar one L3. Shape factor values are not too variable and transversely cut capillaries are in a range of 0.79–0.89. It means that the shape of capillaries is moderately oval, not too different from the regularly spherical one.

Capillary on the longitudinal section were evaluated separately, because the maximal dimension does not correspond to the maximal diameter, but to the length of it, shown in the section (Table 2). The differences between them are also not remarkable; even the length is somewhat longer in all dorsal segments of the gray matter. There are no remarkable dif-

ferences between various segments, however, in both dorsal and ventral parts of the gray matter of the lumbar segment the length is longer than in the other parts.

Dimensions of blood capillary in the white matter

Substantial differences between diameters of capillaries in the transversal section and between capillary lengths in the longitudinal section in various parts of the white matter funiculi were not found. The diameters of capillaries were ranged from 13.5 μm to 24.0 μm and were mostly less than comparable values in the gray matter. The values of the shape factor were rather homogenous: 0.80–0.88.

Capillary lengths on longitudinal sections were, on the contrary, higher than in the same situation in the gray matter. Values measured in the various areas were ranged from 34.6 μm to 102.4 μm. These values were nearly two times higher than those of the gray matter. The cause of it is the radial entrance of vessels to the white matter from the vasocorona.

Blood capillaries density per area unit

The capillary network in the gray matter is much richer than in the white matter. The average values of capillary numbers per 1 mm^2 are given in Table 3 for the white matter and in Table 4 for the gray matter. Graphically, the values are shown in Figures 1, 2 and 3.

In the cervical segment C7, a higher number of white matter capillaries is found in the anterior funiculus and in the ventral part of the lateral funiculus. The highest values in the gray matter are found in the region of the ventral columna, then in the lateral one and in the dorsal part of the columna posterior. Extremely high numbers of blood capillaries were found in the central gray of all segments. This pattern is logical because the central gray is a region of entrance of aa. sulcocommissurales and their abundant branching into smaller arteriae supplying both, ventral and dorsal areas of the gray matter.

In the thoracic segment Th5, the white matter capillary density is similar to the one in the cervical segment. The highest number is found

Table 3. Average values of blood capillaries number per unit of area 1 mm^2 in various regions of the white matter of three examined segments of the spinal cord. (Description indications 1–7 see in the text).

Region of the white matter examined	C7	Th5	L3
Indication Funiculus			
1 Funiculus anterior	33.66	41.65	36.83
2	32.18	29.64	32.33
3	28.10	24.59	32.86
4 Funiculus lateralis	23.95	26.88	29.87
5	25.13	22.31	44.01
6	28.34	28.32	44.54
Funiculus posterior			
7 Fasciculus gracilis	25.91	26.93	32.58
8 Fasciculus cenеatus	24.57	26.90	28.34

Table 4. Average values of blood capillaries number per unit of area 1 mm^2 in various regions of the gray matter of three examined segments of the spinal cord.

Region of the gray matter examined	C7	Th5	L3
A	67.01	66.41	64.89
B	54.59	88.69	68.06
C	61.50	104.08	74.82
D	68.93	98.00	75.91
E	133.63	112.31	125.09

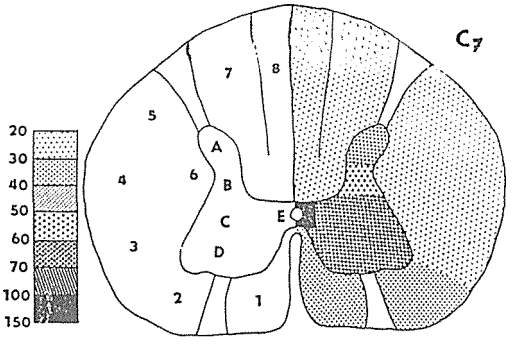

Figure 1. Density of blood capillaries per area of 1 mm^2 in various regions of gray and white matters in the seventh cervical (C7) segment.

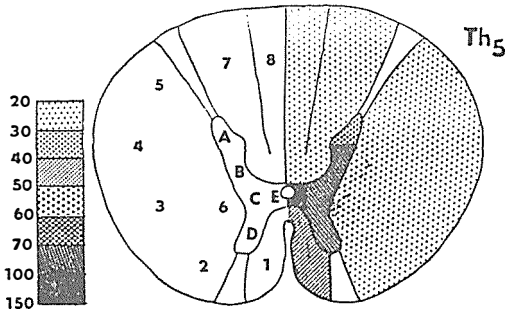

Figure 2. Density of blood capillaries per area of 1 mm^2 in various regions of gray and white matters in the fifth thoracic (Th5) segment.

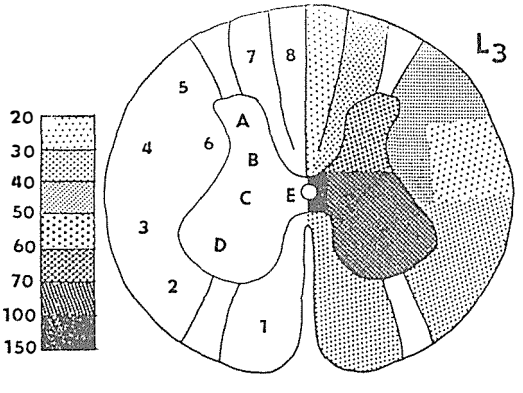

Figure 3. Density of blood capillaries per area of 1 mm^3 in various regions of gray and white matters in the third lumbar (L3) segment.

in the anterior funiculus. This number is higher than that in the cervical and lumbar segments. In the gray matter, the capillary density is significantly higher than in the other two segments except of the dorsal part of columna grisea posterior, where the density is similar.

In the lumbar segment L3, the density of capillaries is higher in both, gray and white matters. The dorsolateral and central parts of lateral funiculus have the highest density of all white matter areas studied. Density values in fasciculus gracilis and in the lateral part of the lateral funiculus were similar to the ones in the other two segments. In the gray matter of the lumbar segment a very high capillary density was found and accordingly it is divided into two parts (with Rexed' s lamina V as the border), the ventral one having a higher density value.

In all segments studied, the richest blood supply has been found in the ventral areas of the gray matter and the highest density is in the thoracic segment, next in the lumbar and relatively small number of capillaries in the cervical segment. In the white matter, no significant difference has been found between cervical and thoracic segments. On the contrary, the lumbar segment has higher values than the previous ones.

Relative area of the blood network

By the next criterion, evaluating the grade of vascularisation of various areas, the parameter of the relative area of the blood network was used. The parameter is the value expressing a proportion of all capillary areas in the whole studied area.

Results obtained for the gray matter (Table 5) confirm the previous observation. In all studied regions, the relative area of blood network increased from dorsal (A) to ventral (D) sites. The minimum value is in the cervical segment and the maximum one in the lumbar segment.

The same measurement proved the maximal proportion of the blood network in the anterior funiculus and the minimal one in the posterior

funiculus (Table 6). By comparison of segments, the highest grade of vascularisation was found in the white matter of the lumbar segment and the minimal in the cervical one.

Discussion

The given results have revealed a quantitative morphological evaluation of spinal cord capillaries and the blood vessel network is a suitable method for studying the differences in blood supply of various regions. Measurements were carried out post mortem, therefore, absolute values do not respond to those in vivo values. We suggest postmortal changes as being proportional and thus the mutual comparison, which was the main goal of the paper, is possible and gives a real picture.

The gained results have confirmed a marked difference between the rich vascularisation of the gray matter and relatively not so rich in the white one. In the gray matter significant differences have been found between ventral and dorsal columns and the grade of vascularisation is increasing to the ventral parts of anterior columns. In the white matter, the vascularisation grade is usually somewhat higher in ventral funiculi. By the quantitative measurement significant diferences have been shown in various spinal cord segments as well. In the gray matter the vascularisation grade is increasing from the cervical to the lumbar and to the thoracic segment. The relative area of the vascularisation in the white matter is increasing from the cervical to the lumbar segment. The lumbar segment has significantly higher blood supply of the white matter than cervical and thoracic segments.

The papers dealing with the spinal cord radiosensitivity have claimed that the cervical cord is more sensitive than the thoracic one.[5–7, 14] More prominent histopatological changes are usually found in the white matter than in the gray one.[14–16] By lower radiation the dose changes are usually described in dorsal funiculi of the white matter, presumably in deeper parts. Further predillect regions are posterolateral superficial regions.[16] In the early stadium of late changes, the decreasing of oligodendroglial cells has been noticed. During the late phase, vascular changes (progressive changes like wall thickening, complete fibrose capillaries obliteration) become more prominent.[15] These changes could result in the necrosis of the white matter and the gray one as well. It seems that neurones are preserved intact very long.[16] This is surely the stadium when blood supply is sufficient enough to fulfil at least a minimum function. By higher radiation doses and by the late reaction, the coagulative necrosis is a predominant pattern.

Table 5. Average values of relative area of blood capillaries in per cent of the total area examined (= 100 %) in various regions of the gray matter of three given segments of the spinal cord.

Region of the gray matter examined	C7	Th5	L3
A	0.94	1.74	1.54
B	1.20	2.15	2.10
C	1.30	2.18	2.51
D	1.51	2.40	2.63
E	2.53	1.97	2.17

Table 6. Average values of relative area of blood capillaries in per cent of the total area examined (= 100 %) in various funiculi of the white matter of three given segments of the spinal cord.

Examined fasciculus of the white matter	C7	Th5	L3
Fasciculus anterior	0.79	0.88	1.21
Fasciculus lateralis	0.69	0.79	0.99
Fasciculus posterior	0.57	0.65	0.94

The different radiosensitivity of various spinal cord segments (cervical and thoracic) and different histopatological findings following the irradiation (more prominent consequences in the white matter and their predilect location in posterior and lateral funiculi) seem to be dependent on different blood supply patterns of these regions. The less is the grade of vascularisation the more radiosensitive the locations. It is contradictory because we know that oxygen sensitizes the effect of the radiation (in nondirect ionisation effect).[17, 18] In this case, one has to keep in mind we are dealing with a normal health tissue that was irradiated, but not the primarily hypoxic tumor. The hyperbaric oxygen irradiation experiments did not prove the spinal cord to be more radiosensitive.[19–21] Normally, tissues are properly supplied with oxygen; that means tissues with minimum vascularisation do not suffer from hypoxia. Where vascular network is richer, the higher need of oxygen is expected, for example by a functional demand. Such a rich capillar network could also have better capacity for the reparative process in tissues irradiated because it sufficiently provides them with oxygen and other substancies to keep them on going process. We know from our clinical practice that some late reactions are positively affected in the hyperbaric oxygen. On the other hand, some pathological situations are known with the vascular network impairment. The risk of the late radiation reaction in the central nervous system is higher by this way[22, 23].

We have to suggest the multidimensional system; the current status of it is a result of many mutually dependent processes. The nervous system is schematically composed of neurones, glial cells and vessels. Each component is a system of cells by itself including many kinds of highly specialized cells. At the same time each system is dependent on the proper function of the other complicated system. Morphologically and functionally, different heterogenous neurones without repopulation capacity are in general dependent on the very heterogenous group of glial cells that can repopulate[24, 25] and both previous systems are dependent on the proper function of the vessel system, which is again completely different from the point of view of the reparation and the repopulation.[26–28] On the other hand, not perfectly understood biological relationship of physical radiation dose, fractions number, total time of irradiation, dose rate and volume effect makes a problem more complicated.

The relation of the vascularization grade to the resultant radiosensitivity is probably only one of many relations in such a complicated and complex system as the human spinal cord, reacting to radiation, is.

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Edge-enhancement performance of the histogram shifting filter

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A quantitative evaluation of the edge-enhancement properties of the histogram shifting (HS) filter is presented, and compared to more common edge-enhancers such as linear high-pass, unsharp masking, homomorphic and statistical differencing filters. Parameters related to noise and edge levels were calculated from simulated noise-free and noisy test images to determine the relative merits of the various filters. The HS edge-enhancer tends to change the relative intensities of the upper and lower level of an edge which may cause difficulties when absolute intensity levels are required. However, the HS filter appears to offer good edge enhancement with the lowest noise amplification when compared to results of other filters, and may thus be very beneficial in practical situations where, in general, noise amplification is not desired.

Key words: radiographic image enhancement; filter comparison, image processing, algorithm performance

Introduction

Various forms of image enhancement techniques exist to improve the visual appearance of a medical image. Edge enhancement is one of these forms and accentuates edges of an image to make it more subjectively pleasing to the human eye.[1] These edge-enhancers may include linear high-pass filters,[2, 3] unsharp masking,[4, 5] homomorphic filtering[6] and statistical differencing[7, 8] techniques. We have recently developed an algorithm which we have labeled histogram shifting (HS) that is capable of edge-detection or edge-enhancement depending on the value of a parameter.[9] In this paper, we will quantitatively evaluate the HS algorithm as an edge-enhancer by comparing its performance with that of the other four edge-enhancers mentioned above.

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Materials and methods

The test images were generated by a "C" program we wrote on a Silicon Graphics Incorporated (Mountain View, Calif. 940939–7311) 4D20 Personal Iris workstation (SGI). We also wrote the high-pass, HS, unsharp masking, statistical differencing and the homomorphic filtering algorithms in "C" on the SGI. Clinical

radiographs were entered into a 386 PC by a VDC3874 (Sanyo Electric Inc., Japan) video camera connected to a Matrox (Montreal, Canada) IM 1280 video digitizer and transferred to the SGI via Ethernet. All images were 256×256 by 8 bits, and filter kernel sizes were all 3×3.

Edge enhancement algorithms

The histogram shifting algorithm was compared to a number of existing edge enhancement algorithms presently used for image processing purposes. As mentioned, these include three kernel-based linear high-pass filters, unsharp masking, statistical differencing, and the homomorphic filters using the previously-mentioned high-pass kernels. A brief overview of the filters studied follows.

Histogram shifting in accomplished by subtracting a fraction, f, of the minimum pixel value of a neighbourhood around each pixel as follows:

$$X'_{k,l} = X_{k,l} - [f * \min (X_{i,j})] \quad (1)$$

where $X'_{k,l}$ is the new grey level of the pixel located at k,l in the image, $X_{k,l}$ is the original value of the pixel located at k,l in the image, and min $(X_{i,j})$ is the minimum of the pixel values located at i,j in the neighbourhood surrounding the target pixel.

The factor, f, controls the amount the histogram local to each pixel is shifted towards the zero value. When $f = 1.0$, the histogram will be shifted such that the minimum value is zero and edge-detection will be produced. The HS algorithm for $f = 1.0$ should then be compared to well known edge-detectors, such as Laplacian and Sobel filters. In this case, however, the HS filter is basically equivalent to the erosion-residue morphological edge detector.[10]

As the value of f is lowered towards zero, edge enhancement will result with lessor degrees of enhancement. In this study, we present images processed with $f = 0.9$, 0.7, and 0.5 labeled HS 0.9, HS 0.7, and HS 0.5, respectively, to represent the useful range of enhancement prodeced by this algorithm.

Three conventional linear high-pass kernels of the following forms:

$$\mathrm{HP1} = \begin{bmatrix} 0 & -1 & 0 \\ -1 & 5 & -1 \\ 0 & -1 & 0 \end{bmatrix}, \quad \mathrm{HP2} = \begin{bmatrix} -1 & -1 & -1 \\ -1 & 9 & -1 \\ -1 & -1 & -1 \end{bmatrix},$$

$$\mathrm{HP3} = \begin{bmatrix} 1 & -2 & 1 \\ -2 & 5 & -2 \\ 1 & -2 & 1 \end{bmatrix} \quad (2)$$

were evaluated.

We also implemented the above kernels (Eq. 2) in a homomorphic fashion by taking the logarithm of the pixel values before applying the kernels and then taking the inverse logarithm of the result. The homomorphic filters are labeled HM1, HM2 and HM3 corresponding to the kernels PH1, HP2 and HP3, respectively.

Unsharp masking is a non-linear edge enhancement technique in which a blurred version of the image is subtracted from the original image itself as follows:[11]

$$X'_{k.l} = \frac{c}{2c-1} X_{k,l} \frac{1-c}{2c-1} X'_{k,l} \quad (3)$$

where c is a weighting factor typically between 0.6 and 0.8 and $X'_{k,l}$ is the value of the pixel located at k,l in the blurred image. For this study, we used a 3×3 averaging kernel to blur the image and values for c of 0.8, 0.7, and 0.6, labeled UM 0.8, UM 0.7 and UM 0.6, respectively.

Statistical differencing[7, 8] is a method of edge enhancement which modifies each pixel value according to the mean and standard deviation of the neighbouring pixels. We have implemented this technique as follows:

$$X'_{k,l} = [X_{k,l} - (X_{k,l})] \left[\frac{a * S_{k,l} - Sd}{a * \mathrm{Sd}} \right] + r * X_{k,l} \quad (4)$$

where $(X_{k,l})$ and $S_{k,l}$ are the mean and standard deviation of the values of the pixels in a neigh-

bourhood centered at k,l in the image, a is a factor between 0.0 and 1.0 controlling the degree of edge enhancement, Sd is a factor representing the desired standard deviation of the resulting image, and r is a scaling factor between 0.0 and 1.0 which varies the degree of contribution of the original image to the processed image. We designed statistical differencing filters with a r of 0.5, an Sd of 85 with an a of 0.1, 0.5 and 0.8 labeled SD 0.1, SD 0.5 and SD 0.8, respectively.

Method of analysis

To analyse the performance of the edge enhancement algorithms is a quantitative manner, we applied two test to images processed by the algorithms. The algorithms were applied to two dimensional step images. The step images were obtained by dividing each test image into two equal-sized sections, one section with uniform low pixel values and the other with uniform high pixel values. The low pixel value in all our test images was 50, while the high pixel value was either 75, 100 or 150. This resulted in three test images with steps from 50 to 100 (50–100), 50 to 75 (50–75), and 50 to 150 (450–150) pixel values, respectively. We only show results obtained with test images of steps 50–100 pixel values, but the results are very similar for the other step values. The step images were corrupted by Gaussian in one set of studies, and uniformly distributed additive noise in another set of studies. The Gaussian noise had a zero mean and a variance of 96 while the uniformly distributed noise had a zero mean and a variance of 123.

For the first test, we measured the increase in noise induced by the enhancement process by determining the mean $\overline{Y}_l$, $\overline{Y}_h$ and standard deviation σ_l, σ_h of the pixel values within a 50-pixel square region of interest in the "low" and "high" regions of the step images (Figure 1), respectively. The subscripts l and h correspond to the "low" and "high" regions, respectively. Each of the square regions are 235 pixels from the edge. The value of the standard deviation indicates the degree of noise amplification within the image caused by the enhancement process.

In the second test, we calculated the degree of edge enhancement by the algorithm in the following manner. The mean of 50-pixel values, $\overline{Z}_{et}$, $\overline{Z}_{eb}$ along a one-pixel thick linear region at the top and bottom of the edge (see Figure 1), respectively were found, and a figure of merit M describing the degree of edge enhancement was calculated:

$$M = \frac{(\overline{Z}_{et} - \overline{Z}_{eb})}{(\overline{Y}_h - \overline{Y}_l)} \tag{5}$$

The figure of merit is the ratio of the edge height to the step height and indicates the amount of overshoot at the edge caused by the algorithm. A larger value of M indicates increased overshoot which implies that the edges are more pronounced.

Results and discussion

A. Noise-free images

Results of our calculation are shown in Tables 1 to 3. In Table 1, which represents a step without noise, the σ_l and σ_h are all zeroes, and $M = 1$ for the original unprocessed image. The HP, UM and HM algorithms do not change the values of $\overline{Y}_l$ and $\overline{Y}_h$ except for the slight changes produced by UM 0.7 ($\overline{Y}_l$, from 50 to 49; and $\overline{Y}_h$, from 100 to 99) . The SD algorithm

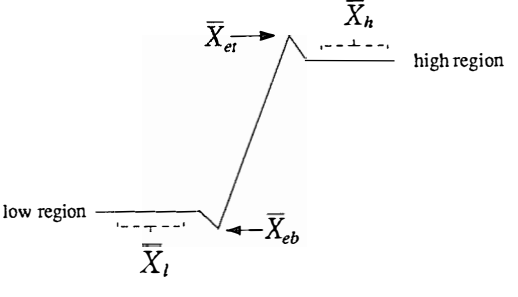

Figure 1. Schematic of the step edge profile with a pictorial definition of the parameters discussed in the text.

Table 1. Results of calculation of the quantitation parameters for a noise-free image composed of a step. The step function is comprised of values of 50 to values of 100.

Algorithm	$\overline{Y}_l$	σ_l	$\overline{Y}_h$	σ_h	M
original	50.00	0.00	100.00	0.00	1.00
HS 0.5	25.00	0.00	50.00	0.00	2.00
HS 0.7	15.00	0.00	30.00	0.00	3.33
HS 0.9	5.00	0.00	10.00	0.00	10.00
HP 1	50.00	0.00	100.00	0.00	3.00
HP 2	50.00	0.00	100.00	0.00	5.00
HP 3	50.00	0.00	100.00	0.00	1.00
UM 0.8	50.00	0.00	100.00	0.00	1.22
UM 0.7	49.00	0.00	99.00	0.00	1.50
UM 0.6	50.00	0.00	100.00	0.00	2.30
MH 1	50.00	0.00	100.00	0.00	3.57
MH 2	50.00	0.00	100.00	0.00	5.08
MH 3	50.00	0.00	100.00	0.00	1.00
SD 0.1	25.00	0.00	50.00	0.00	10.20
SD 0.5	25.00	0.00	50.00	0.00	4.80
SD 0.8	25.00	0.00	50.00	0.00	4.28

Table 2. Results of calculation of the quantitation parameters for a step image with Gaussian noise. The step function is comprised of values of 50 to values of 100.

Algorithm	$\overline{Y}_l$	σ_l	$\overline{Y}_h$	σ_h	M
original	49.13	94,36	99.63	94.02	0.97
HS 0.5	31.38	91.80	56.85	82.19	1.82
HS 0.7	22.44	95.29	39.66	96.07	2.93
HS 0.9	17.36	100.85	22.64	102.48	8.21
HP 1	54.11	1997.39	100.26	2615.39	2.78
HP 2	65.85	4708.23	104.05	6055.23	5.32
HP 3	57.74	2719.52	101.40	3739.26	1.05
UM 0.8	49.23	158.43	99.37	158.27	1.18
UM 0.7	49.37	268.22	99.45	270.11	1.44
UM 0.6	50.66	714.33	100.56	764.96	2.17
MH 1	75.43	4580.86	112.62	3061.84	4.16
MH 2	99.24	9107.73	124.25	6613.41	8.18
MH 3	82.68	6484.45	116.44	4347.08	1.22
SD 0.1	57.39	4970.33	74.43	5841.47	12.19
SD 0.5	29.86	775.10	51.93	993.48	5.86
SD 0.8	27.83	540.0	50.90	674.53	5.09

Table 3. Results of calculation of the quantitation parameters for a step image with white noise (uniformly distributed). The step function is comprised of values of 50 to values of 100.

Algorithm	$\overline{Y}_l$	σ_l	$\overline{Y}_h$	σ_h	M
original	49.37	126.14	99.65	124.99	0.96
HS 0.5	32.13	120.07	57.43	121.47	1.81
HS 0.7	25.16	119.38	40.34	121.98	2.92
HS 0.9	18.48	120.12	23.62	123.58	8.43
HP1	56.29	2519.58	100.13	3570.09	2.73
HP2	72.34	5753.50	107.20	7862.34	5.36
HP3	60.89	3526.19	102.54	5009.62	1.00
UM 0.8	49.08	208.77	99.36	209.87	1.16
UM 0.7	49.25	349.93	99.43	356.38	1.43
UM 0.6	50.38	947.19	100.43	1007.41	2.09
HM 1	83.50	6207.03	116.73	4234.16	4.08
HM 2	107.88	11038.52	128.32	8250.66	9.77
HM 3	90.39	8325.30	120.31	5657.07	1.14
SD 0.1	67.27	6230.23	82.16	7558.15	12.42
SD 0.5	32.52	1035.39	52.77	1493.46	6.31
SD 0.8	29.66	750.62	51.21	1069.39	5.50

halves the values of both $\overline{Y}_l$ and $\overline{Y}_h$, while the HS algorithms significantly decreases $\overline{Y}_l$ and $\overline{Y}_h$ as the shifting factor f approaches the value of 1 when the HS algorithm can be considered a strict edge detector. Except for HP3 and HM3, all algorithms show an increase in the figure of merit M with respect to the unprocessed image. The HP and its related HM filter show virtually identical increase in M. There is marginal increase in M for the UM filters, although greater increase would perhaps have been noted if a weighting factor of $c < 0.6$ would have been used. However, a c of 0.6 is the smallest value commonly used in unsharp masking. The remaining filters, SD and HS show possibilities of producing the greatest edge enhancements with the largest figure of merit (i.e., $M_{HS0.9} \sim M_{SD0.1} \sim 10$). Both these filters achieve this figure of merit by keeping constant at least the relative values of $\overline{Y}_l$ and $\overline{Y}_h$. One should note, however, that the absolute values of $\overline{Y}_l$ and $\overline{Y}_h$ at which this figure of merit is achieved are much lower in the HS algorithm than they are in the SD algorithm.

B. Noisy images

We can see from Tables 2 and 3 that results with a noisy image are quite different, although whether that noise is Gaussian or white does not appear to significantly effect the performance of the algorithms. As expected, the standard deviations σ_l and σ_h of the original and the processed images have significantly increased with respect to the original images. An important observation is that the HS filter has kept the values of σ_l and σ_h very close to those of the original images, while all other filters have significantly increased the value of this parameter with respect to those obtained from the unprocessed images. The filters, HP, HM and SD have increased the values of σ_l and σ_h by much more than an order of magnitude with respect to those calculated from the unprocessed images. Although the UM has not drastically increased the values of σ_l and σ_h the parameters, it generally produced the lowest figure of merit. The HP3 produced the lowest figure of merit with a significant increase in σ_l and σ_k with respect to those calculated from the unprocessed images.

The figure of merits for the HM and SD filters increase with noisy images, but the values have slightly decreased for the others studied, including the HS filter. It appears from these tables that the HS filter delivers reasonably high figure of merits with the lowest amplification of noise. Although, the SD filter offers larger figures of merit, this is produced at the expense of extremely large noise amplification. The HS 0.9 filter delivers a high figure of merit but the relative $\overline{Y}_l$ and $\overline{Y}_h$ values have drastically decreased from the original image, and from that produced in the noise-free image. As discussed, the HS filters, at least keep the relative $\overline{X}_{l,h}$ values constant in the noise-free image but deviates from this value in noisy images, especially when the f factor approaches 1.

C. Clinical images

Typical results of the edge-enhancement processes studied are shown in Figure 2. Although, the images in this figure are only 8 bit deep, the results should be representative to images of greater depth, since we are comparing the results of processing with the original unprocessed 8 bit image. This is a case of bronchiectasis where the number and size of distorted bronchi and vessels is clinicaly important. Results of the three HS filters studied, and the most visually detailed results of the other filters studied are displayed. As expected, the distorted bronchi are more noticeable within the processed images. The significant noise amplification of the HP, HM and SD filters are also well exemplified in these images. The HS in UM filters offer less noise amplification with reasonable edge enhancement. As expected from our discussion above, the images resulting from the HS filters do not appear to exhibit great noise amplification. One notices that although edges are more enhanced within the image resulting from UM processing (Figure 1 (f)) than they are with the image enhanced with HS processing, areas in the images processed from the HS filter (Figure

1 (b)) apear to offer a greater range of intensity. This is especially noticeable in the regions of the heart.

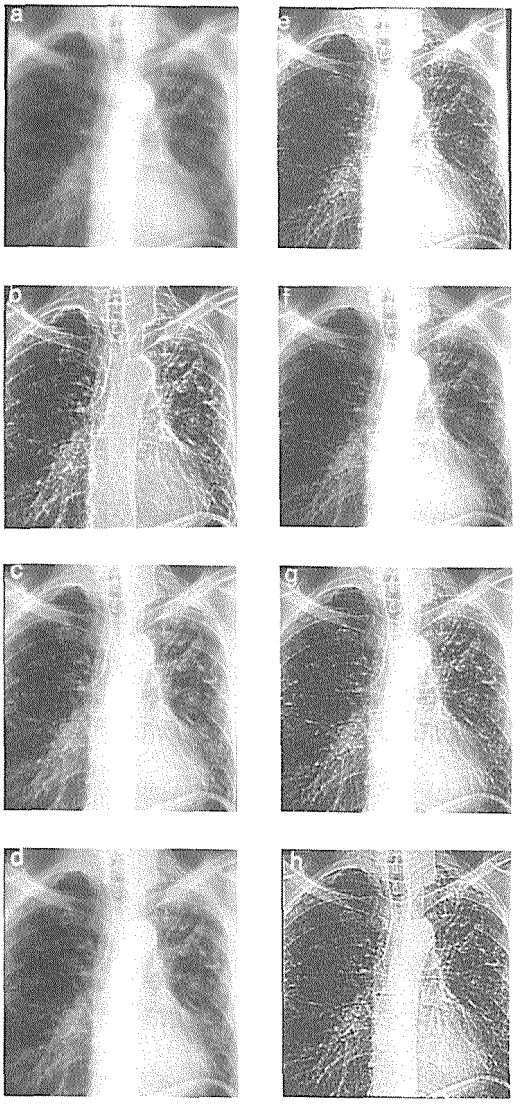

Figure 2. Typical results of the HS algorithm compared to those of others studied. (a) An original chest radiograph of a case of bronchiestasis. Result after processing with histogram shifting of (b) $f = 0.9$, HS 0.9. (c) $f = 0.7$, HS 0.7, and (d) $f = 0.5$, HS 0.5. Result after processing with (e) linear high pass filter, HP2; (f) unsharp masking, UM 0.6; (g) homomorphic; HM2; and (h) statistical differencing, SD 0.1.

Conclusion

The edge-enhancement properties of the HS algorithm have been quantitatively compared with several proven algorithms (linear high-pass, unsharp masking, homomorphoric and statistical differencing). Unsharp masking produced low noise amplification and has the advantage of tending to keep the relative intensity of edges more constant, but with the slight disadvantage of sometimes not offering the same range of grey-level intensities as does the HS process. The HS algorithm appears to offer good edge-enhancement, and has the distinct advantage of accomplishing this with the lowest noise amplification when compared to the other filters studied and without saturating enhanced areas of the image.

Like all edge-enhancement filters, the HS algorithm must be applied judiciously to each particular case, but because of its properties and simplicity of the algorithm can become an important part of an image processing system.

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Parameterization of megavoltage transmission curves used in shielding calculations

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An analytical expression based on six fitting parameters is proposed to model the transmission of megavoltage photon beams through shielding barriers made of standard density concrete, iron, and lead. The model reproduces published transmission curves within ±0.7%, and can be used with excellent accuracy for any beam with nominal energy in the clinical range (5–45 MV). Extrapolation of the model beyond this energy range or to other shielding materials, however, is not recommended.

Key words: radiotherapy, high-energy; radiation protection

Introduction

The design or modification of a radiotherapy department is a complex process beginning with the approval of a budget and progressing to the selection and purchase of treatment units. Once the general layout for an installation has been decided, it is usually the responsibility of a medical physicist to design the shielding necessary to limit the radiation exposure outside the radiation therapy rooms to acceptable levels.

A detailed analysis of the formalism used in shielding calculations can be found in the literature.[1] There are several variables which collectively define the thickness of a shielding barrier that will adequately protect individuals in the vicinity of a radiation therapy treatment machine. These include the maximum permissible dose equivalent incurred annually by any individual, the workload for the therapy machine, the use factor for a given machine orientation, the occupancy factor of the location to be shielded, and the distance between the source of radiation and the shielded area. Once the values of these parameters have been decided, a barrier transmission factor representing the level of transmission through a shield resulting in the maximum permissible dose equivalent in the shielded area can be calculated using a straightforward formalism.[1] The thickness of a shielding barrier corresponding to this transmission factor is then determined using published transmission curves.[1,2] These curves are a function of the shielding material and the energy of the radiation beam to be shielded. Unfortunately, the original transmission curves are small and not very clear. Furthermore, imterpolation betwen energies, which is often necessary to obtain information for beam energies not specifically plotted, is very difficult and imprecise.

In this paper we present an analytical expression which can be used to relate the thickness

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of a shielding barrier to its transmission of radiation. The formalism is valid for radiation beams with a nominal energy in the range most commonly produced by contemporary external beam therapy accelerators (1 MV to 25 MV). The expression reproduces published transmission curves for the standard shielding materials (concrete, iron, and lead) with good accuracy and simplifies interpolation of transmission data between energies.

Materials and methods

A data set containing barrier thickness as a function of radiation transmission was derived from the transmission curves for standard density concrete, iron, and lead given in NCRP Reports #49 and #51,[1,2] to be known below as NCRP #49 and NCRP #51, respectively. The transmission curves in NCRP #49 are plotted for beam energies up to 10 MV. For higher energy beams, one must refer to NCRP #51, where transmission curves are given for beams produced by monoenergetic electrons with energies up to 176 MeV interacting with a target. For this work it was assumed that the nominal energy of the resulting x-ray beam is equal to the energy of the electrons which produce the beam. In other words, the transmission properties of an x-ray beam resulting from, for example, 1.0 MeV electrons hitting a target are identical to those of a 1MV x-ray beam.

The following function was fitted to the data set derived from each transmission curve:

$$t = a \log B, \qquad (1)$$

where t and B are the tickness (in cm) and transmission, respectively, of a shielding barrier and α is a fitting parameter. The fitting was done using a commercially available graphics software package (KaleidaGraph, Abelbeck Software, Reading, PA) which incorporates an algorithm for non-linear curve fitting. The program was implemented on a Macintosh personal computer (Apple Computers, Cupertino, CA) and allows the user to define single variable functions with up to nine parameters to be fit to a data set. The efficiency of the fitting procedure is enhanced with the use of partial derivatives which effectively steer the algorithm towards optimal fits. In Eq. (1) the dependence of shield thickness and transmission is reversed compared to the transmission curves in NCRP #49 and #51. This is because shielding calculations usually require the determination of barrier thickness for a calculated transmission, thus it is more intuitive to analyze the curves with transmission as the independent variable and shield thickness as the dependent variable. Should one require the inverse information, *i.e.*, the transmission of a given thickness of shielding material, the equations can always be inverted with little loss of accuracy.

To facilitate interpolation of transmission curves between energies, an analytical expression was fitted to the relationship between a for a given shielding material and the energy of the beam. The form of the equation is

$$a = c_1 E^{c_2} exp\left(-\frac{E}{c_3}\right) + c_4 E + c_5 \log(c_6 E), \qquad (2)$$

where $c_{1\text{-}6}$ are the fitted parameters and E is the energy of the beam. The functional dependence of Eq. (2) does not arise from any theoretical basis and was chosen only because of its ability to fit the data points for all three shielding materials with minimal error.

Results and discussion

In fitting Eq. (1) to the transmission curves in NCRP #49 and #51, it was assumed that the curves are mono-exponential. Based solely on qualitative shape, there is no question that this assumption is valid for the megavoltage transmission curves given in NCRP #49. However, several transmission curves given in NCRP #51 show a definite shoulder at small shield thicknesses. This shoulder is most pronounced at high beam energy (E > 38 MV) and is a result of selective attenuation by the pair production

interaction of higher energy photons in the first few centimeters of shielding material. The inaccuracy introduced by fitting Eq. (1) to a transmission curve with a shoulder is acceptable since the effect occurs primarily for beam energies higher than those currently used clinically. It is possible to achieve a better fit to these high energy transmission curves by introducing extra terms into Eq. (1) to account for the shoulder region. The point of this analysis, however, is to devise an analytic expression which can be used to interpolate between energies. If more than one parameter were used to describe the transmission curves, the simple analytic expression given by Eq. (2) relating the slope of a transmission curve to beam energy would not be valid.

Table 1 gives a listing of the value of a for photon beams of several nominal energies interacting in concrete, iron, and lead. The values of a determined for energies duplicated in NCRP #49 and #51 are slightly different; those shown in Table 1 are the higher of the two values as determined from either report. The difference is most likely a result of the definition of the photon beam energy used in the two reports. In any case the discrepancies are small and can be ignored. Figures 1 (a–c) are plots of a as a function of beam energy for concrete, iron, and lead, respectively. As expected the value of a decreases with increasing energy for concrete and iron, reflecting the more penetrating power of higher energy photon beams. For lead, however, a first decreases, reaches a minimum around 6 MV, and then increases for higher energies. This behavior is a result of the increase in the probability for

Table 1. Values of the parameter a obtained from a fit of Eq. (1) to the megavoltage transmission curves in NCRP #49 and #51. The error in the parameter resulting from the fit is approximately constant at 0.5%.

Beam Energy (MV)	Concrete (cm)	Iron (cm)	Lead (cm)
1.0	–16.0340	–5.0740	–2.5003
2.0	–20.7205	–7.1715	–3.7617
3.0	–24.5509	–8.2177	–4.6826
4.0	–29.1533	–9.0735	–5.2512
6.0	–34.6469	–9.8341	–5.5817
8.0	–38.0341	–10.3140	–
10.0	–40.0698	–10.5116	–5.2341
20.0	–45.5178	–	–4.8200
31.0	–	–11.1280	–
38.0	–47.9993	–	–
86.0	–	–	–4.2154

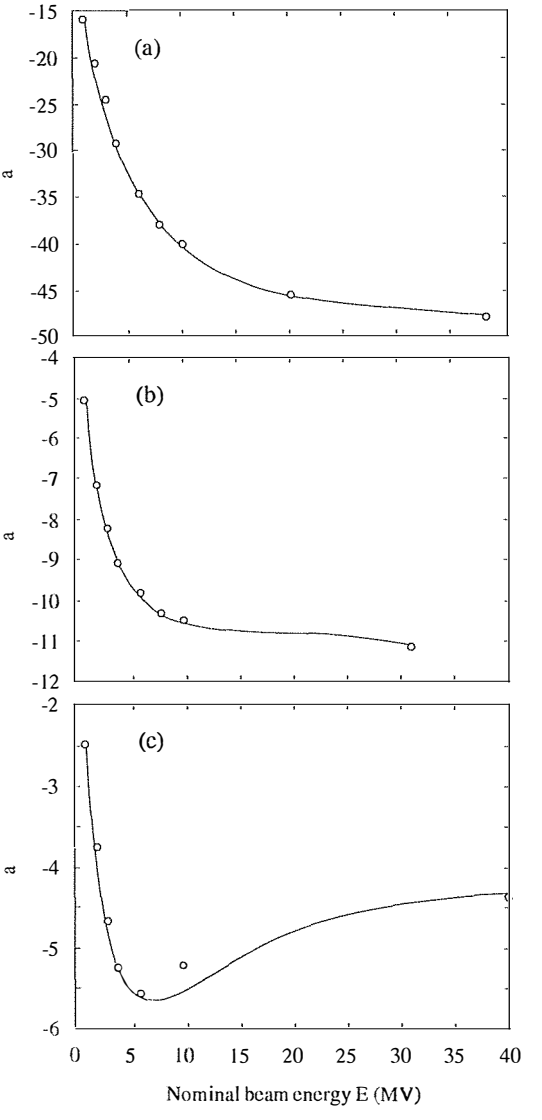

Figure 1. Plots of the parameter a, determined by fitting Eq. (1) to the megavoltage x-ray beam transmission curves for (a) standard density concrete, (b) iron, and (c) lead, as a function of nominal beam energy. The transmission curves used in the determination of a are found in NCRP #49 and #51.[1,2]

pair production and the concurrent dicrease in the Compton effect with increasing beam energy.

The parameters c_{1-6} of Eq. (2) fit to the data points in Figure 1 are given in Table 2. The solid lines in Figure 1 (a–c) represent plots of Eq. (2) with these parameters. It was found that four parameters are sufficient to describe the behavior of concrete, while for iron and lead all six parameters are significant. The correlation coefficient for each fit is 0.995, 0.998 and 0.900 for concrete, iron, and lead, respectively. As demonstrated in Figure 1 and by the value of the correlation coefficients, the fits to the data points are excellent with the exception of the curve for lead, which overestimates by 4% the value of a at 10 MV. In the energy range used most often clinically (4–25 MV), the average error for all sheilding materials is 0.7% with a range of 0.04% to 4%. At lower beam enery (1 MV to 4 MV), the formalism can also be used to provide estimates of transmission, however the accuracy is only ±7%.

Equations (1) and (2) can be combined as follows to determine the shielding thicknes necessary to produce a given transmission of x rays in the energy range (1 MV–25 MV) analyzed in this work:

$$t = \left[c_1 E^{c_2} \exp\left(-\frac{E}{c_3}\right) + c_4 E + c_5 \log(c_6 E) \right] \log B, \quad (3)$$

where t is the shielding barrier thickness (in cm) that will allow a transmision of B and c_{1-6} are given in Table 2. To illustrate the usefulness of this formalism, say, for example, that one must determine the thickness of a shielding barrier that will allow a transmission of 10^{-6} of 18 MV x rays. This photon beam in currently very commonplace in modern radiotherapy departments, yet transmission curves for this energy are not specifically plotted in either NCRP #49 or #51. Equation (3) facilitates interpolation of the curves that are published in the reports to obtain transmission information for an 18 MV beam. Using Eq. (3) with $B = 10^{-6}$ and the values of c_{1-6} found in Table 2, we find that a 270 cm thick concrete barrier will be sufficient to limit the transmission of an 18 MV x-ray beam to 10^{-6}. If the space for shielding is limited to, say 250 cm, then a concrete wall alone cannot be used. In this situation a shielding barrier comprised of a combination of concrete and iron or lead would most likely be designed. As mentioned above, Eq. (3) can be used inversely to calculate the transmission for a given thickness of shielding material. This inverse functional relationship is useful when determining the individual thicknesses of combined layers of two or more shielding materials that will provide adequate shielding yet not violate a given total thickness constraint. For the situation described above, one finds using Eq. (3) that a barrier comprised of 240 cm of concrete and 7.2 cm of iron will result in a transmission of 10^{-6} and also remain within the thickness constraint.

Conslusions

In this work we have presented an analytical expression based on published transmission data which can be used to determine the shielding barrier thicknesses that will result in a given transmission of radiation. The expression

Table 2. Values of the constants c_{1-6} obtained from a fit of Eq. (2) to the data in Figure 1. The errors in the constants were on the order of 1%. The correlation coefficient of each fit is also shown.

Shielding Material	c_1	c_2	c_3	c_4	c_5	c_6	R^2
Concrete	–15.920	0.490	25.833	–0.684	0	–	0.995
Iron	–4.760	0.290	13.579	–0.0541	–5.110	1.244	0.998
Lead	–2.770	0.470	7.706	0.0408	–3.570	1.082	0.900

can also be used inversely to determine the transmission of a given thickness of shielding material. The materials analyzed are those used most often for shielding purposes: standard density concrete, iron, and lead. The equations were derived using transmission data for photon energies in the range of 1 MV to 38 MV, however, they are most accurate in the clinical energy range, *i.e.,* 4 MV to 24 MV. Extrapolations of the curves to energies below 1 MV or above 38 MV is not recommended as this will not provide accurate transmission information. Furthermore, the formalism is valid only for the standard shielding materials: concrete, iron, and lead.

References

1. National Council on Radiation Protection and Measurements. Report No. 49, *Structural Shielding Design and Evaluation for Medical Use of X Rays and Gamma Rays of Energies up to 10 MeV.* Washington, DC: 1976.
2. National Council on Radiation Protection and Measurements. Report No. 51, *Radiation Protection Design Guidelines for 0.1–1000 MeV Particle Accelerator Facilities.* Washington, DC: 1977.

Book review

Radiation therapy in paediatric oncology

Editor: J. Robert Cassady, M.D., Professor, Department of Radiation Oncology, Colegge of Medicine, The University of Arizona, Health Science Center, 1501 North Campbell Ave., Tucson, AZ 85724, USA

ISBN 3-540-54105-5 Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-54105-5 Springer-Verlag New York Berlin Heidelberg

On 385 pages the book gives a review of the role of radiotherapy in the treatment of most childhood tumors. With the contribution of 20 authors from various fields of oncology it features 25 chapter on etiology, radiation tissue effects, molecular biology and genetics, interaction between radiotherapy and chemotherapy in leukemias and lymphomas, CNS tumors, Wilms tumor, Ewing's sarcoma, rhabdomyosarcoma, osteosarcoma, retinoblastoma, Langerhans cell histiocytosis, epithelial carcinomas and unusual childhood tumors. In their field of interest and expertise the contributors introduce their approach to diagnostics and treatment of malignancies. With 77 figures, including diagnostic images and radiotherapy planning schemes, and with 94 tables the contents of the text are well illustrated to the reader.

J. R. Cassady highlights the current trends in the management of neuroblastoma, while L. E. Kun provides an overview on other brain tumors. Together with P. S. Swift's chapters on brain stem and spinal chord tumors the book gives a complete guide in the treatment of CNS malignancies. The effects of therapy on CNS functions are detailed by the two leading European oncologists Van Der Kogel and Van Der Schueren. The volume concludes with the editor's chapter on future prospects in childhood cancer. With each chapter comes an extensive reference list for those who wish to learn more on the subject.

Radiation Therapy in Pediatric Oncology presenets the essential knowledge on the relation of radiotherapy and pediatric oncology. It is of an exceptional value for all physicians who encounter the task of treating children suffering from cancer.

Reviewed by G. Horváth, M.D.
Radiologist
University Scholl of Medicine, Pécs, Hungary

ESTRO teaching course on basic clinical radiobiology

Location: Congress Center and Hotel ILF
Prague, Czech Republic
Time: 16–20 October 1994
Director: Prof. G. Gordon Steel (UK)
Teachers: Dr. Søren M. Bentzen (Denmark)
Prof. Stanley Dische (UK)
Dr. Michael C. Joiner (UK)
Prof. Albert van der Kogel (The Netherlands)
Dr. Fiona A. Stewart (The Netherlands)
Local organiser: Prof. J. Bauer
Course coordinator: Germaine Heeren (Belgium)
Participants: Approximately 90 people; doctors and physicists from 20 different countries

This perfectly prepared and organised teaching course of ESTRO – the European Society for Therapeutic Radiology and Oncology – provided an excellent introduction to radiation biology and its application to radiotherapy.

On the arrival at the hotel all the participants of the course received a textbook with the title: Basic Clinical Radiobiology. Dr. Gordon Steel, being not only one of the authors of this book but also the leading lecturer of the course, the lectures followed the logical order of the chapters of the book. This helped the audience to understand the presented slides and projections. Some of the lecturers were researchers and radiation biologists. Some others were clinicians who are trying to introduce newer and better fractionation schedules into tumour irradiation therapy, based on the most recent results of the radiation biological research.

This teaching course covered the basic ideas of mechanisms of radiation response in tumours and in different types of normal tissues. The linear quadratic (L–Q) approach to time-dose relationships was presented in detail by M. Joiner. The L–Q model gives a good description of radiation response in the low-dose region. The Ellis and the Cohen models turned out to be incorrect when Dr. Søren Bentzen compared them to the linear-quadratic model. A schedule of hyperfractionated accelerated radiotherapy (CHART) calculated using the L–Q formula was presented by Prof. S. Dische. Prof. Kogel gave the audience practical examples on how to use the L–Q formula to calculate the necessary corrections and modify the schedule if an unexpected break or dosimetric error happens during the ongoing fractionated radiotherapy. One of the most interesting and exciting part of the course was the clinical radiobiology workshop lead by Dr. Bentzen, Dr. Dische, Dr. Kogel and Dr. Steel. It was a discussion of clinical cases with therapeutic difficulties leading to different opinions, but in the end well-prepared answers were presented on slides. In his lectures Dr. Steel also explained how to combine radio and chemotherapy and how advantageous brachytherapy is from the radiobiological point of view. Dr. Stewart and Dr. Joiner spoke about the use of particle beams and the future of the Boron neutron capture therapy.

At the end to the course the participants were asked to complete a course evaluation form in order to give some sort of feedback to the tutors. This was followed by an optional examination.

Correspondence to: Géza Sarlós M.D., Department of Obstetrics and Gynaecology, Oncoradiological Centre, University Medical School of Pécs, H-7624 Pécs, Hungary. Phone: + 36-72-324-122/2147. Fax.: + 36-72-314-911.

Géza Sarlós, M.D.

Notices

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

Ecology

The "International Congress on Hazardous Waste. Impact on Human and Ecological Health" will be held in Atlanta, USA, *June 5–8, 1995.*

Contact Dr. John S. Andrews, Jr., ATSDR, 1600 Clifton Rd., NE (E-28), Atlanta, GA 30333; or call + 1 404 639 0708.

Gastroenterology

The "Regional Postgraduate Course" will be offered in Columbus, Ohio, USA, *June 11, 1995.*

Contact ACG, 4900B S. 31st., Arlington, VA 22206-1656; or call + 1 703 820 7400.

Genetics

The course on genomic information and ethical inplications will be offered in Seattle, USA, *June 11–14, 1995.*

Contact Univ. of Washington School of Medical History and Ethics, SB-20, Seattle, WA 98195, USA; or call + 1 206 616 1864.

Nuclear medicine

The "42nd Annual Meeting of the Society of Nuclear Medicine" will be held in Minneapolis, USA, *June 11–14, 1995.*

Contact Society of Nuclear Medicine, 136 Madison Ave, New York, NY 100 166 760, USA. Fax: + 1 212 545 0221.

As a service to our readers, notices of meeting or courses will be inserted free of charge.
Please sent information to the Editorial office, Radiology and Oncology, Vrazov trg 4, 61105 Ljubljana, Slovenia.

Radiology

The "Roentgen Centenary Congress" will take place in Birmigham, U.K., *June 12–16, 1995.*

Contact V. Whitehead, British Institute of Radiology, 36 Portland Place, London WN1, 4AT, U.K.; or call + 44 71 436 7807; Fax: + 44 71 255 3209.

Dyspnea

The "Internartional Symposium" will be held in Amsterdam, The Netherlands, *June 22–23, 1995.*

Contact Simon Rietveld, Univ. of Amsterdam, Rm. 820, Roetersstraat 15, 1018 WB Amsterdam, the Netherlands; or call + 31 20 525 6228.

Gynecology

The "Annual Clinical Meeting" of Society of Obstetricians and Gynecologists of Canada will be offered in Calgary, Alta., Canada, *June 24–28, 1995.*

Contact Linda Huskins, SOGC, 774 Echo Dr., Ottawa, ON K1S 5N8, Canada; or call + 1 613 730 4192.

Immunology

The "6th International Workshop on Antineutrophill cytoplasm antibodies" will take place in Paris, France, *June 28–July 1, 1995.*

Contact P. Lesavre, Dept. de Nephrologie, Hopital Necker, 161 rue de Sevres, 75743 Paris Cedex 15, France.

Gastroenterology

The "8th International Workshop on Gastroduodenal Pathology and *Helicobacter Pylori*" will be offered in Edinburgh, Scotland, U.K., *July 7–9, 1995.*

Contact Confrex, 145 Islingword. Rd., Brighton BN2, 2SH, U.K.; or call + 44 273 623 123.

Oncology

The "11th Annual Meeting of the British Oncological Association" will be held in Heslington, U.K., *July 9–11, 1995.*

Contact British Oncological Association, Congress House, 55 New Cavendish Street, London W1M 7RE, U.K. Fax: + 44 71 935 7559.

Science editors

The "8th International Conference", entitled "Science, Culture and Communication for the 21st Century", organised by International Federation of Science Editors, will be offered in Barcelona, Spain, *July 9–13, 1995.*

Contact Secretariat, IFSE-8, Apartado 16009, E-08080 Barcelona, Spain.

Immunology

The "8th International Congress of Mucosal Immunology" will be held in San Diego, California, USA, *July 16–21, 1995.*

Contact Proffesional Conf. Management, 7916 Convoy Court, San Diego, CA, 92111; or call + 1 619 569 9921.

Chemotherapy

The "19th International Congress of Chemotherapy" will be offered in Montreal, Canada, *July 16–21, 1995.*

Contact 19th ICC Secr., 205 Viger Ave West, Suite 207, Montreal, Quebec H2Z 1G2, Canada. Fax: + 1 514 871 2870.

Epidemiology and Biostatistics

The conference of Canadian Society for Epidemiology and Biostatistics will be held in St. John's, Newf., Canada, *August 16–19, 1995.*

Contact CSEB Conference'95 Office, c/o Health Research Unit. P.O. Box 23068, St. John's, NF A1B 4J6, Canada; or call + 1 709 737 6720.

Cancer treatment

The "9th International Confereence on Chemical Modifiers of Cancer Treatment" will be held in Oxford, UK. *August 22–26, 1995.*

Contact Mrs V. Fielden, Conference Secretariat, MRC Radiobiology Unit – Experimental Oncology, Chilton, Didcot, Oxon OX11 0RD, United Kingdom; or call + 44 235 834 393. Fax: + 44 235 834 776.

Esophagus

The 6th Word Congress of the Initiation Society for Diseases of the Esophagus will be offered in Milan, Italy, *August 23–26, 1995.*

Contact Organizing Secretariat, ECON, Via della Moscova, 16, 20121 Milan, Italy; or call + 39 2 29 005 745; Fax: + 39 2 29 005 790.

Pharmacoepidemiology

The "11th International Conference on Pharmacoepidemiology" will be held in Montreal, Canada, *August 27–30, 1995.*

Contact ISPE, Univ. of Kansas Medical Ctr., Robinson 4004, 3901 Rainbow Blvd., Kansas City, KS 66160-7313, USA; call + 1 913 588 2790.

Radiology

The "10th International Congress of Radiation Research" will be held in Wuerzburg, Germany, *August 27–September 1, 1995.*

Contact Prof. C. Streffer, Inst. of Medical Radiobiology, Universitätsklinikum, Essen, 45122 Essen, Germany; or call + 49 201 72234152; Fax: + 49 201 723 5966.

Degestive Endoscopy

The course will be offered in Amsterdam, the Netherlands, *September 7–8, 1995.*

Contact Helma Stockmann, European Postgrad. Gastro-Surgical School, Rm. G4 109.3 Academic Medical Ctr., Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands; or call + 31 20 566 3924.

Gastroenterology

The "Regional Postgraduate Course" will be offered in Cooperstown, N.Y., USA, *September 9, 1995.*

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The "Regional Postgraduate Course" will be offered in San Antonio, Tex., USA, *September 16 and 17, 1995.*

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Bone and joint diseases

The "22nd Annual Refresher Course of the International Skeletal Society" will take place in New Orleans LA, USA, *October 18–21, 1995.*

Contact Ryals and Associates, P.O. Box 1925, Roswell, GA 30077-1925 or Byron Gilliam Brogdon, M. D., Professor of radiology, USA Medical Center, 22451 Filling Street, Mobile, AL 36617, USA.

Ethics in medicine

The "6th International Congress" will be held in New York, USA, *October 22–25, 1995.*

Laser in Dermatology

The "2nd International Symposium" will be offered in Ulm, Germany, *October 27–28, 1995.*

Contact Inst. für Lasertechnologien in der Med., Helmholtzstr. 12, 89081 Ulm, Germany; or call + 49 731 1429 0.

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S Fondacijo "Docent dr. J. Cholewa" želimo zato pomagati sposobnim raziskovalcem dopolnjevati znanje in udejanjati lastne ideje ter s tem pospeševati njihova prizadevanja pri premagovanju te hude bolezni. Da bomo lahko izkoristili naš znanstveno-raziskovalni potencial in obogatili zakladnico znanja o rakastih boleznih, vas pozivamo, da s svojimi prispevki omogočite Fondaciji uresničevanje njenega poslanstva.

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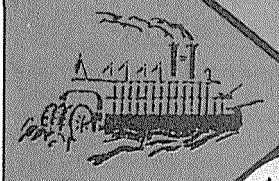

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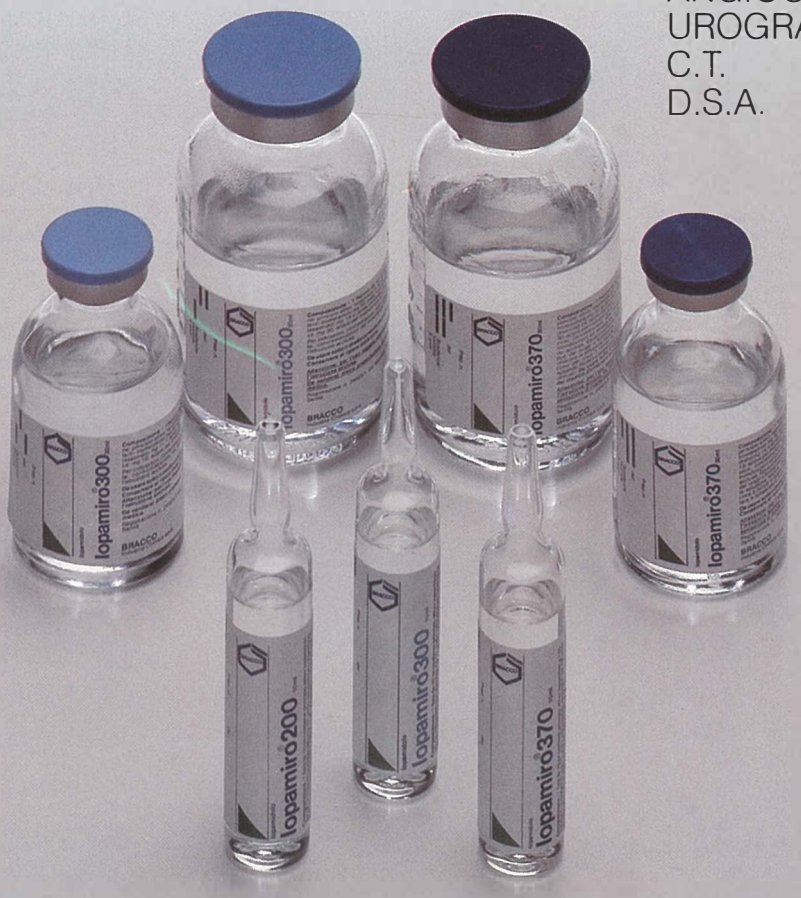

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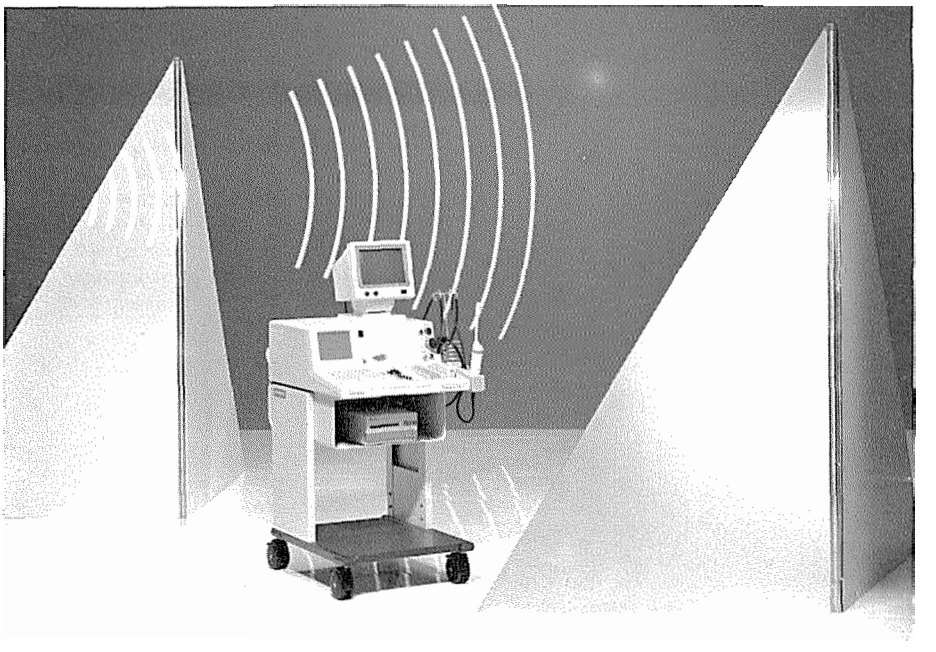

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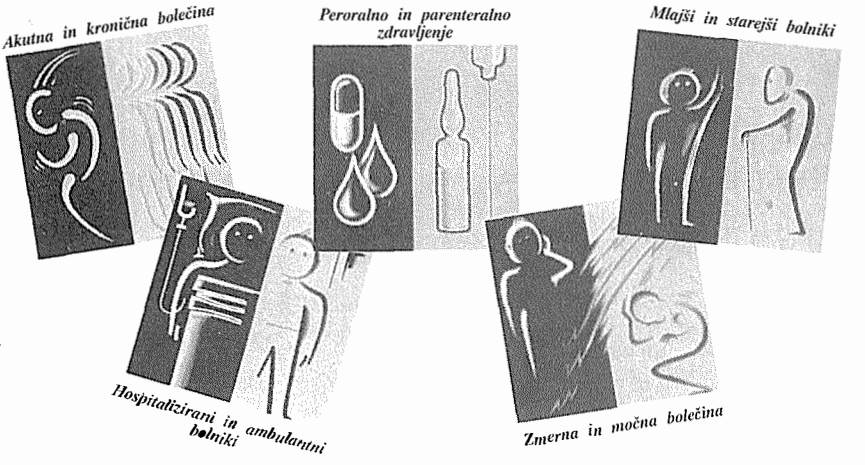

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2. Chapman S, Nakielny R. *A guide to radiological procedures.* London: Bailliere Tindall, 1986.

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

Nepotrebno je, da bolezen spremlja bolečina

Moč opioidnega analgetika brez opioidnih stranskih učinkov

- **centralno delujoči analgetik za lajšanje zmernih in hudih bolečin**
- **učinkovit ob sorazmerno malo stranskih učinkih**

Indikacije: Srednje močne do močne akutne ali kronične bolečine. ***Po tristopenjski shemi Svetovne zdravstvene organizacije za lajšanje bolečin pri bolnikih z rakavim obolenjem tramadol odpravlja srednje hudo bolečino ali bolečino druge stopnje.*** **Kontraindikacije:** Zdravila ne smemo dajati otrokom, mlajšim od 1 leta. Tramadola ne smemo uporabljati pri akutni zastrupitvi z alkoholom, uspavali, analgetiki in drugimi zdravili, ki delujejo na osrednje živčevje. Med nosečnostjo predpišemo tramadol le pri nujni indikaciji. Pri zdravljenju med dojenjem moramo upoštevati, da 0,1 % zdravila prehaja v materino mleko. Pri bolnikih z zvečano občutljivostjo za opiate moramo tramadol uporabljati zelo previdno. Bolnike s krči centralnega izvora moramo med zdravljenjem skrbno nadzorovati. **Interakcije:** Tramadola ne smemo uporabljati skupaj z inhibitorji MA●. Pri sočasni uporabi zdravil, ki delujejo na osrednje živčevje, je možno sinergistično delovanje v obliki povečane sedacije, pa tudi ugodnejšega analgetičnega delovanja. **Opozorila:** Pri predoziranju lahko pride do depresije dihanja. Previdnost je potrebna pri bolnikih, ki so preobčutljivi za opiate, pri starejših osebah, pri miksedemu in hipotiroidizmu. Pri okvari jeter in ledvic je potrebno odmerek zmanjšati. Bolniki med zdravljenjem ne smejo upravljati strojev in motornih vozil. **Doziranje in način uporabe:** ●*drasli in otroci, starejši od 14 let:* Injekcije: 50 do 100 mg i.v.,i.m.,s.c.; intravensko injiciramo počasi ali infundiramo razredčeno v infuzijski raztopini. Kapsule: 1 kapsula z malo tekočine. Kapljice: 20 kapljic z malo tekočine ali na kocki sladkorja; če ni zadovoljivega učinka, dozo ponovimo čez 30 do 60 minut. Svečke: 1 svečka; če ni učinka, dozo ponovimo po 3 do 5 urah. ●*troci od 1 do 14 let:* 1 do 2 mg na kg telesne mase. Dnevna doza pri vseh oblikah ne bi smela biti višja od 400 mg. **Stranski učinki:** Znojenje, vrtoglavica, slabost, bruhanje, suha usta in utrujenost. Redko lahko pride do palpitacij, ortostatske hipotenzije ali kardiovaskularnega kolapsa. Izjemoma se lahko pojavijo konvulzije. **Oprema:** 5 ampul po 1 ml (50 mg/ml), **5 ampul po 2 ml (100 mg/2 ml)**, 10 ml raztopine (100 mg/ml), 20 kapsul po 50 mg, 5 svečk po 100 mg.

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